BioOne COMPLETE

Abstracts for the 3rd International Congress on Stress Responses in Biology and Medicine and the 2nd International Congress of Stress Research, 2007

Source: Cell Stress & Chaperones, 12(2)

Published By: Cell Stress Society International

URL: https://doi.org/10.1379/1466-1268(2007)12[e1:AFTRIC]2.0.CO;2

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Module 1 – Oral lectures:

1A_01_S

HSP27 (HSPB1) AS A THERAPEUTIC TARGET

Arrigo André-Patrick

Stress, Chaperons and Cell Death Laboratory, CGMC, CNRS UMR 5534, Claude Bernard University LYON 1, 16 Rue Dubois, Bat. Gregor Mendel, 69622 Villeurbanne, France, arrigo@univ-lyon1.fr

Human Hsp27(HspB1) is a molecular chaperone which is constitutively expressed in several mammalian cells, particularly in pathological conditions. This protein has functions as diverse as protection against toxicity mediated by aberrantly folded proteins or oxidative-inflammation conditions. In addition, it has anti-apoptotic properties and is tumorigenic when expressed in cancer cells. Hsp27(HspB1) has implications, either positive or deleterious, in pathologies such as neurodegenerative diseases, asthma, and cancers. Moreover, mutations in *hsp27* gene have been detected which are responsive of hereditary motor neuropathies. Hsp27(HspB1) is therefore an active determinant in health and disease and not just a passive storage device. Approaches as well as preliminary results towards therapeutic strategies aimed at modulating the expression and/or the activities of Hsp27(HspB1) will be presented.

1A_02_S

HSPB8 FORMS WITH BAG3 A CHAPERONE COMPLEX STIMULATING MACROAUTOPHAGY: POSSIBLE INVOLVEMENT OF SHSP IN PROTEIN QUALITY CONTROL

Jacques Landry

Centre de recherche en cancérologie de l'Université Laval, L'Hôtel-Dieu de Québec, Québec, Canada, G1R 2J6, jacques.landry@med.ulaval.ca

Intracellular protein aggregation can occur upon proteotoxic stress or genetic mutations and represents a major threat in the crowded environment of the cell. The Hsp70/Hsp90 proteins and their associated co-chaperones form a protein quality control system which can recognize such proteins and either assist them in renaturation or target them for degradation. Proteins from the small Hsp family, and in particular HspB1 (Hsp27) and HspB8, may similarly act as molecular chaperones as evidenced by their association with human diseases featuring protein conformation defects. Diverse types of activities are associated with each of these proteins. On one hand, HspB1 (Hsp27) but not HspB8 potentiates

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

the renaturation of damaged proteins in the cells. In this activity, HspB1 recognizes the substrate and then recruits a renaturating machinery likely involving Hsp70 family members. On the other hand, HspB8, but not HspB1, forms in cells a stable protein complex with Bag3, a Bag family member previously identified as a Hsp70 co-chaperone. In association with Bag3 but independently Bag3 binding to Hsp70, HspB8 efficiently limits the accumulation of aggregation-prone protein substrates by targeting them to destruction by macroautophagy, a lysosome-based protein degradation system capable of degrading large structures and insoluble protein aggregates. In this complex HspB8 likely acts as a molecular chaperone responsible for the recognition the damaged substrates whereas Bag3 is responsible for recruiting and stimulating the macroautophagy machinery. Accordingly, HspB1 engineered to interact with Bag3 can also target unstable proteins to degradation. Hence HspB proteins can, like Hsp70/Hsp90, function in protein quality control by recognizing protein substrates, the fate of which being determined by associated co-chaperones that are specific to individual chaperones.

1A_03_S

RECOVERY OF MACROMOLECULAR SYNTHESIS AFTER STRESS: THE ROLE OF SMALL HEAT SHOCK PROTEIN

Nicolette H. Lubsen, N.Lubsen@science.ru.nl

Cells stressed by heat downregulate protein synthesis. During recovery from heat stress protein synthesis slowly recovers. In the presence of either αB-crystallin or Hsp27, both small heat shock proteins, the rate of recovery is enhanced. Using fluorescence recovery after photobleaching we showed that Hsp27, but not αB-crystallin, increased the pool of mobile stress granule-associated EGFP-eIF4E in heat shocked cells. Hsp27 also partially prevented the sharp decrease in the pool of mobile cytoplasmic EGFP-eIF4G, supporting other evidence for a direct interaction between eIF4G and Hsp27. sHsps did not prevent the phosphorylation of eIF2α by a heat shock, but promoted dephosphorylation during recovery. Blocking the endogenous heat shock response by expressing a dominant negative HSF1 mutant during recovery from a heat shock slows the rate of recovery of protein synthesis and blocks the restorative effect of sHsps, showing that sHsps need to cooperate with other Hsps of which the synthesis is induced by heat shock. Translational recovery 24 hours after heat shock does not differ between cells expressing dnHSF1 and control cells. However, if cells express dnHSF1 as well as the C-terminal fragment of GADD34, which causes constitutive dephosphorylation of eIF2α, translation does not recover. These data show that two partially

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

redundant pathways are involved in translational recovery from a heat shock.

1A_04_S

REGULATION OF SMALL HEAT SHOCK PROTEINS IN AGING AND RESISTANCE TO STRESS

Robert M. Tanguay, Geneviève Morrow, Hyun-Ju Kim, Sébastien Michaud

Lab Cellular Developmental Genetics, CREFSIP, Dept Medecine, Pav. Marchand, Université Laval, Québec, Canada G1K 7P4. E-mail: Robert.tanguay@rsvs.ulaval

Small HSP are involved in the refolding and/or disposal of protein aggregates, a feature of many age-associated diseases. In *Drosophila melanogaster*, there are 4 main small Hsps each residing in a different intracellular compartment. Targeting the expression of the mitochondrial Hsp22, to different cell types can increase lifespan by more than 30%. Long-lived flies expressing Hsp22 in motorneurons have an increased resistance to oxidative stress and maintain their locomotor activity longer. Over expressing the cytosolic Hsp23 in a pan-neuronal fashion (transgenic GAL4/UAS system) also increases longevity by 15%. Conversely, a strain carrying an insertion in the promoter of *hsp23* which downregulates its expression in specific cells of the embryonic CNS and in adults has a decreased lifespan. The action of these chaperones on lifespan likely involves different pathways as suggested by the longevity curves, and their respective site and developmental pattern of expression. Microarrays analysis was used to unveil the mechanisms involved in lifespan. The transcriptional changes brought by Hsp22 overexpression occur early in adulthood and are associated with genes involved in energy metabolism, protein biosynthesis, and protein folding. The relation between the insulin/IGF signaling pathway and the heat shock response has also been examined using flies with mutations in the heat shock factor HSF and dFOXO. Altogether, these results confirm a beneficial role of the expression of small chaperones and corroborate the pivotal role of the nervous system and the insulin/IGF pathway in the ageing process. Supported by CIHR (Canada) and the EU 6th Framework Programme MiMage.

1B_01_S

FUNCTIONAL INTERPLAY AMONG THE MAJOR CHAPERONE MACHINES OF *E. COLI*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Ronald S. Ullers[1], Debbie Ang[1], Françoise Schwager[1], Pierre Genevaux[2], and Costa Georgopoulos[1]

[1]Département de Microbiologie et Médecine Moléculaire, Centre Médical Universitaire, 1, Rue Michel-Servet, CH-1211 Geneva, Switzerland; and [2]Laboratoire de Microbiologie et Génétique Moléculaires, Institut de Biologie Cellulaire et de Génétique, Centre National de la Recherche Scientifique, Université Paul-Sabatier, 118 Route de Narbonne, 31062 Toulouse Cedex 09, France

Polypeptides emerging from the ribosome are assisted by a pool of molecular chaperones and targeting factors, which enable them to efficiently partition as cytoplasmic, integral membrane, or exported proteins. In *Escherichia coli*, the chaperones SecB, Trigger Factor (TF), and DnaK are key players in this process. Here, we report that, as with *dnaK* or *dnaJ* mutants, a *secB* null strain exhibits a strong cold-sensitive (Cs) phenotype. Through suppressor analyses, we found that inactivating mutations in the *tig* gene encoding TF fully relieve both the Cs phenotype and protein aggregation observed in the absence of SecB. This antagonistic effect of TF depends on its ribosome-binding and chaperone activities but unrelated to its peptidyl-prolyl *cis/trans* isomerase's (PPIase) activity. Furthermore, in contrast to the previously known synergistic action of TF and the DnaK/DnaJ chaperone machine above 30°C, a *tig* null mutation partially suppresses the Cs phenotype exhibited by a compromised DnaK/DnaJ chaperone machine. The antagonistic role of TF is further exemplified by the fact that the *secB dnaJ* double mutant is viable only in the absence of TF. Finally, we show that, in the absence of TF, more SecA and ribosomes are associated with the inner membrane, suggesting that the presence of TF directly or indirectly somehow interferes with the process of cotranslational protein targeting to the Sec translocon. Various models to explain our results (occasionally seemingly contradictory) will be offered.

1B_02_S

ROLE OF HSP110 IN THE FUNCTIONAL NETWORK OF HSP70 CHAPERONES

Jocelyne Fiaux, Claes Andréasson, Heike Rampelt, Heather Sadlish, Matthias Mayer and Bernd Bukau

ZMBH, University of Heidelberg, Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany, bukau@zmbh.uni-heidelberg.de

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Eukaryotic cells express Hsp110 proteins that constitute a diverged branch of the Hsp70 molecular chaperone superfamily. Recently, both the yeast Hsp110 member Sse1p and the mammalian orthologue Hsp105 were found to act as potent nucleotide exchange factors for cytosolic Hsp70 chaperones. We set out to characterize the steps of the nucleotide exchange cycle of the yeast Hsp70, Ssa1p, catalyzed by Sse1p using H/D exchange and mass spectrometry. The mechanism was found to involve formation of a stable but nucleotide-sensitive complex. Furthermore, Sse1p itself displays nucleotide binding and hydrolysis and adopts conformations dependent on the nucleotide binding status. Interestingly, nucleotide binding by Sse1p appears to regulate its activity as a nucleotide exchange factor. We also used H/D exchange and mass spectrometry to map the interaction surface of the Sse1p-Ssa1p complex. Based on these results, we can now propose a detailed model for the Sse1p-catalyzed nucleotide exchange cycle.

1B_03_S

NETWORKS OF HSP70 AND J-PROTEIN MOLECULAR CHAPERONES

Elizabeth A. Craig, Alison Meyer and Chandan Sahi

Department of Biochemistry, University of Wisconsin, Madison WI, 53706, USA ecraig@wisc.edu

Highly conserved molecular chaperones function in a wide variety of cellular processes, including protein folding, translocation of proteins across membranes and remodeling of protein complexes. J-proteins are obligate partners of Hsp70s that act via their J-domains to stimulate Hsp70's ATPase activity, stabilizing their interactions with client proteins. In addition many J-proteins contain additional domains, some of which are capable of binding client proteins and allowing J-proteins to "deliver" them to their partner Hsp70. Both Hsp70s and J-proteins are encoded by large multigene families. Our results indicate that certain Hsp70s and J-proteins have evolved to function in specialized cellular processes and/or have "nontraditional" functions. For example, Ssz1 forms a stable heterodimer with the ribosome-associated J-protein Zuo1 and is required for Zuo1's efficient stimulation of the ATPase activity of its partner Hsp70 Ssb. Jjj1, is an example of a specialized J-protein. It plays an important role in the biogenesis of 60S ribosomal subunits, likely facilitating the dissociation of factors from preribosomes to generate subunits competent for translation. In contrast, other J-proteins are multifunctional. The most abundant J-protein of the yeast cytosol, Ydj1, functions in multiple cellular processes. Surprisingly, however, expression of only a J-domain at normal levels is capable of rescuing the severe growth defect caused by the absence of Ydj1. Thus many functions carried out by this highly conserved and

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

complex J-protein require only the capacity to stimulate Hsp70s ATPase activity, not the "delivery" of client proteins.

1B_04_S

HSP110 PROTEIN CHAPERONE FUNCTION IN YEAST

Kevin Morano

University of Texas Medical School, Micro and Molecular Genetics, 6431 Fannin, JFB 1.765, Houston, TX 77030, USA
Kevin.a.morano@uth.tmc.edu

SSE1 and *SSE2* encode the essential yeast members of the Hsp70-related Hsp110 molecular chaperone family. Both mammalian Hsp110 and the Sse proteins functionally interact with cognate cytosolic Hsp70s as nucleotide exchange factors, and in yeast Sse1 is required for Hsp90 chaperoning. We demonstrate that Sse1 forms high affinity heterodimeric complexes with both yeast Ssa and mammalian Hsp70 chaperones, and that ATP binding to Sse1 is required for binding to Hsp70s. The nucleotide binding domains (NBD) of both Sse1/2 and the Hsp70s dictate interaction specificity, and are sufficient to mediate heterodimerization with no discernable contribution from the peptide binding domains (PBD). However, the PBD is required for NEF activity. To better understand the roles of the Hsp110 chaperones, we are investigating the participation of Sse1 in cellular processes requiring Hsp70. To that end, we have generated a novel temperature sensitive allele of SSE1 that should shed light on the essential functions of this intriguing chaperone family.

1C_01_S

MULTI-SITE POST-TRANSLATIONAL MODIFICATIONS AND FUNCTIONAL INTERPLAY OF HSF1 AND HSF2

Lea Sistonen

Turku Centre for Biotechnology and Department of Biology, Åbo Akademi University, BioCity, Tykistökatu 6, 20520 Turku, Finland. Email: lea.sistonen@btk.fi

Heat shock factors, HSFs, are specific transcriptional regulators of heat shock genes encoding heat shock proteins, Hsps, that function as molecular chaperones in protecting cells against proteotoxic stress. The

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

activity of HSFs is under stringent, mainly post-translational control, e.g. acetylation, phosphorylation, sumoylation, and ubiquitylation. Among the functional domains of HSFs, the amino-terminal helix-turn-helix DNA-binding domain is the most conserved and it also designates membership to the HSF family. The activation-induced trimeric assembly of HSFs is mediated by hydrophobic heptad repeats and is unusual, as proteins containing leucine zippers often form dimers. Four HSFs have been identified in vertebrates, HSF1 and HSF2 being ubiquitously expressed and well conserved throughout evolution, whereas HSF3 has been found only in avian species and HSF4 only in mammals. The different members of the mammalian HSF family have been considered to be functionally distinct; HSF1 is essential for the heat shock response, whereas HSF2 and HSF4 are refractory to stress stimuli but are important for differentiation and development, including corticogenesis, spermatogenesis, and maintenance of sensory organs, e.g. lens and olfactory epithelium. We have, however, evidence for a functional interplay between HSF1 and HSF2. These factors can interact through their trimerization domains, and in response to stress, they can bind to hsp promoters as well as to satellite III repeats at locus 9q12. In both cases, an intact HSF1 is required, suggesting that HSF1 influences the DNA-binding activity of HSF2. At the transcriptional level, HSF2 is able to modulate HSF1-mediated expression of the target genes in a gene-specific manner.

1C_02_S

HSF1 ACTIVATION AND THE CONTROL OF APOPTOSIS IN CHEMORESISTANT CANCERS

G. Belardo, A. Rossi, S. Ciafre', A. Ciucci, P. Gianferretti, S. Roberts and M.G. Santoro

Department of Biology, University of Rome Tor Vergata and Institute of Neurobiology and Molecular Medicine, CNR, Rome, Italy; Department of Chemistry, University of Manchester, Manchester, UK.

Activation of the heat shock response (HSR) via HSF1 contributes to preserve cellular function and homeostasis under stress conditions, and to establish a cytoprotective state in several human diseases. In cancer, however, HSR activation has been associated with both anti- and pro-apoptotic responses. Heat-induced expression of cytoprotective and antiapoptotic heat shock proteins (HSP) is a known complication of hyperthermia, resulting in cancer cell thermotolerance and chemoresistance. In some instances, however, HSF1 activation may result in apoptosis induction. We have developed a library of novel potent inducers of HSF1 which are characterized by pro-apoptotic activity in several types of chemoresistant cancers. We have previously shown that

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

HSF1 induction prevents TNFα- and mitogen-induced activation of NF-κB, a nuclear factor playing an important role in promoting inflammation, as well as cell proliferation and survival. NF-κB has been found to be constitutively activated in several types of chemoresistant cancers where it suppresses cell death pathways by switching on genes that dampen pro-apoptotic signals. We now show that activation of HSF1 by diverse chemical inducers, as well as by hyperthermia itself, results in inhibition of constitutive NF-κB activity and rapid down-regulation of the expression of NF-κB-dependent survival genes, triggering apoptosis in aggressive cancers presenting aberrant NF-κB regulation. The results suggest that the block of anti-apoptotic signaling pathways utilizing the IκB kinase IKK may play an important role in modulating HSR pro-apoptotic effects in chemoresistant cancers.

1C_03_S

ROLES OF HSF1 IN INFLAMMATORY AND IMMUNE RESPONSE

Akira Nakai

Department of Biochemistry and Molecular Biology, Yamaguchi University School of Medicine, Ube, Japan, e-mail: anakai@yamaguchi-u.ac.jp

Inflammatory cytokines such as IL-1, IL-6, and TNF-α are induced in response to bacterial infection and diseases. These cytokines elicit the febrile response that is a complex physiological reaction to disease including cytokine-mediated rise in body temperature and activation of inflammatory systems. Fever plays beneficial roles on disease prognosis clinically. Experimentally, pretreatment of animals with heat shock also increases survival in a LPS-injected endotoxic model. These beneficial roles of fever are mediated partly by suppressing pyrogenic and inflammatory cytokines as expression of TNF-α, IL-1β, and IL-6 reduces in heat-shocked cells and in whole body exposed to high temperature. It was shown that HSF1 inhibits expression of cytokines by binding directly to TNF-α promoter, or by physically interacting with NF-IL6, an activator for IL-1β. However, molecular mechanisms underlining fever-mediated suppression of cytokine gene expression are uncovered yet. Here we show that heat shock suppresses LPS-induced induction of IL-6 by activating HSF1 that induces ATF3, a negative regulator of IL-6. In vivo analysis using HSF1-null and ATF3-null mice reveals that HSF1 as well as ATF3 acts as a negative regulator of IL-6 expression in whole body and is required to inhibit fever. Unexpectedly, overexpression of ATF3 into cells has no effect on IL-6 expression in the absence of HSF1. HSF1 also binds directly to IL-6 promoter, and is required not only for a repressor ATF3, but also an activator NF-kB to bind to IL-6 promoter by partially opening

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

chromatin structure. Taken together with the effects on expression of TNF-α and IL-1β, these results indicate that HSF1 plays a major role in feedback regulation of the febrile response.

1C_04_S

HSF2 INFLUENCES THE DECISION BETWEEN PROLIFERATION AND MIGRATION FOR NEURAL CORTICAL PROGENITORS

Diane Trouillet, Anne Le Mouël, Rachid El Fatimy, Laurence Denis and Valérie Mezger

CNRS UMR8541, Ecole Normale Supérieure, 46 rue d'Ulm 75005 Paris

Heat Shock Factors (HSFs) are not only responsible for response to environmental stress, but are involved in developmental processes. We showed in collaboration with Lea Sistonen's lab that HSF2 is involved in meiosis in both genders and in brain development (Kallio et al 2002; Chang et al 2006). We will describe how HSF2 influences the radial migration of young postmitotic neurons during cortical development, but also the proliferation of their neural progenitors (NPCs), using loss and gain of function models : *Hsf2*$^{-/-}$ mice and the overexpression of HSF2 in the chick neural tube, by *in ovo* electroporation. The identification of new HSF2 target genes suggests us that HSF2 directly regulates genes that are involved in the control of microtubule dynamics, either in mitosis or during migration. The question is how HSF2, which is active in NPCs as well as in migrating postmitotic neurons, might differentially regulate distinct pools of target genes in these cell populations. The ability of HSF2 to differentially recruit transcription factors and chromatin modifiers as well as its distinct biochemical properties in NPCs versus postmitotic neurons has been investigated by biochemical and chromatin immunoprecipitation (ChIP) analyses and will be discussed. Such a combination of events and properties, coupled with the geography of HSF2 binding sites and of other transcription factors sites –characteristic of a given target gene – should allow HSF2 to induce or repress transcription and therefore to allow the decision for a NPC to continue to divide or to exit the cell cycle and start to migrate. Grant supports : ARC (Association pour la Recherche contre le Cancer) and ANR Neurosciences (Agence nationale pour la Recherche).

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1D_01_S

HSP RELEASE: PASSIVE VS ACTIVE RELEASE MECHANISMS

Alexzander Asea
Division of Investigative Pathology, Scott & White Clinic and Texas A&M University System Health Science Center College of Medicine, 2401 South 31st Street, Temple, TX 76508 United States
E-mail: asea@medicine.tamhsc.edu

Thus far, two mechanisms are recognized by which heat shock proteins (HSP) are released from cells into the extracellular milieu and subsequently into systemic circulation; a passive release mechanism as a result of necrotic cell death, severe blunt trauma, surgery and following infection with lytic viruses, and an active release mechanism which involves the non classical protein release pathway by which HSP70 is released as free HSP72 and within highly immunologically potent exosomes. This presentation covers the most recent findings on the mechanisms by which stress induces the release of HSP72 into the systemic circulation and addresses the biological significance of circulating HSP72 to host defense against disease.

1D_02_S

HSP70 SECRETION AND BINDING: ITS PLACE IN THE IMMUNE RESPONSE

Stuart K. Calderwood, Salamatu S Mambula, Jimmy Theriault and Jianlin Gong

Beth Israel Deaconess Medical center, Harvard Medical School and Center for Molecular Stress Response, Boston University Medical School.

Hsp70 plays a significant extracellular role within the immune response and can carry out both pro-inflammatory / pro-immune functions and anti-inflammatory effects depending on cellular and tissue context. However, understanding its place in the immune response requires deciphering the mechanisms by which it is released and the cell surface structures which it binds on target cells in the immune system.
We have examined mechanisms of Hsp70 release from tumor cells and macrophages and have found evidence for an active secretion mechanism. Our experiments indicate that hsp70 secretion employs a similar pathway to that used by other "leaderless" proteins such as interleukin 1β (IL-1β) involving the entry of Hsp70 into secretory lysosomes and ATP dependent release. Our evidence suggest however, that the trigger for hsp70 release

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

is different from that which releases IL-1β: for instance, in macrophages exposed to *E. coli*, the trigger for IL-1β release is lipopolysaccharide, while Hsp70 release is triggered by a different signal. In addition, IL-1β release is independent of Hsp70 release and Hsp70 blocking does not alter release IL-1β.
Hsp70 released from cells binds to adjacent cells. Our experiments indicate that such receptors may include a diverse group of structures. Hsp70 signaling can be mediated by Toll Like receptors (TLR), CD40 and LRP/CD91. However, our recent studies indicate that Hsp70 internalization is mediated by scavenger receptors (SR) found on antigen presenting cells. We have found that at least 3 members of the SR including LOX-1, SREC-1 and FEEL-1/CLEVER-1 can bind and internalize Hsp70. As the SR play a profound role in the "cross presentation" of extracellular proteins to CD8+ T lymphocytes, these experiments suggest a pathway along which secreted Hsp70 with chaperoned peptide antigens can be taken up by APC and interact with immune cells.

1D_03_S

EXTRACELLULAR HEAT SHOCK PROTEINS: SEARCHING FOR A ROLE?

John H.H. Williams

Chester Centre for Stress Research, Department of Biological Sciences, University of Chester, Parkgate Road, Chester, CH1 4BJ, UK. E-mail: John.Williams@chester.ac.uk

Hsp70 can be detected in serum, despite being an intra-cellular protein. Hsp70 must therefore be released by damaged cells, or secreted by healthy cells. We have previously demonstrated that Hsp70 and Hsp60 are released from cells. An increasing number of cell types, including peripheral blood mononuclear cells (PBMCs), have been demonstrated to release Hsps, including Hsp70, Hsp60 and Hsp90.
This release of Hsp is stimulated, *in vivo* and *in vitro*, by a wide variety of stressors. Several cell types respond to extracellular Hsp exposure by releasing cytokines, such as TNFα. The type and source (human or bacterial) of Hsp alter the cytokine response. Extracellular Hsp70 protects cells from heat shock without needing to enter the cells. It is unclear whether the interaction of these extracellular hsps with the membrane is directly with lipid or protein.
The aims of this talk are to discuss:

- routes of Hsp release, and through this challenge the paradigm that only necrotic cells release Hsps.
- how extracellular Hsps interact with cells, and through this

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

investigate their potential roles: as danger signals and as cell protectors.

1D_04_S

QUALITY CONTROL OF EXTRACELLULAR PROTEIN FOLDING: AN EMERGING FIELD

Justin J Yerbury, Amy R Wyatt, Stephen Poon and Mark R Wilson

School of Biological Sciences, University of Wollongong, Northfields Avenue, Wollongong. NSW. 2522. Australia.

Many serious human diseases are thought to arise from the effects of intra- or extracellular aggregates of proteins with non-native conformations. Inside cells, chaperone and protease systems regulate protein folding; however, little is currently known about any corresponding mechanisms that operate extracellularly. Knowledge of these mechanisms is important because it may lead to the development of new disease therapies. We have identified a small family of abundant human plasma proteins which have a potent small heat shock protein-like chaperone action. These proteins occur in plasma at levels of 0.1-2.0 mg/ml and thus in this locale are orders of magnitude more abundant than normally intracellular chaperones (e.g. Hsp70) found extracellularly[1]. The abundant extracellular chaperones (ECs) form stable soluble complexes with misfolded and partially unfolded proteins to inhibit amorphous protein aggregation induced by physical or chemical stresses. The ECs also exert potent effects on amyloid formation and toxicity[2]. Recent data suggests that complexes formed between ECs and "damaged" (misfolded/unfolded) proteins are rapidly bound by cell surface receptors *in vivo* and disposed of by receptor-mediated endocytosis and lysosomal degradation. Thus, a model is emerging in which ECs patrol extracellular spaces, bind to damaged proteins when present and mediate their rapid clearance and disposal. Disease pathologies associated with protein aggregation may arise when this system is overloaded.

1. Yerbury, J., Wyatt, A., Stewart, E., and Wilson, M. R. (2005). EMBO Rep. *6*, 1131-1136.
2. Yerbury, J., Poon, S., Meehan, S., Thompson, B., Kumita, J., Dobson, C., and Wilson, M. R. (2007). FASEB J. (published on-line April 5, 2007)

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Module 1 – Poster lectures:

1A_01_P

INHIBITION OF APOPTOTIC CELL DEATH BY A NOVEL 16.2 KD HEAT SHOCK PROTEIN VIA HSP90 MEDIATED LIPID RAFTS STABILIZATION AND AKT ACTIVATION PATHWAY

Balazs Sumegi[1], Szabolcs Bellyei[1,2], Eva Pozsgai[1], Andras Szigeti[1,2], Arpad Boronkai[1,2], Ferenc Gallyas Jr.[1]

Departments of Biochemistry and Medical Chemistry[1] , Oncotherapy[2], University of Pécs, Pécs, Hungary
Correspondence to: Balazs Sumegi, PhD, DSci, Department of Biochemistry and Medical Chemistry, University of Pécs, 12 Szigeti Street, Pécs H-7624, Hungary. Tel: +36-72-536-276 Fax36-72-536-277 e-mail: balazs.sumegi@aok.pte.hu

AlphaB-crystallin homology, heat stress induction and chaperone activity suggested that a previously encloned gene product is a novel small heat shock protein (Hsp16.2). Suppression of Hsp16.2 by siRNA sensitized cells to hydrogen peroxide or taxol induced cell-death. While over-expressing of Hsp16.2 protected cells against stress stimuli by inhibiting cytochrome c release from the mitochondria, nuclear translocation of AIF and endonuclease G, and caspase 3 activation. Recombinant Hsp16.2 protected mitochondrial membrane potential against calcium induced collapse *in vitro* indicating that Hsp16.2 stabilizes mitochondrial membrane systems. Hsp16.2 formed self-aggregates and bound to Hsp90. Inhibition of Hsp90 by geldanamycin diminished the cytoprotective effect of Hsp16.2 indicating that this effect was Hsp90-mediated. Hsp16.2 over-expression increased lipid rafts formation as demonstrated by increased cell surface labeling with fluorescent cholera toxin B, and increased Akt phosphorylation. The inhibition of PI-3-kinase-Akt pathway by LY-294002 or wortmannin significantly decreased the protective effect of the Hsp16.2. These data indicate that the over-expression of Hsp16.2 inhibits cell death via the stabilization of mitochondrial membrane system, activation of Hsp90, stabilization of lipid rafts and by the activation of PI-3-kinase - Akt cytoprotective pathway.

1A_02_P

HSPB8 AND BAG3: A NEW CHAPERONE COMPLEX STIMULATING MUTATED HUNTINGTIN DEGRADATION BY MACROAUTOPHAGY

Carra S * (1), Seguin S (1), Vinet J (2), Lambert H (1), Sik A (2) and Jacques Landry (1)

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

(1) Centre de recherche L'Hôtel-Dieu de Québec, Université Laval, 9 rue McMahon, Québec, Canada; jacques.landry@med.ulaval.ca
(2) Centre de recherche Robert-Giffard, 2601 Chemin de la Canardière, Québec, Canada

HspB8 is a member of the small heat shock proteins or HspB family of molecular chaperones, which comprises ten members in mammals (HspB1-10). We previously demonstrated that, in cultured cells, overexpression of HspB8, but not of HspB1 or HspB5, totally blocked the insolubilization and accumulation of a pathogenic aggregation-prone form of Huntingtin (Htt43Q). Here we report that HspB8 stably and sotichiometrically interacts with the co-chaperone Bag3. HspB8 association with Bag3 is essential for both its structural stability and chaperone function, as demonstrated by knocking down Bag3 expression by siRNA technique. We next investigated the chaperone function of the HspB8/Bag3 complex. We found that Bag3 overexpression, like HspB8, facilitated Htt43Q degradation in cultured cells. Treatment with specific macroautophagy inhibitors dramatically decreased the HspB8/Bag3 chaperone activity and lead to the accumulation of aggregated Htt43Q. Moreover, HspB8 and Bag3 both increased the number of cells containing LC3 positive-vacuoles and stimulated the lipidation of LC3, a step which is necessary for LC3 incorporation into the autophagosomes. These results strongly suggest the implication of the macroautophagy process in the HspB8/Bag3 complex mechanism of action. By joining the ability of recognizing endogenous misfolded proteins and of stimulating the macroautophagic vacuole formation, the new HspB8/Bag3 chaperone complex may play a crucial role in the protein quality control system responsible for eliminating potentially harmful aggregating proteins.

1A_03_P

PHOSPHORYLATION OF HUMAN SMALL HEAT SHOCK PROTEIN HSP22 BY CAMP-DEPENDENT PROTEIN KINASE

Anton A. Shemetov, Ivan S. Chernik, Alim S. Seit-Nebi, and Nikolai B. Gusev

Department of Biochemistry, School of Biology, Moscow State University, Moscow 119992, Russia

The data of literature (Benndorf et al J. Biol. Chem.276, 26753, 2001) indicate that small heat shock protein with molecular mass 22 kDa (HSP22, HspB8 or H11 kinase) can be phosphorylated by protein kinase C and p44 MAP kinase. Ser24 and Ser57 of human HSP22 are located in consensus sequence RXS recognized by cAMP-dependent protein kinase

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

and we supposed that this enzyme could also be involved in HSP22 phosphorylation. To check this suggestion we obtained three mutants (S24D, S57D, S24,57D) of HSP22 and analyzed phosphorylation of the wild type protein and these mutants by cAMP-dependent protein kinase. Wild type HSP22 and its S24D mutants were rapidly phosphorylated up to 1 mole of phosphate per mole of protein, whereas S57D and S24,57D mutants were not phosphorylated by cAMP-dependent protein kinase. These data indicate that S57D is the primary site phosphorylated by cAMP-dependent protein kinase. Phosphorylation (or mutations mimicking phosphorylation) does not affect the oligomeric structure of HSP22 analyzed by size-exclusion chromatography and has no effect on chemical crosslinking of HSP22. At the same time mutations mimicking phosphorylation affect accessibility of Trp residues to solvent and increase susceptibility of HSP22 to chymotrypsinolysis. Mutations (or phosphorylation) decrease chaperone-like activity of HSP22 if insulin or rhodanase were used as model protein substrates. It is concluded that HSP22 can be phosphorylated by cAMP-dependent protein kinase and phosphorylation of Ser residues located in the N-terminal domain might affect its structure and chaperone-like activity.
Acknowledgement. This investigation was supported by Russian Foundation of Basic Science.

1A_04_P

NEW IDEAS ON MECHANISM OF PROTEIN AGGREGATION AND MECHANISM OF PROTECTIVE ACTION OF α-CRYSTALLIN

K.A. Markossian and B.I. Kurganov

Bach Institute of Biochemistry, Russian Academy of Sciences, 119071 Moscow, Leninsky pr. 33 e-mail: markossian@inbi.ras.ru, boris@kurganov.com

The kinetics of thermal aggregation of proteins (β-crystallin from calf eye lens, glyceraldehyde-3-phosphate dehydrogenase from rabbit skeletal muscle and mitochondrial aspartate aminotransferase from porcine heart) were studied by dynamic light scattering. On the basis of the study of aggregation kinetics a new mechanism of protein aggregation was developed. The first stage of protein aggregation is the stage of the formation of the start aggregates. The size of the start aggregates was estimated by construction of the light scattering intensity *versus* hydrodynamic radius plots. The hydrodynamic radius of the start aggregates remains constant at variation of temperature and the protein concentration. The second stage of the aggregation process is sticking together of the start aggregates. This stage proceeds in the kinetic regime

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

wherein the rate of aggregation is limited by diffusion of the particles (the regime of "diffusion-limited cluster-cluster aggregation"). Under this regime each collision of the interacting particles results in their sticking together. Suppression of thermal aggregation of proteins in the presence of α-crystallin is due to diminishing of the size of the start aggregates, increase in the duration of the latent period over which the start aggregates are formed, and transition of the aggregation process into the kinetic regime wherein the sticking probability for the colliding particles becomes less than unity (the regime of "reaction-limited cluster-cluster aggregation").

The study was funded by the Russian Foundation for Basic Research (grants 05-04-48691-a and 06-04-39008-a), Program "Molecular and Cell Biology" of the Presidium of the Russian Academy of Sciences.

1A_05_P

MODULATION OF THE CHAPERONE-LIKE FUNCTION OF SMALL HEAT SHOCK PROTEINS BY METHYLGLYOXAL

Ram H. Nagaraj, Ashis Biswas, Manjunatha Bhat. Case Western Reserve

University and Cleveland Clinic Foundation, Cleveland, OH, USA

Alpha-crystallin and Hsp27 belong to the family of small heat shock proteins. They are stress proteins and play an important role in preventing protein aggregation and cell death by external stress. Methylglyoxal is an ubiquitous alpha-dicarbonyl compound produced from the triose phosphate intermediates of glycolysis. It reacts rapidly with arginine, cysteine and lysine residues in proteins and chemically modifies them to form stable adducts. We have studied the effect of methylglyoxal modification on the chaperone-like function of alpha-crystallin and Hsp27. Methylglyoxal modification of these two stress proteins increased their chaperone function on a concentration-dependent manner. Specific arginine modification to argpyrimidine was found to be responsible for the enhanced chaperone function. Site-directed mutagenesis of methylglyoxal-modifiable arginine residues to alanine mirrored the effects of methylglyoxal. Introduction of additional guanidino groups abolished the chaperone-like function of alphaA-crystallin but subsequent modification by methylglyoxal not only revived the chaperone-like function but also made it better than the unmodified protein. Modification of both substrate proteins and alphaA-crystallin by methylglyoxal further enhanced resistance to protein aggregation by thermal and chemical stress. These results suggest that physiological dicarbonyl methylglyoxal promotes the chaperone function of alphacrystallin and Hsp27 and prevents aggregation of proteins and thus may play a vital role in cell

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

response to stress in health and disease.

1A_06_P

TRANSDUCED TAT-HSP40 PROTECTS CELLS AGAINST OXIDATIVE STRESS

Jung-Hoon Yoon[1], Soo-A Kim[2] and Sang-Gun Ahn[1,*]

[1]Oral Biology Research Institute, BK21 projects, Department of Pathology, Chosun University College of Dentistry, Gwangju, KOREA, [2]Department of Biochemistry, Dongguk University College of Oriental Medicine, Gyeongju, KOREA
** corresponding author*

Abstract
Heat shock protein (Hsp) 40 acts as a co-chaperone of Hsp70 by facilitating the ATPase activity of Hsp70 as well as promoting the protein folding and renaturation by Hsp70. In the present study, Hsp40 gene was fused with a gene fragment encoding the HIV-1 TAT protein transduction domain (YGRKKRRQRRR) in a bacterial expression vector pTAT-HA to produce the TAT-fused Hsp40 (TAT-Hsp40). Purified TAT-Hsp40 was effectively transduced into the HEK 293 cells in a time- and dose-dependent manner. To examine the effect of TAT-Hsp40 upon oxidative stress, HEK293 cells were exposed to H_2O_2. Oxidative stress induced the rapid increase of proteasome activity followed by cell death in HEK 293 cells. However, HEK 293 cells transduced by TAT-Hsp40 showed resistance against oxidative stress-induced cytotoxicity. TAT-Hsp40 transduced cells showed decreased proteasome activity and inhibited Hsp70 degradation. These results suggest that Hsp40 might protect cell death from oxidative stress by preserving the cellular level of Hsp70.

Keywords: Hsp40, Hsp70, TAT, proteasome, oxidative stress

1A_07_P

EFFECT OF ALPHA-CRYSTALLIN ON THERMAL DENATURATION AND AGGREGATION OF MITOCHONDRIAL ASPARTATE AMINOTRANSFERASE

N.V. Golub, K.A. Markossian

Bach Institute of Biochemistry RAS, 33 Leninsky av., Moscow, 119071, Russia (ngolub@inbi.ras.ru)

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Thermal denaturation and aggregation of mitochondrial aspartate aminotransferase have been investigated by differential scanning calorimetry (DSC) and dynamic light scattering. The dependence of excess heat capacity of AAT on temperature passes through a maximum at 72.4 degrees C and can be described by a scheme in which the denaturation process is considered as an irreversible first-order reaction. Inactivation of AAT follows the exponential law. The parameters of Arrhenius equation have been determined from the DSC data and temperature dependence of the inactivation rate constant. Calculations show that at 57.5 degrees C the inactivation constant (k_{in}) is 28.9 times higher than the denaturation rate constant (k_{den}) determined from the DSC data. The k_{in}/k_{den} ratio decreases with temperature and becomes equal to 1.3 at 77 degrees C. It has been shown that alpha-crystallin, the protein possessing a chaperone-like activity, reduces thermostability of AAT and accelerates thermoinactivation of the enzyme. Suppression of thermal aggregation of AAT in the presence of alpha-crystallin has been demonstrated. The protective effect of alpha-crystallin is due to the decrease in the size of the start aggregates and transition of the aggregation process from the regime of diffusion-limited cluster-cluster aggregation (the sticking probability for the colliding particles is equal to unity) to the regime of reaction-limited cluster-cluster aggregation (the sticking probability for the colliding particles is less than unity). The study was supported by the Russian Foundation for Basic Research (grants 05-04-48691-a and 06-04-39008), Program "Molecular and Cell Biology" of the Presidium of the Russian Academy of Sciences and INTAS (grant 03-51-4813).

1A_08_P

HSP26 FROM *SACCHAROMYCES CEREVISIAE* IS A POLYDISPERSE SMALL HEAT-SHOCK PROTEIN.

JA Aquilina, JLP Benesch, RA Lindner, A Rekas, E Vierling, CV Robinson, JA Carver

University of Wollongong, Northfields Avenue, Wollongong, NSW, Australia 2522, email: aquilina@uow.edu.au

Here we report investigations into the oligomeric organization and dynamics of the *Saccharomyces cerevisiae* small heat-shock protein *Sc*HSP26. Using both multi-angle light scattering and mass spectrometry (MS) approaches we have shown that at ambient temperature this protein is exists as a polydisperse assembly, comprising numerous oligomeric states. Relative quantification of these oligomeric states using a tandem MS technique demonstrated the 24mer to be anomalously abundant, consistent with previous reports which have culminated in a 3D structure

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

for this oligomeric state. Significantly, we demonstrate for the first time, that *Sc*HSP26 also forms larger oligomers, up to 40 subunits in size at room temperature. The oligomers observed were exclusively composed of an even number of subunits. This strongly suggests there to be a basic dimeric 'building block', an observation consistent with the 3D structure of the *Sc*HSP26 24mer, as well as studies performed on sHSPs from bacteria, plants and mammals. Using an online thermo-controlled device we investigated the effect of heat stress on the oligomeric structure of *Sc*HSP26. We found that as the temperature was increased, dissociation of the oligomers into dimers and monomers was observed. Such dissociation has previously been observed for *Sc*HSP26 and other sHSPs. The remaining *Sc*HSP26 existed in forms 32 subunits or larger, with the majority having assembled into a 40mer. There is a possibility that these larger forms are unusually resistant to thermal stress, or *Sc*HSP26 preferentially associates into these forms at elevated temperatures.

1A_09_P

TWO SMALL HEAT SHOCK PROTEINS OF A FISSION YEAST FUNCTION IN DIFFERENT MANNER TO COPE WITH WIDE RANGE OF TEMPERATURES AND VARIOUS DENATURED PROTEINS

Masafumi Yohda[1*], Chika Sugino[1], Maya Hirose[1], Ryo Iizuka[1], Masafumi Shimizu[2], Shun-ichi Kidokoro[3], Noriyuki Ishii[4],

[1]Tokyo University of Agriculture and Technology, Koganei, Tokyo, [3]Tokyo University of Technology, Hachioji, Tokyo, [4]Nagaoka University of Technology, Nagaoka-shi, Niigata, [5]National Institute of Advanced Industrial Science and Technology, Tsukuba-shi, Ibaraki, Japan
[*]Corresponding author, yohda@cc.tuat.ac.jp

There exist two small heat shock proteins (SpHsp15.8 and SpHsp16.0) in the fission yeast, *Schizosaccharomyces pombe* (*S. pombe*). At the elevated temperatures, both SpHsp15.8 and SpHsp16.0 dissociate into small oligomers and then interact with denatured substrate proteins. SpHsp16.0 exhibited clear enthalpy change for denaturation at over 60 ºC in differential scanning calorimetry. In addition, there was another small enthalpy change at about 50 ºC, which is likely to correspond to oligomer dissociation. The oligomer dissociation and interaction with denatured protein of SpHsp15.8 and SpHsp16.0 were analyzed by fluorescence polarization analysis (FPA). Both sHsps exhibited temperature dependent decrease of fluorescence polarization, which correlates with the dissociation of large oligomers to small oligomers. The dissociation of SpHsp15.8 oligomer started to occur at about 35 ºC and proceeds gradually. On the contrary, SpHsp16.0 oligomer was stable up to about 45 ºC, but dissociate to small oligomers abruptly at the temperature.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Interaction between sHsps and denatured CS at the elevated temperature was also examined by FPA. Interestingly, SpHsp16.0 is likely to interact with denatured CS in the dissociated state, but SpHsp15.8 in the large complex in contrast. The results suggest that *S. pombe*, utilizes two sHsps to cope with wide range of temperature and various denatured proteins.

1B_01_P

THE PAM18/TIM14-PAM16/TIM16 COMPLEX OF THE MITOCHONDRIAL TRANSLOCATION MOTOR: THE FORMATION OF A STABLE COMPLEX FROM MARGINALLY STABLE PROTEINS

Ohad Iosefson, Abdussalam Azem, Ran Levy, Milit Marom, Olga Slutsky-Leiderman

Department of Biochemistry, Tel Aviv University, Tel Aviv, Israel

The majority of the mitochondrial proteins encoded in the nucleus, synthesized in the cytosol and transported into the mitochondria. The presequence translocase associated import motor is required in order to import matrix-targeted proteins across the mitochondrial inner membrane. This transport machinery is composed of the following constituents: mitochondrial Hsp70, the nucleotide exchange cofactor-GrpE, Tim44, Tim14 and Tim16. All of these proteins are essential to the eukaryotic cell viability. However, in order to shed light on the accurate cooperation of this intriguing motor, in-vitro analysis of the purified components needs to be done. Therefore, our work is focused on in-vitro characterization of the structure-function relationship of the two motor constituents: Tim14 and Tim16. We characterized the interaction between these proteins using two different methods: fluorescence polarization and heat thermal denaturation. In order to examine the stability of the Tim14-Tim16 complex by plotting their heat thermal denaturation curve Circular dichroism spectrophotometer was used. In addition, by using fluorescence polarization approach, we measured the affinity of these two proteins and determined the dissociation constant (K_d) of their complex.

1B_02_P

HSP70 IS DIFFERENTIALLY EXPRESSED IN CATTLE AND SHEEP LEUKOCYTES

Linda L Agnew, Ian G Colditz

CSIRO Livestock Industries, Armidale, NSW, Australia

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Flow cytometry is a rapid and quantitative method of determining intracellular heat shock protein (hsp) 70 expression in individual cells from a heterogeneous population such as peripheral blood mononuclear cells. Hsp70 expression is induced *in vivo* in a range of leukocyte types in response to a variety of stressors. The application of a mild, transient, non-lethal, *in vitro* heat shock is a technique that allows the researcher to examine the cellular stress response which is a highly conserved defence mechanism. Thus constitutive and in vitro induced leukocyte hsp70 expression may be a useful indicator of an organism's adaptation to environmental/physiological stress. In the current studies, flow cytometric methods were used to demonstrate that hsp70 is constitutively expressed in ovine and bovine leukocytes but that the level of expression varies considerably between different leukocyte types and between species. The optimum temperature for heat shock of leukocytes from sheep and cattle without a loss of cell viability is 43.5°C. In sheep, the magnitude of upregulation of hsp70 expression by in vitro heat shock was CD14+ cells > $\gamma\delta$ T cells > neutrophils > B cells > CD4 = CD8. A similar ranking was observed in cattle. Best results were obtained from fresh samples; after storage at room temperature for 24 hours upregulation was highly variable between animals and less than in fresh samples. These studies demonstrate that evaluation of leukocyte hep70 expression by flow cytometry is a robust, reproducible method for use in the evaluation of stress responses in livestock species.

1B_03_P

EVOLUTION AND DIVERSITY OF HUMAN HSP70: IMPLICATIONS FOR HEALTH AND DISEASE

Luciano Brocchieri[1], Everly Conway de Macario[2], Alberto J. L. Macario[2]

[1]University of Florida, Department of Molecular Genetics and Microbiology and UF Genetics Institute, Gainesville, FL, USA; [2]Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD, USA.

Defective chaperones are implicated in disease, the chaperonopathies, and senescence. Defective chaperone identification and elucidation of pathogenetic role in disease and ageing requires full gene identification in the genome; characterization of genes and proteins *in silico*, *in vitro* and *in vivo*; and clinicopathological studies. We applied a series of complementary bioinformatics and evolutionary methods (chaperonomics) to the study of human Hsp70, and identified 47 loci encoding Hsp70-like sequences: 16 were functional genes, encoding proteins conserved at the

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

N-terminal but not at the C-terminal domain, of which eight encoded typical Hsp70s (65-80kDa) with nucleotide-binding and substrate-binding domains, two encoded products lacking most or all of the C-terminal domain, and six encoded heavier proteins ($\geq$ 81 kDa) with atypical C-terminal domains. Also, 31 *hsp70*-related pseudogenes were identified. Evolutionary analyses showed that ER-residing Hsps evolved by duplication while the six typical genes whose products reside in the cytosol/nucleus, and the majority of the pseudogenes, originated from retrotransposition of one gene, HSPA8. These evolutionary processes generated a group of genes with wide diversity further amplified by the occurrence of multiple mRNA variants and protein isoforms. The variety of the Hsp70 proteins is reflected in the diversity of their patterns of expression and localization in an assortment of tissues, cell types and sub-cellular compartments, through the stages of development and ageing. Consequently, the pathogenetic impact of defective Hsp70s should be widespread.

1B_04_P

LEPTIN AND CERULENIN DIFFERENTLY REGULATE HEAT-SHOCK PROTEIN-70 (HSP-70) GENE EXPRESSION IN CHICKEN TISSUES

Denise Figueiredo, Arieh Gertler, Eddy Decuypere, Johan Buyse, Gérard Cabello, Sami Dridi*

Catholic University of Leuven, 3001 Heverlee, Belgium
sami.dridi@biw.kuleuven.be

Lines of evidence suggested that systems involved in the regulation of the stress responses and of energy homeostasis are highly integrated. Since leptin and cerulenin (the natural fatty acid synthesis inhibitor) have been shown to affect food intake and energy homeostasis, and since the stress biormarker Hsp-70 gene was found to interact directly with fatty acids, we hypothesized that leptin or cerulenin may regulate Hsp-70 gene expression. Therefore, the present study was undertaken to examine this issue.
Leptin and Cerulenin significantly ($P<0.05$) decreased food intake in broiler chickens. Leptin significantly down regulated HSP-70 mRNA levels in chicken liver and hypothalamus, but not in muscle. In contrast, cerulenin significantly induced Hsp-70 gene expression in chicken muscle, but not in liver or hypothalamus. These results indicated that the regulation of Hsp-70 gene expression in normal chickens, as estimated by oxidative stress indices [thiobarbituric acid reacting substances (TBARS), ferric reducing/antioxidant power (FRAP), and Caeruloplasmin oxidase activity] levels, is tissue-specific. In attempt to discriminate between the effect of these products and the effect related to food intake reduction on

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Hsp-70 gene expression, we also evaluated the effect of food deprivation on the same cellular responses. Food deprivation for 16h did not affect Hsp-70 gene expression in all tissues examined, indicating that the effects of leptin and cerulenin are independent of the inhibition of food intake. To ascertain whether these effects are direct or indirect, we carried out *in vitro* studies. Leptin and cerulenin treatments did not affect Hsp-70 gene expression in Leghorn Male Hepatoma (LMH) and Quail Myoblasts (QM7) cell lines suggesting that the observed effects *in vivo* may be mediated through the central nervous system.
The present study suggest that cerulenin does not act as leptin at least for the regulation of HSP-70 gene, however it does mimic some effects of leptin to reduce food intake. Although these products altered HSP-70 gene expression in normal conditions, it remains to be determined whether they would have similar effects in chickens following stress events.
Key words: leptin, cerulenin, HSP-70, food intake, cell culture, oxidative stress

---

1B_05_P

HSP70 AND ITS PEPTIDE FRAGMENTS UP-REGULATE IFN-γ PRODUCTION AND MODULATE THE EXPRESSION OF SURFACE MARKERS IN HUMAN NK CELLS

Kovalenko E.I., Kanevski L.M., Nekrasov A.N., Alekseeva L.G., Vlaskin P.A., Strelnikova Yu.I., Sapozhnikov A.M.

Shemyakin & Ovchinnikov Inst. of Bioorganic Chemistry, Moscow, Russia, *lenkovalen@mail.ru*

Hsp70, potential activator of cell-mediated immunity, can be released by tumor cells to extracellular space. Earlier we showed co-stimulating effects of exogenous Hsp70 on IFN-γ production induced by IL-2 or IL-12, though Hsp70 itself did not evoke IFN-γ production in non-activated NK cells. In this work we analyzed the effects of Hsp70 on NK cells using Hsp70 peptide fragments. NK cells of high purity were isolated with magnetic separation and cell sorting. Intracellular IFN-γ production and surface expression of CD16, CD56 and CD94 were measured by flow cytometry. Six peptide fragments of the substrate-binding domain of inducible Hsp70 were chosen on the base of information structure analysis of the protein sequence [1]. Influence of recombinant Hsp70 and the peptides on IFN-γ production and marker surface expression was estimated upon 24 h incubation of the cells with IL-2. Hsp70 as well as peptides 399-408 and 411-424 co-stimulated IFN-γ production in IL-2-activated NK cells suggesting that these peptides may contain Hsp70 sites responsible for the interaction with NK cells. The same peptides modulated NK cell

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

surface antigen expression in Hsp70-like manner. $CD3^-CD16^-CD56^+$ cell population was more susceptible to Hsp70 action comparing with $CD3^-CD16^+CD56^+$ population. The Hsp70 effects in the experimental system did not depend on LPS. In sum the results testify that exogenous Hsp70 affects directly human NK cells, leading to the strengthening of IFN-γ production and modulation of antigen surface expression in conditions of cell stimulation by activating cytokines. 1. Nekrasov A.N., Entropy of protein sequences: an integral approach, J Biomol Struct Dyn 20: 87-92, 2002.

1B_06_P

PRIMARY CULTURES OF BOVINE INTERVERTEBRAL DISC CELLS HAVE A REDUCED RESPONSE TO THERMAL STRESS

SJ Owen, S Roberts, CA Sharp

RJAH Orthopaedic Hospital, Gobowen, UK sharon.owen@rjah.nhs.uk

Intervertebral discs (IVD) are avascular tissues that separate the bony vertebral bodies and act as shock absorbers for the spinal column. At the centre of each disc is the nucleus pulposus (NP), a region rich in proteoglycan and type II collagen produced and maintained by chondrocyte-like NP cells. *In vivo*, NP cells have adapted to a low oxygen environment and constant mechanical loading. We have studied the response of NP cells to thermal stress. NP cells were isolated from bovine coccygeal IVDs. Primary cells were cultured up to passage 4 (P4). Type I and II collagen (Col I/II), aggrecan and GAPDH gene expression were determined by PCR at each passage. Cells were cultured for 3 days following isolation or passage (P0, 2 and 4) then heat shocked at 45°C for 1h and allowed to recover at 37°C for 6, 24 and 48h. Total cell number at each time point was determined using propidium iodide uptake after fixation. At P2 Col I expression was up-regulated and by P3 Col II expression was down-regulated whereas aggrecan expression remained unchanged. Primary NP cell HSP70 production was maximal (0.5ng/1000 cells) after 24h recovery whereas those at P2 and P4 were maximally elevated (2.8 and 1.7ng/1000 cells respectively) after 6h. Total cell number at P0, 2 and 4 were only marginally affected by heat shock (110, 94 and 102% respectively) compared with controls. Viability was unaffected. Freshly isolated bovine NP cells have an attenuated response to thermal stress and dedifferentiate in culture losing their chondrocyte-like phenotype. NP cell dedifferentiation is accompanied by increased HSP70 production after heat shock. *In vivo*, NP cell phenotype reflects their tissue environment which is modified by cell culture.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1B_07_P

HEAT SHOCK PROTEINS IN PATHOLOGICAL HUMAN INTERVERTEBRAL DISCS

C. A. Sharp, H. Evans, S. Roberts, S. J. Owen

RJAH Orthopaedic Hospital, Gobowen, UK chris.sharp@rjah.nhs.uk

Back pain is associated with intervertebral disc (IVD) degeneration, resulting from the failure of disc cells to maintain a functional tissue matrix. IVDs are avascular and exchange of nutrients & waste product is slow. This environment may be detrimental to disc cell function & survival. Disc degeneration begins with loss of proteoglycans & fluid, resulting in loss of disc height & functional impairment. Disc herniation begins with the protrusion of the nucleus pulposus (NP) into the surrounding annulus fibrosus (AF). If the AF ruptures the NP can extrude into the spinal canal & become separated from the disc. Scoliosis is a spinal deformity with lateral curvature of the spine. Hsp27&72 have been identified in "normal" post-mortem (PM) human IVDs & Hsp72 appeared to increase with degeneration. Little is known of Hsps in discs from patients with disc pathologies. We have studied HSF1, Hsp27&72 in IVDs from 33 patients with herniated (n=19, mean age 43y) & degenerate (14, 45y) discs, 5 with scoliosis (15y) removed at surgery & 5 control discs (55y) removed at PM. IHC was performed using specific mAbs to Hsp27&72 & a pAb to HSF1 & labeled with peroxidase and DAB. On each section of disc 200 cells were counted. Data are presented as the mean % of +vely stained cells. More cells were +ve for Hsp27 & 72 in herniated discs (49% & 38%) than in degenerate (37%&26%), scoliotic (26%&21%) or controls (33%&30%). Hsp27+ve cells were greater in herniated than scoliotic discs (p=0.02). HSF1 staining was greater in controls (24%) than in herniated (16%), degenerate (6%) or scoliotic (6%) discs. There were no trends with age for any of the antigens. HSF1, Hsp27&72 are detectable in IVD tissue. Overall Hsp levels were greatest in herniated and lowest in scoliotic discs that may imply less capacity to mount a stress response.

1B_08_P

POLYAMINE COMPOUND DEOXYSPERGUALIN INHIBITS HEAT SHOCK PROTEIN-INDUCED ACTIVATION OF IMMATURE DENDRITIC CELLS

Sugawara Atsushi, Toshihiko Torigoe, Yasuaki Tamura, Kenjiro Kamiguchi, Kyuichi Nemoto, Noriyuki Sato

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

(OBJECTIVES)Deoxyspergualin (DSG),a spermidinyl,α- hydrocyglycyl, 7-guanidinoheptanoyl peptidomimetic, is a potent immunosuppressive agent. for rejection of organ transplant., whose mechanism of action remains unknown..
Dendritic cells (DC) are considered the most potent antigen-presenting cells (APC) and they can stimulate $CD4^+$and $CD8^+$T cells.. HSP 70 bind to DC and induce DC maturation and activated DC to elicit immune responce. It has been reported that DSG and DSG analogs bind to Hsp70 and Hsp90. We report here DSG inhibit Hsp70 binding to DC by flow cytometry analysis and prevent Hsp70 induced DC activation.by TNF-alpha releasing assey.These data indicate that interaction of DSG and Hsp70 involve in immunosupresive ability of DSG.
(MARERIALS and METHODS) Bone marrow-derived immature DCs were generated from the femurs and tibia of C57BL/6 mice,which were incubated in complete RPMI-1640 with 10%heatinactivated FCS and 20 ng/ml GM-CSF . Flow cytometry analysis was performed to comfirm Hsp70 binding to DCs. Immature DCs were incubated with Alexa 488-labeled Hsp70 at 4°C for 15 minutes with DSG, DSGanalog(which does not have immunosuppressive activity). To evaluate the effect of immunosuppresion of DSG, tumor necrosis factor α (TNF-α) release assay was done.Immature DCs of mice were incubated for 12hours with Hsp70 alone, with DSG or DSG analog.
(Result) Binding of Hsp70 to DCs decreased under DSG presence.Hsp70 induced TNF-α release of DCs was suppresed by DSG.
(Conclusion) These results supposed that Hsp70 and Hsp70 family was involved in DSG immunosuppresive function.

1B_09_P

RECOMBINANT EXPRESSION, PURIFICATION AND CHARACTERISATION OF THE COMPLEX BETWEEN HSP70 PROTEINS AND THEIR HSP40 CO-CHAPERONE PARTNERS.

A.Vydyanath, S.Smerdon, M.Odell

School of Biosciences, University of Westminster, London, W1W 6UW,
Email of corresponding author: anu.keshav@gmail.com

Nascent polypeptides emerging from the ribosome are exposed to a complex medium in the cytoplasm crowded with macromolecules such as cellular proteins and RNA. The formation of unwanted interactions can pose a threat to the proper folding of the nascent chain and can lead to aggregation. Assistance to prevent this process comes in the form of molecular chaperones, Heat shock proteins. The 70kDa members of the

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

heat shock protein family (eg. Hsp70) function as molecular chaperones by binding to exposed hydrophobic patches on nascent polypeptides forming non-covalent interactions, thereby preventing their aggregation and facilitating their proper folding. The folding reaction comprises of cyclic binding and release of the unfolded substrate powered by ATP hydrolysis. Hsp70 requires the assistance of a co-chaperone, generally provided by the Hsp40 group of proteins, for the cycle of protein folding. Biochemical analyses have mapped the possible sites of interaction between the Hsp70 and Hsp40 proteins and predicted a bipartite mode of interaction amongst the components of the chaperone cycle, Hsp70, Hsp40 and the substrate. However, structural investigations into the mechanistic features of the folding cycle have been hampered by the transient nature of interaction. We have devised a cloning strategy for the expression and purification of a recombinant human Hsp70/Hsp40 complex from *E.coli* .The complex was purified using Ni-NTA affinity chromatography and gel filtration column. The gel filtration elution profile matched neither of the individual Hsp70 or Hsp40 components. We are analysing the stability and behaviour of the recombinant complex with both nucleotides and substrates. The recombinant complex will be used to understand the mechanistic differences in the interactions of the Hsp70 with Hsp40 and Hsp70 with substrate during the stages of the folding cycle. We will present our human Hsp70/Hsp40 complex cloning, expression and purification strategy and its preliminary biochemical characterisation.

1B_10_P

HEAT SHOCK COGNATE PROTEIN HSC70 AS A REGULATOR OF THE ACTIVIN/NODAL/TGF-ß-SMAD2 SIGNALLING PATHWAY DURING ZEBRAFISH DEVELOPMENT

Michiaki Yamashita, Misako Hojo, and Takeshi Yabu

National Research Institute of Fisheries Science, Fukuura, Yokohama 236-8648, Japan, mic@affrc.go.jp

The cytosolic heat shock protein 70 (HSP70) and heat shock cognate protein 70 (HSC70) are thought to combine to function as a molecular chaperone. The protein binds transiently to nascent polypeptides and unfolded proteins, and prevents intramolecular and intermolecular interactions, which can result in misfolding or aggregation. Precise control of Nodal proteins, which are members of the transforming growth factor-ß (TGF-ß) superfamily, has been identified as a key endogenous mesoderm inducer in vertebrates. In the present study, we report that the HSC70 molecular chaperone promotes the mesoderm induction activities of Nodal

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

signalling. To study the biological function of HSC70, the endogenous expression of HSC70 was knocked down by the injection of a specific antisense morpholino oligonucleotide (HSC-MO). The HSC-MO-injected embryos showed reduced phosphorylation of Smad2 by Nodal signalling, and this phenotype was rescued by co-injection of the HSC70 protein. However, the HSP70 mutant that lacked the C-terminal tetrapeptide (EEVD) motif showed no activity upon induction of Nodal signalling. The Activin type IIB receptor (ActRIIB) was immunoprecipitated with HSC70, and its autophosphorylation was enhanced in the presence of HSC70, which suggests that HSC70 binds directly to the receptor and modulates its activity. Therefore, HSC70 plays essential roles in the formation and activation of the type IIB receptor upon Nodal signalling.

1B_11_P

SURFACE HSP70 EXPRESSION AND APOPTOSIS IN NORMAL AND MALIGNANT LEUCOCYTE POPULATIONS

Francesca Leoni, Nina C. Dempsey, Claire, Hunter-Lavin, Christine Hoyle, John H.H. Williams

Chester Centre for Stress Research, Department of Biological Science, University of Chester, Parkgate Road, Chester, United Kingdom, CH1 4BJ, f.leoni@chester.ac.uk

Hsp70 is normally an intracellular protein, however there is an increasing amount of data demonstrating their release from cells and interaction with membrane lipids. Surface Hsp70 expression has been shown to be restricted to tumour cells and has an established role as a target for tumour immunity.

We have initially examined surface expression of Hsp70 in leukemic patients finding an increased expression of surface Hsp70 in malignant cells as expected. Jurkat cells do not express large levels of surface Hsp70 at normal growing temperatures. However, we have shown an increase of surface Hsp70 expression at 42°C but not at 45°C. Pro caspase-2 and the effector caspase-3 were both activated after heat shock, with a peak of caspase-3 after 2.5 hours and a decrease in viability and necrosis after longer time periods and higher temperatures. The time course of the appearance of surface Hsp70 correlated with phosphatidilserine (PS) externalisation (detected using annexin V) after 42°C heat shock. This association is in accordance with previous studies showing the localisation of Hsp70 on the cell surface within the lipid raft structures which are partly comprised of PS.

We have also found surface expression of Hsp70 in neutrophils and monocytes from normal lysed whole blood, and observed an increase in Hsp70 surface expression in these populations after heat shock at 42°C

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

but not at 45°C. Again these data were associated with the increased externalisation of phosphatidilserine (PS) on the outside of the cells, detected with Annexin-V. We will present further data on the relationship between insertion of Hsp70 into the plasma membrane and the secretion of Hsp70 from cells.

1B_12_P

CHARACTERIZATION OF NUCLEOTIDE-INDUCED CHANGES IN THE QUATERNARY STRUCTURE OF HUMAN COGNATE 70 KDA HEAT SHOCK PROTEIN (HSP70) BY SMALL-ANGLE X-RAY SCATTERING AND ANALYTICAL ULTRACENTRIFUGATION

Cristiano L.P. de Oliveira, Júlio C. Borges, Iris L. Torriani and Carlos H.I. Ramos*

*Universidade Estadual de Campinas, PO Box 6154, Campinas SP, 13084-862, Brazil. *: chiramos@gmail.com*

Abstract
The 70 kDa heat shock protein (Hsp70) can be divided into an N-terminal nucleotide binding domain (NBD) that binds and catalyzes the hydrolysis of ATP, and a C-terminal substrate binding domain (SBD) that interacts with polypeptide targets. Hsp70 proteins assist the protein folding process through nucleotide-controlled cycles of substrate binding and release by alternating between an ATP-bound state in which the affinity for substrate is low and an ADP-bound state in which the affinity for substrate is high. It has been long recognized that the two-domain structure of Hsp70 is critical for these regulated interactions. Therefore, it is important to gather essential information about conformational changes caused by nucleotide binding both in each domain and in the spatial position of one domain related to the other. In this work we used analytical ultracentrifugation and small angle X-ray scattering to characterize the effect of both ADP and ATP binding into human cognate Hsc70 protein. The results showed that ATP causes a higher global conformational change in the quaternary structure of Hsp70 than ADP does. In conclusion, ATP binding and hydrolysis trigger the largest changes in conformation.
Research in the laboratory of CHIR is supported by grants from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Pesquisa e Desenvolvimento (CNPq), and NIH-R03TW007437 funded by the Fogarty International Center.

1B_13_P

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

SHORT TERM TREADMİLL EXERCİSE-İNDUCED ADRENAL HSP70 EXPRESSİON İS DEPEND UPON EXERCİSE-RELATED ELEVATİON OF BODY TEMPERATURE

Akin S, Naito H, Ogura Y, Sekine-Ichinoseki N, Kurosaka M, Kakigi R, Katamoto S, Demirel H.

Hacettepe University School of Sports Sciences and Technology, Beytepe Ankara, TURKEY: senaya@hacettepe.edu.tr

It is well-known that physiological stress created by treadmill exercise can result the inductions of HSP70 in skeletal muscle, heart, liver, and adrenal glands. Heat production during exercise has been shown to be the main factor for the increased levels of HSP expression in myocardium. On the other hand, ACTH release subsequent to hypothalamic-pituitary-adrenal activation (HPA) following various stresses is being held responsible for adrenal HSP induction. Therefore, exercise-induced adrenal HSP expression may be differently regulated than that of myocardium. This study tested the hypothesis whether treadmill exercise results in inductions of HSP70 independently of an elevation of body temperature. Twenty-six female Sprague Dawley rats (13 weeks old) were randomly assigned either to a sedentary control group (CON; n=8), or one of two exercise training groups: (1) cold exercise (CE; n=9); (2) normal temperature exercise (NE; n=9). All animals were individually housed in a climate-controlled room (25°C). During exercise training, NE exercised in the aferomentioned room while CE did in a cold room (4°C). Animals started to run on a treadmill at 25m/min for 10 min and running time was increased 10 min per day by reaching 50 min on day 5th. After that, rats ran 60 min a day at 30m/min for 4 consequtive days. Rectal temperatures were measured before and after each training session. Following last exercise bout the animals were anesthetized and both heart and adrenal were immediately removed for immunoblotting of HSP70 and HSC70 expression. Exercise resulted in a significant elevation of body temperature only in NE (3.0 ± 0.5°C, $p<0.05$). In NE group, HSP70 expression was significantly ($p<0.05$) higher than those of CON and CE groups in both myocardium and adrenal. Although there was a trend to increased levels of HSP70 in adrenal in CE group, it was not significantly higher than that of CON ($p>0.05$). The levels of HSC70 were not diferent among the three groups in both tissues. This study has shown that exercise-related elevations of body temperature could be the main factor for the inductions of adrenal HSP70 expression.

1C_01_P

CRYSTAL STRUCTURE OF *PYROCOCCUS FURIOSUS* HEAT SHOCK

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

REGULATOR, A MOLECULAR CHIMERA REPRESENTING EUKARYAL AND BACTERIAL FEATURES

Wei Liu[1], Gudrun Vierke[2], Ann-Kathrin Wenke[2], Michael Thomm[2] and Rudolf Ladenstein[1]

[1] *Karolinska Institutet, Novum, Center for Structural Biochemistry, 141 57 Huddinge, Sweden*
[2] *Archaea Center, University of Regensburg, 93053 Regensburg, Germany*
E-mail address for the corresponding author: wei.liu@biosci.ki.se

All living organisms share a common molecular stress response upon rapidly up-shifted environmental temperature. The heat shock response is characterized by a dramatic change in gene expression patterns and elevated syntheses of a family of heat shock protein, most of which function as molecular chaperones in preventing the aggregation of denatured proteins and/or helping protein refolding. The expression of most heat shock genes is strictly repressed under normal conditions, but activated once stress response is triggered. The mechanism of heat shock regulation differs among the three kingdoms. Compared to bacteria and eukaryotes little is known on heat shock regulation in archaea. The first transcriptional regulator selectively inhibiting cell-free transcription of archaeal heat shock promoters has been recently identified from the hyperthermophilic archaeon *P. furiosus* (1). The 24 kDa protein named as Phr forms a homodimer and specifically inhibits the transcription *in vitro* and *in vivo* by binding to a 29-bp DNA sequence overlapping the transcription start site in heat shock promoters. Phr established a novel protein family with non-homologous amino acid sequence with eukaryotic HSFs. The regulator specifically represses the expression of heat shock genes at physiological temperature *in vitro* and *in vivo* but is released from the promoters upon heat shock response. We report here the crystal structure of Phr which represents the first characterized heat shock transcription factor in archaea (2). Structure analysis revealed a stable homodimer, each subunit consisting of a N-terminal winged helix DNA-binding domain (wH-DBD) and a C-terminal antiparallel coiled coil helical domain. The overall structure shows as a molecular chimera with significant folding similarity of its DBD to the bacterial SmtB/ArsR family, while its C-terminal part was found to be a remote homologue of the eukaryotic BAG domain. The dimeric protein recognizes a palindromic DNA sequence. Molecular docking and mutational analyses suggested a novel binding mode in which the major specific contacts occur at the minor groove interacting with the strongly basic wing containing a cluster of 3 arginine residues. These results argue for an unprecedented DNA binding mode.

Reference

1. Vierke, G., Engelmann, A., Hebbeln, C., and Thomm, M. A novel

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

archaeal transcriptional regulator of heat shock response. (2003) J Biol Chem 278, 18-26

2. Liu, W., Vierke, G., Wenke, AK. Thomm, M. & Ladenstein R. (2007). Crystal structure of the archaeal heat shock regulator from *Pyrococcus furiosus*. A molecular chimera representing eukaryal and bacterial features. *J. Mol. Biol.* (in press)

1C_02_P

GLOBAL CHIP-CHIP PROMOTER ANALYSIS IN MOUSE TESTIS REVEALS HSF2 BINDING TO THE Y CHROMOSOME

Malin Åkerfelt [1,2]*, Eva Henriksson [1,2]*, Asta Laiho [1], and Lea Sistonen [1,2]

[1] Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Turku, Finland
*[2] Department of Biology, Åbo Akademi University, Turku, Finland. * Equal contribution.*
E-mail of corresponding authors: malin.akerfelt@btk.fi, eva.henriksson@btk.fi

Heat shock factor 2, HSF2, is a member of an evolutionary conserved HSF transcription factor family, which in mammals consists of three genes, *Hsf1*, *Hsf2* and H*sf4*. The HSFs were originally found as regulators of the heat shock response, but mutant analyses revealed HSFs also to be important developmental factors. The *Hsf2*$^{-/-}$ mice show defects in the development of the CNS and in the function of the reproduction systems of both genders. In males, increased apoptosis in testis as well as reduced sperm count are detected. Further, the structure of the seminiferous tubules is altered, displaying less differentiating spermatocytes and extensive vacuolization of the tubules. The molecular mechanisms causing these defects have remained obscure. Thus, we performed a chromatin immunoprecipitation on promoter microarray (ChIP-chip) screen on wild type mouse testis to find HSF2 target genes. We have now identified a large set of novel target promoters of HSF2 in mouse testis. In addition to DNA binding, we have verified that HSF2 is transcriptionally active as the corresponding mRNAs are altered in *Hsf2*$^{-/-}$ testis. Many of the novel target genes of HSF2 are indeed involved in spermatogenesis. The ChIP-chip screen surprisingly revealed a significant accumulation of HSF2 on genes located on the Y chromosome and important in sperm differentiation. Taken together, our results provide strong evidence for HSF2 playing significant role in spermatogenetic processes.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1C_03_P

DEVELOPMENTAL ACTIVITY OF HSF1 REVEALED BY LOSS OF FUNCTION EXPERIMENTS

Elisabeth Christians

Traditional description of HSF1 activity is based on stress induction of molecular modifications which enable DNA binding and transcription of target genes. This view seems to imply that HSF1 is mainly inactive in normal, physiological situation.
Previously published data and the work presented here strongly suggest that this description needs to be revised.
Loss of function by gene targeting of Hsf1 in mice revealed a complex phenotype including HSF1 maternal requirement in the oocyte. Hsf1-/- females produce oocyte but they appear to be unable to properly accomplish expected developmental steps such as meiotic maturation, fertilization and activation, which are mandatory for embryonic development.
In order to better understand the link between HSF1 and those developmental steps, we searched for known and unknown targets of HSF1 in oocytes. Using RT combined with real time PCR, we show that Hsp genes are differentially expressed and regulated by HSF1 in oocytes under normal, physiological conditions.
Among HSF1 known targets, Hsp86 (Hsp90alpha), which is described as the inducible form of Hsp90, is significantly reduced in immature oocyte before meiotic maturation.
Additional experiments will be discussed to show how Hsp90 alpha can be one of the key link between HSF1 and early developmental steps undertaken by the oocytes.

1C_04_P

COREST REPRESSES HEAT SHOCK RESPONSE MEDIATED BY HSF1

Gómez A.V[1]., Battaglioli E.[2], Mass J.C.[3], Mandel G[4]., Kukuljan M.[3] and Andrés M.E[1].

[1]Department of Cellular and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Catolica de Chile, Santiago, Chile. [2]Department of Biology and Genetics for Medical Sciences, University of Milan, Milan, Italy. [3]Centro de Neurociencias Integradas, Iniciativa Científica Milenio y Programa de Fisiología y Biofísica, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile. [4]Howard Hughes Medical Institute, Vollum Institute at the Oregon Health & Science University, Portland, Oregon, USA.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

The transcriptional corepressor CoREST is a main component of a chromatin modifying complex that recruits the histone deacetylases, HDACs1/2, and the histone lysine specific demethylase, LSD1. First characterized as a corepressor of the RE1 Silencing Factor/Neural restrictive silencing factor (REST/NRSF), its transcriptional regulator role has been already demonstrated in the control of the expression of neuronal genes. The high evolutionary conservation of CoREST complex, allows suspecting that it might control the expression of another subset of genes. By a yeast two hybrid screening assay, using CoREST as bait, we identified the molecular chaperone heat shock protein 70 (Hsp70) as a CoREST interacting protein. We corroborated the association between both proteins, and delimited the interacting region of CoREST to its both SANT domains. Considering that Hsp70 is involved in the heat shock response as a transcriptional corepressor of Heat Shock Factor 1 (HSF1), we evaluated whether CoREST regulate the heat shock response. Our results show that CoREST overexpression represses the heat shock induction of a reporter gene driven by the human Hsp70 promoter. Moreover, CoREST represses HSF1 transcriptional activity in both control and heat shock treatment. Next, we analyzed if Hsp70 repressor effect depend on CoREST expression. Reducing CoREST expression by using a short hairpin RNA induces a significant increase over the activity of the Hsp70 promoter mediated by HSF1. Moreover, overexpressing Hsp70 is not able to repress the activity of HSF1 in cells with reduced CoREST expression. In conclusion, we demonstrated that CoREST regulates the heat shock response at transcriptional level repressing HSF1 activity.
Supported by FONDECYT 1030496

1C_05_P

HEAT SHOCK TRANSCRIPTION FACTOR 1 PLAYS A ROLE IN FEEDBACK REGULATION OF THE FEBRILE RESPONSE

Sachiye Inouye[1], Mitsuaki Fujimoto[1], Tamami Nakamura[1], Eiichi Takaki[1], Naoki Hayashida[1], Tsonwin Hai[2] and Akira Nakai[1]

[1]Department of Biochemistry and Molecular Biology, Yamaguchi University School of Medicine, Minami-Kogushi, Ube, Japan, [2]Department of Molecular and Cellular Biochemistry, Ohio State University, Columbus, Ohio, USA, e-mail: sinoue@yamaguchi-u.ac.jp

Febrile response is a complex physiological reaction to disease including cytokine-mediated rise in body temperature and activation of inflammatory systems. Importantly, fever plays beneficial roles on disease prognosis experimentally and clinically, probably by suppressing pyrogenic and inflammatory cytokines in cells and in whole body. However,

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

molecular mechanisms underlining fever-mediated suppression of cytokine gene expression are uncovered yet. Here we show that heat shock suppresses LPS-induced induction of IL-6 by activating HSF1 that induces ATF3, a negative regulator of IL-6. In vivo analysis using HSF1-null and ATF3-null mice reveals that HSF1 as well as ATF3 acts as a negative regulator of IL-6 expression in whole body and is required to inhibit fever. Unexpectedly, overexpression of ATF3 into cells has no effect on IL-6 expression in the absence of HSF1. HSF1 also binds directly to IL-6 promoter, and is required not only for a repressor ATF3, but also an activator NF-κB to bind to IL-6 promoter by partially opening chromatin structure. These results indicate that HSF1-ATF3 pathway is involved in feedback regulation of the febrile response.

1C_06_P

HEAT SHOCK FACTOR (HSF) 4A INHIBITS HEMIN-INDUCED ACTIVATION OF HSF2

Sang-Gun Ahn[1], Jung-Hoon Yoon[1], and Soo-A Kim[2,*]

[1]Oral Biology Research Institute, BK21 projects, Department of Pathology, Chosun University College of Dentistry, Gwangju, KOREA, [2]Department of Biochemistry, Dongguk University College of Oriental Medicine, Gyeongju, KOREA
** corresponding author*

Abstract
The inducible regulation of heat shock gene transcription is mediated by a family of heat shock factors (HSFs) that respond to diverse forms of physiological and environmental stress including temperature, heavy metals, oxidative stress. Although HSFs have been extensively studied with respect to their regulation by molecular chaperones, regulatory mechanism between HSF family members are poorly understood. In this study, we identified the interaction of HSF2 with HSF4A but not with HSF4B under non-stressed conditions. Using immunoprecipitation assay, we found that leucine zipper domain 1-3 of HSF4A was directly interacts with HSF2 and inhibits transcriptional activity of HSF2. Interestingly, HSF4A was accumulated to the nucleus upon hemin treatment and inhibited trimerization and following target gene transcription of HSF2. These observations suggest HSF4A as an active repressor of HSF2-mediated transcription.

Keywords: HSF2, HSF4A, leucine zipper, hemin, immunoprecipitation

---

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1C_07_P

GENETIC EVIDENCE FOR A PROTECTIVE ROLE OF HEAT SHOCK FACTOR 1 AGAINST GASTRIC ULCER AND COLITIS RELATED TO HUMAN INFLAMMATORY BOWEL DISEASE

Ken-ichiro Tanaka, Tohru Mizushima

Graduate School of Medical and Pharmaceutical Sciences, Kumamoto University

Gastric lesions result from an imbalance between aggressive and defensive factors. Indirect lines of evidence suggest that heat shock factor 1 (HSF1)-dependent induction of heat shock proteins (HSPs) by various aggressive factors provide a major protective mechanism. On the other hand, inflammatory bowel disease (IBD) involves infiltration of leukocytes into intestinal tissue and both pro-inflammatory cytokines (such as TNF-α) and cell adhesion molecules (CAMs) play an important role in this step. However, the role of HSF1 in development of IBD has remained unknown. In this study, we examined the production of gastric lesions and DSS-induced colitis, animal model of IBD, using HSF1-null mice lacking HSF1. The production of gastric lesions by ethanol or hydrochloric acid was stimulated in HSF1-null mice. Ethanol administration up-regulated gastric mucosal HSPs, in particular HSP70, in an HSF1-dependent manner, and more apoptotic cells were observed in the gastric mucosa of HSF1-null mice than in wild-type mice. Geranylgeranylacetone (GGA), a clinically used anti-ulcer drug with HSP-inducing activity, suppressed ethanol-induced gastric lesions in wild-type mice but not in HSF1-null mice. The results suggest that the production of irritant-induced gastric lesions in HSF1-null mice is due to their inability to up-regulate HSPs, leading to apoptosis. It is also suggested that the HSP-inducing activity of GGA contributes to the drug's anti-ulcer activity. On the other hand, the DSS-induced colitis was worsened in HSF1-null mice. DSS-administration up-regulated HSP70 at colonic tissues in an HSF1-dependent manner. Comparing to wild-type mice, levels of pro-inflammatory cytokines (such as TNF-α) and CAMs at colonic tissues with DSS-administration were increased in HSF1-null mice. Macrophages prepared from HSF1-null mice showed higher activity for lipopolysaccharide-stimulated generation of TNF-α. Suppression of *hsf1* expression stimulated lipopolysaccharide-induced up-regulation of CAMs. These results suggest that HSF1 play a protective role against DSS-induced colitis. Furthermore, this protective role seems to involve suppression of expression of TNF-α and CAMs. This study provides the first direct genetic evidence that HSF1 confer protection against the development of gastric lesions and colitis related to human IBD.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1C_08_P

CHARACTERIZATION OF THE DNA-BINDING SITES OF HSF4 THAT CONSTITUTIVELY FORMS A TIMER

Mitsuaki Fujimoto, Eiichi Takaki, Naoki, Hayashida, Sachiye Inouye, and Akira Nakai

Department of Biochemistry and Molecular Biology, Yamaguchi University School of Medicine, Minami-Kogushi, Ube, Japan, e-mail: yoji530@yamaguchi-u.ac.jp

Heat shock transcription factors (HSFs) regulates expression of heat shock genes and genes related to development. HSFs bind to heat shock element (HSE), which is composed of at least three inverted repeats of consensus sequence nGAAn. Under normal condition, HSF1 and HSF2 exist as a monomers and a dimmer, respectively. HSF1 is converted to a trimer upon heat shock. In contrast, HSF4 constitutively stays a trimer that binds to the HSE under normal condition. Recent observations showed that HSFs plays roles in physiology including developmental processes and complements or competes with each other. However, we do not understand how HSFs do so. To examine their cooperativity, here we examined specificity of DNA-binding by HSFs. We firstly determined the HSE-binding specificity of HSF4 by a random oligonucleotide selection method. We found that the G nucleotide in 5'-nGAAn-3' in all of the selected sequences is conserved, but there is little preferences for the nucleotides in As. Mutations in the A nucleotides in the nGAAn caused decreased binding of HSF1 and HSF2 whereas those affected HSF4 binding only a little. There results indicate that the HSF4-binding sequence is an inverted repeats of nGnnn. We next performed in vivo chromatin immunoprecipitation (CHIP) assay, and 71 candidate target genes were identified. Consensus sequence of the putative HSF4-binding sites was the same as that identified by a random oligonucleotide selection method. These results indicate that HSF4 possesses stronger affinity for a set of DNA-binding sites, whose sequences weakly matched with a canonical HSE sequence, than HSF1 and HSF2.

1C_09_P

HSFS, A MOLECULAR BASIS FOR FETAL ALCOHOL SYNDROME (FAS)?

Rachid EL FATIMY[1], Anne LE MOUËL[1], Leslie SCHWENNDIMANN[2], Pierre GRESSENS[2], Valérie MEZGER[1]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1. CNRS UMR8541, École Normale Supérieure, 46 rue d'Ulm, 75005 PARIS, France.
2. INSERM 676 et service de Neuropédiatrie, Hôpital Robert Debré, 48 bd Sérurier, 75019 PARIS, France

The transition from normal growth conditions to stress conditions induces the cell protective heat shock proteins (HSPs). *Hsp* genes are activated by Heat Shock Factors (HSFs). HSF1, the major stress-responsive transcription factor in mammals, is activated through a multi-step pathway including posttranslational modifications and binding to Heat shock Elements (HSE) upstream of *Hsp* genes. Although reported to be desactivated by heat shock (HS), HSF2 contributes to *Hsp* induction through interplay with HSF1.
HSFs are also involved in development. Cerebral cortex consists in 6 layers of neurons arising from migration. We showed that *Hsf2*$^{-/-}$ cerebral cortices display mispositioning of neurons due to disturbances in the expression of target genes which control neuronal migration signaling pathways (*Chang Y, 2006 Genes & Dev)*.
We are interested by the intriguing role of HSF1 and HSF2 as coordinators between stress and development. Chronic alcoholic intoxication (CAI) causes neuronal migration defects in brain development, which are part of the fetal alcohol syndrome (FAS), but whose molecular basis remain elusive. Since alcohol induces HSPs in cultured cells, we hypothesized that CAI disturbs HSF1/HSF2 activities in fetal cortices. Such alterations could disturb the expression of target genes that contain HSE and are regulated by HSF2 only during normal neuronal migration.
We showed that HSF1 and HSF2 activities are disturbed both in *ex vivo* alcoholic exposure of cultured cells and *in vivo* CAI of fetal cortices. Differential HSF1 posttranslational modifications were induced by alcohol versus HS. Modifications of HSF activities correlated to disturbances in the expression levels of various HSF2 target genes involved in neuronal migration. By ChIP (chromatin immunoprecipitation), we characterized the differential i*n vivo* occupancies by HSF1 versus HSF2 on these genes. Therefore HSFs provide a molecular explanation to the FAS aspect of neuronal migration defects.

1C_10_P

DUAL ROLES OF HEAT SHOCK FACTOR 1 (HSF1) IN CARDIOPROTECTION, PATHOLOGIC HYPERTROPHY AND HEART FAILURE IN TRANSGENIC MICE

András Orosz and Ivor Benjamin

Division of Cardiology, University of Utah, Salt Lake City, USA, 84112
andras.orosz@hsc.utah.edu

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Heat shock factor 1 (HSF1), the major stress-inducible transactivator binds to heat shock elements (HSE) embedded in the promoter of heat shock proteins (HSPs) under stressful conditions and up-regulates the synthesis of HSP genes. In principle, HSF1 activation in the heart provides protection against ischemic insults and other stressful episodes through the increased synthesis and chaperone activities of HSPs. HSF1 level is also up-regulated in physiological cardiac hypertrophy following strenuous exercise regimens. To study HSF1 functions in the heart, we generated a transgenic mouse model expressing cardiac specific, inducible, but constitutively active HSF1 whose transcriptional competency does not require stressors. Two transgenic mouse lines were characterized, termed mHSF1(+) Low Tg and High Tg, with transgene expression ranging between equivalent and ~5-10 fold of the endogenous HSF1 level, respectively. In mHSF1(+) Low Tg mice, 7 days expression of mHSF1(+) induced ~3-fold upregulation of each major HSP groups, which conferred significantly increased ischemic protection in an *ex vivo* heart injury model. In contrast, doxycyclin feeding of mHSF1(+) High Tg line triggered ~5-10-fold increase in mHSF1(+) and target HSPs levels and ~30% cardiac hypertrophy in 7 days. Interestingly, 3 weeks mHSF1(+) expression resulted in decompensated cardiac hypertrophy, massive cell death and fibrosis along with greatly decreased cardiac function and overt heart failure in transgenic mice. Mechanistically, we hypothesize that upregulated class I and class II histon deacetylase family members, direct or indirect targets of mHSF1(+), may potentiate cardiomyocyte hypertrophy, whereas decreased expression of PGC-1α, a transcriptional co-activator of mitochondrial biogenesis accelerates the metabolic demise of mHSF1(+) High Tg hearts. Our studies have clearly established an intriguing dichotomy, based on low and high levels of HSF1 transactivation in both protective and pathological cardiac gene expression programs and, perhaps, human pathologies.

1D_01_P

PROTECTION OF CELLS: A POSSIBLE ROLE FOR EXTRA-CELLULAR HSP70

Helen Williams, Claire Hunter-Lavin & John H.H. Williams

Chester Centre for Stress Research, Department of Biological Sciences, University of Chester, Parkgate Road, Chester, United Kingdom, CH1 4BJ
helen.williams@chester.ac.uk

Hsps have been typically regarded as intra-cellular molecules that mediate a range of essential housekeeping and cytoprotective functions but increasingly there has been accumulating evidence which suggests that

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

these molecules have importance as extra-cellular proteins. Hsps have been detected on both the cell surface and outside of cells under physiological conditions. We added soluble Hsp70 (bovine and recombinant) to U937 cells and erythrocytes to determine whether this Hsp70 provided added protection to these cells. A time and concentration dependent effect was observed in both cell types. A one hour treatment with 0.1 µg/ml Hsp70 protected U937 cells from apoptosis induced by heat stress at 42.6°C, determined by annexin-V, caspase-3 activity and visually using fluorescence microscopy. One hour incubation with 0.5 µg/ml Hsp70 protected U937 cells from necrosis induced by 46.2°C heat shock, measured by propidium iodide, and 1 hr of 2 µg/ml Hsp70 maintained cellular viability measured by MTS assay compared to control populations. Erythrocytes required 3 hr of 10µg/ml Hsp70 to protect against cell lysis induced by heat shock at 42°C, treatment with BSA showed no effect. These data support a possible role for extra-cellular Hsp70 in the protection against heat-induced cell death. Further characterisation of this response will be determined by flow cytometry and fluorescence microscopy.

1D_02_P

HSP70 IS SECRETED FROM CELLS IN VESICLES THAT RESEMBLE LIPID RAFTS

A. De Maio[1, 2], M. Rodríguez[1], T. Frey[2], M. Gehrman[3], D. Nino[1], J.C. Diaz [4], C. Steinem[5], N. Arispe[4], G. Multoff[3], and V. Vega[1]

[1]Department of Surgery, University of California San Diego, La Jolla, California, USA; [2]Johns Hopkins University, Baltimore, Maryland, USA; [3]Klinikum Rechts der Isar, Technical University, Munich, Germany;[4]Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA;[5]Institute for Organic and Biomolecular Chemistry, University of Goettingen, Göttingen, Germany

Heat shock proteins (hsp) play a major role in a variety of cellular processes during normal conditions as well as during the restoration of homeostasis after stress. Recently, Hsp70, the major stress-induced hsp, has been found in the extracellular medium. Moreover, Hsp70 has been observed to activate cells of the immune system. Thus, circulating Hsp70 is thought to act as a danger signal that triggers the response to injury. We have previously reported that Hsp70 is capable of interacting with lipid membranes and has a high selectivity for phosphatidylserine (PS). The incorporation of Hsp70 within PS-containing membranes was enhanced by the presence of spingolipids (GM1) and cholesterol, which are major components of lipid rafts. In fact, Hsp70 was detected in the lipid raft fraction isolated from Triton X-100 solubilized HepG2 cells after heat

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

shock (HS). The presence of Hsp70 in this fraction was reduced by depletion of cellular cholesterol by treatment with β-cyclodextrin. Hsp70 was visualized within the plasma membrane of HepG2 cells after HS, co-localizing with lipid raft markers. Analysis of the extracellular medium of HepG2 cells after heat shock revealed the presence of Hsp70 within membranes that can be isolated by high speed centrifugation. These membranes containing Hsp70 were rich in GM1 and cholesterol, but depleted of other cellular components, such as actin. Moreover, the Hsp70 within these extracellular membranes was resistant to Triton X-100 solubilization. Thus, extracellular membranes containing Hsp70 resemble lipid rafts. We propose that part of the extracellular Hsp70, which may be involved in the activation of immune cells, is present in the membrane derived from lipid rafts.

1D_03_P

EXTRACELLULAR HSP72: A DOUBLE-EDGED SWORD FOR HEALTH

Monika Fleshner

Dept. Integrative Physiology, University of Colorado, Boulder, CO 80309-0354, USA
fleshner@Colorado.edu

Environmental or emotional challenge triggers a cascading series of physiological responses which are collectively termed the "stress response". The stress response can be assessed at the behavioral, neural, hormonal, immunological and single cell, levels and evolved to benefit an organism's chance of survival during times of acute challenge. The stress response has been studied for many years, however, its impact on specifically immune function has only recently been appreciated. Acute activation of the stress response has both inhibitory and stimulatory effects on immunity. The focus of this presentation is on a novel mechanism for the immunostimulatory effects of stress. Specifically, we propose that an endogenous, ubiquitous cellular stress protein, heat shock protein 72, when found in the extracellular environment may contribute to stress-induced potentiation of innate immunity. ***We develop the hypothesis that the release of extracellular heat shock protein 72 (eHsp72) is a normal feature of the acute stress response that can have either positive or negative consequences for host defense depending on several factors, including the nature of the eHsp72 (naked versus antigen-associated), and host health status (absence or presence of pre-existing inflammatory disease).*** Thus, stress-induced eHsp72 release may be a double-edged sword for host defense.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1D_04_P

THE ROLE OF CLUSTERIN IN EXTRACELLULAR PROTEIN QUALITY CONTROL

Wyatt, A. R.[1] Wilson, M. R. [1] Katsifis, A. [2] Greguric, I. [2] Mattner, F. [2] and P. Berghofer[2]

[1]Department of Biological Sciences, University of Wollongong, Northfields Avenue, Wollongong, NSW, Australia
[2]Radiopharmaceuticals Research, The Australian Nuclear Science and Technology Organisation (ANSTO), Lucas Heights, NSW, Australia

Processes to attain and maintaining the correct three-dimensional shape, or 'native conformation' of proteins are vital. However, certain conditions including thermal and oxidative stress cause proteins to unfold and aggregate. Intracellular and/or extracellular protein aggregates have been identified in a large number of diseases, including Alzheimer's disease, arthritis and type II diabetes. While several intracellular quality control mechanisms for the folding state of proteins have been characterized, corresponding mechanisms for extracellular protein quality control have yet to be identified. Clusterin is an extracellular chaperone that can stabilise proteins and prevents their precipitation during exposure to high temperatures or oxidative stress. We have demonstrated that clusterin stabilizes proteins by forming high molecular weight (HMW) complexes (> 4 x 10^7 Da) with them. Using an animal model, the fate of blood-borne ^{123}I -HMW complexes was investigated. ^{123}I -HMW complexes were rapidly cleared from circulation and targeted primarily to the liver and spleen. In the absence of specific extracellular proteolytic mechanisms it appears likely that extracellular proteins are targeted and transported intracellularly for degradation. Receptor-mediated endocytosis is one possible route for the clearance of HMW complexes. Clusterin affinity chromatography of cell membrane proteins from liver and spleen may identify potential receptors for HMW complexes. These findings suggest an important role for clusterin in extracellular protein quality control.

1D_05_P

THE EXTRACELLULAR PROTEASE INHIBITOR ALPHA$_2$-MACROGLOBULIN HAS CHAPERONE-LIKE PROPERTIES.

Justin J Yerbury, Katie French and Mark R Wilson

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

School of Biological Sciences, University of Wollongong, Northfields Avenue, Wollongong, NSW 2522, Australia. Email: jyerbury@uow.edu.au

α_2-Macroglobulin (α_2M) is a secreted glycoprotein, found at high levels in human blood, best known for its ability to inhibit a large array of proteases by a unique trapping method. Protease binding promotes a change in structure of α_2M yielding an "activated" conformation which exposes a binding site for the low density lipoprotein receptor. This process facilitates the clearance of α_2M-protease complexes from the body. We report here that α_2M also has potent chaperone properties, making it the first known protein to combine both these activities. We show that α_2M inhibits the heat induced aggregation of citrate synthase and creatine phosphokinase, and the oxidative stress induced aggregation of lysozyme and α-synuclein. Protease-mediated activation of α_2M abolishes its chaperone-like activity. However, we show that native α_2M is able to form soluble complexes with stressed proteins and then subsequently become activated by interacting with a protease. This activation of α_2M in complex with misfolded proteins provides a potential mechanism for the *in vivo* clearance of α_2M/stressed protein/ protease complexes. We propose that α_2M is a member of a small group of abundant extracellular proteins with chaperone properties that patrol extracellular spaces for unfolded/misfolded proteins and facilitate their disposal.

1D_06_P

BACTERIAL BINDING AND OPSONIZING EFFECT OF EXTRACELLULAR HSP70

Minh Tu Nguyen, Tibor Károly Fábián, Mahavir Singh, Péter Csermely, Csaba Sőti

Semmelweis University Department of Medical Chemistry, Budapest, P.O.Box 260; H-1444 Hungary
Email: minhtu@freemail.hu

Stress (or heat shock) proteins form an ancient defense system of our cells. They were considered to be intracellular, but cells under stressful conditions can express stress proteins on their surface and release them to the extracellular space, where these proteins funtion as a „danger signal" and induce apoptosis of target cells as well as activate both humoral and cellular immune responses. One of the most important stress protein implicated in extracellular signalling is Hsp70, the inducible cytosolic isoform of the 70 kDa heat shock protein family.
In the present experiments we investigated the effect of extracellular

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Hsp70 on the interaction of bacteria with the immun system.
Our results demonstrated that extracellular Hsp70 was able to bind both to the surface of Gram-positive *Streptococcus mutans and mitis* and Gram-negative *Escherichia coli* bacteria. Acidic milieu (pH 5.5) and high temperature (42°C) facilitated while ATP partially inhibited the formation of the Hsp70-bacterium complex. We found that extracellular Hsp70 exerted an opsonizing effect, it was able to activate the killing activity of polymorphonuclear neutrophil leukocytes.
Our results provide a novel mechanism by which extracellular Hsp70 may induce the activation of the innate immune response. These findings may lead to the deeper understanding of the relationship between stress and immunity, as well as may promote the development of immune stimulatory drugs.

1D_07_P

EXTRACELLULAR HSP70-INDUCED SIGNALING EVENTS IN A431 CARCINOMA CELLS

Anton L. Evdonin, Natalia D. Medvedeva

Institute of Cytology, RAS, S-Petersburg, Russia
evdonin@mail.ru

Recent studies demonstrated that Hsp70 is being released into blood or the conditioned medium of cultured cells under stressful conditions. Exogenously added purified Hsp70 was shown to induce signal transduction in monocytes and macrophages. However, whether Hsp70 can induce signal transduction events in non-immune cells remains unclear. The aim of this study was to investigate the signaling events triggered by extracellular Hsp70 in carcinoma cells. At initial steps of heat stress Hsp70 was shown to appear in the conditioned medium of heated A431 carcinoma cells due to its secretion. Both heat shock and extracellular Hsp70 induce association of TLR2/4 with downstream adaptor protein MyD88 which confirms the activation of TLR2/4. Simultaneously, heat shock, extracellular Hsp70 and conditioned medium from heated A431 cells stimulate ligand-independent EGFR activation and activation of EGFR-dependent signaling pathways including Erk1/2, PLCγ1 and STAT3. The depletion of Hsp70 from such medium abolished EGFR transactivation. The neutralizing antibody to TLR2 and 4 attenuated both EGFR and ERK1/2 phosphorylation which confirms the cross talk between TLRs and EGFR signaling systems in A431 cells. Moreover, extracellular Hsp70 and heat shock induce association of TLR2 and 4 with EGFR. While the protective function of intracellular Hsp70 is well documented, the present study provide evidence that Hsp70 secreted from cells at initial steps of

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

heat stress might play the same function by mediating EGFR transactivation, which is known to prevent stress-induced apoptosis.

1E_01_P

THE INTERACTION OF HELICAL PROTRUSIONS IS IMPORTANT IN THE PROTEIN FOLDING CYCLE OF GROUP II CHAPERONINS

Taro KANZAKI[1], Ryo IIZUKA[2], Kazunobu TAKAHASHI[3], Kosuke Maki[3], Noriyuki ISHII[4], Muhamad Sahlan[1], Masahiro FURUTANI[5], Kunihiro KUWAJIMA[3], Masafumi YOHDA[1]

[1]Tokyo Univ. of Agri. & Technol., Tokyo; [2]Univ. of Tokyo, Tokyo; [3]Inst. for Mol. Sci., Aichi; [4]Adv. Ind. Sci. & Technol., Ibaraki; [5]Minase Res. Lab., Sekisui Chem. Co. Ltd, Osaka, Japan
email: kanzaki@bel.bio.tuat.ac.jp

Group II chaperonins, found in the archaeal and eukaryotic cytosol, function independently of the cofactor corresponding to GroES of group I chaperonins. Rather, the helical protrusion at the tip of the apical domain, which forms a built-in lid of the central cavity, substitutes for the cofactor. We have been studying the protein folding mechanism of group II chaperonins using the chaperonin from a hyperthermophilic archaeum, *Themococcus* sp. strain KS-1. Previous studies have shown that ATP drives the conformational change of group II chaperonins, from the open-lid, substrate binding conformation, to the close-lid conformation to encapsulate unfolded protein in the central cavity. However, the details of the ATP-driven reaction remain obscure. It has been considered that the conformational change is driven in the cooperative manner. However, the mechanism of the cooperation between subunits has not been studied. To elucidate this issue, we constructed and characterized the chaperonin complexes which are composed of wild and mutated subunits in the ordered fashion by connecting them. Although the complexes containing mutations around ATP binding sites retained protein folding activity, the complex with the helical protrusion deletion had lost ATP dependent conformational change and also protein folding activity. Thus, we concluded that not cooperative action of ATP hydrolysis but the interaction of helical protrusions in the ring is important in the protein folding cycle of group II chaperonins.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1E_02_P

GENE STRUCTURE AND SPATIAL DISTRIBUTION OF HSP60 CHAPERONIN OF *LUCILIA CUPRINA*: IMPLICATIONS IN GERM CELL DIFFERENTIATION AND NUCLEAR FUNCTIONS

P. V. J. Reddy, Sunita Sharma and P. K. Tiwari

School of Studies in Zoology, Jiwaji University, Gwalior – 474 011. India.
Email: pk_tiwari@hotmail.com

The HSP60 chaperonin is widely appreciated for its role in protein folding. However, the detailed information on its cellular functions still remained elusive. The present study is a follow-up of our studies on the functional characterization and significance of HSP60 protein in sheep blowfly, *Lucilia cuprina*, which revealed interesting results. The immunocytochemical and immunoflourescence studies showed the expression of *Lucilia* HSP60 protein to be generally constitutive, whose level rises upon heat stress. In a few cell types, it appears tissue- or developmental stage-specific and even heat inducible. More striking is its presence in several nuclei of larval and adult polytene cell types while gene or protein analysis revealed an apparent absence of NLS, strongly suggesting its involvement in both cytoplasmic and nuclear functions. The expression pattern in germline tissues is more interesting, particularly in ovarian tissues. The oocytes showed a stage-specific expression of the protein, varying from 1st – 14th stages. The spatial pattern of HSP60 expression in mature eggs indicates a distinct role for it. The complete *L. cuprina hsp60* gene was cloned using genomic PCR strategy and RACE. The major portion of the coding sequence of the *hsp60* gene was isolated using the primers designed from *Drosophila melanogaster* cDNA sequence. The sequence analysis revealed a 2190 bps long sequence with a 1730 bps coding region and 5' and 3' UTRs of 189 and 271 bases respectively. Sequence alignment of nucleotide and amino acid sequences confirmed a significant homology among divergent species. The i*n-silico* analysis of the protein structure revealed significant structural homology with GroEL. The real time PCR analysis showed that the copy number and expression levels of *hsp60* gene are almost ten fold lesser than its compatriot *Drosophila*. RNA-RNA *in-situ* hybridizations showed transcript expression to be more in differentiating cell types than the differentiated cell types, further pointing its functional significance to be differential during various life cycle stages. Details of the possible mechanism/s of the nuclear localisation of HSP60 and its functional significance in cell nuclei and in differentiating (germ) cells will be discussed.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1E_03_P

HSP90 IN *C. ELEGANS* AND *BRUGIA*: HIGHLY CONSERVED YET DIFFERENT

Eileen Devaney, Vicki Gillan, Kirsty Maitland and Nik A.I.I. Nik Him

The molecular architecture of Hsp90 is highly conserved throughout evolution. However, *C. elegans* Hsp90 is unique amongst eukaryotes because of its resistance to Geldanamcyin. This finding was originally interpreted as an example of adaptive evolution as both *C. elegans* and the micro-organism that synthesizes GA live in the same ecological niche of the soil. In contrast Hsp90 from the parasitic nematode *Brugia pahangi* specifically binds to GA. Using a comparative genomics approach we have investigated whether the GA-resistant phenotype of *C. elegans* is unique or is shared with other nematodes. From our analysis to date additional nematodes that express either a GA-resistant or a GA-sensitive Hsp90 have been identified and we are using this information in an attempt to dissect the molecular basis of GA resistance in *C. elegans*. In addition we are expressing the GA sensitive Bp-Hsp90 in *C. elegans* to determine whether this confers GA sensitivity on the free-living species. Our results suggest that additional factors beyond the primary amino acid sequence and predicted structure determine the GA sensitivity of Hsp90.

1E_04_P

AMINO ACID SUBSTITUTIONS OBSERVED IN HUMAN HSP90 ISOFORMS THAT IMPEDE THE DIMERIC INTERACTION

Takayuki K. NEMOTO, Takeshi KOBAYAKAWA, Toshio ONO, Shin-ichi YAMADA, Akio MIZUNO, Tomomi T. BABA, Yuko OHARA-NEMOTO

It has been proposed that Hsp90 captures a client protein like a molecular clamp. The dimeric structure of Hsp90 mediated by the C-terminal region appears to be essential as a joint of the clamp. We here examined the effects of the amino acid substitutions present between human Hsp90 isoforms, i.e., Hsp90α and Hsp90β, and a single nucleotide polymorphism (SNP) found in Caucasians on the dimeric interaction. The dimer-forming potential of Hsp90β is less than that of Hsp90α due to the 16 amino acid substitutions at the 561-685 amino-acid region of the C-terminus. Bacterial two-hybrid system demonstrated, among the 16 amino acids, the conversions of Thr566 and Ala629 of Hsp90α to Ala558 and Met621 were primarily responsible for impeded dimerization of Hsp90β. Yeast expression system revealed that the SNP (Gln488>His) of the Hsp90α observed in Caucasians disrupts the function of Hsp90 (MacLean, M.J., et

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

al 2006). To investigate its molecular mechanism, we evaluated the domain-domain interaction of Hsp90α with the missense mutation. Human Hsp90α 401-732 interacts with both Hsp90α 1-400 and 401-732 through the intra-molecular and dimeric interactions, respectively. The two-hybrid system revealed that the mutation selectively decreased the latter interaction. The present study defined the amino acid substitutions affecting the dimerization of Hsp90 and demonstrated that the dimeric interaction is prerequisite for the *in vivo* function of Hsp90.

1E_05_P

SIGNIFICANCE OF THE N-TERMINAL DOMAIN FOR THE FUNCTION OF CHLOROPLAST CPN20 CHAPERONIN

Anat L. Bonshtien, Celeste Weiss, Abdussalam Azem

Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel
anatgree@post.tau.ac.il

Chaperonin proteins cpn60 and cpn10 are essential proteins involved in cellular protein folding. Plant chloroplasts contain a unique version of the cpn10 co-chaperonin, cpn20, which consists of two homologous cpn10-like domains (N-cpn20 and C-cpn20) that are connected by a short linker region. Although cpn20 seems to function like other single-domain cpn10 oligomers, the structure and specific functions of the domains are not understood. We mutated amino acids in the "mobile loop" regions of either N-cpn20, C-cpn20 or both: a highly conserved glycine, which was shown to be important for flexibility of the mobile loop and a leucine residue shown to be involved in binding of co-chaperonin to chaperonin. The mutant proteins were purified and their oligomeric structure validated by gel filtration, native gel electrophoresis and circular dichroism. Functional assays of protein refolding and inhibition of GroEL ATPase both showed that i) Mutation of the conserved glycine reduced the activity of cpn20, whether in N-cpn20 (G32A) or C-cpn20 (G130A). The same mutation in the bacterial cpn10 (GroES G24A), had no effect on activity. ii) Mutants of the highly conserved leucine of N-cpn20 (L35A) were inactive, as was the corresponding mutant of GroES (L27A) iii) Mutants of the conserved leucine in C-cpn20 (L133A) retained 55% activity. We conclude that the structure of cpn20 is much more sensitive to alterations in the mobile loop than is the structure of GroES. Moreover, only N-cpn20 is necessary for activity of cpn20. However, full and efficient functioning requires both domains.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1E_06_P

KNOCKDOWN OF THE HSP90 CO-CHAPERONE CDC37 CHEMOSENSITIZES AND ARRESTS THE GROWTH OF PROSTATE CANCER CELLS BY INHIBITING MULTIPLE SIGNALING CASCADES

Phillip J. Gray, Jr., Mary Ann Stevenson and Stuart K. Calderwood

Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215. Correspondence: pjgray@bidmc.harvard.edu

In the fight against prostate cancer, the second leading cause of cancer death in men, delineation of the complex molecular interactions within the prostate cancer cell will be critical to improving outcomes for patients. The HSP90 chaperone system is already known to interact with and maintain a wide variety of cell signaling pathways commonly dysregulated in cancer. An important part of this system is the kinase and steroid receptor targeting subunit Cdc37. Using RNA interference by lentivirus-delivered shRNA constructs we were able to determine the effects of Cdc37 knockdown on prostate cancer cells. Our data indicate that loss of Cdc37 function induces growth arrest and sensitizes prostate cancer cells to chemotherapeutic drugs such as Paclitaxel. Although loss of Cdc37 function does not significantly alter the intracellular levels of most known HSP90/Cdc37 clients, it does markedly inhibit their activity and ability to function in signal transduction. We show that loss of Cdc37 leads to reduced flux through multiple signaling pathways including the Erk pathway, the Akt pathway, the mTOR pathway and the androgen receptor pathway. We have also discovered synergistic interaction between the knockdown of Cdc37 and the inhibition of HSP90 by the anticancer drug 17AAG. Thus Cdc37 is important for maintaining the tumor phenotype in cancer cells and represents a novel target in the search for multi-targeted therapies based on the HSP90 chaperone system.

1E_07_P

INTERACTION OF PREFOLDIN WITH GROUP II CHAPERONIN IN THE PRESENCE OF VARIOUS NUCLEOTIDES

Tamotsu ZAKO[1,*], Yosuke MURASE[1, 2], Andrei ZHUKOV[3], Robert KARLSSON[3], Masafumi SHIMIZU[4], Ryo IIZUKA[2], Mizuo MAEDA[1] and Masafumi YOHDA[2]

*[1]RIKEN, [2]Tokyo Univ. of Agri. and Tech, [3]BIAcore AB, [4]Tokyo Univ. of Tech. * Address: Bioengineering Lab. RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198 Japan, E-mail:zako@riken.jp*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Prefoldin is a molecular chaperone that captures a protein-folding intermediate and transfers it to a group II chaperonin for correct folding. Previously we have shown that the binding rate of prefoldin to chaperonin correlates with the transfer rate of a substrate between them and electrostatic interaction is important for their interaction. However, the manner in which the prefoldin interacts with its substrates and cooperates with the chaperonin is poorly understood. In this study, we examined affinities of *Pyrococcus* prefoldin (PhPFD) and a chaperonin mutant from *Thermococcus* sp. strain KS-1 (CPNαK250E/K256E) in the presence of various nucleotides using fluorescent anisotropy and SPR sensor. CPNαK250E/K256E takes an open conformation in the nucleotide-free or ADP bound states and changes to a closed conformation upon binding of ATP. Our results indicate that PhPFD binds more tightly to CPNαK250E/K256E in the nucleotide-free state or in the presence of ADP than in the presence of AMP-PNP, unhydrolyzable ATP analogue. These results agree well with the substrate protein transfer model suggesting the prefoldin binds to the open-state chaperonin. The kinetic experiments using BIAcore T100 have shown that PhPFD-CPNαK250E/K256E interaction involves two steps. The fast encounter step is followed by a slower docking step whereby the complex undergoes a slow conformational transformation.

1E_08_P

CHARACTERIZATION OF THE ACTIVATION MECHANISM OF HTRA PROTEASE FROM *ESCHERICHIA COLI*.

Anna Sobiecka-Szkatula[1]*, Joanna Skorko-Glonek[1], Andrea Scire[2], Fabio Tanfani[2], and Barbara Lipinska[1]

1. University of Gdansk, Gdansk, Poland.
2. Universita Politecnica delle Marche, Ancona, Italy.
** sobiecka@biotech.ug.gda.pl*

HtrA from *E. coli* is a periplasmic serine protease responsible for degradation of misfolded proteins. The proteolytic activity of HtrA is regulated in a temperature-dependent manner. According to the HtrA's crystal structure below 30°C the access to the active center is restricted by three interacting loops: LA, L1 and L2. At higher temperatures the protease is activated and this process most probably requires the disruption of the loop trio. The way this process occurs is still not clear. To examine the importance of the LA-L1-L2 interaction on HtrA stability we introduced a tryptophan residue into each of the loops. As the wt HtrA does not contain tryptophan, the introduced Trp served as internal probe for studying conformational changes within the loops. Using Fourier

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Transform-Infrared Spectroscopy we confirmed that the mutations did not affect the secondary structure or the thermal stability of the mutants; thus the proteins were suitable for further analysis. The conformational changes induced by temperature were studied by circular dichroism (CD) technique at wavelengths 260-320 nm. We monitored the CD spectra at temperature range 25- 50°C and focused particularly on the spectrum of tryptophan (wavelength range 280 –300 nm). We observed that significant changes in the CD signal intensity occurred at temperature ranges 30-32,5°C and 37–45°C. The observed changes correlate well with the changes of the HtrA proteolytic activity: at 30°C HtrA becomes proteolytically active and at temperature above 40°C the activity significantly rises to reach maximum at 50-55°C.
The obtained results were verified further by spectrofluorimetric methods.

1E_09_P

ANTI-KU-POSITIVE AUTOIMMUNE DISEASES: POTENTIAL FUNGAL TRIGGERING

F. Guarneri, M. Vaccaro, C. Guarneri

Institute of Dermatology - University of Messina (Italy)
AOU Policlinico "G. Martino" - Via Consolare Valeria - Gazzi – Messina (Italy)
E-mail of the corresponding author: f.guarneri@tiscali.it

Molecular mimicry (MM) has been postulated as a trigger of autoimmune diseases (AID). Ku is a heterodimer of 70 kDa (p70) and 80 kDa (p80) protein subunits and has a key role in multiple processes, particularly in stress conditions: DNA repair, chromosome maintenance, transcription regulation, V(D)J recombination. Ku is also part of a group of DNA-associated antigens targeted by autoantibodies in systemic lupus erythematosus and related disorders. It is found in 5% of sclerodermic patients, and is suggestive of systemic sclerosis/polymyositis overlap syndrome. Since a possible role of MM in the generation of autoimmunity to this antigen has been suggested, we used *in silico* techniques to identify potentially responsible microbial proteins. We used BLAST to compare amino acid sequences of p70 and p80, with all known sequences (as of April 18, 2007) of bacteria (n=5,229,868), viruses (n=629,582) and fungi (n=511,126). The cut-off for the E parameter was 0.001. We found no homologies with bacterial or viral proteins. Homologous fungal proteins were 51 for p70 and 24 for p80. In both cases, only 12 belonged to human pathogens (*A. clavatus, A. fumigatus, A. nidulans, A. niger, A. terreus, C. glabrata, C. globosum, C. immitis, C. neoformans, N. fischeri, Y. lipolytica,* and *D. hansenii* for p70 and *C. albicans* for p80). Amino acid identity range was 20-30% and 20-27%, similarity range was 39-50%

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

and 40-48%, E values were between $2x10^{-66}$ and $3x10^{-4}$ and between $2x10^{-35}$ and $3x10^{-4}$, respectively. p70-homologous segments spanned at least one of the 3 T-cell epitopes of this molecule, while p80-homologous segments spanned at least one of the 3 T-cell epitopes of the autoantigen in 8 cases (in the others, overlap was partial). Our data suggest that some fungi could trigger anti-Ku-positive AID via MM. Further research is needed to verify this hypothesis.

1F_01_P

EXTRACELLULAR HSP70-INDUCED SIGNALING EVENTS IN A431 CARCINOMA CELLS

Anton L. Evdonin, Natalia D. Medvedeva

Institute of Cytology, RAS, S-Petersburg, Russia
evdonin@mail.ru

Recent studies demonstrated that Hsp70 is being released into blood or the conditioned medium of cultured cells under stressful conditions. Exogenously added purified Hsp70 was shown to induce signal transduction in monocytes and macrophages. However, whether Hsp70 can induce signal transduction events in non-immune cells remains unclear. The aim of this study was to investigate the signaling events triggered by extracellular Hsp70 in carcinoma cells. At initial steps of heat stress Hsp70 was shown to appear in the conditioned medium of heated A431 carcinoma cells due to its secretion. Both heat shock and extracellular Hsp70 induce association of TLR2/4 with downstream adaptor protein MyD88 which confirms the activation of TLR2/4. Simultaneously, heat shock, extracellular Hsp70 and conditioned medium from heated A431 cells stimulate ligand-independent EGFR activation and activation of EGFR-dependent signaling pathways including Erk1/2, PLCγ1 and STAT3. The depletion of Hsp70 from such medium abolished EGFR transactivation. The neutralizing antibody to TLR2 and 4 attenuated both EGFR and ERK1/2 phosphorylation which confirms the cross talk between TLRs and EGFR signaling systems in A431 cells. Moreover, extracellular Hsp70 and heat shock induce association of TLR2 and 4 with EGFR. While the protective function of intracellular Hsp70 is well documented, the present study provide evidence that Hsp70 secreted from cells at initial steps of heat stress might play the same function by mediating EGFR transactivation, which is known to prevent stress-induced apoptosis.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1F_02_P

STRESS PROTEIN ACTIVATION BY THE OMEGA-3 FATTY ACID DOCOSAHEXAENOIC ACID IN HUMAN COLON CANCER CELLS

Hilde Bremseth[1*], Anne Gøril Lundemo[2] , Caroline Hild Jakobsen[2], Gro Leite Størvold[2,3], Hans Einar Krokan[1] and Svanhild Arentz Schønberg[2]

[1] Department of Cancer Research and Molecular Medicine, NTNU
Erling Skjalgssons gate 1, N-7006 Trondheim, Norway
[2] Department of Laboratory Medicine, Children's and Women's Health, NTNU
Erling Skjalgssons gate 1, N-7006 Trondheim, Norway
[3]Department of Medical Genetics, Ullevål University Hospital, Faculty of Medicine, University of Oslo, 0318 Oslo, Norway
**Correspondence: hilde.bremseth@ntnu.no*

Docosahexaenoic acid (commonly known as DHA; 22:6(ω-3)) is an omega-3 essential fatty acid. We have earlier shown that DHA affects the proliferation and survival of colon cancer cells. In order to evaluate the molecular mechanisms involved in the anti-cancer action of this fatty acid, gene expression analysis were performed at several time points. The results showed activation of a stress response after treatment of the cells with DHA. Heat shock protein 70 (HSP70) and the Nrf2-dependent stress protein heme oxygenase-1 (HO-1) were both found to be highly up-regulated at the protein level. These results suggest that stress proteins are key mediators or modulators of the effects of DHA. Genes encoding redox enzymes were also found to be up-regulated after DHA treatment. The work presented here will try to answer if the observed stress protein activation in colon cancer cells treated with DHA is related to changes in the cellular redox state and involves modification of redox-sensitive transcription factors.

1F_03_P

HANS SELYE'S "DISEASE OF ADAPTATION"--TYPE 2 DIABETES MELLITUS—A BREAKDOWN IN THE STRESS RESPONSE

Philip L. Hooper, Paul L. Hooper

University of Colorado Health Sciences Center, Glen Haven, CO, USA
University of New Mexico, Albuquerque, NM, USA
phoopermd@gmail.com

Hans Selye identified type 2 diabetes as a "disease of adaptation", a pathology that occurs as the stress response itself breaks down in the face

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

of prolonged, chronic stress. His writings pondered the etiology of such diseases, suggesting that there was a finite energy of adaptation that, when depleted, resulted in disease, early aging and ultimately death. Our thesis is that type 2 diabetes is indeed the product of a breakdown in the stress response that results, not from general energetic constraints, but from a specific self-perpetuating cycle that cripples the body's ability to respond to stress via loss of insulin signaling. This breakdown occurs in the following way: chronic metabolic stress (from high fat diet, inactivity, aging, genetic predisposition) leads to the production of cytokines and counter-regulatory hormones (cortisol, epinephrine, growth hormone), which impair insulin signaling. Reduction of insulin signaling activates glycogen synthase kinase-3 (GSK-3) which deactivates Heat Shock Factor-1, resulting in lower heat shock proteins (Hsps)-- a state which further promotes cytokine activity and augments defects in insulin signaling. Proof of concept for this thesis is supported by these observations: 1) Reduction of pain and stress in surgery improves outcomes. 2) Insulin infusion at the time of surgery or in critically ill patients (with no diabetes) raises Hsp levels and improves morbidity and mortality. 3) Agents that improve insulin signaling (like GSK-3 inhibitors) raise Hsp levels, thus reducing inflammation which further improves insulin signaling and survival. 4) When insulin signaling is damaged (pre-diabetes and diabetes), tissue Hsp levels are low and tissues are vulnerable to stress. Thus, Hans' concern that the stress response itself can lead to disease and death is valid, not due to energy exhaustion, but due to loss of cytoprotective Hsps and unabated inflammation.

1F_04_P

EFFECT OF GELDANAMYCIN ON TRANSCRIPTION OF HEAT-SHOCK PROTEIN 90 ISOFORMS, HSP90ALPHA AND HSP90BETA, IN BRAIN OF GOLDFISH

Nao Kagawa and Kohta Morishige

Kinki University, Osaka, Japan. kagawa@life.kindai.ac.jp

Geldanamycin (GA) is a specific inhibitor of chaperoning function of Hsp90 protein. We recently reported that intracerebral GA treatment drastically reduced basal level of Hsp90 protein in the brain of goldfish (*Carassius auratus*). To understand mechanisms involved in this finding, we examined mRNA levels of two isoforms of Hsp90, α and β, after GA administration in the brain of goldfish at a dose of 0.05 or 1.0 μg/g-body weight. Hsp90α and Hsp90β are stress-inducible and constitutive protein, respectively. Levels of Hsp90α mRNA, Hsp90β mRNA and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) mRNA in the brain were determined by RT-PCR method at allotted time from 4 to 72 h after GA treatment. The

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

OD ratio of Hsp90α mRNA or Hsp90β mRNA to GAPDH mRNA was calculated as an index of transcription level for Hsp90α or Hsp90β gene. The OD ratio for Hsp90α after GA treatment at 1.0 and that at 0.05 μg/g both showed a 1.5 fold significant increase in comparison with GA-untreated control as determined at 12 and 20 h post-treatment; then, they peaked 20 and 28 h after treatment, respectively. Thereafter, the ratio values both decreased to the control level at 36 h post-treatment. In contrast, the OD ratios for Hsp90β were almost constant throughout the time course used after treatment without significant deviation from the control value. These results suggest that GA caused reduction in the abundance of Hsp90 protein in the brain of goldfish is a post-translation event, most probably, by proteosome-mediated degradation of GA-bound Hsp90α protein, which in turn upregulate transcription of Hsp90α gene.

1F_05_P

DESIGN OF NOVEL STRESS PROTECTORS BASED ON SHORT SYNTHETIC PEPTIDES

Navolotskaya E.V.[1], Kovalitskaya Y.A.[1], Kolobov A.A.[2], Sadovnikov V.B.[1]

[1]Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Pushchino, Moscow Region, Russia; [2]State Research Center for Institute of Highly Pure Biopreparations, St. Petersburg, Russia

The work is directed towards the solution of one of the most important problems of modern biology and medicine – the creation of novel effective and safe agents capable of elevating adaptation potential of the human body under the influence of various extreme factors. The objective of the work is synthesis of a set of short corticotropin-like peptides and study of their effect on non-specific resistance of laboratory animals subjected to various stress factors. It was synthesized 75 of corticotropin-like peptides, which contained from 13 to 2 amino acid residues. The ability of the synthesized peptides to inhibit the specific binding of tritium labeled corticotropin fragment 11-24 to rat adrenal cortex in vitro was investigated. On the base of the obtained results there were selected 6 peptides (KKRR, KKRRP, VKKPGSSVKV, KKRRLLLL, LLKKRRLLLL, ac-KKRR-NH_2) with the highest inhibitory activity for in vivo tests. The influence of the selected peptides on the level of glucocorticoids and catecholamines in the adrenals and blood of rats in the experiments on acute hemorrhage and hypobaric hypoxia were studied. It was established that intravenous injection of peptides KKRR, KKRRP or ac-KKRR-NH_2 at the dose of 1 μg/kg could correct the changes in the content of corticosterone, adrenaline and noradrenaline in the adrenals and plasma of rats that were subjected to hemorrhagic shock or hypobaric hypoxia. It has been shown that the

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

stress-protective activity of these peptides is mediated by the corticotropin receptor in cortex of the adrenals.

1F_06_P

PROTECTIVE EFFECT OF A NOVEL MOLECULAR CHAPERONE INDUCER, PAEONIFLORIN, ON THE HCL-INDUCED GASTRIC MUCOSAL INJURY

Kenzo Ohtsuka, Daisuke Kawashima, Midori Asai

Laboratory of Cell & Stress Biology, Department of Environmental Biology, Chubu University, 1200 Matsumoto-cho, Kasugai, Aichi 487-8501, Japan (Corresponding author: Ohtsuka, K., email: kohtsuka@isc.chubu.ac.jp)

Moderate overexpression of heat shock proteins (HSPs) or molecular chaperones could confer cells and tissues stress tolerance and provide beneficial effects on various pathological states associated with protein misfolding and protein aggregation. Recently, we found a novel chaperone inducing compound, paeoniflorin, which is one of the major constituents of a herbal medicine derived from *Paeonia lactiflora* Pall. Paeoniflorin could induce Hsp70, Hsp40, and Hsp27 in cultured HeLa cells. Treatment of cells with paeoniflorin resulted in enhanced phosphorylation and acquisition of DNA binding ability of heat shock factor 1 (HSF1), as well as the formation of characteristic HSF1 granules in the nucleus, suggesting that the induction of these HSPs by paeoniflorin is mediated by the activation of HSF1. Also, thermotolerance was induced by the treatment with paeoniflorin. Paeoniflorin had no apparent toxic effect at concentrations as high as 500 μM (*Cell Stress & Chaperones* 9: 387-389, 2004).

Also, we investigated the effect of paeoniflorin on the induction of Hsp70 in mouse stomach and the HCl-induced gastric mucosal injury. Administration (i.p.) of paeoniflorin resulted in the induction of Hsp70 in mouse stomach detected by western blot and immunohistochemical staining. Severe gastric mucosal injury was caused 3 hr after oral administration of HCl. Prior administration of paeoniflorin could protect the HCl-induced gastric mucosal injury as demonstrated by an overview and histological HE staining. The extent of protection was well correlated with the expression level of Hsp70. No apparent systemic side effect of paeoniflorin was observed so far. Hsp70 was also induced in liver, heart and brain by paeoniflorin (manuscript in prepararion). From these results, it is suggested that paeoniflorin and paeoniflorin-containing herbal medicines might be used clinically as chaperone inducers for the prevention and treatment of various pathological states, such as stress ulcers and ischemia-induced injuries, and of diseases associated with protein misfolding and protein aggregation.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

This work was supported in part by a Grant-in-Aid for the High-Tech Research Center Establishment Project (2002-2006, Chubu University) from the Japanese Ministry of Education, Culture, Sports, Science and Technology.)

1F_07_P

ADAPTOGENS – MODIFIERS OF STRESS RESPONSE

Panossian A.*, Wikman G.
Swedish Herbal Institute Research and Development, Prinsgatan 12, SE-413 05 Göteborg, Sweden

Hambartsumyan M., Hovhanissyan A.
"ExLab" Expert Analytical Laboratory of Armenia Drug Agency, Komitas Ave. 49/4,
375051 Yerevan, Armenia

Adaptogens possess anti-fatigue and anti-stress activities that can increase mental and physical working performance against a background of fatigue or stress. A characteristic feature of adaptogens is that they act as eustressors or challengers and give rise to stimulating or stress-agonising effects following single administration. The stress-protective action achieved by multiple administration of adaptogens is not, therefore, the result of inhibition of the stress response of an organism, but of adaptive changes in the organism in response to the repeated stress-agonistic properties of the drug. The aim of the present study was to ascertain which mediators of stress response are significantly involved in the mechanisms of action of adaptogens, and to determine their relevance as biochemical markers for evaluating anti-stress effects in rabbits subjected to immobilisation stress. Blood levels of stress-activated protein kinase (SAPK/JNK), the phosphorylated kinase p-SAPK/p-JNK, nitric oxide (NO), cortisol, testosterone, prostaglandin E2, leukotriene B4 and thromboxane B2 were determined in groups of animals prior to daily oral administration of placebo or rhodioloside or extracts of Eleutherococcus senticosus, Schisandra chinensis, Rhodiola rosea, Bryonia alba or Panax ginseng over a 7 day period. Ten minutes after the final treatment, animals were immobilised for 2 hours and blood levels of the markers re-determined. In the placebo group, only p-SAPK/p-JNK, NO and cortisol were increased significantly (by 200-300% cf basal levels) following immobilisation stress, whilst in animals that had received multiple doses of adaptogens/stress-protectors, the levels of NO and cortisol remained practically unchanged after acute stress. It is known that NO can strongly inhibit the production of cellular energy through two mechanisms: (i) inhibition of mitochondrial respiration by reversible and irreversible

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

inhibition of cytochrome P450, and (ii) the inhibition of glycolysis through modification of the SH-groups of glyceraldehyde-3-phosphate dehydrogenase. The inhibition of stress-induced NO production demonstrated in the present study may provide an explanation of the energy bursting effects of adaptogens that result in a prolonged phase of endurance and postponed fatigue. Rhodioloside and extracts of S. chinensis and R. rosea (adaptogens containing only phenolic compounds) were the most active inhibitors of stress-induced p-SAPK/p-JNK. B. alba and P. ginseng (stress-protectors containing only cortisol-like substances) exerted little effect on p-SAPK/p-JNK levels. It is suggested that the inhibitory effects of R. rosea and S. chinensis on SAPK/JNK activation may be associated with their anti-depressant activity as well as their positive effects on mental performance under stress.

*E-mail of corresponding author: ap@shi.se

1F_08_P

INHIBITION OF ER STRESS-INDUCED XBP1 ACTIVATION BY TRIENE-ANSAMYCINS

Etsu Tashiro, Yushi Futamura, Naoka Hironiwa and Masaya Imoto

Faculty of Science and Technology, Keio University, Yokohama 223-8522, Japan
E-mail: tashiro@bio.keio.ac.jp

Accumulations of unfolded proteins in ER cause ER stress, leading to activation of several transcription factors. Among them, XBP1 is one of the major transcription factors of ER stress. Under ER stress condition, a 26-nt intron of XBP1 mRNA is spliced out by activated IRE1, leading to a frame shift. This splicing event creates translational frameshift in XBP1 mRNA to produce an active transcription factor. Recently, XBP1 is considered to be much correlated with tumor progression, because it has been reported that Xbp1-knockdown cells did not form tumors in mice and the expression of XBP1 was increased in a several types of tumor cells. Therefore, XBP1 inhibitor would be a new class of anti-cancer drug. To screen inhibitors of ER stress-induced XBP1 activation, we first constructed pcDNA3/XBP1-luc plasmid that is fused the luciferase cDNA downstream of XBP1 cDNA. Then, we transfected pcDNA3/XBP1-luc plasmid into HeLa cells to generate HeLa/XBP1-luc cells that are stably transcribed XBP1-luciferase mRNA. Under normal condition, the XBP1-luciferase mRNA is not spliced and its translation is terminated at the stop codon near the splice site of XBP1 mRNA, resulting in that the luciferase protein is not produced. In contrast, under ER stress condition, the 26-nt intron is excised, leading to frame shift of XBP1-luciferase mRNA and its

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

translation is then terminated at the stop codon of the luciferase mRNA. Thus, we can easily detect the XBP1 activation as the luciferase reporter signals in HeLa/XBP1-luc cells. In the course of our screening for the inhibitor of thapsigargin-induced XBP1 activation from microbial origin by using HeLa/XBP1-luc cells, we found and isolated natural small molecules, triene-ansamycins such as trienomycin A. Triene-ansamycins showed a marked inhibitory activity toward thapsigargin-induced XBP1 activation, as evaluated by both luciferase assay and RT-PCR. Here, we will present the isolation, structure determination, and biological activities of Triene-ansamycins.

1F_09_P

EFFECT OF DOBUTAMINE AND NOR-EPINEPHRINE ON TNF-A AND IL-6 RELEASE OF WHOLE BLOOD AFTER LPS STIMULATION

V. Papandreou, P. Myrianthefs, G. Fildissis, J. Portolos, G. Baltopoulos

Athens University School of Nursing ICU at « KAT » Hospital, Nikis 2, 14561, Kifissia, Greece, email: vpapandr@gmail.com

The purpose of the study was to examine the effect of dobutamine (D) and nor-epinephrine (NE) on cytokine release of whole blood after ex vivo LPS stimulation. Heparinized blood samples were collected from 10 healthy volunteers, (mean age 33.6±1.5, all men) at 08.00 AM and transferred to the lab for processing. One hundred μL of blood was added in 900 μL of RPMI 1640 culture medium for final volume of 1 ml and placed in plastic culture dishes. Samples were then added to wells and maintained at 37° C in a 5% CO_2 atmosphere and LPS was added (500 pg/ml). After incubation of 4 hours the culture plates were centrifuged (1800 rpm, 5 min) and supernatants were collected for cytokine measurements using the ELISA method. We studied the effect of D and NE at different doses (10^{-6}, 10^{-5}, 10^{-4}) on cytokine production after whole blood LPS stimulation. We repeated the experiment by adding metoprolol (b-blocker) at a dose of 10^{-5}. Total WBCs were 6730 ± 242.2; lymphocytes were 2461 ± 395.7 and monocytes 359.2 ± 38.5 per μl. Dobutamine and NE significantly ($p<0.01$) decreased TNF-α production at doses of 10^{-5}, 10^{-4} but not at dose of 10^{-6} compared to controls. This effect was restored by adding metoprolol (10^{-5}) for dose of 10^{-5} but not for dose of 10^{-4} of D and NE. Dobutamine and NE significantly ($p<0.05$) decreased IL-6 production at a dose of 10^{-4} but not at doses of 10^{-5} and 10^{-6}. This effect was restored by adding metoprolol (10^{-5}). Dobutamine and NE decrease pro-inflammatory cytokine release of whole blood stimulated with LPS in a dose dependent fashion which is restored by the use of b-blockers. Dobutamine and NE seem to have equivalent cytokine inhibition effect.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1F_10_P

STABILIZATION OF IGFBP-1 MRNA BY ETHANOL IN HEPATOMA CELLS INVOLVES THE JNK PATHWAY

Michele GARLATTI, Laurent Magne, Etienne Blanc, Alexandre Marchand, Pierre Fafournoux, Robert Barouki, Helene Rouach

Insulin-like growth factor-binding protein-1 (IGFBP-1) modulates cell growth and metabolism and IGFBP-1 induction is suggested to be a physiological mechanism to restrict growth process under stress conditions in order to preserve the energy for survival functions. The aim of our study was to determine the molecular mechanisms involved in IGFBP-1 upregulation by ethanol. The human hepatoblastoma HepG2 cell line do not expressed ethanol-metabolizing enzymes. Exposure of these cells to varying concentrations of ethanol (35 to 150 mM) induced the IGFBP-1 mRNA and protein up to 5-fold in a dose-dependent manner. A similar effect was observed using primary cultures of human hepatocytes. This effect of ethanol in HepG2 cells was not prevented by various inhibitors of ethanol metabolism and by the anti-oxidant N-acetylcysteine. While ethanol did not modify the IGFBP-1 gene promoter activity, it triggered a 2- to 3-fold increase in the IGFBP-1 mRNA half-life and this stabilisation required the 5’ and the 3’ untranslated region of the mRNA. Ethanol elicits a rapid and transient activation of JNK in HepG2 cells and IGFBP-1 induction was partially prevented by a specific inhibitor of the JNK pathway. This study reveals a novel pathway of gene regulation by ethanol which involves the activation of JNK and the consequent mRNA stabilisation. These data improve the current understanding of the mechanisms involved in the control of gene expression by ethanol.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Module 2 – Oral lectures:

2A_01_S

P53 FAMILY IN APOPTOSIS

Eleonora Lapi[1], Silvia Di Agostino[1], Hilah Gal[3], Sara Donzelli[1], Eytan Domany[4], Gideon Rechavi[5], Sabrina Strano[1], David Givol[3], Xin Lu[5] and **Giovanni Blandino**[1,2]

[1]Regina Elena Cancer Institute, Rome , Italy - blandino@ifo.it
[2]Rome Oncogenomic Center, Rome, Italy.
[3] Weizmann Institute of Science, Rehovot , Israel.
[4] Weizmann Institute of Science, Rehovot , Israel.
[5] Sheba Medical Center, Tel-Hashomer, Israel.
[6]Ludwig Institute for Cancer Research, London, UK

The p53 family is known to be involved in the transcriptional control of growth arrest and apoptosis. Despite the recent identification of specific p73-target genes by genome-wide expression profile techniques, p73-mediated apoptosis occurs mostly through the activation of a set of genes that were originally found to be activated by p53. This suggests that promoter selectivity by both p53 and p73 might be the result of biochemical events such as post-translational modifications and specific protein-protein interactions.
We have already shown that the transcriptional co-activator Yes-associated protein (YAP) interacts with p73 and determines p73 gene targeting in response to DNA damage. We have also found that YAP localizes into the PML nuclear bodies and requires PML to exert its function as a specific co-activator of p73. Here we show the existence of a pro-apoptotic auto-regulatory feedback loop, during the apoptotic response, between p73, YAP and PML. We demonstrate that the p73/YAP complex is required for PML induction after cisplatin treatment and that PML exerts a vital role in the execution of the apoptotic process regulating YAP stability. YAP is becoming a very intriguing protein due to its critical role in regulating p73 accumulation and function following DNA damage, but very little is known about its regulation. Here we show that YAP is polyubiquitinylated and degraded through the ubiquitin-proteasome pathway. We also show that YAP and PML physically interact and that PML regulates YAP half-life, preventing its ubiquitinylation and subsequent degradation.

2A_02_S

HEAT SHOCK PROTEINS ORCHESTRATE DECISION DIFFERENTIATION VERSUS APOPTOSIS

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Celine Didelot, Jean-Antoine Ribeil, Yael Zermati, D. Lanneau, Olivier Hermine and Carmen Garrido

INSERM UMR 866, Faculty of Medicine, Dijon, 21000, France and Necker Hospital, PARIS 75743 Cedex 15, France

Heat shock proteins, particularly HSP90, HSP70 and HSP27, are well known regulators of apoptosis by interfering with key apoptotic proteins. Apoptosis and cell differentiation are two physiological processes that share common features like chromatin condensation and the need of the proteases called caspases. Little is known about the role of HSPs in the differentiation process. Here we show that HSP70, during terminal blood red cells differentiation and at the onset of caspase activation, translocates into the nucleus where colocalizes and interacts with GATA-1, a ttranscription factor essential for red cells progenitors (erythroblasts) differentiation. *In vitro* and *in vivo* assays demonstrate that HSP70 inhibits caspase-3-mediated proteolysis of GATA-1, allowing the differentiation of the erythroblasts. If the amount of nuclear HSP70 is reduced, GATA-1 is cleaved and the cells die by apoptosis (Ribeil et al, Nature 2007).
Another HSP needed in the differentiation process is HSP90, and more specifically the isoform beta. During monocytic and epithelial cells' differentiation, HSP90β accompanies the protein c-IAP1 (inhibitor of apoptosis protein-1) that is translocated from the nucleus to the cytosol. This translocation is needed for the cells to differentiate (Plenchette et al, Blood 2004). Depletion or neutralization of HSP90β blocks c-IAP1 cytosolic translocation and the differentiation process. We conclude that HSPs, like HSP70 or HSP90β, by their presence in a given cellular compartment and their cytoprotective properties, direct the cells to differentiate.

2A_03_S

NOVEL MECHANISMS OF HEAT SHOCK-INDUCED APOPTOSIS

Rania S. Milleron, Indra M. Mahajan, and Shawn B. Bratton

Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin.

Adaptive responses to mild heat shock are among the most widely conserved and studied in nature. More intense heat shock, however, induces apoptosis through mechanisms that remain largely unknown. Recently, we have observed that heat shock activates an apical protease, which stimulates mitochondrial outer membrane permeabilization (MOMP) and processing of the effector caspase-3 in a zVAD-FMK (polycaspase

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

inhibitor) and Bcl-2-inhibitable manner. Surprisingly, however, heat shock did not require any of the known initiator caspases (-2, -8, -9, or -12) or their activating complexes to promote apoptotic cell death, but instead relied upon the activation of an apparently novel apical protease with caspase-like activity. Current efforts in the laboratory are now focused on unraveling the mechanisms through which heat shock induces MOMP, and our most recent findings will be discussed.

2A_04_S

LYSOSOMAL CONTROL OF TUMOR CELL DEATH BY HSP70

Thomas Kirkegaard-Sørensen, Nicole Fehrenbacher, Jesper Nylandsted and Marja Jäättelä.

Apoptosis Department and Centre for Genotoxic Stress, Institute of Cancer Biology, Danish Cancer Society, Strandboulevarden 49, DK-2100 Copenhagen, Denmark. mj@cancer.dk

Tumor invasion and metastasis are associated with altered lysosomal trafficking and increased expression of the lysosomal proteases termed cathepsins. Emerging experimental evidence suggest that such alterations in lysosomes may form an "Achilles heel" for cancer cells by sensitizing them to death pathways involving lysosomal membrane permeabilization and the release of cathepsins into the cytosol. Here, I will highlight our recent unpublished results on cancer-related changes in the composition and function of lysosomes, focusing on the mechanisms by which lysosomal Hsp70 inhibits cancer cell death and emerges as a putative target for future cancer therapy.

Reference for background information:
Kroemer G, Jäättelä M. Lysosomes and autophagy in cell death control. *Nature Rev Cancer* 5:886-897, 2005

2A_05_S

UNDERSTANDING THE DEMOLITION PHASE OF APOPTOSIS

Seamus J. Martin

Molecular Cell Biology Laboratory, Department of Genetics, The Smurfit Institute, Trinity College, Dublin 2, Ireland
Email: martinsj@tcd.ie

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Apoptosis (programmed cell death) is coordinated by a family of cysteine proteases—the caspases—that dismantle cells by targeting numerous proteins for limited proteolysis. The mammalian caspase family contains 3 members, some of which participate in apoptosis. Caspases normally exist as dormant precursor enzymes in healthy cells but can be activated at the onset of apoptosis via a number of distinct activation pathways. Here we discuss the caspase activation pathways that are initiated by the cytotoxic T cell/Natural Killer cell protease granzyme B, as well as by cytotoxic drugs and diverse stress stimuli. How caspase activation results in the controlled demolition of the cell will also be explored.

2B_01_S

CELL SIGNALLING OF STRESS VIA CERAMIDE AND ITS METABOLITES

Antonio Gómez-Muñoz, Patricia Gangoiti, María H. Granado, and Monika González

Department of Biochemistry and Molecular Biology, Faculty of Science and Technology, University of the Basque Country, 48080-Bilbao, Spain. E-mail: antonio.gomez@ehu.es

Cellular stress has been defined as the threat of damage to macromolecules. Since many lipids, enzymes and signalling pathways contribute to the cellular stress response it is necessary to identify the key players that are located at major nodes within the stress response network. Many types of stresses including UV or ionizing radiation, oxidative stress, chemotherapeutic drugs, or starvation cause DNA or protein damage. This can result in growth arrest, apoptosis, or inflammatory responses. One of the mechanisms involved in these actions is the sphingomyelin pathway. Ceramide, the central molecule in this pathway, is an important second messenger that engages different downstream effectors depending on the concomitant activation of other second messengers and the activity of enzymes that convert ceramide to other related metabolites such as sphingosine, sphingosine 1-phosphate (S1P) or ceramide 1-phosphate (C1P). Whilst ceramide is pro-apoptotic and can induce cell cycle arrest, S1P or C1P are anti-apoptotic and have mitogenic properties. Ceramide and C1P can be interconverted in cells by kinase and phosphatase activities. An appropriate balance between the levels of these metabolites is crucial for cell and tissue homeostasis. Switching this balance towards accumulation of one or the other can result in metabolic dysfunction or disease. Therefore, the activity of the enzymes that are involved in C1P and ceramide metabolism must be efficiently coordinated to ensure normal cell functioning.
Supported by “Ministerio de Educación y Ciencia” (Grant BFU2006-13689),

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Madrid, Spain, and "Universidad del Pais Vasco, UPV/EHU (Grant 9/UPV 00042.310-15852/2004), Bilbao, Spain.

2B_02_S

TEMPERATURE STRESS: REACTING AND ADAPTING – LESSONS FROM POIKILOTHERMS

John L. Harwood

Cardiff University, School of Biosciences, Cardiff, CF10 3US, U.K. : Harwood@Cardiff.ac.uk

Poikilotherms, which represent most of the species on Earth, have to be able to adapt to changing environmental temperatures. In particular, the correct functioning of their membranes is critical. In order to ensure this, poikilotherms need to modify membrane lipids – sometimes very rapidly. We have studied this process in a number of organisms but, in particular, in the soil protozoon *Acanthamoeba castellanii*. The latter reacts to low temperatures by increasing activity of a fatty acid delta -12 desaturase, mainly through gene expression. Following low temperature stress, *A. castellanii* shows increased desaturase activity within minutes. This leads to conversion of oleate to linoleate and more 20C polyunsaturated metabolities. As soon as membrane fluidity is returned to normal, phagocytosis and growth recommence. Interestingly, desaturase activity is also regulated independently by oxygen concentrations. We have isolated a gene coding for a delta-12 desaturase which is bifunctional and also catalyses delta-15 desaturation. Aspects of temperature adaptation in *A. castellanii* and other organisms will be discussed.

2B_03_S

ABNORMAL INTERACTION OF MUTANT HSP22 (HSPB8) WITH THE RNA HELICASE DDX20 (GEMIN3, DP103)

Xiankui Sun[1], Jean-M. Fontaine[1], Adam D. Hoppe[2], Serena Carra[3], Cheryl DeGuzman[1], Jody L. Martin[4], Stephanie Simon[5], Patrick Vicart[5], Michael J. Welsh[1], Jacques Landry[3], Rainer Benndorf[1]

Departments of [1]Cell and Developmental Biology and [2]Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI, USA; [3] Centre de recherche en cancérologie de l'Université Laval, L'Hôtel-Dieu de Québec, Québec, Canada; [4]Department of Medicine, Cardiovascular Institute, Loyola University Chicago, Maywood, IL, USA; and [5]EA 300

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Stress et pathologies du cytosquelette, Université Paris 7, France.
rbenndo@umich.edu

Eight mutations in the small heat shock proteins (sHSP) Hsp22 and Hsp27 have been associated with the motor neuron diseases (MND) distal hereditary motor neuropathy and Charcot-Marie-Tooth disease. Hsp22 and Hsp27 interact with each other, suggesting that these two etiologic factors may act in the same pathway. In an effort to learn about the role of Hsp22 in MND, we screened a human cDNA library by the yeast two-hybrid method for potential binding proteins. One identified protein was the RNA helicase Ddx20, a core component of the survival-of-motor neuron (SMN) complexes. This interaction was verified by independent methods including FRET. Both mutant Hsp22 forms showed abnormally increased binding to Ddx20. Interestingly, Ddx20 itself binds to the SMN protein, and mutations in the *SMN1* gene cause spinal muscular atrophy, another MND. Thus, these protein interaction data have linked the etiologic factors Hsp22, Hsp27, and SMN, and mutations in any of these genes cause the various forms of MND. SMN complexes are involved in RNP processing. The mutant Hsp22/Ddx20 interaction was sensitive to treatment with RNase suggesting involvement of RNA in this interaction and a potential role of sHSPs in RNP processing.

2B_04_S

ROLES OF MOLECULAR CHAPERONES IN QUALITY CONTROL OF MEMBRANES AND MEMBRANE ASSOCIATING PROTEINS IN PROKARYOTES.

Hitoshi Nakamoto

Department of Biochemistry and Molecular Biology, Saitama University, Saitama 338-8570, Japan. Email: nakamoto@post.saitama-u.ac.jp

Molecular chaperones play important roles in protein quality control. Ample evidence has accumulated to show that they associate with membranes although they do not contain transmembrane domains or signal sequences. Here, I present an overview of function of molecular chaperones, especially those from cyanobacteria, in quality control of membrane and membrane-associating proteins.
In contrast to heterotrophic organisms such *E. coli*, cyanobacteria have layers of green membranes called thylakoid membranes where photosynthesis takes place. Thylakoid membranes possess membrane-embedded protein complexes such as photosystem II as well as peripheral soluble protein complexes such as phycobilisomes. Both photosystem II and phycobilisomes are thermolabile elements of the thylakoid membrane. Small Hsp, GroEL (Hsp60), DnaK (Hsp70), and HtpG (Hsp90) have been

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

shown to associate with (thyakoid) membranes. Among them, small Hsp has been studied most extensively in terms of cellular localization and physiological relevance of small Hsp thylakoid association.
Genetic studies indicated that small Hsp confers thermostability to photosystem II and light-harvesting phycocyanins, the major component of phycobilisomes. Constitutive expression of a small Hsp in cyanobacterial cells stabilized subcellular structures such as thylakoid membranes under elevated temperature or intensive light stress. These results are consistent with *in vitro* studies by Vígh's group that showed that small Hsp possesses an ability to stabilize the lipid phase of membranes. Thus, small Hsp possesses not only an activity to protect proteins located either in cytosol or in membranes, but also an ability to stabilize membranes *in vivo*.

2B_05_S

POTENTIAL LIPID SENSORS REFINING THE HEAT SHOCK PROTEIN RESPONSE

László Vígh

Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, H-6726, Szeged, Temesvari krt. 6, Hungary
Vigh@nucleus.szbk.u-szeged.hu

Cancer, diabetes type two, some neurodegenerative and numerous other diseases are known to be associated with elevated HSP levels and membrane abnormalities. The present study aims to establish a mechanism for the connection between lipid composition, fluidity, microdomain organization of plasma membrane and expression of HSPs in mammalian cells. Our data show that exposure of cells to various membrane perturbation modulate the heat shock protein expression without inducing protein-unfolding. We examined alterations in the size, dynamics and distribution of microdomains on the cells surface upon membrane perturbing treatments(heat stress, benzyl-alcohol, cholesterol depletion). Microdomains traced by different fluorescent lipid analogues (bodipy-GM1, bodipy-SM and fPEG-Cholesterol) and the "bulk" membrane regions (labeled with bodipy-C5-PC) were followed with ultrasensitive TIRF video and confocal microscopy. These studies made us capable to observe relationships between cellular distribution and movements of lipid rafts and the level and profile of HSP response. Our observations may lead to the development of non-proteotoxic compounds to target specific membrane microdomains involved in generation/transduction or modulation of stress protein signals which could have considerable therapeutic benefit.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2C_01_S

DECREASED EXPRESSION OF THE MITOCHONDRIAL MATRIX PROTEASES CLPP AND LON IN CELLS FROM PATIENT WITH HEREDITARY SPASTIC PARAPLEGIA (SPG13).

Jakob Hansen[1], Thomas J. Corydon[2], Alexandra Dürr[3,4], Marit N. Nielsen[1], Jane H. Christensen[1], Niels Gregersen[1], Bertrand Fontaine[5] and Peter Bross[1].

[1]Research Unit for Molecular Medicine, Aarhus University Hospital, Skejby, Denmark.
[2]Institute of Human Genetics, University of Aarhus, Aarhus, Denmark.
[3]INSERM U679, [4]Departement de genetique, [5]INSERM U546, Hôpital de la Salpêtrière, Paris, France.
Jakob.hansen@ki.au.dk

The mitochondrial Hsp60 chaperone promotes folding of proteins in the mitochondrial matrix space, and plays a crucial role in protein quality control. A mutation in the *HSPD1* (SPG13) gene encoding the mutant Hsp60-(p.Val98Ile) protein has been associated with a dominantly inherited form of spastic paraplegia. The Hsp60-(p.Val98Ile) protein is functionally impaired and displays a reduced efficiency in mediating folding of the malate dehydrogenase substrate protein possibly related to a reduced ATP-ase activity of the chaperonin complex, but the molecular defect involved in axonal degeneration in spastic paraplegia is unknown. We have investigated mitochondrial function and gene expression levels of key mitochondrial chaperones and proteases in cultured lymphoblastoid and fibroblast cells from SPG13 patient cells. We found that impaired Hsp60-(p.Val98Ile) function is not related to a severe mitochondrial dysfunction phenotype as indicated by assessment of mitochondrial membrane potential, cell vitality, and sensitivity towards oxidative stress insults. However, a decreased expression of protein quality control proteases Lon and ClpP in SPG13 patient cells was demonstrated. We propose that decreased protease levels may represent an adaptive change of protein quality control giving more time to the folding of proteins whose folding is impaired due to a reduced activity of Hsp60-(p.Val98Ile).

2C_02_S

STRESS-INDUCED NUCLEAR BODIES AND TRANSCRIPTION OF REPEATED SEQUENCES

Caroline Jolly, Angéline Eymery, Sabrina Fritah, and Claire Vourc'h

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Institut Albert Bonniot, CRI INSERM-UJF U823, Team "Stress and Dynamics of Genome Organization", Site Santé La Tronche, BP170, 38042 Grenoble cedex 9, France.
Corresponding author : caroline.jolly@ujf-grenoble.fr

Exposure of cells to stress induces dramatic changes in gene expression, activating the expression of certain genes such as those encoding the heat shock proteins or HSPs, and inactivating others. In paralell to the activation of hsp gene expression, we have shown that heat shock also induces the formation of particular nuclear structures termed nuclear stress bodies or nSBs. These structures form principally on the pericentromeric region of human chromosome 9 (9q12) through a direct binding of HSF1 with satellite III repeated sequences. We have shown that heat shock induces the transcription of these repeated sequences into non-coding RNAs termed satellite III transcripts. This transcription is RNA-polymerase II- and HSF1-dependent. The function of the satellite III transcripts is still unknown, but several hypotheses can be considered. Since they remain associated with chromosome 9 for several hours after synthesis, they may play a role in chromatin structure. Alternatively, since several splicing factors remain associated to the sat III transcripts, they could play a role in the regulation of alternative splicing, a function which is indeed altered during heat exposure. I will present our latest findings concerning satellite III transcripts and discuss their possible function during stress exposure.

2C_03_S

EUKARYOTIC RNA THERMOSENSOR

Ilya Shamovsky, June Hyung Lee, Maria Vera, Konstantin Shatalin, and Evgeny Nudler

Department of Biochemistry, New York University School of Medicine, New York, NY, 10016, USA

The heat shock transcription factor (HSF1) plays a central role in the heat shock (HS) response in eukaryotes by inducing the expression of heat shock proteins (HSPs) and other cytoprotective proteins. HSF1 is present in unstressed mammalian cells in an inactive monomeric form and becomes activated by heat and other stress stimuli. HSF1 activation involves trimerization and acquisition of a site-specific DNA-binding activity, which is negatively regulated by interaction with certain HSPs. We have shown that HSF1 activation by HS is an active process that is mediated by a ribonucleoprotein complex containing translation elongation factor eEF1A and a novel non-coding RNA, which we termed HSR1 (Heat

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Shock RNA-1). Both HSR1 and eEF1A are required for HSF1 activation *in vitro*. Antisense oligonucleotides or siRNA against HSR1 impair the HS response *in vivo*, rendering mammalian cells thermosensitive. We also show that non-coding RNAs homologous to mammalian HSR1 are present in other eukaryotic species including *Xenopus*, *Drosophila* and *C.elegans.* HSR1 is constitutively expressed in all these organisms and its homologues are functionally interchangeable. Our results suggest a general model for eukaryotic HS genes activation whereby HSR1 serves as a cellular thermosensor that determines the temperature threshold for the HS response; while eEF1A links HSP expression to major cellular perturbations during HS, such as translational shutdown and cytoskeleton collapse. The central role of HSR1 during HS implies that targeting this RNA could serve as a new therapeutic mode for cancer, inflammation and other conditions associated with HSF1 deregulation.

2C_04_S

NON-CODING HSRω RNA AND POST-TRANSCRIPTIONAL PROCESSING IN STRESSED CELLS

Moushami Mallik and Subhash C. Lakhotia

Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005, India (lakhotia@bhu.ac.in)

Every cell needs a large variety and number of proteins for transcription and processing of nascent transcripts (splicing, other RNA processing, transport etc). Stress or non-permissive conditions, which largely inhibit transcriptional and RNA processing machineries, result in high surplus of unengaged RNA processing proteins. Since these proteins are not degraded but must be available upon recovery, they need to be reversibly sequestered. Heat stress induced formation of nuclear stress bodies/granules in human cells and clustering of the varieties of nuclear speckles (IGCs, paraspeckles, omega speckles etc) in different cell types appear to reflect such sequestration. A major focus of studies in our laboratory is on the developmentally expressed and stress-inducible non-coding *hsrω* gene in *Drosophila*. The large nuclear transcript of this gene, hsrω-n, is known to be required for organizing the unengaged nuclear hnRNPs and related RNA-binding proteins in nucleoplasmic omega speckles in nearly every cell type of *Drosophila*. Using transgenic lines designed to either over-express or ablate the hsrω-n transcripts, we reconfirm that the large nuclear hsrω-n transcript is required for formation of omega speckles and show that this non-coding transcript plays crucial roles in normal development and is also essential for survival of the organism following stress. We believe that such non-coding RNA species

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

perform a wide range of functions in cells through their ability to interact with a great variety of proteins and thus act as "hubs" to integrate complex networks of gene activity during development and under conditions of stress.

2D_01_S

STRESS IN-AND-OUT

Gábor Bánhegyi

Department of Medical Chemistry, Semmelweis University, 1444 Budapest, POB 260, Hungary. E-mail: banhegyi@puskin.sote.hu

Glucocorticoids are main actors in the pathomechanism of stress. In the original context formulated by Hans Selye in his stress theory, glucocorticoids are produced in the adrenal cortex upon the activation of the hypothalamic – pituitary – adrenal axis. Their increased production mediates alarm reactions in acute stress, facilitating metabolic alterations in the general adaptation syndrome allowing the individual to attempt countermeasures such as the „fight or flight" response. Recent observations show that active glucocorticoids can be also formed from their inactive counterparts in various tissues, including liver and adipose tissue. This prereceptorial activation takes place in the lumen of the endoplasmic reticulum (ER) and depends on the activity of the glucose-6-phosphate transporter – hexose-6-phosphate dehydrogenase – 11β-hydroxysteroid dehydrogenase type 1 triad. Increased prereceptorial glucocorticoid activation is accompanied by the signs of ER stress in certain human diseases (obesity, metabolic syndrome, type 2 diabetes). These diseases are more common among socio-economically disadvantaged individuals and are associated with lifestyle factors and chronic stress. It has been recently suggested that the ER can function as a sensor for electron donors and acceptors, *i.e*. nutrients and oxygen. In the current social environment of high energy input and minimal physical activity, the ER encounters a nutrient (electron) overload, leading to a redox imbalance in the lumen, which is the most frequent cause of ER stress and consequent apoptosis. Furthermore, reductive effects favor the increased prereceptorial glucocorticoid activation. In conclusion, glucocorticoid response can be initiated by an autonomous sensing of (nutrient) stress in cellular level beside the central neuroendocrine mechanism.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2D_02_S

ER STRESS INDUCTION OF UPR REGULATOR GRP78: ROLE IN DEVELOPMENT AND DISEASE

Amy S. Lee, Peter Baumeister, Changhui Mao, Miao Wang, Risheng Ye, Min Ni, Yong Fu, Jianze Li and Dezheng Dong

Department of Biochemistry and Molecular Biology and the USC/Norris Comprehensive Cancer Center, Keck School of Medicine of the University of Southern California, 1441 Eastlake Ave., Los Angeles, CA 90089-9176. E-mail: amylee@usc.edu

Mammalian cells have evolved multiple adaptive pathways, referred to as the unfolded protein response (UPR), that allow them to respond to perturbations in endoplasmic reticulum (ER) homeostasis. One major pro-survival mechanism is mediated by the ER chaperone GRP78/BiP, an anti-apoptotic protein which also regulates ER stress signaling. To probe the physiologic function of GRP78/BiP, mouse models were recreated targeting the Grp78 allele. This lead to the discovery that complete depletion of GRP78 results in early embryonic lethality due to proliferation defects and apoptosis of the inner cell mass which is the precursor of embryonic stem cells. Our results show that reduction of GRP78 level by half is sufficient to maintain cellular homeostasis during development with no major consequence in ER stress signaling. This implies that an elevated GRP78 level is more critically needed in cells undergoing physiological or pathological stress, as exemplified by protection of vulnerable neuronal cells and allowing cancer cells to evade the host defense system and cancer therapies. In this lecture, we will discuss the consequence of conditional knockout of GRP78 in specific neuronal cells. Due to hypoxic conditions and glucose deprivation caused by poor vascularization, the microenvironment of tumors represents physiological ER stress and the UPR is activated for tumor cell survival. In this lecture, we will discuss how GRP78 deficiency will affect cancer progression and the underlying mechanisms responsible for the phenomenon.

2D_04_S

MANAGING AND EXPLOITING STRESS IN THE ANTIBODY FACTORY

Roberto Sitia

Università Vita-Salute San Raffaele Scientific Institute, 20132 Milano, Italy, Fax +39 02 2643 4723, E-mail: r.sitia@hsr.it

Upon encounter with antigen, long-lived B lymphocytes differentiate into

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

short-lived plasma cells, the terminal effectors of the humoral immune response. Plasma cells are specialized in immunoglobulin (Ig) secretion, each of them being capable of releasing thousands molecules per second. How do plasma cells achieve such an efficiency? How do they cope with metabolic and redox imbalances that exuberant protein secretion can cause? Is plasma cell death linked to Ig production, such as to limit antibody responses? We have dissected terminal plasma cell differentiation through dynamic imaging, proteomics and genomic analyses. Our results show that waves of functionally related proteins are produced to increase the capacity of the antibody factory, and shed some light in the signalling pathways utilised to orchestrate massive *de novo* ER biogenesis. As to the mechanisms that lead to plasma cell death, we showed that in the late phases of plasmacytic differentiation, when antibody production becomes maximal, proteasomal activity unexpectedly decreases. The excessive load for the reduced proteolytic capacity correlates with accumulation of polyubiquitinated proteins, stabilization of endogenous proteasomal substrates (including Xbp1s, Ik-Bα and Bax), onset of apoptosis, and sensitization to proteasome inhibitors. A developmental program seems therefore to link plasma cell death to protein production, explaining the peculiar sensitivity of normal and malignant plasma cells to proteasome inhibitors.

2E_01_S

GAS SENSING IN THE NERVOUS SYSTEM: HYPOXIA AND POTASSIUM CHANNELS

P.J. Kemp, S.P. Brazier, N. Baban, D. Riccardi, C.T. Müller & [1]S.J. Williams.

School of Biosciences, Cardiff University, U.K. Kemp@cf.ac.uk.

Whether part of the normal intra-uterine development, at high altitude or in pathological conditions, hypoxia is one of the most common stresses to which an organism may be exposed and the ability to adapt to such changes in blood gases is crucial for optimal delivery of molecular oxygen to respiring tissues. The principal sensory component of this homeostatic mechanism is the carotid body. Ideally situated in the bifurcation of the common carotid artery, they respond muliplicatively to hypoxia, hypercapnia, pH and hypoglycaemia. At the cellular level, hypoxia promotes inhibition of plasma membrane the Ca^{2+}-activated, K^+ channel (BK_{Ca}) of carotid body glomus cells which leads to Ca^{2+} influx and transmitter release. Functional proteomics has recently demonstrated that hemeoxygenase-2 (HO-2) is an O_2 sensor linking hypoxia to BK_{Ca} inhibition (Williams *et al.*, 2004 *Science* **306**, 2093-2097), 2004; a

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

process which depends upon carbon monoxide (CO) as the second messenger. The mechanism of such gas/channel interactions is complex, and may involve interactions with heme (Jaggar *et al. Circ Res* **97**, 805-812, 2005. Using chemical and molecular modifications of the BK_{Ca} α-subunit in combination with channel chimera studies, we are now beginning to appreciate the kinetic and structural basis of the dynamic regulation of BK_{Ca} by endogenous production of CO. Taken together, we have proposed a model of how HO-2 functions as a sensor of acute reductions in environmental O_2. Thus, in normoxia, the protein partnership of HO-2 and BK_{Ca} optimizes the permissive effect of CO. However, during the stress of hypoxia, the balance between intracellular heme concentration and the evolution of cellular CO is altered, thereby promoting channel inhibition and activation of the carotid body.

2E_02_S

OXYGEN, A SOURCE OF LIFE AND STRESS – WHEN HYPOXIA MEETS CANCER

E. Berra, M. C. Brahimi-Horn, J. Chiche, F. Dayan, R. Garcia-Medina, A. Ginouvès, N. Mazure, D. Roux, M-P. Simon and **J. Pouysségur**

Institute of Signaling, Developmental Biology and Cancer Research, CNRS-UMR 6543, Centre Antoine Lacassagne, 33 Avenue de Valombrose, 06189 Nice, France. pouysseg@unice.fr

The development of an oxygen-rich atmosphere has been one of the most important events in the history of life on Earth. Oxygen with its exceptional reactivity, represents the far most potent natural pollution on our planet, promoting Life or Death. About 2.4 billion years ago the high amount of dissolved and free oxygen, produced by photosynthesis, in the oceans and atmosphere has driven to extinction most anaerobic organisms. Over the past 500 million years, oxygen levels fluctuated between 15 and 35% imposing constant stress on and subsequent stringent evolution of living organisms.
During mammalian embryonic development or in the context of tumor expansion, proliferating cells rapidly outstrip the supply of nutrients. Although cells sense and respond to variations in concentrations of all nutrients, oxygen sensing has emerged as a central control mechanism of vasculogenesis. Whereas a decrease in the pO_2 (hypoxic stress) induces angiogenesis, an increase in pO_2 (hyperoxic stress) induces vascular pruning. Oxygen concentrations 'sculpt' the blood vascular network of vertebrates. At the heart of this regulatory system is the Hypoxia-Inducible Factor, HIF, which interestingly controls, among other gene products, the expression of VEGF-A and Angiopoïetin-2 (Ang-2), two key

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

angiogenic factors. This finding has therefore placed the hypoxia-signaling pathway at the forefront of nutritional control. Rapidly activated upon a hypoxic stress, HIF induces a vast array of gene products inducing cell-, tissue-, and organismal-survival. Among the HIF-controlled functions are inhibition of ATP-consuming processes (protein and lipid synthesis), inhibition of mitochondrial respiration (to save O_2), increase in anaerobic glucose metabolism, regulation of intracellular pH, increased angiogenesis and cell migration, and so HIF has become recognized as a strong promoter of tumor growth. This pro-oncogenic feature is only one facet of the dual action of HIF. Besides being a 'guardian' of oxygen homeostasis, HIF is capable of inducing pro-apoptotic gene products (BNIP3, BNIP3L) that are in fact pro-survival by inducing autophagy. The molecular mechanism leading to this survival process that is strictly controlled by a drop in pO_2 will be presented.

2E_03_S

HYPOXIA AND INFLAMMATION

Cormac T. Taylor

UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland. cormac.taylor@ucd.ie

The chemical reduction of molecular oxygen which occurs during mitochondrial oxidative phosphorylation is the main source of metabolic energy for virtually all eukaryotic cells. Decreased tissue oxygen supply (leading to hypoxic stress) is a common feature in a range of disease states where inflammation occurs including inflammatory bowel disease (IBD), arthritis, cancer, atherosclerosis and stroke. Recent studies indicate that hypoxia promotes inflammatory signaling pathways through specific mechanisms involving altered hydroxylation of specific residues on key transcription factors including (but likely not limited to) the hypoxia inducible factor (HIF-1) and nuclear factor kappa B (NF-κB). Thus it appears that hypoxia plays an important modulatory role in inflammatory disease development. It appears that a family of prolyl- and asparaginyl-hydroxylases are key common oxygen sensors in conferring hypoxic sensitivity to these pathways. These hydroxylases are absolutely dependent upon the presence of molecular oxygen for activity and are thus inhibited in hypoxia leading to derepression of transcriptional effectors. A greater understanding of the oxygen sensing and signaling mechanisms leading to the activation of these transcriptional responses to hypoxia will allow the development of novel therapeutics in a range of disease states where hypoxia and inflammation are co-incidental events.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2E_04_S

STRESS REGULATED BHLH/PAS TRANSCRIPTION FACTORS: THE DIOXIN RECEPTOR AND HYPOXIA INDUCIBLE FACTORS

M.L Whitelaw, S. Linke, A. Chapman-Smith, T. Wallis, K. Dave, J. Gorman and D.J. Peet

School of Molecular and Biomedical Sciences and ARC Centre for the Molecular Genetics of Development, University of Adelaide, South Australia.
murray.whitelaw@adelaide.edu.au

The basic Helix-Loop-Helix / Per-Arnt-Sim (bHLH/PAS) family of transcription factors perform essential functions during early development and help maintain homeostasis in the adult. For example, the Hypoxia Inducible Factors (HIF-1a and HIF-2a proteins) play a major role in angiogenesis and cellular adaption to low oxygen stress. At normoxia, two oxygen dependent hydroxylases posttranslationally modify specific proline and asparagine residues of the HIFs, severely dampening their activity. During hypoxia, the HIFs exhibit dramatic increases in both protein stability and intrinsic transactivation capacity, due to attenuation of these two hydroxylases. The Dioxin Receptor (or Aryl hydrocarbon receptor) responds to the stress of xenobiotic infiltration by inducing a battery of genes for xenobiotic metabolism. The Dioxin Receptor (DR) is also the mediator of toxic responses to dioxins and PCBs. Both HIFs and the DR need to heterodimerise with a central bHLH/PAS partner protein, termed Arnt, to form active transcription factor complexes. The PAS domain provides a critical protein interaction surface during dimerisation and in the case of the DR, functions as a signal regulated domain. During our studies of stress induced activation mechanisms of the HIFs and DR, we have found recurring themes of posttranslational modification and an important role of the PAS domain in allowing the bHLH to bind to non-canonical E-box sequences of DNA. The presentation will present data to illustrate and expand upon these themes.

2F_01_S

THYLAKOID PROTEASES IN HIGHER PLANTS - ROLES IN PROTEIN QUALITY CONTROL

Zach Adam

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

The Hebrew University, The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, Rehovot 76100, Israel. E-mail: zach@agri.huji.ac.il

The dependence of photosynthesis on light is obvious. The higher the light intensity, the higher is the rate of photosynthesis, up to a level where light energy is no longer limiting and photosynthesis remains constant. However, further increase in the intensity of light may lead to a decrease in photosynthesis rate, a phenomenon known as 'photoinhibition'. Photoinhibition is attributed to oxidative damage, primarily to photosystem II (PSII) and its reaction center protein D1. A number of mechanisms have been evolved during evolution to minimize oxidative damage, but if PSII is damaged after all, a PSII repair cycle operates to allow photosynthesis to proceed. A key component of this cycle is the proteolytic removal of damaged D1 protein, prior to its replacement by a newly synthesized one. Degradation of the D1 protein has been a central question in the field of photosynthesis for the past 20 years or so, but only in recent years the identity of the proteases involved has started to unravel. Recombinant FtsH, a thylakoid ATP-dependent metalloprotease, was first shown to participate in D1 degradation in an *in vitro* study. Later on, *in vivo* analysis of Arabidopsis FtsH mutants revealed that they were more sensitive to photoinhibition than wild type, and that damaged D1 protein was stabilized in them. Further analysis of different mutants suggested that the chloroplast FtsH complex is composed of two essential types of subunits, each one of them is encoded by two redundant genes. More recently, analysis of knock-down mutants of the lumenal serine protease Deg1 suggested that this protease is also involved in the process of D1 degradation. Wider implications to questions of chloroplast biogenesis and maintenance will be discussed.

2F_02_S

PROTEIN FOLDING, QUALITY CONTROL AND DEGRADATION IN THE ER: THE ROLE OF *N*-GLYCANS

Tatiana Soldà, Tito Cali, Carmela Galli, Maurizio Molinari

Institute for Research in Biomedicine, Bellinzona, Switzerland, maurizio.molinari@irb.unisi.ch

The ER is the site of folding and assembly of proteins destined for the plasma membrane, the secretory and endocytic organelles and the extracellular space. Most of the proteins synthesized in the ER are covalently modified by co-translational addition of pre-assembled glucose$_3$-mannose$_9$-*N*-acetylglucosamine$_2$- (Glc$_3$-Man$_9$-NAcGlc$_2$) core oligosaccharides. Protein-bound oligosaccharides are exposed to several

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

ER-glycanases that sequentially remove terminal glucose or mannose residues. Rapid generation of a mono-glucosylated ($Glc_{\underline{1}}$-Man_9-$GlcNAc_2$) trimming intermediate is required to enter the calnexin chaperone system in which protein folding progresses with highest efficiency. Removal and re-addition of the innermost glucose residue activate cycles of dissociation/re-association with calnexin that may facilitate, and in some cases is required for acquisition of the polypeptide's native structure. Slower removal of terminal α1,2-bonded mannose residues from *N*-linked glycans occurs upon persistent polypeptide retention in the ER, which is symptom of defective folding. Substrate de-mannosylation eventually interrupts *futile* folding attempts, results in substrate exclusion from the calnexin chaperone system and promotes retro-translocation into the cytosol for degradation operated by the 26S proteasome. De-glucosylation and de-mannosylation activities must be tightly regulated because the *N*-glycan composition will determine if the associated protein will be subjected to folding-attempts in the ER lumen or if it will be retro-translocated into the cytosol and degraded.

2F_03_S

PROTEASE AND CHAPERONE FUNCTIONS IN THE MAINTENANCE OF MITOCHONDRIAL PROTEIN HOMEOSTASIS

Claudia Leidhold, Tom Bender, and Wolfgang Voos

Institute for Biochemistry and Molecular Biology, University of Freiburg, Herrmann-Herder-Str. 7, D-79104 Freiburg, Germany

Apart from supplying ATP, mitochondria are involved in crucial biosynthetic and signaling processes in a eukaryotic cell. Mitochondrial protein homeostasis is determined by the import of newly synthesized proteins and proteolytic removal of excess or damaged polypeptides. Damaged polypeptides, generated under environmental stress conditions, are first recognized by chaperones, stabilized and refolded to the functional state. If this fails, the proteins are transferred to the proteolytic system for their removal. The coordinated activities of chaperones and proteases form a protein quality control system that is required for the maintenance of organellar function. Mitochondrial proteases belong to the AAA+ protein family and can be separated into soluble and membrane-integrated types. We used a proteome analysis of isolated mitochondria to determine the native substrate selectivity of these proteases. We were able to identify a group of specific substrates for the matrix protease Pim1 that were distinguished by an intrinsic low structural stability and the presence of small molecule cofactors. Cells lacking mitochondrial proteases showed a higher sensitivity to high levels of reactive oxygen

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

species (ROS). A specific subgroup of mitochondrial proteins showed enhanced degradation rates in the presence of ROS. Enzymes containing Fe/S cluster exhibited a high sensitivity to increased ROS levels. Interestingly, proteins that belonged to the ROS detoxification system showed the highest relative degradation rates. We conclude that the protein quality control system contributes prominently to the maintenance of mitochondrial protein functions under stress conditions.

2F_04_S

PROTEIN QUALITY CONTROL AND DEGRADATION AT THE ENDOPLASMIC RETICULUM

Robert Gauss, Ernst Jarosch, Christian Hirsch, and Thomas Sommer

Max-Delbrück-Center for Molecular Medicine, 13092 Berlin, Germany

A quality control system surveys the lumen of the endoplasmic reticulum (ER) for terminally misfolded proteins. Polypeptides singled-out by this system are ultimately degraded by the cytosolic ubiquitin proteasome pathway. This process is termed ER-associated protein degradation (ERAD). A central ERAD component is the ubiquitin ligase Hrd1/Der3. This ligase forms a complex with its partner protein Hrd3 and with the ER-membrane protein Der1. Our data imply that Hrd3 is the major substrate receptor of this heterogenic ligase complex in the ER-lumen. Although Hrd3 and Der1 bind to soluble substrate proteins independently, both proteins are essential to trigger substrate dislocation. At the cytosolic face of the ER the Hrd1-complex associates with the AAA-ATPase Cdc48/p97. Cdc48p binding depends on it’s membrane receptor Ubx2, but most importantly also on substrate processing by the Hrd1-complex, suggesting that ubiquitination precedes substrate mobilization by the Cdc48/p97-complex.In addition, we were able to detect an interaction between the ER quality control lectin Yos9p and Hrd3p. We have identified designated regions in the luminal domain of Hrd3p that interact with Yos9p and Hrd1p. Binding of misfolded proteins occurs via Hrd3p, suggesting that Hrd3p recognises proteins which deviate from their native conformation while Yos9p ensures that only terminally misfolded polypeptides are degraded.

2G_01_S

VARIATION IN STRESS RESPONSES WITHIN A BACTERIAL SPECIES AND THE INDIRECT COSTS OF STRESS RESISTANCE

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Thomas Ferenci[a], and Beny Spira

[a] *School of Molecular and Microbial Biosciences, The University of Sydney, NSW 2006 Australia*

ABSTRACT: Bacteria can exhibit high levels of resistance to one or more environmental stresses such as temperature, osmolarity, radiation, pH, starvation as well as resistance to noxious chemicals and antibiotics. Yet evolution has not optimized stress resistance in all bacteria to all stresses. Even within a species like *Escherichia coli*, stress resistance is not constant between strains, suggesting that selection for stress resistance is under counter-selection in some environments. The trade-offs associated with stress resistance in *E. coli* are due to more than the direct cost of resistance mechanisms. A significant indirect cost is that high stress resistance is associated with a reduced ability to compete for poor growth substrates like acetate or even good substrates like glucose at sub-optimal concentrations. High stress resistance also decreases the ability to use inorganic nutrients like phosphate. This trade-off between self preservation and nutritional competence, called the SPANC balance, is likely to be major selective influence in natural populations. Another cost of high stress resistance in *E. coli* is an elevated mutation rate and the increased generation of deleterious mutations. Directional adaptations in SPANC balance and mutation rate are environment-dependent. The most common variations in SPANC are due to polymorphisms in the levels of global regulators RpoS and ppGpp between different strains. High levels favour stress resistance, lower levels allow better nutrition. The intimate association of RpoS/ppGpp with stress resistance and SPANC balancing influences numerous cellular processes and bacterial properties, including virulence.

2G_02_S

MECHANISMS OF TRANSLATION REGULATION DURING COLD-SHOCK IN *ESCHERICHIA COLI*

Claudio O. Gualerzi and Anna Maria Giuliodori

Laboratory of Genetics, Dept. of Biology MCA, University of Camerino, 62032 Camerino (MC), Italy.
e-mail: claudio.gualerzi@unicam.it

Cold-shock (cs) translational bias, namely the condition which favors translation at low temperature of cs mRNAs, is one of the main mechanisms by which *Escherichia coli* cells ensure the selective expression of its cs genes after cold stress. The bias is partly due to intrinsic features of cs mRNAs, which make them prone to translation at low temperature,

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

and to a cold stress-induced transient increase of the Initiation Factors (IFs)/ribosome ratio. In this study we have undertaken the task of: i) identifying the mechanism generating the stoichiometric imbalance of the IFs/ ribosome ratio; ii) unraveling the role of the IFs in the translation bias; iii) elucidating the secondary structure of the paradigm cs mRNA, namely the *E. coli cspA* mRNA and iv) detecting possible temperature-dependent variations of its structure.
The results obtained indicate that: i) transcription and translation of *infA* and *infC* which encode IF1 and IF3, respectively, are activated *de novo* by cs while ribosomal subunits assembly is slowed down; ii) at low temperature IF3 stimulates the rate of "30S initiation complex" formation with cs mRNAs while inducing the formation of non-productive 70S initiation complexes with non-cs mRNAs; iii) the increased level of IF1 and IF3 during cs is essential to provide a sufficient pool of dissociated 30S ribosomal subunits capable of "70S initiation complex" formation and iv) the structure of cspA mRNA, as determined by chemical and enzymatic probing, changes upon temperature down-shift exposing the translation initiation region.

2G_03_S

MECHANISMS OF TRANSLATION REGULATION DURING COLD-SHOCK IN *ESCHERICHIA* COLI IMPLICATION OF STRESS IN THE LOSS OF VIRULENCE FACTORS IN UROPATHOGENIC ESCHERICHIA COLI

S.M. Soto, J. Vila

Microbiology Department, Hospital Clinic of Barcelona. Villarroel 170. 08036. Barcelona, Spain. E-mail: sarasotog@yahoo.es

Escherichia coli is by far the most common cause of urinary tract infections (UTI). Uropathogenic *E. coli* (UPEC) strains possess several virulence determinants that allow them to colonize the urinary tract, avoid host defenses, and cause damage to the uroepithelium, which may, in some cases, lead to passage of the bacterium into the blood-stream. Several genes encoding urovirulence factors, such as hemolysin, cytotoxic necrotizing factor type 1 (*cnf1*), P-pili F13 (*pap*), S-family adhesins, iron systems, some capsule factors and the autotransporter toxin sat1 are located in the chromosome and/or plasmids forming clusters named pathogenicity islands (PAIs). Several studies have demonstrated that quinolone resistant *E. coli* strains have fewer virulent factors than quinolone susceptible strains. Thus, the aim of this work was to study the possible relationship between bacterial stress produced by quinolones and the loss of virulence factors located in PAIs in UPEC. Three UPEC quinolone-susceptible and hemolytic clinical strains were submitted to

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

subinhibitory concentrations of ciprofloxacin. A sample of the well showing growth at the highest quinolone concentration was spread onto large blood Columbia agar plates. The nonhemolytic colonies were analyzed to determine the loss of the hemolysin gene (*hly*) and other factors related to PAIs. The three strains lost hemolytic capacity between passages 1-4 in the presence of ciprofloxacin. The loss rate was between $1x10^{-4}$ and $5x10^{-3}$. No colonies without hemolytic capacity were found after 15 passages of wild-type strains using antimicrobial-free culture medium. In conclusion, these findings suggest that quinolones produce bacterial stress the response of which is a loss of virulence factors.

2G_04_S

OSMOTIC STRESS AND OTHER STRESSORS AS INDUCERS OF MULTIDRUG RESISTANCE

María M. Tavío

University of Las Palmas de Gran Canaria. Faculty of Health Sciences. Las Palmas G.C.- Spain, mtavio@dcc.ulpgc.es

In order to survive under and adapt to different conditions bacteria have systems that are able to sense and respond to environmental stimuli. A complex network of regulatory systems ensures a coordinated and effective answer to different stresses that can act on a bacterium simultaneously. Hyperosmolarity and some chemicals as fluoroquinolones, salicylate, non-antimicrobial medicaments as diazepam, anti-inflammatory drugs, among others, can induce an increased active efflux and organic solvent tolerance, loss of porins and multidrug resistance, both in wild type strains and clinical isolates of enterobacteria. Besides the role of efflux systems in multidrug resistance phenotypes, they seem to have a natural function exporting signals for cell–cell communication. AcrAB-TolC is an efflux system that exudes fluoroquinolones and is up-regulated by SdiA, a quorum-sensing transcriptional regulator. Another transcriptional regulators that are also involved in bacterial stress response such as *marA* or *soxS,* also activate AcrAB-TolC. Sigma factors, and the two-component systems CpxAR and BaeSR are also key pieces in the regulation of gene expression in response to stress conditions. Thus, the response regulator CpxR can lead to an increase of the mRNA level of several drug exporter genes and the mutation level as well as to a diminished OmpF assembly. From this point of view the development of intrinsic multidrug resistance might be understood as part of the bacterial response to stress. The *in vitro* induction of multidrug resistance has been associated with high levels of inducers, those that are close to their minimal inhibitory concentrations. Therefore, bacterial response to osmotic stress might be linked to multidrug resistance phenotypes.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Module 2 – Poster lectures:

2A_01_P

OSTEOPROTEGERIN AS AN ANTI-APOPTOTIC PROTEIN

Nina C. Dempsey, Claire Hunter-Lavin, Michael J. Marshall & John H.H. Williams

Chester Centre for Stress Research, Department of Biological Science, University of Chester.
Parkgate Road, Chester, United Kingdom, CH1 4BJ
n.dempsey@chester.ac.uk

The role of OPG in bone turnover through its interaction with RANKL is well established, however this protein has now been shown to have anti-apoptotic properties which may contribute to the survival of cancer cells. As confirmation of this potential role we show data that OPG can interact with both TRAIL and TNFα using ELISA and BIAcore. We have then demonstrated that OPG will protect cells against TRAIL or TNFα induced apoptosis.
We have then focused on the release of OPG under stress conditions from MG63 cells and looked at the existence of OPG feedback mechanisms. We show that a number of stressors that induce apoptosis also result in an increase in the release of OPG from cells. These stressors include – UV exposure and exposure to TNFα or TRAIL. The increase in release of OPG occurs prior to indicators of apoptosis – caspase 3 activation or Annexin V binding. Interestingly, induction of apoptosis by elevated temperature coincides with a total cessation in OPG release.
Production of OPG can be regulated by a feedback mechanism as release can be increased by lowering the ambient concentration of OPG, and conversely that by increasing this ambient concentration, the release of OPG is inhibited. The changes are mirrored by alterations in gene expression.
We discuss the potential role of OPG in the treatment of tumours and strategies that can be used to bypass this protection.

2A_02_P

HYPEROSMOTIC STRESS RESPONSE

Roberta R Alfieri, Mara Bonelli, Andrea Cavazzoni, Angelo F Borghetti, Pier Giorgio Petronini

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Dipartimento di Medicina Sperimentale Università degli Studi di Parma Via Volturno 39 Parma E-mail: roberta.alfieri@unipr.it

Many types of mammalian cells can survive a moderately hypertonic environment due to a specific adaptation process that results in the cellular accumulation of compatible osmolytes. This adaptation process, involves early responses, occurring over milliseconds to minutes, and later responses, requiring hours to days. The virtually instantaneous reduction in cell volume due to the osmotic efflux of water induced by acute hypertonic stress is rapidly corrected by what is referred to as regulatory volume increase (RVI). This early process is mediated by pre-existing ion transport systems that produce increases in the intracellular concentrations of potassium, sodium and chloride ions, and the accompanying influx of water causes RVI. The later phase is characterised by increased production of heat shock proteins (HSPs) and either the synthesis or the uptake and cellular accumulation of compatible osmolytes. In mammalian cells the latter include neutral amino acids or their derivatives, polyols such as sorbitol and myo-inositol, and methylamines such as betaine. The usual explanation of this phenomenon is the need to replace the early cellular accumulation of inorganic ions with small organic molecules that do not affect cell function even at relatively high intracellular concentrations. Accumulation of compatible osmolytes within the cell thus maintains intracellular water homeostasis without impairing normal biochemical functions such as protein synthesis. Cells do not adapt and die by apoptosis when hypertonic medium has been depleted of these molecules. Compatible osmolytes thus enable cells to survive under hypertonic conditions, protecting them from apoptosis and modulating the adaptive response.

2A_03_P

GRP94 IS ESSENTIAL FOR MUSCLE DIFFERENTIATION AND HAS ANTI-APOPTOTIC ACTIVITY BECAUSE IT CONTROLS THE SECRETION OF INSULIN-LIKE GROWTH FACTORS

Olga Ostrovsky, Sherry Wanderling, Birgitte B. Simen and Yair Argon

Division of Cell Pathology, Children's Hospital of Philadelphia, Philadelphia PA, 19104.
Email: yargon@mail.med.upenn.edu

Because only few of its client proteins are known, the physiological roles of the ubiquitous endoplasmic reticulum chaperone GRP94 are poorly understood. Using targeted disruption of the murine GRP94 gene we show that it has essential functions in embryonic development. *Grp94* -/-

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

embryos die on day 7 of gestation, fail to develop mesoderm, primitive streak or proamniotic cavity. *Grp94* -/- ES cells grow in culture and are capable of differentiation into cells representing all three germ layers. However, these cells do not differentiate into cardiac, smooth or skeletal muscle.
Grp94 -/- embryonic stem (ES) cells are also hyper-sensitive to serum deprivation and die rapidly. The death is apoptotic, involving changes in mitochondrial potential and activation of caspase 3. Media conditioned by wild type ES cells, or by the myogenic cell lines C2C12 and RD, protect *grp94*-/- cells from apoptosis in the absence of serum. The active component in these media was identified as insulin-like growth factor II (IGF-II). GRP94 interacts physically with pro-IGF-II intermediates, as shown by co-immunoprecipitation. These results suggest, therefore, that pro-IGF-II is a client of GRP94 which requires the chaperone activity for secretion.
The muscle differentiation defect of *grp94*-/- ES cells is also related to control of IGF production, because differentiation of mutant ES cells can be partially restored by provision of exogenous IGF. In addition, the role of GRP94 in muscle differentiation was demonstrated by silencing its expression in the myogenic cell line C2C12 with RNAi. C2C12 cells that express 10% of normal GRP94 levels are incapable of fusion into myotubes and fail to express contractile proteins. We suggest that the importance of GRP94 for embryonic development and its known anti-apoptotic activity are both due to its control of the production of insulin-like growth factors.

2A_04_P

GLUTATHIONE S-TRANSFERASE PI REGULATES UV-INDUCED JNK SIGNALING IN SH-SY5Y CELLS

M Castro-Caldas[1,2], E Rodrigues[1], MC Lechner[1], MJ Gama[1]

[1]*UBMBE. Faculdade de Farmácia, Universidade de Lisboa*
[2]*SABT. Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa*

Activation of c-Jun N-terminal kinase (JNK) signaling pathway is a key event in neuronal apoptosis. Previous studies demonstrated that in SH-SY5Y cells UV-induced apoptosis is associated with activation of JNK. The cellular mechanisms underlying the control of JNK activity before and immediately after stress are not completely understood. Under resting conditions the basal activity of JNK is low, since JNK is kept inactive by the presence of one or more repressors. Inactivation of JNK may be mediated by binding through protein-protein interactions to non-substrate proteins, including Glutathione S-Transferase pi (GSTpi). GSTpi belongs to a multigene family of isozymes catalyzing detoxification reactions. Although

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

it has previously been shown that over expression of GSTpi protects cells from JNK-mediated apoptosis, the mechanisms underlying regulation of JNK signaling by GSTpi in neuronal cells have never been described. In this work, SH-SY5Y cells were treated with UV to evaluate the regulation of JNK signaling by GSTpi. The relative concentrations of the GSTpi, p-JNK/JNK and apoptotic proteins were estimated by Western blot. Direct interaction of GSTpi and JNK was determined by co-immunoprecipitation assays. Evaluation of GSTpi dimers and multimeric complexes formation was performed by SDS-PAGE under non-reducing conditions. Our results show that UV treatment induces apoptosis in SH-SY5Y cells and the transient activation of JNK. Furthermore, the increase of JNK enzymatic activity correlates with changes of GSTpi-JNK complexes and the concentration of GSTpi multimer forms. Taken together our results suggest that GSTpi may act as a regulator of the UV-induced cellular stress response, controlling JNK activity by protein-protein interactions.

2A_05_P

THE ROLE OF HSP72 IN NEURONAL CELL SURVIVAL AND THE RESISTANCE OF NEURONE-LIKE SH-SY5Y CELLS TO APOPTOTIC INSULTS

Cheng, L., [1*] Smith, DJ., [1] Nagley, P[1] and Anderson, RL[2]

1. Dept of Biochemistry and Molecular Biology, Monash University, Victoria, Australia
2. Peter MacCallum Cancer Centre, Victoria, Australia
**Lesley.Cheng@med.monash.edu.au*

Heat shock proteins have been implicated in neuronal cell survival. We studied here the anti-apoptotic effects of heat-inducible hsp72 in the human SH-SY5Y neuroblastoma cell line, propagated in an undifferentiated form and able to be differentiated into neurone-like cells using retinoic acid with brain-derived neurotrophic factor. Mild heat stress (43C for 30 min) was applied to induce hsp72, subsequently referred to as thermal pre-conditioning treatment. It was observed that thermal pre-conditioning protects cells against apoptosis induced by a subsequent treatment with staurosporine (50 nM). Neurone-like SH-SY5Y cells displayed reduced Bax activation, cytochrome c release and nuclear fragmentation when thermally pre-conditioned compared to non-pre-conditioned control cells (all monitored by immunocytochemistry and confocal microscopy). The suggestion that hsp72 may be involved in blocking apoptosis and that the block by hsp72 may be upstream of Bax was tested by constructing stable transfectants over-expressing hsp72 (5YH72.1). Such cells maintained levels of hsp72 comparable to those seen in untransfected undifferentiated SH-SY5Y cells exposed to thermal

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

pre-conditioning; similar levels of hsp72 were also found in neurone-like untransfected cells without heat shock, conditions that also induce hsp72. 5YH72.1 cells showed enhanced thermotolerance, at significantly higher temperatures than neurone-like untransfected cells (themselves more thermotolerant than their undifferentiated counterparts). Moreover, neurone-like 5YH72.1 cells treated with 50 nM of staurosporine failed almost completely to display Bax activation and nuclear fragmentation. Undifferentiated 5YH72.1 cells were also protected but to a lesser extent against the molecular effects of staurosporine treatment. The data support the proposition that hsp72 is responsible for the thermoprotection observed in SH-SY5Y cells. Further, hsp72 acts upstream of the mitochondria to prevent apoptosis in these cells when expressed in moderately high quantities.

2A_06_P

UPREGULATION OF ANTI-APOPTOTIC BCL-2 BY A NOVEL ENDOPLASMIC RETICULUM PROTEIN SIGMA-1 RECEPTOR

Teruo Hayashi and Tsung-Ping Su

Intramural Research Program, NIDA, NIH, DHHS, 333 Cassell Drive, Baltimore, MD 21224. thayashi@intra.nida.nih.gov

Extreme cellular stress induces apoptosis, a deliberate life-relinquishment of a cell. Bcl-2 serves as a powerful antidote against apoptotic cell death by preventing the mitochondrial permeability transition that causes the release of caspase activators. Although the level of Bcl-2 has been shown to be altered under a variety of cellular stresses, signaling pathways that regulate the Bcl-2 expression are not fully understood. Sigma-1 receptors (Sig-1Rs) are endoplasmic reticulum (ER) proteins that are activated by steroids and psychotropic drugs. Sig-1Rs express highly in the nervous system as well as in carcinomas. Activation of Sig-1Rs is known to promote a robust neuroprotective action, whereas inhibition of Sig-1Rs promotes cell death such as seen in cancer cells. Recent studies have proposed, therefore, the Sig-1R as a potential therapeutic target in treatments of ischemia and cancer. Here, we reported that the Sig-1R is a novel ER protein regulating Bcl-2 expression. Sig-1Rs co-localized with Bcl-2 at the ER, but not at mitochondria in CHO cells. Overexpression of Sig-1Rs significantly increased Bcl-2 proteins in mitochondria, whereas knockdown of Sig-1Rs by siRNAs caused a prominent downregulation of Bcl-2. RT-PCR and Northern blotting revealed that knockdown of Sig-1Rs downregulated the bcl-2 mRNA, indicating a transcriptional activation and/or mRNA degradation of bcl-2 by Sig-1Rs. In keeping with these findings, knockdown of Sig-1Rs potentiated cell death when CHO cells were under apoptotic stimuli. Our findings suggest that the novel ER

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

protein Sig-1R is intrinsically regulating the level of mitochondrial Bcl-2, and, thus, Sig-1R agonists such as pregnenolone sulfate and DHEAS may exhibit cell protective action, a least in part, by increasing the expression and thus augmenting the anti-apoptotic activity of Bcl-2. (Sponsored by IRP/NIDA/NIH/DHHS)

2A_07_P

HTRA2 IS UP-REGULATED IN THE HEAT-STRESSED RAT TESTIS

TETSUO HAYASHI, NOBUYUKI ISHII, TOSHIYA TERAO, MAKOTO MOROZUMI AND TAKUMI YAMADA

Department of Urology, Saitama Medical Center, Saitama Medical University, Kawagoe, Saitama, Japan

Aim: The aim of the present study was to elucidate the role of the high temperature requirement A 2 (HtrA2) for the germ cell loss in the heat-stressed testis.
Methods: We examined the expression of HtrA2, caspase-9 activity and proteolytic activity of HtrA2 in the rat testis and their in vivo responses to experimental cryptorchid treatment.
Results: Northern analysis revealed the expression of HtrA2 mRNA peaked at days 1 and 7 after cryptorchid treatment. While the expression of HtrA2 mRNA was recognized in the spermatogonium, spermatocytes and some spermatids in normal adult rat testis, the experimental cryptorchidism treatment resulted in a marked increase in its signal intensity in spermatocytes and some spermatids and the layers of spermatogonium and early primary spermatocytes became negative at days 1 and 7 after the treatment. However, the spermatogonium, Sertoli cells and interstitial cells appeared to have strong intensities at days 14, 28 and 56 after the treatment. Western analysis revealed the expression of HtrA2 protein peaked at days 2 and 28, Caspase-9 activity peaked at day 2 and HtrA2 proteolytic activity peaked at day 28. Consequently, the first peak of HtrA2 mRNA expression was followed by the peak of caspase-9 activity and the second peak was followed by the peak of proteolytic activity.
Conclusion: These findings suggest the probabilities that the heat stress results in germ cell death by caspase-independent manner with the elevation of proteolytic activity of HtrA2 as well as caspase-dependent manner with the elevation of caspase-9 activity.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2A_08_P

FUNCTIONAL STUDIES ON UBIQUITIN RECEPTOR PROTEIN, HFAF1, IN STRESS RESPONSES.

Jejean Lee, Young-Mee Kim, Kong-Joo Lee

Center for Cell Signaling and Drug Discovery Research, College of Pharmacy and Division of Life & Pharmaceutical Sciences, Ewha Womans University, Seoul 120-750, South Korea
kjl@ewha.ac.kr

Human Fas-associated protein, hFAF1, was identified as Fas-associating molecule and a member of the apoptosis signaling complex. We identified that hFAF1 acts as a scaffolding protein by unveiling the interacting proteins and newly found N-terminal ubiquitin-associated (UBA) domain. N-terminal UBA domain was identified to recruit K48- and K63-linked polyubiquitinated proteins and plays a role to accumulate the ubiquitinating proteins as a ubiquitin receptor. One ubiquitin like (UBL) domain interacts with Hsp70 and negatively regulates its chaperone activity. C-terminal ubiquitin regulatory X (UBX) domain was identified to interact with AAA ATPase p97/VCP which is involved in the ubiquitin-proteasome pathway. These interactions of hFAF1 to two chaperone proteins (Hsp70 and VCP) suggest that FAF1 as a ubiquitin receptor plays important roles in stress response and apoptotic cell death. In this study, we will demonstrate the regulation mechanism by examining the post-translational modifications using proteomic tools, and the biological functions by examining the effects of VCP binding defect mutants and RNAi of hFAF1 in response to stress. This can suggest the biological role and regulation of ubiquitin receptor hFAF1 in stress-induced ubiquitin-proteasome system. [Supported by KOSEF NCRC for CCS & DDR and FPR05A2-480. J Lee and YM Kim supported by BK21]

2A_09_P

PPARγ AGONISTS AND HSP70 RENDERS THE RESISTANCE TO APOPTOSIS IN γ-IRRADIATED CANCER CELLS

Piotr Pierzchalski[1], Agata Krawiec[1], Wiesław Pawlik[1], Maciej Gonciarz[2]

[1]Jagiellonian University Medical College, Grzegórzecka 16, 31-531 Kraków, POLAND
[2] Endoscopy Unit, St. Barbara District Hospital, Plac Medyków 1, 41-200 Sosnowiec, POLAND

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

The involvement of peroxisome proliferator-activated receptors (PPARs) in the cancer cell apoptosis is a generally accepted fact. However, some reports indicate that the activation of PPARγ is directly responsible for carcinogenesis.
It is well known that the high level of heat shock proteins (HSPs) in cancer cells is associated with metastasis, the poor prognosis and the resistance to radio as well as chemotherapy. HSP70 as a part of the most important systems for maintaining the viability of the cell, is known to counteract against the apoptosis. We report here the involvement of HSP70 in anti-apoptotic action of activated PPARγ in γ-irradiated human colon cancer cells.
We have used Caco-2 cells (human colon adenocarcinoma) as an experimental model. In this system PPAR-γ agonists induced nuclear translocation of PPAR-γ as well as HSF-1. This translocation was followed by the increase of HSP70 mRNA and protein expression.
Cells subjected to γ-radiation (photons) with therapeutic dose of 2,5 Gy, manifested pattern of PARP degradation typical for apoptosis, showing both the native 112 KD and digested 85 KD forms. It suggests activation of caspases 3 or 6. Stimulation of the cultures with PPARγ agonists prior to the irradiation eliminated altogether the process of PPAR-γ nuclear translocation and PARP degradation. PPARγ remained in the complexes with AKT-1 in cytoplasmic as well as in nuclear pool. However this treatment did not affect HSF-1 translocation and HSP70 expression.
According to our elucidation, in γ-irradiated cells nuclear translocation of PPARγ is abolished and PPARγ-AKT-1 complexes are conserved in which PPARγ remains insensitive for its agonists treatment. Most likely, at the same time PPARγ agonists directly activate HSP 70. The process is undisturbed by the γ-irradiation what renders the colon cancer cells resistance to apoptosis.

2A_10_P

RELATIONSHIP BETWEEN APOPTOSIS AND HSP70 EXPRESSION IN LYMPHOCYTES EXPOSED TO STRESS MEDIATORS

Sapozhnikov A.M., Kovalenko E.I., Murashko D.A., Alekperov E.A., Shustova O.A.

Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia, amsap@mail.ru

It has been shown that adrenaline in contrast to glucocorticoids can activate immune system. However existing data indicate that catecholamines like glucocorticoids induce apoptosis in lymphocytes. Earlier we demonstrated that lymphoid cell apoptosis is accompanied by

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

increase of HSP expression. In this work we carried out a study of effect of adrenaline and dexamethasone on apoptosis and HSP70 expression in cultures of mouse lymphocytes. Obtained results confirmed apoptogenic effect of catecholamine and dexamethasone. However, the stress mediators had opposite effects on level of HSP70 expression: adrenaline enhanced intracellular content of the protein whereas dexamethasone had small but significant inhibitory action on HSP70 expression. The catecholamine-induced increase of HSP70 expression was mediated by α-adrenoreceptor because the effect was suppressed by phospholipase C inhibitor but was not decreased by PKA inhibitor. In addition, we have revealed that α-adrenergic antagonist prazosin reduce adrenaline-induced HSP70 expression while β-adrenergic antagonist propranolol decrease the apoptogenic effect of catecholamine. Phenylephrine – specific α-adrenergic agonist had no apoptogenic effect in contrast to adrenaline interacting with both α- and β-adrenoreceptors. We suppose that the difference between immunomodulating activity of catecholamines and glucocorticoids may be connected with their opposite influence on lymphocyte HSP system. The results suggest that effect of catecholamines on apoptosis and HSP70 expression are initiated by the same hormonal signal perceived by means of different adrenoreceptors.
This study was supported by grants from RFBR (06-04-49568) and BTEP/ISTC (73/2627).

2A_11_P

EXPRESSION OF HEAT SHOCK PROTEINS IN PRIMARY PORCINE MYOTUBES EXPOSED TO STRESSORS

Straadt, I. K.[1*]; Young, J. F.[1]; Bertram, H. C.[1]; Gregersen, N.[2]; Bross, P.[2]; Oksbjerg, N.[1]

University of Aarhus, [1]Department of Food Science, [2]Research Unit for Molecular Medicine, DK
**Cor. author: ida.straadt@agrsci.dk*

Stress exposure to animals prior to slaughter is causing big variations in the meat quality. Using primary porcine myotubes as a model, exposure to different stressors was investigated. When myotubes were exposed for 1 h to 50-250μM H_2O_2 the peak in expression of mRNA for HSP70 and HO1, measured 18 h after stress exposure, was seen at a concentration of 200 μM H_2O_2. At 250 μM the expression decreased substantially, indicating that the cells were no longer capable of expressing heat shock proteins at a high level. Also the appearance of the cells evidenced the toxic effect of H_2O_2 levels above 200 μM. When testing the viability this was confirmed as the survival of myotubes exposed to more than 100 μM H_2O_2 for 1 h

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

showed a decreased cell viability determined by WST-1. Based on these observations a level of 100 μM H_2O_2 was chosen for investigating the development in expression levels of heat shock proteins over a time period in the myotubes. A significant increase in expression of both HSP70 and HO1 mRNA was observed after exposure to H_2O_2 for 1 h and measuring expression up to 18 h after exposure. No change in the cell viability determined by WST-1 was observed when the myotubes were exposed to heat (42°C or 45°C) for 1 h. However, comparing cells exposed to 42°C and 45°C the expression was approximately 10 times higher when exposed to 45°C. No change in the expression level of HO1 mRNA was observed when exposed to heat. Hence, primary porcine cells are more sensitive to H_2O_2 compared to myotubes and muscle cell lines from other species. In contrast to this, primary porcine muscle cells are not very sensitive to heat stress when determining the cell viability by WST-1.

2A_12_P

APOPTOSIS AND HEAT SHOCK PROTEIN HSP70 IN AGED MAMMALIAN OOCYTES.

P. Esponda [1] and H. Díaz [2]

[1] *Centro de Investigaciones Biológicas. CSIC. Madrid. Spain.*
[2] *Facultad de Ciencias. Universidad de Valparaíso. Valparaíso. Chile*

The fertile life of the mammalian is short and a few hours after ovulation it loses its capacity to be fertilized. Ageing affect oocyte fertilizing ability and aged females produce numerous infertile and/or abnormal oocytes. We used normal and aged mouse oocytes to analyze initial apoptosis (using Anexin V), late apoptosis (using the TUNEL method) and the presence of the stress protein HSP70 (using antibodies and RT-PCR). We induced the ovulation by hormones (PMSG-HCG) to collect oocytes from young females (3 months old). Gametes were also aged in the oviduct after ovulation during 20 hours (postovulatory ageing) and other oocytes were collected from 12-15 months old females (preovulatory ageing).
Anexin V showed that a high percentage (39.2%) of initial apoptosis appeared in oocytes ovulated by old females, and in oocytes aged in the oviduct during 20 hours (31.6%). TUNEL results showed that the high percentages (56.5%) of apoptosis occurred when oocytes were aged in the oviduct during 20 hours. Oocytes from young or old females showed a similar degree of damage (12.9-16.1%). Nevertheless, young oocytes recovered from females that were not treated with hormones, show a lower percentage of apoptosis (3.7%). TUNEL procedure was also applied to histological sections from ovaries of different ages (3; 11; 14 and 24 months old). Results showed that apoptotic cells were progressively

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

increased in the insterticial tissue of the organ.
Normal oocytes do not showed the presence of the stress protein HSP70, but this protein appeared in the cytoplasm after an exposure to 40ºC during 4 hours, and in oocytes after postovulatory ageing. RT-PCR showed HSP70 gene expression in aged oocytes.
These results indicated that ageing increase apoptosis of the mouse oocyte and of the ovarian tissue. Apoptosis and HSP70 are principally produced by environmental conditions, as the long storage of the oocyte in the oviduct or the employ of hormones used to induce ovulation.

2A_13_P

STRESS HORMONES INDUCE IN CANCER CELLS ATP DEPLETION VIA PERTURBATION OF OXIDATIVE PHOSPHORYLATION

Eliezer Flescher

Tel Aviv University, Tel Aviv 69978 Israel, flascher@post.tau.ac.il

The jasmonate family of plant stress hormones exhibits selective cytotoxic activities towards cancer cell lines as well as leukemic cells from chronic lymphocytic leukemia patients. The aim of this project was to elucidate the jasmonate mechanism of action. We found that jasmonates induce a rapid depletion of ATP in cancer cells, preceding any signs of cell death. Furthermore, we found a positive correlation between the susceptibility of a given cell type to the cytotoxic effect of jasmonates and the degree of ATP depletion induced in that cell. The two major sources of cellular ATP, oxidative phosphorylation and glycolysis, determine the steady state levels of ATP. Experiments using modulators of ATP synthesis via glycolysis or oxidative phosphorylation suggest that the latter is the pathway suppressed by jasmonates. Consequently, the direct effects of jasmonates on mitochondria were evaluated. Jasmonates induced cytochrome c release and swelling in mitochondria isolated from cancer cells but not from normal ones. Thus, the selectivity of jasmonates against cancer cells is rooted at the mitochondrial level, and probably exploits differences between mitochondria from normal versus cancer cells. The permeability transition pore complex (PTPC) regulates movement of compounds across the mitochondrial membrane. Abnormally long opening of this pore can be associated with cytochrome c escape into the cytosol, resulting eventually in cell death. Jasmonate-induced release of cytochrome c from mitochondria isolated from cancer cells was inhibited by inhibitors of PTPC opening, suggesting that the mitochondrial permeability transition induced by jasmonates is PTPC-mediated. These findings position jasmonates as promising anti-cancer drugs acting via energetic depletion in neoplastic cells.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2B_01_P

COMMON PHYSICOCHEMICAL PROPERTIES OF POLAR LIPIDS UNDERLYING THERMAL ADAPTATION OF MARINE HYDROBIONTS AND THEIR VULNERABILITY TO HIGH TEMPERATURE

Nina Sanina

Far Eastern National University, Vladivostok, Russia

Changes of thermotropic behavior and fatty acid composition of the major polar lipids from different species of marine invertebrates and macrophytes underlying their warm-acclimation and seasonal acclimatization were studied by GLC, DSC and polarizing microscopy to estimate the capacity of these phylogenetically different ectothermic organisms to adapt in conditions of global temperature changes. Common strategy of thermal adaptation of studied animals and plants was revealed in spite of substantially different composition of polar lipids and their fatty acid (FA) pools. The seasonal changes of thermotropic behavior and FA composition of phosphatidylcholine (PC) (or DGTS) and phosphatidylethanolamine (PE) were consisted with homeoviscouse adaptation conception. However T_{max} of photosynthetic lipids (glycolipids and phosphatidylglycerol) did not change or even increased from summer to winter that may induce the low-temperature inhibition of photosynthesis. Irrespective to different thermotropic behavior, the unsaturation of both lipid groups rose due with increasing n-3/n-6 PUFAs ratio. The reciprocal conversion n-6↔n-3 was shown to regulate not only the effect of their derivatives but the viscosity of membrane lipids. The most share of n-6 in PC and PE seems to connect with enhanced role of plasma membrane lipids in communication with environment in summer. Whereas increased level of n-3 in photosynthetic lipids appears to maintain photosynthesis and respiration at low temperatures. High vulnerability of hydrobionts to the sharp temperature elevation in winter or superoptimal temperatures in summer were shown to be caused by the absence of effective compensation in physical state of membrane lipids and proximity of their isotropic transition to these temperatures.

2C_01_P

HEAT-INDUCED GLOBAL DEACETYLATION OF CORE HISTONES INVOLVES HDAC1 AND HDAC2

Sabrina Fritah, Cyril Boyault, Susanna Chiocca, Saadi Khochbin, Caroline

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Jolly, Claire Vourc'h

CRI Inserm U823, Institut Albert Bonniot, Grenoble, France, Sabrina.fritah@e.ujf-grenoble.fr

Heat shock induces major changes in gene expression. Besides the up-regulation of specific genes, among which hsp genes, heat shock also induces a general shut-down of gene expression underlined by a global chromatin remodelling. While the mechanisms involved in the heat-induced activation of hsp genes have been investigated in details, the molecular events associated with the global down-regulation of the genome are poorly understood. By using a combination of in situ and molecular approaches, we have shown that heat shock induces a massive and reversible deacetylation of core histones affecting specific epigenetic marks of histones H3 and H4. This deacetylation of core histones is a conserved phenomenon. Moreover it is correlated with the intensity of stress. We have characterized the molecular mechanisms underlying the heat-induced deacetylation, and have identified HDAC1 (Histone Deacetylase 1) and HDAC2 as the key actors of this event. Interestingly, we found that HDAC1 and HDAC2 are complexed with HSF1 (Heat shock factor 1), in a stress dependent manner. We thus identify HDAC1 and HDAC2 as novel important actors of the heat shock response. Our last results concerning the mechanisms involved in the regulation of HDACs activity during stress will be also presented and discussed.

2C_02_P

STRESS-INDUCED NUCLEAR BODIES AND TRANSCRIPTION OF REPEATED SEQUENCES

Caroline Jolly, Angéline Eymery, Sabrina Fritah, and Claire Vourc'h

Institut Albert Bonniot, CRI INSERM-UJF U823, Team "Stress and Dynamics of Genome Organization", Site Santé La Tronche, BP170, 38042 Grenoble cedex 9, France.
Corresponding author: caroline.jolly@ujf-grenoble.fr

Exposure of cells to stress induces dramatic changes in gene expression, activating the expression of certain genes such as those encoding the heat shock proteins or HSPs, and inactivating others. In paralell to the activation of hsp gene expression, we have shown that heat shock also induces the formation of particular nuclear structures termed nuclear stress bodies or nSBs. These structures form principally on the pericentromeric region of human chromosome 9 (9q12) through a direct binding of HSF1 with satellite III repeated sequences. We have shown that heat shock induces the transcription of these repeated sequences into

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

non-coding RNAs termed satellite III transcripts. This transcription is RNA-polymerase II- and HSF1-dependent. The function of the satellite III transcripts is still unknown, but several hypotheses can be considered. Since they remain associated with chromosome 9 for several hours after synthesis, they may play a role in chromatin structure. Alternatively, since several splicing factors remain associated to the sat III transcripts, they could play a role in the regulation of alternative splicing, a function which is indeed altered during heat exposure. I will present our latest findings concerning satellite III transcripts and discuss their possible function during stress exposure.

2D_01_P

APOLIPOPROTEIN B MISFOLDING AND ER STRESS: EVIDENCE THAT GLUCOSAMINE-INDUCED MISFOLDING OF APOB LEADS TO ER STRESS AND ER-ASSOCIATED PROTEASOMAL DEGRADATION

Wei Qiu, Rita Kohen, Angela Rutlege, and Khosrow Adeli

Molecular Structure and Function, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, M5G 1X8, Canada

Glucosamine treatment of HepG2 cells increased levels of the ER chaperones, 78-kDa glucose-regulated protein (Grp78) and Grp94, in a dose-dependent manner and led to significant decreases in both cellular and secreted apolipoprotein B100 (apoB100) by up to 97% ($p<0.01$). In contrast, no changes were observed in ER resident (ER60, PTP-1B) or secretory (albumin, apoE) control proteins. Glucosamine-induced apoB degradation was similarly observed in primary hamster hepatocytes and McA-RH7777 cells. Glucosamine treatment led to reduced tranlocational efficiency of apoB100 in the ER and enhanced its ubiquitination and proteasomal degradation. Adenoviral overexpression of Grp78 also led to significantly decreased levels of newly synthesized apoB100 in a dose-dependent manner ($p<0.01$). Grp78-induced downregulation of apoB100 was sensitive to inhibition by the proteasome inhibitor, lactacystin, but not lysosomal protease inhibitors, E64 and leupeptin, suggesting that overexpression of Grp78 selectively induced proteasomal degradation of apoB100. We then examined the mechanisms linking glucosamine-induced ER stress and apoB-lipoprotein biogenesis. Trypsin sensitivity studies suggested glucosamine-induced changes in apoB100 conformation. Endoglycosidase H studies of newly-synthesized apoB100 revealed glucosamine induced N-linked glycosylation defects resulting in reduced apoB100 secretion. Taken together, these data suggest that co-translational glucosamine treatment may cause defects in apoB100 N-linked glycosylation and folding, resulting in ER stress and enhanced proteasomal degradation. Binding and retention by Grp78 may play a

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

critical role in proteasomal targeting and the ER quality control of misfolded apoB. Interaction with core lipoprotein lipids may facilitate apoB transport out of the ER by reducing Grp78-mediated ER retention.

2D_02_P

CHARACTERISATION OF THE ENDOPLASMIC RETICULUM OXIDOREDUCTASE ERO1-LB

Adam Benham, Sanjika Dias Gunasekara, Marcel Van Lith, Gareth Williams, Ritu Kataky

Disulfide bond catalysis is an required of protein production in the secretory pathway, from yeast through to man. In the endoplasmic reticulum (ER), protein disulfide isomerase (PDI) catalyses the oxidation and rearrangement of disulfide bonds, and is re-oxidised by an endoplasmic reticulum oxidoreductase (ERO). The elucidation of ERO function was greatly aided by the genetic analysis of two *S. cerevisiae ero* mutants. These strains arise from point mutations in the FAD binding domain of the Ero protein. The *ero1-1* and *ero1-2* yeast have conditional and DTT sensitive phenotypes, but the effects of the mutations on the behaviour of Ero proteins has not been reported. We therefore analysed these mutations in the context of Ero1b, a mammalian orthologue of Ero1p. Ero1b is upregulated by the UPR, and is strongly expressed in secretory tissues. The Gly to Ser and His to Tyr mutations do not prevent the dimerisation of Ero1b, or the non-covalent interaction of Ero1b with PDI. However, the Gly to Ser mutation abolishes disulfide-dependent PDI-Ero1β heterodimers. Both the Gly to Ser and His to Tyr mutations make Ero1b susceptible to misoxidation and aggregation, particularly during a temperature or redox stress. We conclude that the Ero FAD binding domain is critical for conformational stability, allowing Ero proteins to withstand stress conditions that cause client proteins to misfold.

2D_03_P

ENDOPLASMIC RETICULUM-STRESS SENSOR IRE1 IS ACTIVATED VIA TWO REGULATORY STEPS INVOLVING CLUSTER FORMATION AND DIRECT BINDING TO UNFOLDED PROTEINS.

Yukio KIMATA and Kenji KOHNO

Nara. Inst. Sci. Tech., 630-0192 Nara, Japan kimata@zero.ad.jp

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

With the exception of the commonly accepted fact that the ER chaperone BiP downregulates it, it is unclear how endoplasmic reticulum (ER) stress is sensed by the signal transducer Ire1. The crystal structure of the core stress-sensing region (CSSR) of yeast Ire1 luminal domain has been controversially suggested to indicate that the molecule can bind to unfolded proteins. Here we demonstrate that Ire1 clusters upon ER stress and actually binds to unfolded proteins. Phenotypes of Ire1 mutations that affect these phenomena demonstrate a scenario in which Ire1 is fully activated via two steps, both of which are regulated by ER stress but in different ways. In the first step, BiP dissociation from Ire1 leads to clustering, while in the second step, direct binding of unfolded proteins to the CSSR arranges the orientation of the cytosolic effector domains of clustered Ire1 molecules.

2D_04_P

ENDOPLASMATIC RETICULUM AND ENERGY STRESS RESPONSE MECHANISMS IN INTESTINAL EPITHELIAL CELLS UNDER CHRONIC INFLAMMATION: INHIBITORY EFFECTS OF INTERLEUKIN 10

Messlik A., Ruiz P. A., Kim S. C., Sartor R. B., Haller D.*

Technical University of Munich, Experimental Nutrition Medicine, Am Forum 5, 85350 Freising, Germany
** corresponding author*
email: haller@wzw.tum.de; phone: ++49-(0)8161-712026; fax: ++49-(0)8161-712097

The initiation of endoplasmic reticulum (ER) stress responses and energy deficiency in intestinal epithelial cells (IEC) may contribute to the pathogenesis of chronic intestinal inflammation. The aim of this study was to characterize anti-inflammatory mechanisms of interleukin 10 (IL10) using functional epithelial cell proteomics.
Proteome analysis from primary IEC of *Enterococcus faecalis*- and *Escherichia coli* monoassociated IL10 deficient (IL10-/-) mice revealed increased expression levels of the glucose-regulated ER stress proteins (grp)78 under conditions of experimental colitis. Interestingly, the induction of ER stress response mechanisms under conditions of chronic inflammation was associated with decreased expression levels of the mitochondrial creatine kinase and increased activation of the AMP kinase system in primary IEC, suggesting dysregulation of the cellular energy homeostasis. Most importantly, IL10 inhibited grp78 expression in IL10 receptor reconstituted epithelial cells. Chromatin immunoprecipitation analysis revealed that IL10-mediated p38 signaling inhibited TNF-induced recruitment of the ER-derived activating transcription factor (ATF)-6 to the grp78 promotor likely through the blockade of ATF-6 nuclear

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

translocation.
The failure of energy homeostasis in primay IEC from inflamed IL10-/- mice was associated ER stress responses in the intestinal epithelium. In addition, IL10 inhibits inflammation-induced ER stress response mechanisms by modulating ATF-6 nuclear recruitment to the grp78 gene promotor

---

2D_05_P

PROTEASOMAL DEGRADATION IS TRANSIENTLY ARRESTED DURING INHIBITION OF TRANSLATION IN ER STRESS

Marina Shenkman and Gerardo Z. Lederkremer

Department of Cell Research and Immunology, George Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel
E-mail: gerardo@post.tau.ac.il

The unfolded protein response (UPR) activates transcription of genes involved in proteasomal degradation. However, we found that in its early stages the UPR leads to a transient inhibition of proteasomal disposal of cytosolic substrates (p53 and p27Kip1) and of those targeted to ER-associated degradation (uncleaved precursor of asialoglycoprotein receptor H2a). Degradation resumed soon after the protein synthesis arrest that occurs in early UPR subsided. Consistently, also protein synthesis inhibitors blocked ubiquitin/proteasomal degradation. Ubiquitination was inhibited during the translation block, suggesting short-lived E3 ubiquitin ligases as candidate depleted proteins. This was indeed the case for p53 whose E3 ligase, MDM2, when overexpressed,restored the degradation, whereas a mutant MDM2 in its acidic domain restored the ubiquitination but not completely the degradation. Inhibition of proteasomal degradation early in UPR may prevent depletion of essential short-lived factors during the translation arrest. Stabilization of p27 through this mechanism may explain the cell cycle arrest in G1 when translation is blocked by inhibitors or by the UPR.

2D_06_P

THE APOPTOTIC EFFECT OF N-3 FATTY ACIDS ON THE LEUKEMIA CELL LINE HL60 IS LIKELY TO BE MEDIATED THROUGH ER STRESS.

J. E. Slagsvold, S. A. Schønberg.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Dept of Laboratory Medicine, Women's and Children's Health, the Norwegian University of Science and Technology, N-7489 Trondheim, Norway. E-mail: slagsvol@stud.ntnu.no

Background - Numerous studies indicate an inverse relationship between dietary intake of n-3 polyunsaturated fatty acids (PUFAs) and cancer. Cell culture studies show that n-3 PUFAs inhibit cell proliferation and induce programmed cell death, apoptosis. However, the molecular mechanisms behind these effects are complex and unclear. **Aim** – To investigate signaling pathways involved in EPA-induced growth arrest/apoptosis in HL-60 cells. **Materials and methods** – Gene expression profiling of HL60 cells treated with EPA was performed by Affymetrix GeneChip System and key markers of relevant pathways verified at protein level by Western blotting. **Results** - Several transcripts involved in ER homeostasis, cell cycle and apoptosis were affected. Transcripts involved in the unfolded protein response (UPR) pathway, chaperones, folding proteins and transcripts involved in apoptosis, were upregulated, whereas cyclin D1 and cellcycle/progression transcripts were downregulated. Proteins in the UPR like eIF2α-P, ATF4, SQSTM-1 and cyclin D1 were verified by western blotting. **Conclusion** - The data clearly show that the n-3 PUFA EPA induces apoptosis in HL 60 cells and that the mechanism may be mediated through ER stress.

2D_07_P

ERAD AND ER QUALITY CONTROL COMPARTMENTALIZATION DURING ER STRESS

Bella Groisman, Edward Avezov, Julia Leitman, Efrat Ron and Gerardo Z. Lederkremer

Dept. of Cell Research and Immunology, Tel Aviv University,
bellagr@post.tau.ac.il

Protein homeostasis in mammalian cells is tightly controlled by protein synthesis and degradation. An efficient quality control machinery is required for proper cell function, which is particularly important in conditions of stress. Disruption of homeostasis, e.g by restriction of oxygen or nutrient supply or interfering with calcium balance in the ER results in overloading of the ER, causing ER stress and activation of a protective cellular mechanism termed the Unfolded Protein Response (UPR). During the UPR there is an induction of genes encoding for chaperones and E3 ubiquitin ligases targeted to reduce ER protein overload through ER-Associated Degradation (ERAD).
Using a model ERAD glycoprotein substrate and its non-glycosylated version we found that several of these UPR induced factors, such as the

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

putative mannose lectin EDEM and the E3 RING ubiquitin ligase HRD1 are required for degradation of both substrates, whereas the cytosolic E3 member of the F-box family Fbs2 participates in degradation only of the glycoprotein. We found that upon ER stress HRD1 and Fbs2 accumulate along with the ERAD substrate in the pericentriolar ER-derived quality control compartment (ERQC), previously discovered in our lab. Other additional key factors implicated in ER quality control and the ER stress response, are also found in the ERQC and their localization depends on the function of one of the branches of the UPR. These and other findings suggest that protein recruitment to in the ERQC is involved in coping with ER stress.

2D_08_P

LOW-IRON DIET ABROGATES CHRONIC INTESTINAL INFLAMMATION IN TNFDARE/WT MICE

Werner T, Schulz N, Kollias G, Schuemann K and Haller D*

Technical University of Munich, Experimental Nutritional Medicine, Am Forum 5, 85350 Freising, Germany
** Corresponding author:*
Email: haller@wzw.tum.de; Phone: ++49-(0)8161-712026; Fax: ++49-(0)8161-712102

Patients suffering from intestinal bowel disease (IBD) have decreased anti-oxidant and glutathione levels which are indicative of oxidative stress. High concentrations of iron have been shown to increase the oxidative status on the cell leading to the induction of endoplasmic reticulum (ER) stress and protein oxidative damage. We studied the role of low-dietary iron on molecular mechanisms of intestinal epithelial cell (IEC) ER stress responses under conditions of chronic experimental ileitis in TNFDARE/WT mice.
Histological analysis of normal-iron fed mice showed severe intestinal inflammation with a score of 7.67 +/- 1.53 (pathological range from 0-10), whereas low-iron fed mice presented a significant reduction of inflammation in proximal and distal ileum segments with a score of 2.30 +/- 0.68. Western blot analysis in primary IEC from TNFDARE/WT and wild type mice showed reduced NF-κB activation and MAPK signalling including phosphorylation of RelA, cJun and Erk. The activation of ER stress responses including expression of glucose regulated protein 78, grp58, phosphorylation of eukaryotic initiation factor 2 alpha as well as cleavage of caspase 12 and 3 were decreased under low-iron diet. Furthermore, proteome analysis identified 40 differentially regulated proteins including the iron-regulated pro-inflammatory mediator intelectin. This study presents evidence for the beneficial effects of low-iron intake in

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

a mouse model of chronic ileitis including regulation of oxidative and ER stress response mechanisms at the epithelial cell level and therefore pointing to possible therapeutic use of a low-iron diet in patients of IBD.

2D_09_P

TRANSCRIPTIONAL PROFILING REVEALS INTEGRATION OF ER- AND OSMOTIC-STRESS PATHWAYS.

André S. T. Irsigler[1,¶], Maximiller D. L. Costa[1], Ping Zhang[2], Pedro A. Braga[1], Ralph E. Dewey[2], Rebecca S. Boston[3] and Elizabeth P. B. Fontes[1]

[1]Departamento de Bioquímica e Biologia Molecular, BIOAGRO, Universidade Federal de Viçosa, 36571.000 Viçosa, Minas Gerais, Brazil
[2]Department of Crop Science and [3]Department of Plant Biology, North Carolina State University, Raleigh, North Carolina 27695

Despite the potential of the endoplasmic reticulum (ER) stress response to accommodate adaptive pathways, its integration with other environmental-induced responses is poorly understood in plants. Here, we performed global expression profiling on soybean leaves exposed to polyethylene glycol treatment or to unfolded protein response (UPR) inducers to identify integrated networks between osmotic and ER stress-induced adaptive responses. The results unmasked the major branches of the ER-stress response, which includes enhancing protein folding and degradation in the ER, as well as specific osmotically regulated changes linked to cellular responses induced by dehydration. However, a small proportion (5.5%) of total up-regulated genes represented a shared response that seemed to integrate the two signaling pathways. These co-regulated genes were considered downstream targets based on similar induction kinetics and a synergistic response to the combination of osmotic- and ER-stress-inducing treatments. Genes in this integrated pathway with the strongest synergistic induction encoded proteins with diverse roles. Two of them contained a plant-specific development and cell death (DCD) domain while another had homology to proteins with an ubiquitin-associated (UBA) domain. A NAC domain-containing protein exhibited robust early kinetics of induction consistent with a role as a transfactor. This integrated pathway diverged further from characterized ER-specific branches of UPR as downstream targets were inversely regulated by osmotic stress. Collectively, our results describe a novel branch of the ER stress response that integrates the osmotic signal to potentiate transcription of shared target genes.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2E_01_P

NF-KB FUNCTIONS THE DIVERSITY OF CELLULAR IGF-I/IGFBP-1 EXPRESSION BY HYPOXIA IN TIBETAN PLATEAU MAMMALS

X. Q. Chen, S. J. Wang, Y. Liu and J. Z. Du

Department of Physiology, Divi. Neurobiology and Physiology, School of Medicine, Zhejiang University, Hangzhou 310058, China
dujz@cls.zju.edu.cn

Ochotona curzoniae, Microtus oeconomus and Myospalax baileyi are all native mammals that reside at Qinghai-Tibetan plateau in China and well acclimatized to environmental hypoxia. The present paper addresses the NF-kB's, a nuclear transcriptional factor, involvement in hypoxia stress-induced diversity of IGF-I/IGFBP-1 expression in hepatic and brain cells of Tibetan Plateau mammals. The IGF-I/IGFBP-1 from the prefrontal cortex and the liver cells was tested 6 h after hypoxia exposure (by CoCl2 injection i.p. 20, 40 mg/kg or by normobaric hypoxia, 16.0%, 10.8%, 8.0 %O2) of the Plateau native mammals and mice. PDTC, an inhibitor of NF-κB, was used and preinjected before the hypoxia to evaluated NF-kB action. The results showed that 1) the IGF-I expression in mice hepatic cells of M. oeconomus and M. baileyi markedly increased after the hypoxia exposure, but there was no response in the liver of O.curzoniae; 2) the IGFBP-1 expression in mice hepatic cells of O. curzoniae and M. baileyi markedly enhanced, but no response occurred in M. oeconomus after the hypoxia; 3) PDTC pretreated before hypoxia reversed the hypoxia-enhanced IGF-I in M. oeconomus and M. baileyi; 4) PDTC treatment also reversed the hypoxia-enhanced IGFBP-1 in O. curzoniae and M. baileyi; 5) hypoxia increased the IGF-I mRNA in brain of M. oeconomus and O. curzoniae but not of mice; 6) hypoxia did not induce changes of IGF-I levels in the brain cells of both plateau mammals and laboratory mice. The data suggest that 1) different pattern in IGF-I/IGFBP-1 expression induced by hypoxia represents a diversities in hormone regulation and cell protection from damage in Tibetan native mammals; 2) NF-κB mediates the transcription of IGF-I/IGFBP-1 in liver cells subjecting to hypoxia; Together, the diversity of target-gene phenotype expression may contribute to the multi-model in cell protection from hypoxia damage.Acknowledgement: This work was supported by the NSFC: Projects (No. 30393130;30470648; 30570227), and by The National Basic Research Program "973" No. 2006CB504100).

2E_02_P

PRENATAL HYPOXIA STRESS INDUCED THE HIGH SENSITIVITY OF HPA IN ADULT OFFSPRING RATS

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

X.Q Chen,* J.Z. Du* and J.M. Fan,

Department of Physiology, Divi. Neurobiology and Physiology, School of Medicine, Zhejiang University, New Campus, Hangzhou, 310058, China
**Corresponding authors: X.Q Chen and J.Z. Du, dujz@cls.zju.edu.cn or chewyg@zju.edu.cn*

The hypothalamo-pituitary-adrenal (HPA) axis is affected intensively by environment stress in early life. We invistigated whether CRFR1 or CRFR2 are involved or not in modification of the offspring's HPA activity and behavior response after maternal hypoxia stress (MHS). The pregnant mother rats were exposed to different stressors: simulated hypobaric hypoxia (5km altitude, 10.8% O_2), restraint, cold (4ºC), and in combination stress (Hypoxia+Restraint, Hypoxia+Cold, Hypoxia+Restraint+Cold) throughout the pregnant period 4h per day for 22 days, and the growth parameters of postnatal rats, HPA axis activity and neuroendocrine function during growing and late age of puberty rats were determinated, anxiety-like behavior of adult male rats was evaluated by elevated-plus maze (EPM), and the changes of CRFR1 mRNA and CRFR2 mRNA expression in pituitary of both late age of puberty and adult male rats were measured by in situ hybridizaition. The plasma corticosteron, ACTH, were tested using RIA. the NE, and DA levels in Locus Coeruleus (LC) determinated by HPLC. The research found that MHS significantly decreased the birth weight and delayed the growth rate in postnatal rats, enhanced the basal activity of HPA axis, the expression of CRFR1 mRNA /CRFR2 mRNA in pituitary of late age of puberty rats, induced anxiety-like behavior, elevated the level of NE and DA in the LC. These data suggest that perinatal stress significantly infuluences HPA axis activity and behavior of adult offspring rat, and the CRFR1 and CRFR2 may play a role in modification of these activities by pregnent maternal hypoxia and combnated stress. Acknowledgement:This work was supported by the National Science Foundation of China: Major Project (No. 30393130) and Projects (Nos. 30270232; 30470648; 30570227), and by the China Ministry of Science and Technology (The National Basic Research Program "973" No. 2006CB504100).

2E_03_P

DEFENSE OF THERMAL AND OXYGEN STRESS IN *DAPHNIA MAGNA*

Bettina Zeis, Sabine Zumbrägel, Rüdiger J. Paul

Institut für Zoophysiologie, Hindenburgplatz 55, 48143 Münster, Germany
zeis@uni-muenster.de

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Small planktonic animals like Daphnia experience high fluctuations of environmental oxygen content and temperature, resulting in changes of oxygen supply or demand, respectively. Hypoxia or exposure to high temperatures may lead to the formation of oxygen radicals (reactive oxygen species, ROS) and changes in the cellular redox state. This may activate a biphasic stress response system. A fast component of the stress response aims to reduce radical damage until cellular homeostasis is restored by the slower component (Kültz, 2005, Annu Rev Physiol 67:225).
In the microcrustacean Daphnia magna, hemoglobin (Hb) is the central effector protein of the homeostasis response to restore aerobic metabolism after oxygen- or temperature-induced impairment of tissue oxygenation. Differential expression of a set of Hb orthologs enhances both, concentration and oxygen affinity of the respiratory protein. We studied the induction process of Hb following oxygen or temperature stress on the mRNA and protein level. We also detected changes in the oxygen-sensitive transcription factor HIF (hypoxia-inducible factor), which is under control of ROS.
Analysing the radical defense system under defined oxygen and temperature conditions (Po2: 2, 21, and 60 kPa at 20 °C and 10, 20, and 30 °C at normoxia: 21 kPa), we found that the activity of catalase was slightly increased under hypoxia and at elevated temperature. The concentration of the redox buffer glutathione (GSH, GSSG) was increased at hyperoxic conditions and high temperatures; the same was true for the activity of glutathione-S-transferases. Comparing thresholds for the induction of the stress response system for different clonal lines allows us to discriminate genetically determined components from acclimation effects.

2E_04_P

HYPOXIC STRESS DISRUPTS CELL PROLIFERATION AND APOPTOSIS IN NON-CANCER TISSUES VIA TELOMERASE: A WHOLE FISH ANIMAL MODEL

D.W.T. Au*, E.X.H. Chen, R.M.K. Yu, M.W.L. Chiang, H.O.L., Mok, B.W.P. Yip, R.Y.C. Kong

Biology & Chemistry Department, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong
(email: bhdwtau@cityu.edu.hk)

We have employed the small fish species marine medaka (*Oryzia melastigma*) as a vertebrate model for studying whole animal response under chronic hypoxia, with special interest to investigate regulation of telomerase (TERT) expression via hypoxia-inducible factor and to link

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

telomerase expression to cell proliferation and apotptosis in non-tumor tissues in vivo under hypoxia. The *O. melastigma* exhibits telomere length resembling those of humans. The full-length TERT cDNA, *omTERT*, encodes a protein of 1086 amino acids and contains all of the functional motifs that are conserved in other vertebrate TERTs. The *omTERT* gene is ubiquitously expressed in all fish tissues under normoxia, where highest expression was observed in gonads and the lowest in liver. *In vivo* expression of *omTERT* was significantly upregulated in testis and liver in response to hypoxia (at 96 h and 48 h, respectively), where concomitant induction of the hypoxia inducible factor-*1α* (*omHIF-1α*) and erythropoietin (*omEpo*) genes was also observed. Analysis of the 5'-flanking sequence of the *omTERT* gene identified two HRE (hypoxia-responsive element; nt. –283 and –892) cores. Overexpression of the HIF-1α induced *omTERT* promoter activity as demonstrated using transient transfection assays. The *in vivo* effects of TERT expression on tissue homeostasis (involving a balance of cell proliferation and apoptosis) in whole vertebrate are poorly known. We have optimized a fixation and tissue processing protocol for the adult medaka fish which has proven successful for quantitative *in situ* hybridization (Q-ISH) and immuno-histochemisty (Q-IHC). This whole fish model allows us to study the co-expression profiles of *omTERT* gene and TERT protein in multiple organs of marine medaka simultaneously, and to relate TERT expression to cell proliferation (by PCNA) and apoptosis (TUNEL) *in vivo.* We found that hypoxia induces cell/tissue specific changes in expression of *omTERT* mRNA and protein, telomerase activity, cell proliferation, apoptosis in adult medaka. The findings of our whole fish model will shed light on hypoxic stress and telomerase biology in vertebrates *in vivo*.

2E_05_P

CLONING AND EVOLUTION OF HIF-1A CDNA FROM TIBETAN VERTEBRATES: *PANTHOLOPS HODGSONI*, *MYOSPALAX BAILEYI, MYOSPALAX CANSUS*, *MICROTUS OECONOMUS*, *GYMNOCYPRIS PRZEWALSKII*, AND EPO RESPONSIVNESS UNDER HYPOXIA

XQ Chen [a]*, JZ Du [a]*, YB Cao [a], Y Liu[a], X-C Chen[b] , G Cai [c] and PH Xiao[c]

[a]*Dept.Physiology, School of Medicine, Zhejiang University, Hangzhou 310058, China*
[b]*Northwest Institute of Plateau Biology, The Chinese Academy of Sciences*
[c]*Qinghai Kekexili National Nature Reserve,*
**Corresponding authors:XQ Chen and JZ Du, dujz@cls.zju.edu.cn*

Hypoxia-inducible factor 1α(HIF-1α) is an essential mediator of oxygen homeostasis. Tibetan antelope(*P.hodgsoni*), Plateau pika(*O.curzoniae)*,

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Plateau Zokor(*M.baileyi,M.cansus*), Root vole(*M. oeconomus*), and naked carp(*G.przewalskii*) are native vertebrates at Qinghai-Tibetan plateau and well acclimatized to hypoxia. To better understand the adaptive mechanisms to hypoxia, cDNA encoding HIF-1α was isolated and characterized from those vertebrates. The deduced HIF-1α sequences of *P. hodgsoni, M. oeconomus*, *M. baileyi and M.cansus* showed 90-99% identities with those of the human, rat, yak, respectively; *G.przewalskii* 89-57% with that of rainbow trout and common carp. The conservational and phylogenic clustering of vertebrates HIF-1α sequences was consistent with the vertebrate classification. We estimated codons under positive selection. All positive selection site were not in key domain, but between the key domain of TAD-N and TAD-C (594L, 600L, 604T, 605E, 606E, 607L, 610V, 615T, 628T, 646Q), and another was 8E in *M. baileyi*. The evidence show that plateau animals could have peculiarities for hypoxia, which linked to changes of Tibetan Plateau growing. HIF-1 increased in cortex and liver of mice, *M. Baileyi, and M. oeconomus* under hypoxia not in *O. curzoniae;* EPO increased in cortex and kidney of mice under hypoxia, but only in kidney of *M. oeconomus* under hypoxia. Additionally, under $CoCl_2$, EPO increased in cortex and kidney of three Tibetan mammals not in mice. The differences of HIF and EPO in Tibetan animals and lowland mice suggest diversive strategies are involved in hypoxia. HIF-1 may play an role in mice and *M. Baileyi* not in Tibetan's *O.curzoniae* for hypoxia. The work was supported by the NSFC: (No. 30393130;30470648; 30570227 and by The Program "973" No. 2006CB504100).

2E_06_P

SPECIFIC FKBP38 INHIBITOR REDUCES HYPOXIA-INDUCED APOPTOSIS IN VENTRICULAR MYOCYTES FROM ADULT AND NEONATAL RAT HEARTS

S. Rau[2], B. Husse[1], D. Wildemann[2], M. Gekle[1], G. Fischer[2]

[1]Dept. of Physiology, University Halle and [2]Max-Planck Research Unit for Enzymology of Protein Folding, Halle, Germany
britta.husse@medizin.uni-halle.de

Hypoxia/reoxygenation causes cell death of cardiomyocytes by a mitochondrion-dependent pathway. The Ca^{2+}/CaM activated FK506-binding protein 38 (FKBP38) can interact with Bcl-2 through its PPIase active site and participates in the promotion of apoptosis. This study investigated the effect of specific FKBP38 inhibition with DM-CHX (N-(N',N'-dimethylcarboxamidomethyl)-cycloheximide) on the hypoxia/reoxygenation-induced apoptosis. Ventricular myocytes from adult or neonatal rates were cultured and subjected to hypoxic conditions

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

(0.2% O_2) for 18 or 24 hrs (adult/neonatal) followed by a reoxygenation period (21% O_2) of 24 hrs. Hypoxic condition was proved by HIF1-alpha expression using western blots. Apoptotic cell analysis was determined by using the ViaCount assay (Guava Technologies). In adult myocytes, hypoxia caused 34% and hypoxia/reoxygenation 31% apoptotic cells. Application of DM-CHX (5μM) resulted in 22% (hypoxia) and 11% (hypoxia/reoxygenation) apoptotic cells. In neonatal myocytes, under both conditions 64% apoptotic cells were analysed, reduced to 34% after DM-CHX (5 μM) treatment. As positive control, caspase-inhibition and cyclosporin A showed apoptosis inhibition in both types of myocytes. Hypoxia (2.5-fold) and hypoxia/reoxygenation (1.4-fold) caused injury of adult myocytes measured by relative lactate dehydrogenase activity, which was reduced by DM-CHX (5 μM) treatment. Our results suggest that DM-CHX, a specific inhibitor of FKBP38, reduces apoptosis in cardiomyocytes in a dose-dependent manner. Such a specific drug could be used to decrease the loss of myocytes after damaged injury resulting in an improved cardiac function.

2E_07_P

COMBINED CASPASE AND CALPAIN INHIBITION OF PC12 AND NGF TREATED CELLS AFTER GLUCOSE AND OXYGEN DEPRIVATION INDUCED STRESS

C. Pourzitaki[1], I. Klagas[1], A. Sioga[2], A. Kaidoglou[2], and A. Kritis[3]

[1]Laboratory of Pharmacology, [2]Laboratory of Histology and Embryology and [3]Laboratory of Physiology, Medical School, Aristotle University, Greece. Kritis@med.auth.gr

Hypoxia-induced dysfunction of the central nervous system may be caused by neuronal cell death or by changes in its neurochemical implicated in the regulation of cell death. PC12 cells may serve as a useful model for studying the interaction between hypoxia, and cell death. Programmed cell death (apoptosis) plays an important role in a wide variety of physiological processes, as well as in pathological cellular insults. Abnormal elevation of intracellular Ca^2 is thought to be a critical trigger of neuronal damage associated with hypoxia or ischemia. Several lines of evidence suggest that calcium-activated proteolysis plays a pivotal role in hypoxic-ischemic neuronal damage. Indeed, calpain-mediated proteolysis appears to be one of the earliest biochemical changes occurring after a dense ischemic challenge. In the present study, we tested the effects of z-VAD-fmk as a specific caspase inhibitor combined with MDL28170 a new and more permeable calpain inhibitor during oxygen and glucose deprivation in naïve and NGF treated PC12 cells. Our

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

results show that both protease inhibitors confer comparable cell death resistance to both naive and NGF treated PC12 cells at a concentration of 10μM. It seems that once the death signal is released the cell has to employ either apoptosis or necrosis in order to effect death. Since both classes of proteases are implicated in cell death in our system under the conditions studied, we hypothesize that the two processes, necrosis and apoptosis, are not exclusive to themselves and that at some stages they can share functions of their machinery to one another.

2E_08_P

BIOLOGICAL MARKERS OF STRESS IN COLONIC HEALING AFTER PORTAL ISCHEMIA-REPERFUSION. EXPERIMENTAL STUDY

Schanaider A, Rolim M, Cotta-Pereira G, Eleutherio ECA, Silva PC, Aratanha CB

Article and Research from the Center of Experimental Surgery of the Department of Surgery - Faculty of Medicine of Federal University of Rio de Janeiro

PRUPOSE: To develop the knowledge about the pathophysiology of colonic healing in the presence of portal congestion and to identify biological markers of this oxidative stress.
METHODS: Seventy male Wistar albino adult young rats (*Rattus novergicus albinus*), weighting 250-350g were enrolled. Anesthetic procedure consists of Sevoflurane inhalation. Four groups of experiment were established: G1 - Control (n = 10), G2 - Colonic anastomosis (n = 20); G3- Total portal vein occlusion for 30 minutes (n =20) G4- Association of both procedures as described for groups 2 and 3. Blood samples from all groups were obtained 24 hours and 5 days following surgery in order to assess levels of malondialdehyde (MDA) and protein carbonyl groups by TBA and Slot-blotting reaction, respectively Intestinal fragments stained with Red picrosirius red were also evaluated with polarization. Data were analyzed statistically by Anova and Tukey tests.
RESULTS: Levels of MDA (Figure 1A,B) and carbonyl protein (Figure 2A,B) increased significantly either after 24 hours ($p < 0.002$) or 5 days ($p < 0,0001$), only in those rats belonging to group. Five animals of G4 presented suture dehiscence. In G2 at day 5 it was observed collagens types III and I (Figure 3A,B). There were slight inflammatory infiltrate and no extra cellular matrix production at G3. G4 showed no collagen production (Figure 4A,B).
CONCLUSION: Portal reperfusion interferes with colonic anastomosis healing and biological marker produced may be useful to characterize the oxidative stress.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

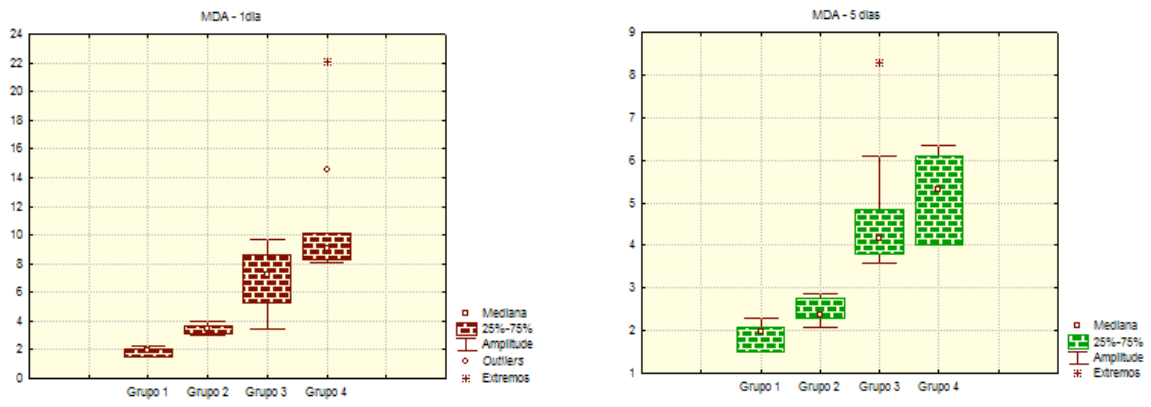

Figure 1 -MDA levels after one (A) and five days (B)

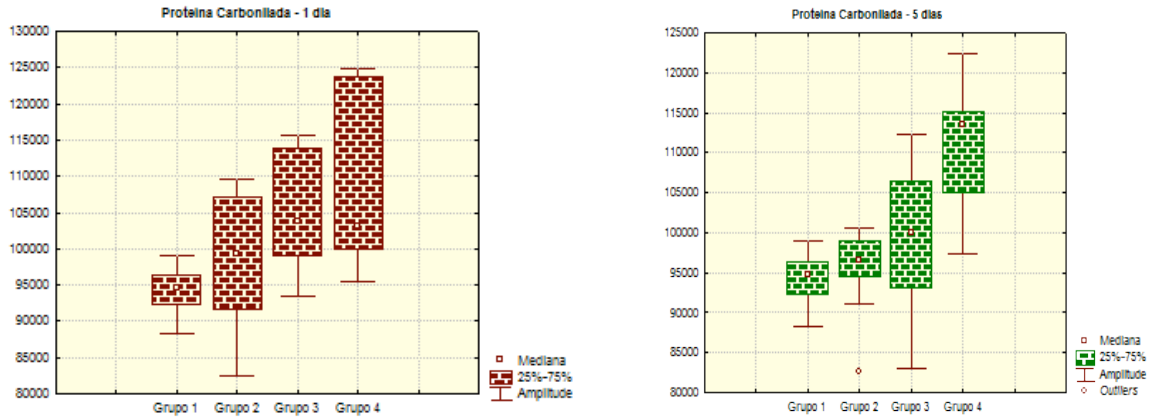

Figure 2- Carbonyl protein levels after one and five days

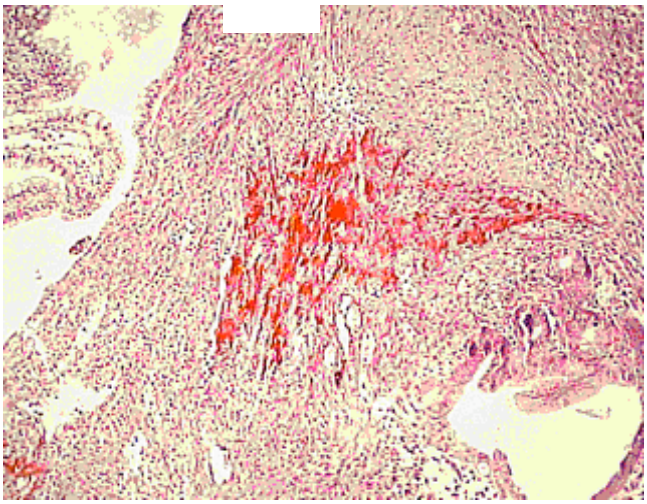

Figure 3A. G 2, day 5, 100 X Picrosirius: It was seen an inflammatory response with fibroblasts immersed in the collagen matrix. -

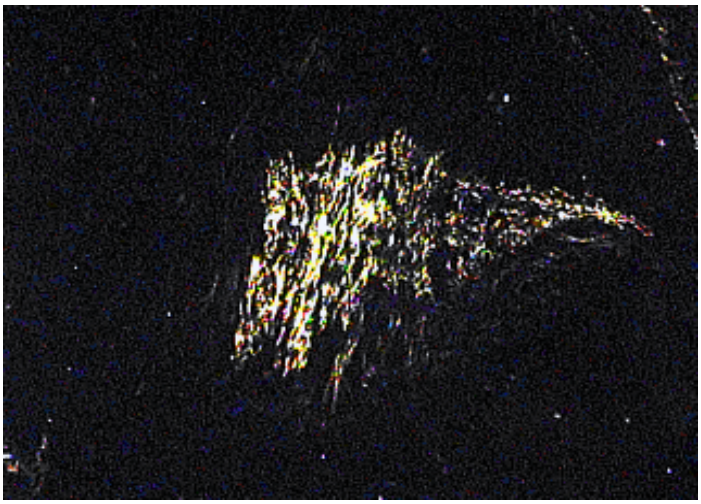

Figure 3B. G2, day 5, 100x Polarization: It was observed the presence of collagen type III (green refringence) and collagen type I (yellowish and reddish refringence)

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

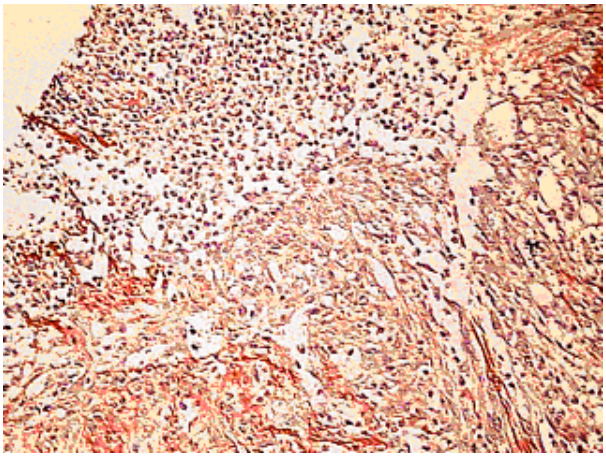

Figure 4A. G4, day 5, Picrosirius X100:An important inflammatory reaction was seen at one of the anastomotic borders but without tissue repair.

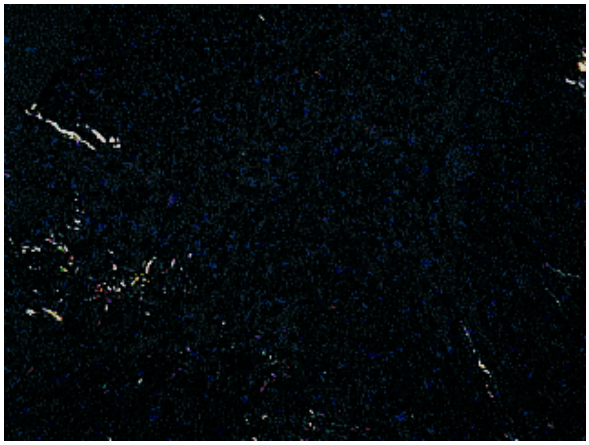

Figure 4B.G4, day 5, Polarization X100: There was no collagen production.

2F_01_P

THE STRESS PROTEOME: 2D-PAGE OF YEAST MITOCHONDRIA UNDER DIFFERENT OXIDATIVE STRESS CONDITIONS

Claudia Leidhold, Tom Bender and Wolfgang Voos

Institute of Biochemistry and Molecular Biology, University of Freiburg, Germany;
Wolfgang.Voos@biochemie.uni-freiburg.de

Besides fulfilling further important metabolic functions, mitochondria are also the place of cellular respiration. During this process, as a side reaction, a continuous production of reactive oxygen species (ROS) occurs. Because of their proximity to the place of ROS formation, mitochondrial proteins are at high risk of being irreversibly damaged and misfolded. This implies an important function of proteolysis in mitochondrial stress defense. Indeed, yeast cells lacking Pim1, the major protease of the mitochondrial matrix, show a significantly higher sensitivity to ROS than wild-type cells. In order to get a general overview about mitochondrial proteins affected by oxidative stress we performed an extensive proteome analysis. We compared the yeast mitochondrial 2D-pattern after treatment with stress-inducing chemicals, such as H_2O_2, antimycin A or menadione, to that of untreated mitochondria. Under each stress condition we identified a number of proteins as candidate substrates for proteolysis, with some of them even being sensitive to all

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

conditions tested. Proteins affected the most by ROS are either themselves involved in oxidative stress defense or contain oxidant-sensitive structures such as Fe-S-clusters. *In vitro* degradation assays with selected candidate proteins could confirm the results obtained from 2D studies. For most of the proteins degraded in a stress-specific manner we could identify Pim1 as the protease responsible for their turnover.

2F_02_P

CHAPERONE-DEPENDENT AND INDEPENDENT ROLE OF N-GLYCAN IN CFTR FOLDING, STABILITY AND MEMBRANE TRAFFICKING

R. Glozman, T. Okiyoneda, H. Barriere and G. L. Lukacs

Hospital for Sick Children, Cell Biology, Toronto, Ontario, Canada, glukacs@sickkids.ca

Although N-glycan-mediated recruitment of lectin chaperones (e.g. calnexin/calreticulin) can assist the folding and suppress the aggregation tendency of newly synthesized glycoproteins at the ER, the glycan-dependent stabilization of plasma membrane protein remains elusive. Here we used the cystic fibrosis transmembrane conductance regulator (CFTR), a PKA-activated chloride channel, to investigate the role of N-glycan in its biogenesis and trafficking. CFTR glycosylation was prevented by genetic and pharmacological means, while chaperone-CFTR interaction was disrupted in calnexin-depleted cells. Calnexin downregulation by siRNA or glucosidase I inhibition decreased CFTR folding efficiency by ~30% in the ER. In contrast, non-glycosylated CFTR folding was attenuated by >80%, suggesting a chaperone-independent role of N-glycans in the channel conformational maturation. The structural destabilization of cell-surface resident, non-glycosylated CFTR was documented by limited proteolysis and conceivable constitutes the trigger for the ubiquitination of the destabilized channel. Ubiquitin conjugation signals to reroute the channel from the constitutive recycling pathway towards lysosomal degradation by the ubiquitin-dependent endosomal sorting machinery. This mechanism appears to account for the >4-fold accelerated turnover of the mutant CFTR in post-Golgi compartments. Our results, jointly, indicate that N-glycans are critical determinant of CFTR conformational maturation/stability in a chaperone-independent manner and outline a possible paradigm for the rapid degradation of glycosylation-deficient membrane proteins from the cell surface.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2F_03_P

GENETIC DISSECTION OF THE PROTEIN QUALITY CONTROL IN *ESCHERICHIA COLI*

Elena García-Fruitós[1], Monica Martínez-Alonso[1], Nuria Gonzàlez-Montalbán[1], Minoska Valli[2,3], Diethard Mattanovich[2,3], Antonio Villaverde[1*]

[1] *Institut de Biotecnologia i de Biomedicina and Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain*
[2] *University of Natural Resources and Applied Life Sciences Vienna, Department of Biotechnology, Institute of Applied Microbiology, Vienna, Austria,*
[3] *School of Bioengineering, University of Applied Sciences FH-Campus Vienna, Austria.*

Keywords: *E. coli*; proteolysis; protein folding; inclusion bodies; chaperones

Abstract:
The protein quality control system is an evolutionary conserved complex mechanism based on a network of cellular proteins with overlapping foldase, disaggregase and protease activities. In bacterial cells, the coordinated activity of all these elements promote proper protein folding or digestion of folding-reluctant, potentially toxic species, what is expected to keep misfolding-prone protein species in a soluble state and cells free from aggregates. However, recent insights on the biology of protein misfolding and aggregation strongly suggest that solubility and conformational quality are not matching events, since at least in some examples, protein aggregates might contain properly folded species. By using *Escherichia coli* cell models, we have finely explored both protein solubility and quality, through the fluorescence emission of green fluorescent protein (GFP) engineered variants, in mutant cell strains lacking defined components of the quality control system, including main chaperones, small heat-shock proteins and proteases. In absence of either chaperones DnaK, ClpB or ClpA and proteases ClpP or Lon, GFP-producing cells are significantly more fluorescent than wild type cells (up to more than two fold), while the solubility of GFP is clearly higher in wild type cells exhibiting a fully functional quality control network (up to around two fold). In all the identified mutants, the enhanced emission of recombinant cells is clearly linked to an elevated intracellular content of highly fluorescent GFP molecules, resulting from inhibition of its proteolytic degradation and the significant expansion of its half life (from 2 to up to 7 h, at 37ºC). Interestingly, the excess of functional protein is overstocked as highly fluorescent inclusion bodies containing properly folded protein species. Overall, these results indicate that the *E. coli* quality control system is governed by an over-committed, chaperone-mediated

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

proteolytic machinery that acts on protein species that are either functional or can reach functional forms when proteolytically stabilized. Intriguingly, the occurrence of molecular determinants of aggregation does not require complete protein unfolding, and in fact, solubility and fluorescence emission are inversely correlated. Therefore, selected genetic deficiencies in the quality control system dramatically enhance the intracellular pool of biologically active although insoluble, misfolding-prone proteins, a fact that might be specially relevant in the context of high quality recombinant protein production.

2F_04_P

LECTIN-DEFICIENT CALRETICULIN RETAINS FULL FUNCTIONALITY AS A CHAPERONE AND QUALITY CONTROL COMPONENT DURING THE BIOGENESIS OF CLASS I HISTOCOMPATIBILITY MOLECULES.

Breanna S. Ireland, Ulf Brockmeier and David B. Williams

Depts. of Biochemistry and Immunology, University of Toronto, Toronto, Canada, M5S 1A8, david.williams@utoronto.ca

Calreticulin (Crt) is a soluble chaperone of the endoplasmic reticulum (ER) that interacts with newly synthesized glycoproteins through a lectin site with specificity for $Glc_1Man_9GlcNAc_2$ oligosaccharides as well as through a polypeptide binding site that recognizes non-native protein conformers. The relative contribution of each site to the overall functions of Crt remains unknown. To address this issue, we created two point mutants, D317A and Y128A, that ablate the lectin function of Crt but do not alter its tertiary structure. We then examined their abilities to support the biogenesis of mouse class I histocompatibility molecules. Class I molecules function to present peptide antigens to cytotoxic T cells. They consist of a glycosylated transmembrane heavy chain, a soluble subunit termed β_2-microglobulin and an 8-9 residue peptide ligand. In Crt-deficient cells, the surface expression of class I molecules is reduced 2- to 3-fold, loading of peptide ligands is inefficient, and peptide-deficient class I molecules are prematurely exported from the ER (defective quality control). We expressed wild type Crt as well as both lectin-deficient Crt mutants in Crt-deficient cells. Remarkably, the lectin-deficient mutants were just as effective as wild type Crt in upregulating class I surface expression, enhancing peptide loading and preventing premature export from the ER. The mutants were also capable of binding to many newly synthesized glycoproteins in addition to class I. We conclude that in the absence of lectin-based interactions, Crt can utilize polypeptide-based interactions to effect its chaperone and quality control functions.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2G_01_P

THE THIOREDOXIN SYSTEM IN THE PSYCHROPHILIC EUBACTERIUM *PSEUDOALTEROMONAS HALOPLANKTIS*

R. Cotugno[1], G. Salomone[1], M.R. Ruocco[1], P. Grimaldi[1], F. Cecere[1], P. Falasca[2], G. Raimo[2], M. Masullo[3], E. De Vendittis[1]

[1]Dept. Biochemistry & Medical Biotechnologies, University of Naples Federico II, [2]Dept. Science & Technology of the Environment & Territory, University of Molise, [3]Dept. Pharmacobiological Sciences, University of Catanzaro Magna Graecia, Italy
E-mail: cotugnoroberta@libero.it

The thioredoxin system, involved in the preservation of the reduced state of cytoplasmic proteins, includes two ubiquitous key components of the intracellular redox balance: thioredoxin (Trx) and thioredoxin reductase (TrxR). Either Trx or TrxR contain a conserved CXXC sequence, switching between disulfide and free dithiol. Trx is a small monomeric protein; TrxR is a NADPH-dependent homodimeric flavoenzyme. The redox cycle in the Trx/TrxR system also involves oscillation between oxidized and reduced form of FAD, using NADPH as electron donor.
This report describes the biochemical characterization of the Trx/TrxR system in *Pseudoalteromonas haloplanktis* (*Ph)*, a psychrophilic eubacterium isolated from marine Antarctic sediments. *Ph*TrxR and *Ph*Trx were obtained as recombinant His-tagged proteins, or isolated from *P. haloplanktis* cells. The activity of recombinant *Ph*TrxR was evaluated by both DTNB- and thioredoxin-reduction methods and compared with that of endogenous *Ph*TrxR. After exogenous FAD caption, recombinant *Ph*TrxR shows the same activity of the endogenous enzyme. The thermal denaturation of the recombinant enzyme was followed by fluorescence melting curves in the temperature range 5-75°C. Heat inactivation experiments gave a half-life of 10 min at 60°C. However, when studying the thermophilicity with the DTNB-reduction method, maximum activity was reached at 30°C. Like the endogenous counterpart, recombinant *Ph*Trx reduces the insulin disulfides in the presence of either DTT or *Ph*TrxR/NADPH.

2G_02_P

VARIATION IN STRESS RESPONSES WITHIN A BACTERIAL SPECIES AND THE INDIRECT COSTS OF STRESS RESISTANCE

Thomas Ferenci[a], And Beny Spira

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[a] *School of Molecular and Microbial Biosciences, The University of Sydney, NSW 2006 Australia*

ABSTRACT: Bacteria can exhibit high levels of resistance to one or more environmental stresses such as temperature, osmolarity, radiation, pH, starvation as well as resistance to noxious chemicals and antibiotics. Yet evolution has not optimized stress resistance in all bacteria to all stresses. Even within a species like *Escherichia coli*, stress resistance is not constant between strains, suggesting that selection for stress resistance is under counter-selection in some environments. The trade-offs associated with stress resistance in *E. coli* are due to more than the direct cost of resistance mechanisms. A significant indirect cost is that high stress resistance is associated with a reduced ability to compete for poor growth substrates like acetate or even good substrates like glucose at sub-optimal concentrations. High stress resistance also decreases the ability to use inorganic nutrients like phosphate. This trade-off between self preservation and nutritional competence, called the SPANC balance, is likely to be major selective influence in natural populations. Another cost of high stress resistance in *E. coli* is an elevated mutation rate and the increased generation of deleterious mutations. Directional adaptations in SPANC balance and mutation rate are environment-dependent. The most common variations in SPANC are due to polymorphisms in the levels of global regulators RpoS and ppGpp between different strains. High levels favour stress resistance, lower levels allow better nutrition. The intimate association of RpoS/ppGpp with stress resistance and SPANC balancing influences numerous cellular processes and bacterial properties, including virulence.

2G_03_P

ESCHERICHIA COLI HEAT SHOCK PROTEINS IBPA/B ARE INVOLVED IN RESISTANCE TO OXIDATIVE STRESS INDUCED BY COPPER IONS.

Ewelina Matuszewska, Joanna Kwiatkowska, Dorota Kuczyńska-Wiśnik, Ewa Laskowska

Department of Biochemistry, University of Gdańsk, Kładki 24, Gdańsk, Poland, lasko@biotech.ug.gda.pl

E. coli IbpA/B proteins in cooperation with other molecular chaperones systems prevent the aggregation of thermally denatured proteins and support their refolding to the native state. Several data suggest that IbpA/B participate in the protection of *E. coli* cells against oxidative stress: IbpA/B inhibits inactivation of some *E. coli* enzymes by superoxide

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

radicals *in vitro*. Furthermore, overproduced IbpA/B increase *E. coli* resistance to superoxide stress. We demonstrate that the IbpA/B participate in the protection of *E. coli* against oxidative stress induced by copper ions. The transition metal copper is essential to a variety of cellular functions, however even moderately increased level of copper may be toxic for the cell. The toxicity results from the Fenton or Haber-Weiss reaction in which copper ions catalyze the production of OH radicals from hydroperoxide. We show that the lack of IbpA/B causes increased *E. coli* sensitivity to copper ions. IbpA/B proteins inhibited copper-catalyzed oxidation of a model enzyme – alcohol dehydrogenase (AdhE) both i*n vivo* and *in vitro.* We suggest that the overall ability of IbpA/B to protect cells from copper -induced damage may result from the metal chelation and direct binding to the protected proteins. Similar activity has been proposed for alfa-crystallin, a mammalian molecular chaperone homologous to the *E. coli* IbpA/B.

2G_04_P

THE ROLE OF HEAT SHOCK PROTEINS IBPA/B IN PROTECTION OF *E.COLI* PROTEINS AGAINST THERMAL DENATURATION UNDER ANAEROBIC GROWTH CONDITIONS.

Ewelina Matuszewska, Dorota Kuczynska-Wisnik, Joanna Kwiatkowska, Elzbieta Ratajczak, Ewa Laskowska

Department of Biochemistry, University of Gdansk, Kladki 24, Gdansk, Poland, ewelinam@biotech.ug.gda.pl

The *E. coli* IbpA/B proteins belong to a group of molecular chaperones which under heat shock protect polypeptides from irreversible denaturation and facilitate their refolding to the native structure in cooperation with the ATP-dependent chaperones: DnaK/DnaJ/GrpE and ClpB. The role of this chaperone machinery has been thoroughly investigated, however it is very little known about their function under anaerobic conditions, despite the fact that the gastrointestinal tracts are a natural anoxic environment for *E. coli*. We have focused our interest on the role of IbpA/B in protection of alcohol dehydrogenase (AdhE). The AdhE is a Fe^{2+} dependent protein with three enzymatic activities: alcohol dehydrogenase, acetaaldehyde –CoA dehydrogenase and pyruvate formate lyase deactivase. AdhE converts acetyl-coenzym A to acetaldehyde and then to ethanol in reactions coupled to oxidation of two NADH molecules. The *adhE* gene is very highly expressed under anaerobic conditions. We demonstrated that IbpA/B in cooperation with DnaK system prevented heat - inactivation and aggregation of AdhE under anaerobic conditions, both *in vitro* and *in vivo*. ClpB inhibited aggregation of cellular proteins in anaerobically growing *E. coli* submitted to heat

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

stress, but was not required for the AdhE protection.

2G_05_P

STARVATION INDUCED PROTEIN FROM *MYCOBACTERIUM SMEGMATIS*

Saraswathi Ramachandran, Dipankar Chatterji

MBU, IISc, Bangalore 560012, India
rs@mbu.iisc.ernet.in

Stationary phase is characterized by scarcity of nutrients, which is essentially the condition in which wild-type bacteria are considered to be found in nature.
A remarkable feature of bacteria is their ability to remain viable during prolonged periods of starvation and their capacity for rapid resumption of growth when nutrients become available again.
Bacteria have evolved sophisticated mechanisms towards this end, some of the dramatic examples being spore-formation and multicellular organization in certain bacteria. In contrast, many other bacteria, among them *E.coli* and *Mycobacterium sp.,* respond by entering a stress-induced program of lowered metabolic activity and increased resistance to various kinds of environmental assaults.
The transition from exponential growth to stationary phase conditions is accompanied by physiological and morphological changes along with changes in gene-expression patterns, with a generalized shutdown of protein synthesis and up-regulation of genes with important functions during the stationary phase.
The Dps (DNA binding Protein from Starved cells) protein binds to DNA without an apparent sequence-specificity and is considered to protect DNA by direct binding, as well as by its ferroxidation activity that protects the cell from Fenton-reaction mediated damage.
Upon entry into carbon-limited stationary phase, *M.smegmatis* also undergoes physiological changes. In order to study the stationary phase response in more detail, we decided to focus on the *M.smegmatis* Dps (DNA-Binding Protein from Starved Cells) that was identified by previous work in the lab as a protein whose expression was up regulated under conditions of carbon deprivation.
The aim of the present study is to investigate the *in vivo* function of Dps in *Mycobacterium smegmatis* as well as its structural features.

2G_06_P

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

IMPLICATION OF STRESS IN THE LOSS OF VIRULENCE FACTORS IN UROPATHOGENIC ESCHERICHIA COLI

S.M. Soto, J. Vila

Microbiology Department, Hospital Clinic of Barcelona. Villarroel 170. 08036. Barcelona, Spain. E-mail: sarasotog@yahoo.es

Escherichia coli is by far the most common cause of urinary tract infections (UTI). Uropathogenic *E. coli* (UPEC) strains possess several virulence determinants that allow them to colonize the urinary tract, avoid host defenses, and cause damage to the uroepithelium, which may, in some cases, lead to passage of the bacterium into the blood-stream. Several genes encoding urovirulence factors, such as hemolysin, cytotoxic necrotizing factor type 1 (*cnf1*), P-pili F13 (*pap*), S-family adhesins, iron systems, some capsule factors and the autotransporter toxin sat1 are located in the chromosome and/or plasmids forming clusters named pathogenicity islands (PAIs). Several studies have demonstrated that quinolone resistant *E. coli* strains have fewer virulent factors than quinolone susceptible strains. Thus, the aim of this work was to study the possible relationship between bacterial stress produced by quinolones and the loss of virulence factors located in PAIs in UPEC. Three UPEC quinolone-susceptible and hemolytic clinical strains were submitted to subinhibitory concentrations of ciprofloxacin. A sample of the well showing growth at the highest quinolone concentration was spread onto large blood Columbia agar plates. The nonhemolytic colonies were analyzed to determine the loss of the hemolysin gene (*hly*) and other factors related to PAIs. The three strains lost hemolytic capacity between passages 1-4 in the presence of ciprofloxacin. The loss rate was between $1x10^{-4}$ and $5x10^{-3}$. No colonies without hemolytic capacity were found after 15 passages of wild-type strains using antimicrobial-free culture medium. In conclusion, these findings suggest that quinolones produce bacterial stress the response of which is a loss of virulence factors.

2G_07_P

OSMOTIC STRESS AND OTHER STRESSORS AS INDUCERS OF MULTIDRUG RESISTANCE

María M. Tavío

University of Las Palmas de Gran Canaria. Faculty of Health Sciences. Las Palmas G.C.- Spain, mtavio@dcc.ulpgc.es

In order to survive under and adapt to different conditions bacteria have systems that are able to sense and respond to environmental stimuli. A

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

complex network of regulatory systems ensures a coordinated and effective answer to different stresses that can act on a bacterium simultaneously. Hyperosmolarity and some chemicals as fluoroquinolones, salicylate, non-antimicrobial medicaments as diazepam, anti-inflammatory drugs, among others, can induce an increased active efflux and organic solvent tolerance, loss of porins and multidrug resistance, both in wild type strains and clinical isolates of enterobacteria. Besides the role of efflux systems in multidrug resistance phenotypes, they seem to have a natural function exporting signals for cell–cell communication. AcrAB-TolC is an efflux system that exudes fluoroquinolones and is up-regulated by SdiA, a quorum-sensing transcriptional regulator. Another transcriptional regulators that are also involved in bacterial stress response such as *marA* or *soxS,* also activate AcrAB-TolC. Sigma factors, and the two-component systems CpxAR and BaeSR are also key pieces in the regulation of gene expression in response to stress conditions. Thus, the response regulator CpxR can lead to an increase of the mRNA level of several drug exporter genes and the mutation level as well as to a diminished OmpF assembly. From this point of view the development of intrinsic multidrug resistance might be understood as part of the bacterial response to stress. The *in vitro* induction of multidrug resistance has been associated with high levels of inducers, those that are close to their minimal inhibitory concentrations. Therefore, bacterial response to osmotic stress might be linked to multidrug resistance phenotypes.

2G_08_P

FUNCTION OF DNAK IN STREPTOCOCCUS INTERMEDIUS

Toshifumi Tomoyasu, Atsushi Tabata and Hideaki Nagamune

Institute of Technology and Science, The University of Tokushima Graduate School. Japan
e-mail: tomoyasu@bio.tokushima-u.ac.jp

Streptococcus intermedius is an anaerobe and belongs to the anginosus group of streptococci (AGS), which constitute a part of the normal flora of the human oral cavity as well as the upper respiratory, gastrointestional, and female urogenital tracts. AGS are recognized as opportunistic pathogens that cause purulent infections and abscess formation. We have previously reported that DnaK chaperone controls the expression of flagella and several pathogenic factors in *Salmonella* Typhimurium. Therefore, we constructed a d*naK* null mutant from *S. intermedius* in order to investigate the possible role of DnaK in pathogenicity. The generation time of a *dnaK* null mutant from *S. intermedius* was approximately twice that of the parent strain. Similar to other gram-negative bacteria, the *dnaK* null mutant exhibited a thermosensitive

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

phenotype and could not grow above 40°C. However, the *dnaK* null mutant did not show acid and H_2O_2 sensitivity, which is characteristic of gram-negative bacteria. Interestingly, GroEL accumulation was observed in the *dnaK* null mutant. The genome sequences from AGS revealed that the heat shock response, including expression of the *groESL* operon, appears to be controlled by the HrcA heat shock gene transcriptional repressor. Our result suggests that DnaK might regulate the activity or cellular amount of HrcA. Neither the *dnaK* mutant nor the parent strain showed a significant difference with regard to the activity of cytolysin (Intermedilysin) and hyaluronidase, and in the ability to form biofilms. These data indicate that DnaK in *S. intermedius* plays a role in the fundamental functions for living (e.g., growth, thermoresistance, and heat shock regulation) but has less functionality in the modulation of expression of pathogenic factors.

2H_01_P

STRESS RESPONSES IN *PROCHLOROCOCCUS*: OXIDATIVE PROTEOLYSIS OF GLUTAMINE SYNTHETASE AND DIFFERENTIAL EXPRESSION OF *CLPP1*, *FTSH1* AND *LON* GENES

Gómez-Baena, G.[1], López-Lozano, A.[1], Rangel, O.A.[1], Donaldson, R.P. [2], García-Fernández, J.M. [1] and Díez, J. [1].

[1]*Departamento de Bioquímica y Biología Molecular. Universidad de Córdoba. Campus Rabanales. Edificio Severo Ochoa. 14071-Córdoba (Spain) v52gobag@uco.es*
[2]*Department of Biological Sciences. George Washington University. 2023 G Street, NW, 20052, Washington, D.C. (USA) robdon@gwu.edu*

Prochlorococcus is a marine cyanobacterium responsible for a significant part of the global primary production as well as one of the most abundant organisms on Earth. Hence, the knowledge of nutrient assimilation in *Prochlorococcus* could provide important information about the physiology of this amazing organism and its ability to cope with limitation of nutrients and light in the oceanic environment. The main limiting nutrient in the ocean is nitrogen, and glutamine synthetase (GS) catalyzes its incorporation into carbon backbones, connecting the nitrogen and carbon metabolism. Our work is focused on the regulation of this important enzyme under stress conditions. Our results show that the degradation of GS is regulated by metal-catalyzed oxidation in *Prochlorococcus*. The proteolysis OF GS IS mediated by proteases that recognize the oxidized form of the enzyme. Starvation for essential nutrients (such a nitrogen and iron) and changes in the redox and energetic state of the cell seem to promote the oxidative modification and degradation of GS. On the other hand, we have measured the expression of the *clpP1*, *ftsH1* and *lon*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

genes, encoding for three proteases, under different stress conditions, finding that ClpP and La proteases seem to play an important role in the response to stress conditions in *Prochlorococcus*.

2H_02_P

HYDROGEN PEROXIDE ENZYMATIC DETOXIFYCATION IN SWINE FOLLICULAR FLUID: FOLLICULAR SIZE-DEPENDENT CHANGES.

Giuseppina Basini, Simona Bussolanti, Sujen Eleonora Santini, Francesca Grasselli

Dipartimento di Produzioni Animali, Biotecnologie Veterinarie, Qualità e Sicurezza degli Alimenti, Sezione di Fisiologia Veterinaria, Università di Parma, 43100 Parma, Italy. E-mail: basini@unipr.it

Follicular fluid (FF) is produced during folliculogenesis, which begins in the ovarian cortex with the recruitment of primordial follicles and ends in ovulation or atresia. FF may be regarded as a biological "window" reflecting metabolic and hormonal processes occurring in the microenvironment of the maturing oocytes before ovulation and also a predictor of the successful fertilization. Therefore, the knowledge of its composition deserves special attention. Oxidative metabolism is essential for energy production and is unavoidably associated with the generation of reactive oxygen species (ROS). The objective of this study was therefore to evaluate, in FF collected from small (<3mm), medium (3-5 mm) and large (>5 mm) swine follicles, hydrogen peroxide (H_2O_2) concentration and the activity of the enzymes involved in H_2O_2 removal, glutathione peroxidase (GSH-Px) and catalase (CAT). Our data evidenced that H_2O_2 levels significantly ($p<0.05$) decresed during follicle development: this is possibly due to a progressively increased ($p<0.05$) CAT activity, since GSH-Px activity resulted unmodified. The reduction of H_2O_2 concentration during the growth of the swine follicles is possibly of physiological impact: at very low concentrations, ROS are important second messengers capable of modulating the expression of genes that govern physiological processes, while an increase in their concentration can result in oxidative stress causing damage to molecules and structures with deleterious effect on oocyte function.

This work was supported by FIL and MIUR-COFIN grants

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2H_03_P

PROTECTIVE ROLE OF NEW NITROGEN COMPOUNDS ON ROS/RNS-MEDIATED DAMAGE TO PC12 CELLS

JP Silva[1], FM Areias[2] and OP Coutinho[1]

[1]Centre of Biology, Department of Biology and [2]Department of Chemistry, University of Minho, 4710-057 Braga, Portugal
Email: jpsilva@bio.uminho.pt

Biological systems are frequently exposed to excessive reactive oxygen (ROS) and nitrogen (RNS) species, causing a disturbance in the cells natural antioxidant defence systems, and resulting in damage to all biomolecules, including nucleic acids. So, there has been a growing interest in developing substances that can act against excessive intracellular free radicals. Here, we studied the protective effects of two new nitrogen compounds, from organic synthesis, FMA762 and FMA796, on ROS/RNS-mediated cell damage. RNS were generated in PC12 cells by the NO donor sodium nitroprusside (SNP). Both FMA762 (37μM) and FMA796 (34μM) effectively decreased SNP-induced cell death (MTT test). This decrease was shown to be mostly due to their RNS scavenging ability, assayed by flow cytometry with the probe DCF. Despite being nitrogenated, the compounds do not work as substrates for NO synthase and do not lead, *per se*, to an extra production of NO. In addition, they didn't have any effect on NO synthase, as they didn't reduce the amount of nitrites produced by SNP (measured by the Griess reaction). On the other hand, the compounds protected cells against *t*-BHP-induced DNA damage, as assessed by the Comet assay. When added 3h prior to *t*-BHP addition, this protection is similar to the one produced by quercetin. Besides, a slight effect in DNA repair also seems to occur. Currently, we are assessing the compounds protection in specific DNA lesions, namely at the endogenous base oxidation level. Altogether, these data suggest a good antioxidant potential for the new compounds, with specific effects at the oxidative DNA damage level.

JPS and FMA are supported by the FCT grants SFRH/BD/17174/2004 and SFRH/BD/3185/2000.

2H_04_P

ISOTHIOCYANATES ENHANCE CYTOTOXIC EFFECT OF PEROXYNITRITE ON PMNS: A CORRELATION WITH NITROSATIVE STRESS BIOMARKER

N. Trakranrungsie[1], P. Yatmark[1], Y. Maneerat[2], K. Kirtikara[3]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[1]*Faculty of Veterinary Science, Mahidol University, Thailand*
[2]*Faculty of Tropical Medicine, Mahidol University, Thailand*
[3]*National Center for Genetic Engineering and Biotechnology, Thailand*

Email: vsntk@mahidol.ac.th

Isothiocyanates (ITCs), a class of phytochemicals available from many cruciferous vegetables, have been reported to have a dichotomous modulating effect on oxidative stress. In addition, it has been suggested that ITCs may display a more cytotoxicity to tumor cells than normal cells. Although the effects of ITCs are intensively studied in cancer cell lines, the experiments on normal cells appear limited. The present study therefore investigated the modulatory role of allyl and benzyl isothiocyanates (AITC and BITC, respectively) on peroxynitrite-induced cytotoxic effect in polymorphonuclear neutrophils (PMNs) and simultaneously evaluated the formation of 8-isoprostane and nitrotyrosine as biomarkers of oxidative and nitrosative stress. 3-Morpholinsydnonimine (SIN-1), a peroxynitrite donor, reduced PMNs viability but increased 8-isoprostane and nitrotyrosine levels in a concentration-dependent manner. AITC or BITC alone at concentration ≤ 3 μM did not cause the cytotoxic effect as compared to untreated control cells. On the contrary, pretreatment with AITC or BITC (.003, .03, .3, 3 μM) further enhanced cytotoxicity in SIN-1-treated cells. It was also observed that the increased formation of nitrotyrosine, but not 8-isoprostane, was depended on AITC or BITC concentration and correlated with the loss of PMNs viability. The results suggest that these ITCs could exacerbate the cytotoxic effect of peroxynitrite in isolated milk PMNs, in part, by enhancing nitrosative stress.

2H_05_P

HSP70 AND HSP32 (HO-1) ARE ESSENTIAL FOR THE FOLATE INDUCED PROTECTION AGAINST HOMOCYSTEINE INDUCED DAMAGE IN ENDOTHELIAL CELLS.

Catherine S. Birch & John H. H. Williams

Chester Centre for Stress Research, University of Chester, Chester, CH1 4BJ UK.
john.williams@chester.ac.uk

Homocysteine (Hcy) is an independent risk factor for vascular disease and Alzheimer's disease. Hcy promotes its harmful effect by promoting the generation of reactive oxygen species (ROS) within the vasculature leading to oxidative damage and initiating inflammatory responses which

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

ultimately lead to endothelial dysfunction. Folate treatment is an established therapeutic strategy to reduce Hcy in patients suffering with vascular disorders. Endothelial cells counteract the harmful effect of inflammation by upregulating a set of protective genes including the heat shock proteins (Hsps). Hsps are a highly conserved group of inducible stress proteins involved in the protection and conservation of protein synthesis. Hsp70 is upregulated when cells are subjected to stressful stimuli, Hsp32 (HO-1) is highly inducible when faced with oxidative stress. Data from qPCR show that Hsp70 and Hsp32 (HO-1) are upregulated in a dose-dependent manner when cells are exposed to Hcy. MTS viability assays indicate that folate (5-MTHF) protects cells from Hcy-induced damage. Inhibition of Hsp70 (quercetin) and Hsp32 (Sn(IX) protoporphyrin) reduce the protective effect of 5-MTHF. RNAi targeted to Hsp70 and Hsp32 (HO-1) support this data. Combined treatment (quercetin plus Sn(IX) protoporphyrin) totally inhibit the protective effect of 5-MTHF. Combined treatment with siRNA for Hsp70 and Hsp32 also totally prevent 5-MTHF protection.
The data support a role for Hsp32 and Hsp70 in the protection against Hcy induced cell death.

2H_06_P

THE BENEFICIAL EFFECTS OF LIPOIC ACID ON PROTEIN OXIDATION AND TOTAL ANTIOXIDANT CAPACITY IN EXPERIMENTAL ADRIAMYCIN (DOXORUBICIN) INDUCED OXIDATIVE CARDIOTOXICITY AND NEPHROTOXICITY

Pinar Atukeren[1], Zeynep Ozturk[1], Ezel Uslu[1], Ibrahim Bayrak[2], M.Koray Gumustas[1], Merve Aldikactioglu[3], Selin Sert[3], Murat Yassa[3], Tuncay Altug[2]

[1]Department of Biochemistry, [2]Animal Production and Research Laboratory, [3]Cerrahpasa Medical School, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey.

E-mail: p_atukeren@yahoo.com

Doxorubicin (DOX) is a widely used anticancer agent whose clinical use is limited on account of its toxicity which have been associated with reactive oxygen species (ROS).Lipoic acid (LA) has cytoprotective potential which has previously been explained by its antioxidant properties distinguishing from other antioxidants:LA neutralizes free radicals in both the fatty and watery regions of cells,and functions in both its reduced and oxidized forms.The present study aimed to investigate the protective efficacy of LA on DOX induced oxidative damage in heart and kidney tissues.40 rats were divided into five groups:1st;control, 2nd;only DOX injected, 3rd;only LA administered (50mg/kg/day,20 days), 4th;LA administered for 20 days+DOX injected, 5th; single dose LA (50mg/kg) injected same time

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

with DOX injection.DOX was injected to the rats (4mg/kg) 48 hours before sacrifization.In heart and kidney tissues; protein oxidation was assesed by the levels of protein carbonyls (PC),also thiobarbituric acid reacting substances (TBARS) were determined and antioxidant status of tissues were determined by evaluating total antioxidant capacity (TAC) and superoxide dismutase (SOD) activity.PC and TBARS levels were elevated in both tissues, in DOX injected groups when compared with the control and LA pretreatment restored TAC levels and SOD activity.The study has highlighted the beneficial effects of lipoic acid pretreatment in reversing the damages caused by doxorubicin.

2H_07_P

OXIDATIVE STRESS DURING PERIPARTUM PERIOD IN MARES: FIELD STUDY

F.Righi, SE.Santini, E. Gervasini, S.Bussolati, G.Basini, A. Quarantelli

Dipartimento di Produzioni Animali, Biotecnologie Veterinarie, Qualità e Sicurezza degli Alimenti. Università di Parma. 43100 Parma, Italy. E-mail: federico.righi@unipr.it.

Oxidative stress has been shown to occur in dairy cattle around calving, but there is no current data on similar phenomenon in periparturient mares. In a previous study we found a decrease of Ferric Reducing Ability (FRAP) in mares' sera around parturition, suggesting non-enzymatic antioxidant molecule consumption during this period. Since this mechanism could lead to redox imbalance, the aim of the present study was to investigate the possible insurgence of oxidative stress in mares around foaling by analyzing the same samples. Levels of Reactive Oxygen Molecules (ROMs) were measured by colorimetric method on blood samples collected from 17 Thoroughbred mares at days -24 (T1), +1 (T2) and +9 (T3) relative to parturition. No significant variation was detected in ROMs concentration at the intervals considered. These findings seem to exclude the occurrence of oxidative stress during the peripartum period in mares. Further research is needed to better define the mechanisms involved in oxidative status regulation around foaling.

2H_08_P

BRAIN OXIDATIVE STRESS IN HYPERTENSIVE RATS

Garrido M. R., Csibi A., Pastorello M., Silva J. A., Ciangherotti C. and Israel A.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

School of Pharmacy, Laboratory of Neuropeptides, Universidad Central de Venezuela, Caracas, Venezuela.
E-mail: mrgarrido11@hotmail.com

Chronic exposure to stress alters prooxidant-antioxidant balance. Genetic hypertension (SHR) is a model of chronic stress which is known to be accompanied with increased oxidative stress and decreased antioxidant enzyme activity. Angiotensin II (ANG) influences blood pressure via its ability to stimulate the NAD(P)H-oxidase and produce reactive oxygen species (ROS) such as superoxide ($0_2^{\cdot -}$), which is sequentially metabolized by the enzymes superoxide dismutase and catalase. In turn, ROS can activate the mitogen-activated protein kinase (MAPK) pathway that is associated with growth and cellular differentiation. Several indirect marker of oxidative stress are increased in the brains of SHR compared with those of Wistar-Kyoto (WKY) rats. There is no direct evidence, however, of changes in oxidative stress and MAPK in the brain in hypertension. Thus, we investigates the status of oxidative stress in hypothalamus (HYP) and subfornical organ (SFO) of WKY and SHR rats, and the involvement of ANG II, by measuring the enzymatic activity of catalase (CAT) and the expression and activation of MAPKs. Male rats, 200-220g, normotensive WKY or SHR were sacrificed and tissues microdissected under stereomicroscopic control. CAT activity was determined spectrophotometrically and ERKs activation by Western blot analysis. In SHR brain structures such as HYP and SFO, CAT activity was decreased when compared with WKY. Likewise, the expression of MAPKs and its activation was diminished in the SHR rats. In both structures ANG II (10^{-7}M) decreased CAT activity. Our results indicate an alteration of oxidative stress and MAPK activation in the brain of hypertensive rats (Grant CDCH PG 06-30-5222-2003).

2H_09_P

ADRENOMEDULLIN, NITRIC OXIDE AND SUPEROXIDE DISMUTASE IN EXPERIMENTAL STRESS ULCER FORMATION

Yesim Temiz[1], Hakan Ekmekci[2], Ezel Uslu[1], Tuncay Altug[3], M.Koray Gumustas[1]

[1]Department of Biochemistry, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey.
[2]Department of Pediatric Hematology/Oncology, Bone Marrow Transplantation Unit, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey
[3]Animal Production and Research Laboratory, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

E-mail: profgumustas@yahoo.com

Stress ulcer is a common cause of gastrointestinal bleeding and a reduction in gastric mucosal blood flow and oxygen derived free oxygen radicals play important role in stress ulcer pathogenesis. Adrenomedullin (ADM) is a vasodilator peptide hormone first discovered in human pheochromocytoma cells. Together with nitric oxide (NO) and endothelin, it is one of the secretuar product of endothel and its vasodilator effect is supposed to depend on NO and effective of protecting the gastric mucosa. Calcium dobesilate (Doxium©) is an angioprotective agent widely used for the treatment of diabetic retinopathy, vascular diseases and chronic venous insufficiency. In our study, we investigated the ADM and NO levels in experimentally induced stress model, in rats. To examine the relation with oxidative stress, we sought TBARS levels and SOD activity together with the effect of calcium dobesilate as well. In ulcerative group; plasma ADM and NO, and in gastric mucosa NO levels were significantly increased ($p< 0,001$). Elevated TBARS levels ($p< 0,01$) and decreased SOD activity ($p<0,001$) were found in gastric mucosa. On the other hand before inducing ulcer, pretreated with calcium dobesilate by tube feeding animals showed lower plasma ADM and elevated NO levels compared to ulcerative animals. No difference were found in comparison between two groups for gastric mucosa NO levels. But TBARS levels were lower in calcium dobesilate pretreated group's gastric mucosa ($p<0,001$). Our results suggests that, in stress ulcer, elevated levels of ADM and NO, may generated to protect organism to a response of increased oxidative stress and decreased gastric mucosal blood flow. Although these results supports that calcium dobesilate has an antioxidant and angioprotective properties, probably related to NO, it is needed to work for further on this molecule.

2H_10_P

ROLE OF OXIDATIVE STRESS IN THE NATRIURESIS INDUCED BY CENTRAL ADMINISTRATION OF ANGIOTENSIN II

Israel A, Arzola J, Varela M, De Jesús S and Toro Y.

Unidad de Neuropéptidos, School of Pharmacy, Universidad Central de Venezuela. E-mail: astern88@hotmail.com

The brain renin angiotensin system has an important role in the regulation of arterial blood pressure and fluid and electrolyte metabolism. Abundant evidence now suggest that a key mechanism by which central angiotensin II (ANG) influences blood pressure it's via its ability to produce reactive oxygen species (ROS) such as superoxide ($0_2^{\cdot -}$). Cerebroventricular (IVT) ANG administration to conscious rats produces antidiuresis and natriuresis, through stimulation of AT_1 receptor. The role of ROS in these

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

actions is unknown. Thus, we assessed the involvement of brain NAD(P)H-oxidase and ROS by the use of the selective NAD(P)H-oxidase inhibitor, Apocynin (APO) and the superoxide dismutase mimetic, Tempol (TEM) administered IVT. Male Sprague-Dawley rats (220 g) were IVT-cannulated and subjected to the following IVT-treatments: saline (5 μl), APO (33 μg/kg/5μl); TEM (50 μmol/10μl). After 30 minutes of each treatment, animals received an IVT-injection of either ANG (50 pmol/5μl) or saline (5 or 10 μl). Animals were water loaded (20 ml/kg, p.o.) and placed into metabolic cages. Urine was collected at 1, 3 and 6 hr. Urinary Na^+ and K^+ was determined by flame photometry. ANG-IVT reduced urine volume at 1 hr and increased sodium and potassium excretion at 1, 3 y 6 hrs. Central administration of APO or TEMP significantly reduced ANG-induced natriuretic effect ($P<0.01$), while did not alter ANG-induced antidiuresis. Our results suggest a role of brain NAD(P)H-oxidase and ROS in the natriuretic action evoked by IVT-ANG (Supported by Grants from CDCH-PI-06-00-6212-2006 and IIF-10-2005).

2H_11_P

PRODUCTS OF OXIDATION AS MEASURABLE INDICATORS OF OXIDATIVE STRESS: VALIDATION OF BIOMARKERS

Maria Kadiiska

National Institute of Environmental Health Sciences/National Institutes of Health, Research Triangle Park, North Carolina, USA.

Oxidation products of lipids, proteins and DNA in plasma and urine of experimental animal model were measured as part of a comprehensive, multilaboratory validation study searching for non-invasive biomarkers of oxidative stress in experimental models, human disease and health. The goal of the study was to find the most sensitive, selective and specific markers of oxidative stress that are applicable to different oxidative insults and stored specimens. Investigators from 24 laboratories worldwide are participating in this study. The focus of this presentation will be on the findings from measurement of oxidative stress in experimental animal models of CCl_4 poisoning and ozone exposure. The time and dose-dependent effects of CCl_4 and ozone exposure on concentrations of lipid hydroperoxides, TBARS, malondialdehyde (MDA) and isoprostanes were investigated with different old and new techniques. In addition, measures of oxidation products of proteins (protein carbonyls, methionine sulfoxidation, tyrosine oxidation products) and DNA (strand breaks, 8-OHdG, M_1G) were carried out as well. The pattern of oxidative stress biomarkers seen in these two exposures will offer insight into the specificity and sensitivity of the markers and will provide evidence that a

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

given product of oxidation may be a marker for some type of oxidative stress but not others.

2H_12_P

ALTERNATIVE OXIDASE FROM THERMOGENIC SKUNK CABBAGE (*SYMPLOCARPUS FOETIDUS*) CONFERS CYANIDE-RESISTANT RESPIRATION TO HUMAN CELLS

Kazushige Matsukawa & Kikukatsu Ito

Cryobiosystem Research Center, Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan, email of corresponding author: kikuito@iwate-u.ac.jp

Alternative oxidase (AOX) plays a pivotal role in cyanide-resistance respiration in the mitochondria of plants, fungi and some animals, which acts as second terminal oxidase bypassing directly electrons of ubiquinol. Here we show that AOX from the thermogenic skunk cabbage, *Symplocarpus foetidus*, successfully conferred cyanide resistant respiration to human cells. To address allotropic expression of skunk cabbage AOX in human HeLa cells, we constructed chimera AOX, which has mitochondrial targeting signature replaced by human cytochrome c subunits VIII. An anti-AOA antibody raised against AOX confirmed mitochondrially-targeted AOX protein. Analysis of O_2 consumption revealed that cyanide insensitive respiration was conferred to AOX-expressing mitochondria, but not mitochondria isolated from control and E217A mutant-expressing cells, whose mutant possesses a failure of active di-iron center. These results suggested that AOX functions as ubiquinol oxidase and confers alternative cyanide-resistant respiration in human cell mitochondria. Flow cytometry analysis demonstrated that AOX-expressed cells were found to have significantly increased mitochondrial membrane potentials and less reactive oxygen species in response to antimycin exposure, a specific inhibitor of respiratory complex III. It is suggested that AOX alleviates some detrimental effects on respiratory chain by antimycin. Our results suggest that *Symplocarpus* AOX is involved in an alternative respiration pathway in humans and thus provides a valuable tool for investigating not only plant AOX in non-plant system, but the dysfunctions in cytochrome respiration that leads to various mitochondrial diseases.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2H_13_P

SOCIAL STRESS INCREASES URINARY EXCRETION OF BIOPYRRINS, OXIDATIVE METABOLITES OF BILIRUBIN, IN MICE

Keiko Motoyama[1], Tomoya Miyashita[1], Tokio Yamaguchi[2], Satoru Hirabayashi[1], Hirotaka Naito[1], Norio Ohashi[1], Keiko Unno[3], Hiroyuki Sakakibara[1], Kayoko Shimoi[1,*]

[1]Graduate School of Nutritional and Environmental Sciences, and [3]Graduate School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan, [2]Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan
**Correspondence: shimoi@smail.u-shizuoka-ken.ac.jp (K. Shimoi)*

In this study, we investigated whether or not urinary excretion of biopyrrins, oxidative metabolites of bilirubin, in mice could be used as a biomarker for psychosocial stress. Male BALB/c mice (4 weeks old) were housed 5 per cage for 10 days. After acclimatization, mice were exposed to two kinds of psychosocial stress; isolation (1 mouse per cage) and confrontation (2 mice per partitioned cage for 10 days and then partition was removed). Mouse blood, urine, and liver were collected after 7 and 30 days of stress. Serum levels of corticosterone and urinary levels of biopyrrins were determined by EIA and ELISA, respectively. An expression of heme oxygenase-1 (HO-1), which is known to be induced by oxidative stress, was analyzed using the methods of RT-PCR and western blot in the mouse liver. Adrenal hypertrophy and significant increases in serum concentration of corticosterone were observed in mice exposed to these types of social stress for 7 and 30 days. Both of the social stress for 7 days markedly induced the expression of HO-1 in the liver, and significantly increased the urinary excretion of biopyrrins. These phenomenons decreased after 30 days, although they were still rather high compared to the control group. These results suggested that social stress causes oxidative stress and that biopyrrins could be useful biomarkers of psychosocial stress.

2H_14_P

BIOCHEMICAL EVALUATION OF THE ROS AND SIALIC ACID LEVELS IN CARDIAC AND RENAL TISSUES OF ADRIAMYCIN-ADMINISTRATED RATS AND THE POSSIBLE ROLE OF LIPOIC ACID TREATMENT.

Zeynep Ozturk, Pınar Atukeren, Ezel Uslu, İbrahim Bayrak, Hüseyin

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Sönmez, Özlem Armay, Merve Aldıkaçtıoğlu, Murat Yassa, Tuncay Altuğ
E-mail: zozturk72@hotmail.com

Department of Biochemistry, Cerrahpasa Medical Faculty, University of Istanbul
Experimental Animals Breeding and Research Laboratory, Cerrahpasa Medical Faculty, University of İstanbul.

Adriamycin (doxorubicin) is an anthracycline antibiotic that has been used for more than 30 years for the treatment of a wide variety of cancers. It is widely accepted that oxidative stress and the production of free radicals are involved in doxorubicin action, both in terms of antitumor effects and cardiotoxicity. Sialic acid; plays an important role in cellular functions and also influences conformation of glycoproteins which is found on the cell membrane.Free radicals are potentially dangerous products of cellular metabolism. Excessive amounts of reactive oxygen species (ROS) can start some lethal chain reactions such as ROS that induces lipid peroxidation. Alpha-lipoic acid is a powerful antioxidant. In this study we investigated the ROS and sialic acid levels in cardiac and renal tissues of doxorubucin- administrated rats and the possible role of lipoic acid.40 Wistar albino rats were divided into five groups: control, ADR group (4mg/kg intraperitoneal), Acute -lipoic acid and ADR (single dose 50mg/kg lipoic acid,4mg/kg ADR intraperitoneal), only lipoic acid (50mg/kg,intraperitoneal), the last group lipoic acid (50mg/kg, intraperitoneal) and ADR(4mg/kg daily intraperitoneal). Sialic acid and TBARS levels were elevated in ADR, LA, LA+DOX groups as compared with the control group in the cardiac tissue .In renal tissue, sialic acid levels declined, in LA and acute LA+ADR, whereas TBARS levels were elevated in ADR and LA+ADR group.

2H_15_P

ACTIVATION OF CELLULAR ANTIOXIDANT DEFENCE SYSTEMS AFTER EXOGENOUS HEAT STRESS IN MEN

Pokora Ilona[1], Kempa Katarzyna[2], Kimsa Elżbieta[2], Pokora Zbigniew[3]

[1]*Department of Physiology, Academy of Physical Education, Katowice, Poland i.pokora@awf.katowice.pl*
[2]*Department of Biochemistry, Academy of Physical Education, Katowice, Poland*
[3]*Department of General and Molecular Biology, and Genetics, Silesian Medical University, Katowice, Poland*

The aim of our study was to examine changes in the blood and cellular antioxidant defense system in response to exogenous heat stress (induced

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

by sauna bath) in young men. At rest prior and after sauna bath tympanic (T_{ty}) and skin temperatures (T_{sk}) were measured and body heat storage (S) was calculated. Before sauna bath, then 60 min, 120 min and 24h post sauna, capillary blood samples were drawn for determinations of: hemoglobin content (HB), hematocrit (HCT), glucose (GLU), blood carbon dioxide (pCO_2), pH and blood oxygen (pO_2), respectively. The changes in blood (%BV), plasma (%PV) and cell (%CV) volumes were calculated. Additionally, venous blood samples were withdrawn to determine plasma: cortisol level, activity of creatine kinase (CK-NAC). Blood antioxidant potential was assessed by determination of antioxidant enzyme activities: glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR). Exogenous heat stress induced an increase in Tty (Δ+2.1^{o}C) and plasma cortisol level, decreased cell (-.05%), blood (-2.97%) and plasma (-4.36%) volumes. Cell volume started to increase after 2h recovery. CK-NAC activity was increased provided evidence slight increase in muscle cell membranes permeability. There was a decrease of activity of GSH-Px and CAT as well as an increase in activities of SOD and GR immediately post sauna. The observations in this study imply that exogenous heat stress was associated with activation of cellular and blood antioxidant defense system.

2H_16_P

PROTECTIVE EFFECTS OF LIPID OXIDATION PRODUCTS AGAINST NEURONAL CELL DEATH INDUCED BY GLUTAMATE AND 6-HYDROXYDOPAMINE: INDUCTION OF ADAPTIVE RESPONSE AND ENHANCEMENT OF CELL TOLERANCE

Yoshiro Saito, Zhi-Hua Chen, Yasukazu Yoshida, Etsuo Niki

Human Stress Signal Res Ctr, Nat Inst Adv Ind Sci Tech (AIST), Ikeda Osaka JAPAN, yoshiro-saito@aist.go.jp

There is increasing evidence to suggest that reactive oxygen species, including a variety of lipid peroxidation products, can induce an adaptive response and enhance cell tolerance; however, the details of the underlying molecular mechanisms have not been clarified. In the present study using both PC12 cells and cultured cortical neurons, we investigated the effect of lipid oxidation products such as 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), 4-hydroxy-2-nonenal (4-HNE) and 7-hydroxycholesterol (7-OHCh) against the cell death induced by 6-hydroxydopamine (6-OHDA) and glutamate. Pretreatment with these lipid oxidation products at sublethal concentrations resulted in a significant protective effect against subsequent oxidative stress, and 15d-PGJ_2, in particular, exhibited a

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

complete protective effect against glutamate-induced neuronal cell death. Pretreatment with 15d-PGJ_2 increased the intracellular glutathione (GSH) as well as the gene expression of glutamate-cysteine ligase (GCL), the rate-limiting enzyme of GSH synthesis. 15d-PGJ_2 protected cells from glutamate-induced GSH depletion, while the inhibition of cellular GSH synthesis by buthionine sulfoximine abolished the adaptive response induced by 15d-PGJ_2. These findings indicate that at low levels, 15d-PGJ_2 acts as a potent survival mediator against glutamate-induced insults via the induction of an adaptive response primarily through the up-regulation of the intracellular GSH synthesis. Furthermore, we observed that the adaptive response induced by 4-HNE was mediated through the elevated thioredoxin reductase activity in an NF-E2-related factor 2 (Nrf2)-dependent manner, while 7-OHCh exhibited the protective effect via the increase of GSH contents in the Nrf2-independent manner. The possible physiological role of adaptive response induced by lipid oxidation products via different mechanism, will be discussed.

2H_17_P

SIMULTANEOUS EVALUATION OF OXIDATIVE AND NITROSATIVE STRESS BIOMARKERS IN PEROXYNITRITE-INDUCED CYTOTOXIC EFFECT ON POLYMORPHONUCLEAR NEUTROPHILS

N. Trakranrungsie[1], P. Yatmark[1], Y. Maneerat[2], K. Kirtikara[3]

[1]Faculty of Veterinary Science, Mahidol University, Thailand
[2]Faculty of Tropical Medicine, Mahidol University, Thailand
[3]National Center for Genetic Engineering and Biotechnology, Thailand
Email: vspym@mahidol.ac.th

Peroxynitrite is a strong oxidant and capable of oxidizing and nitrating several macromolecules. Peroxynitrite reaction can be reflected via the formation of oxidized and nitrated species such as isoprostanes and nitrotyrosine as observed in many animal and human conditions associated with oxidative/ nitrosative damage. Since the function of neutrophils as the first line of defence can be affected by oxidative/ nitrosative stress, the objective of this study was to evaluate the effect of peroxynitrite in milk-derived polymorphonuclear neutrophils (PMNs) and concomitantly monitor the formation of 8-isoprostane and nitrotyrosine as biomarkers of oxidative and nitrosative stress, respectively. 3-Morpholinsydnonimine (SIN-1), a peroxynitrite donor, reduced PMNs viability in a concentration-dependent manner with estimated EC_{50} value of 375 μM. Meanwhile, the increased levels of 8-isoprostane and nitrotyrosine were observed as concentration of SIN-1 increased. The peroxynitrite inhibitors, glutathione, melatonin and uric acid, markedly

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

and concentration dependently attenuated the cytotoxicity of SIN-1, in which a strong inverse relationship between 8-isoprostane and nitrotyrosine levels and cell survival was evident. The data imply that the cytotoxic effect of peroxynitrite on PMNs and the inhibitory action of known peroxynitrite inhibitors were mediated via both oxidative and nitrosative mechanisms.

2I_01_P

CARDIOPROTECTIVE EFFECTS OF POLYPHENOLS EXTRACTED FROM PROPOLIS AGAINST DOXORUBICIN TOXICITY

M. Alyane[1], L. Benguedouar[1] and M. Lahouel[1]

[1-] Laboratoire de Pharmacologie. Faculté des Sciences.
Université de Jijel, 18000, Algérie.
Email: alyanen@yahoo.fr

Propolis (bee glue) is one of the major hive products of bees and is rich in **flavonoids,** which are known for antioxidant activities. It is well known that the chemical properties of phenolic acids or flavonoids, in terms of the availability of the phenolic hydrogens as hydrogen donating radical scavengers, predict their antioxidant properties.
In this study, the flavonoids scavenging activity of propolis has been exploited to obtain protection against the peroxidative damage in rat heart mitochondria which was induced by the administration of an acute dose of doxorubicin (DXR 20mg kg^{-1}, *i.p*). The peroxidative lesions were evaluated biochemically and biophysically, 24^{H} after DXR administration.
Abnormal biochemical changes in heart mitochondria from DXR treated rats including a marked increase in both malondialdehyde (MDA) and anion superoxide production, decrease both of respiratory chain ratio (RCR= V3/V4) and P/O.
Biophysically, collapse of membrane potential of mitochondria, first of the two steps of mitochondrial death pathway, and swelling, responsible of fragmentation and massive cytochrome *c* release, were observed.
Pretreatment of rats with propolis extract, given *per os* (100mg/kg/day) during four days prior to DXR injection, substantially reduced the peroxidative damage in the heart mitochondria: we showed significant reducing both of mitochondrial MDA formation and production of superoxide anion, restoration of RCR and P/O, reducing of rate and the amplitude of mitochondrial swelling and finally restoration of the loss of the mitochondrial membrane potential. The data demonstrate that antioxidants from natural sources may be useful in the protection of cardiotoxicity in patients who receive doxorubicin.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2I_02_P

THE PREVENTIVE ROLE OF PROPOLIS EXTRACT AGAINST MITOCHONDRIAL STRESS INDUCED BY ANTINEOPLASIC AGENTS

Lamia BENGUEDOUAR1[1], Mohammed ALYANE[1] & Mesbah LAHOUEL[1].

1. Laboratoire de Pharmacologie, Faculté des Sciences. Université de Jijel. 18000 Jijel, Algérie
E-mail : benguedouar.lamia@caramail.com

Oxidative stress and mitochondrial dysfunction are associated with disease, toxic process and aging. Because ROS formation is responsible for this dysfunction, we evaluated in vivo and in vitro the prophylactic effect of polyphenols extracted from propolis against mitochondrial oxidative stress induced by two anticancer drugs in female wistar rat using liver and heart mitochondria isolated from rat. RCR, mitochondrial swelling, superoxide radical production, lipid peroxidation, SOD and catalase activities have been measured to assess the mechanisms of protection of propolis extract against doxorubicin and vinblastin toxicity. We find that doxorubicin and vinblastin altered heart and liver mitochondrial functions respectively as attested by the decrease in respiratory control value, the activation of the swelling and the overproduction of SO. Myocardial tissue from doxorubicin treated rats showed a marked increase in MDA production and depletion of reduced GSH contents and an inhibition of catalase and SOD activities. Similar results were also observed in liver tissue. Pretreatment of rats with propolis extract, given intraperitoneally during 4 days prior to doxorubicin and/or vinblastin injection, substantially reduced the peroxidative damage in the myocardium and liver and markedly restored the tissues catalase and SOD activities. The protective effects obtained by propolis, however, were complete and reach those of the control group. Propolis extract at a dosing level equivalent to 10-4M was useful to obtain protective effects. These results strongly suggest that propolis extract protects heart and liver tissues from oxidative stress by protecting the mitochondria.

2I_03_P

THE STUDY OF THE DAMAGE OF MITOCHONDRION INDUCED BY PRENATAL STRESS IN OFFSPRING HIPPOCAMPAL NEURONS

Liang Song[1], Jian-bin Zheng[3], Hui Li[2],Zhi-rong Suo[3], Qing Cai[1], Ning Jia[1], Zhuan-li Bai[1], Rui Chen[2], Qing-hong Li[2], Da-xin Cheng[2] , Zhong-liang Zhu[1,4]*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[1]*Department of Physiology and Pathophysiology, Xi'an Jiaotong University, School of Medicine, Key laboratory of Environment and Gene Related to Diseases (Xi'an Jiaotong University), Ministry of Education, Xi'an, Shaanxi 710061, P. R. China;*
[2]*Department of Pediatrics, Xi'an Jiaotong University, First Hospital, Xi'an, Shaanxi 710061, P. R. China;*
[3]*Institute of Analytical Science Northwest University, Xi'an, Shaanxi 710069, P. R. China;*
[4]*Northwest University College of Life Science, Xi'an, Shaanxi 710069, P. R. China;*

Objective In recent years, more investigator were attracted by the research about the damage of hippocampus induced by prenatal stress. In present study, inner mitochondrial membrane potential (IMMP) in hippocampal neuron and the content of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) in hippocampus of offspring rats were measured to make clear the changes of mitochondrial function and explore the mechanism of the damage of hippocampus induced by prenatal stress.
Methods In this experiment the offspring rats were divided into two groups, CON group and PNS group(those whose dams were exposed to restraint stress on days 14-20 of pregnancy). Offspring (1 month old) were anesthetized with ether and decapitated, and hippocampus were dissected and stored for later experiment: The mitochondrion of hippocampal neurons were labeled with specific fluorescent probe of Rhodamine 123 to measure IMMP by flow cytometry; The extracted and purified mitochondrial DNA of hippocampal neurons were used to measure 8-OH-dG, a typical biomarker of oxidative stress, by the method of high performance liquid chromatography-electrochemical detection (HPLC-ECD).
Results Prenatal stress significantly lowered the IMMP compared with CON group (68.437±10.680 *vs* 124.780±7.336 $P<0.001$). Moreover, IMMP of female offspring in PNS group was lower than that of male offspring in PNS group (61.897± 7.076 *vs* 74.797±9.910 $p<0.05$). The level of 8-OH-dG/10^5dG in PNS group was higher than that in CON group (2.716±0.166 *vs* 1.631±0.138 $p<0.001$). There was significant difference between the female and the male in PNS group alike, the level of 8-OH-dG of the female was higher than that of the male (2.822±0.145 *vs* 2.610±0.114 $p<0.05$).
Conclusion These data suggest that prenatal stress could affect the normal function of mitochondrion in hippocampus of offspring rats, and there exists gender-dependent difference. These probably were involved in the hippocampal neurons oxidative damage induced by prenatal stress.

2I_04_P

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

EVIDENCE OF NMN-AT ACTIVITY, WHICH ALLOWS FOR NAD+ SYNTHESIS FROM NMN AND ENDOGENOUS ATP IN MITOCHONDRIA ISOLATED FROM AGED-DEHYDRATED SLICES TUBERS OF HELIANTHUS TUBEROSUS

Maria Luigia Pallotta Catello Di Martino*

Department of Health Sciences, Department of Animal, Plant and Enviromental Science.*
University of Molise, 86100 Campobasso, ITALY
pallotta@unimol.it

Pyridine dinucleotides are key redox carriers in the soluble phase of all living cells, and both NAD^+ and $NADP^+$ play crucial roles in pro-oxidant and antioxidant metabolism. The capacity of cells to modulate the $NAD(P)H/NAD(P)^+$ ratio is thus critical not only for central redox control of metabolism but also for preemptive management of oxidative stress. Recent studies show that NAD^+ and $NADP^+$ can be utilized to produce a number of metabolites important in cell signalling. Other reactions that consume NAD^+ include protein de-acetylation and poly ADP-ribosylation. These developments bring a significant amount of additional interest to the investigation of cellular NAD^+ biosynthesis and regulation. Relatively little is known about NAD^+ synthesis in plants. Comparative genomics suggests that plants form NAD^+ through *de novo* synthesis from quinolinate, as well as by a salvage pathway that reuses nicotinamide released from NAD^+. $NADP^+$ is produced by phosphorylation of NAD^+ The purpose of our work was to verify the presence of mitochondrial Nicotinamide mononucleotide adenylyl-transferase (NMN-AT), that catalyzes the reversible reaction $NMN + ATP \rightleftharpoons NAD^+ + PP_i$ NMN was added to mitochondria isolated from aged-dehydrated slices tubers of Helianthus tuberosus and $NAD(P)^+$ synthesis was tested by means of HPLC experiments. The dependence of $NAD(P)^+$ synthesis rate on NMN concentration was studied. Hyperbolic dependence of the reaction rate on NMN concentration was found and was inhibited by PPi and AMP. The characterization of NMN-AT provided compelling evidence that NAD^+ biosynthesis pathways may exist in plant mitochondria

---

2J_01_P

THE EFFECTS OF PRENATAL STRESS ON EXPRESSION OF NF-KB□JNK AND P38MAPK IN THE HIPPOCAMPUS OF OFFSPRING RATS

Li Hui[1], Song Tian-bao[2], Zhu Zhong-liang[3*], Chen Rui[1], Cheng Da-xin[1]□Cai Qing[2], Jia Ning[2], Song Liang[2], Li Qing-hong[1]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1. Department of Paediatrics, First Affiliated Hospital of Medical College of Xi'an Jiaotong University, Xi'an, Shaan xi 710061, China
2. Department of Physiology and Pathophysiology, Medical College of Xi'an Jiaotong University, Xi'an, Shaan xi 710061, China
3. College Life of Science Northwest University, Xi'an, Shaan xi 710069, China
**Corresponding Author:*
Professor Zhu Zhong-liang
College Life of Science, Northwest University, Xi'an, Shaanxi 710069, China
*E-mail : **zlzhu@mail.xjtu.edu.cn***

Substantive data has shown that maternal factors, like anxiety, depression and some negative stresses, can affect offspring development. Previous studies revealed that PNS could cause impairment of spatial learning, inhibit cell proliferation in the dentate gyrus and hippocampus and reduce the number of hippocampal neurons in offspring rats. JNK and p38MAPK are members of serine/threonine protein kinases(MAPK). MAPK not only regulates cell proliferation, differentiation and survival, but also plays important roles in synaptic plasticity and memory formation. Nuclear factor (NF-κB) is involved in regulating cell proliferation, differentiation and survival and plays important roles in LTP formation as well.
In this essay, the expression of NF-κB, p38MAPK and JNK in hippocampus of was determined by establishing the model of PNS in order to explore the cell signal transduction pathways concerned. **A**nimals were divided three groups: control (CON), mid-term stress (MS), late term stress (LS). The pregnant rats were exposed to the restraint stress (3 times in a day, 45 minutes each time) during the mid-term or late-term gestation stage. Western-blotting and immunohistochemistry techniques were used to examine the expression of NF-κB and p38MAPK/JNK in hippocamous.The results as follows. Both MS and LS decreased the expression of p65 in female offspring, and there was a significant difference between MS and LS groups ($P<0.01$). The expression of p50 was significantly increased in PNS female offspring hippocampus CA1, CA3 and CA4 region and DG ($P<0.05$, $P<0.01$). The results of male offspring were contrary. In control group the expression of p65 in male offspring was less than that of female CON offspring ($P<0.01$). In LS group the expression of p65 in female offspring was more than that of male offspring ($P<0.01$).The results of Western blotting were similar to those of immunohistochemical experiment results. Both MS and LS increased the expression of p38MAPK in female offspring, and there was a significant difference between MS or LS and CON groups ($P<0.01$). In control group the expression of p38MAPK in male offspring was more than that of female CON ($P<0.01$). In LS group the expression of p38MAPK in male offspring was less than that of male offspring ($P<0.01$). LS decreased the expression of JNK in female offspring compared to MS and CON($P<0.05$□$P<0.01$). In LS group the expression of JNK in female offspring was less than that of male offspring hippocampus ($P<0.01$). In

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

conclusion, PNS had significant effects on the level of NF-κB, JNK and p38MAPK in hippocampus, particularly in LS. Additionally female offspring was more sensitive to PNS on P38MAPK while male offspring was on JNK. These signal transduction pathways may be involved the hippocampal changes induced by PNS in offspring rats.

2J_02_P

ONCOGENIC RAS, VIA PI3K AND MAPK PATHWAYS, TRIGGERS ENDOPLASMIC RETICULUM STRESS-INDUCED APOPTOSIS

Chen Chang-Yan

Boston University School of Medicine, Boston, MA, USA
yanyan@bu.edu

Oncogenic Ras induces apoptosis upon protein kinase C (PKC) suppression. The mechanisms by which Ras regulates apoptosis remain unclear. In this study, we used three *V12-Ha-ras* effector loop mutants to dissect Ras downstream signaling in murine NIH3T3 cells and tested the roles of each mutant and the three possible pairwise combinations in the induction of apoptosis. The expression of a single *ras* mutant can not activate programmed cell death after treatment with GO6976 (a PKC inhibitor). The concurrent activation of PI3K and MAPK pathways increases the susceptibility of the cells to apoptosis in response to PKC suppression. We also showed that although such co-activation alone moderately upregulates ROS production, concurrent suppression of PKC causes a dramatic elevation of ROS which leads to the accumulation of unfolded proteins in the endoplasmic reticulum (ER) and subsequent induction of apoptosis. Thus, our study suggests that under the condition of PKC abrogation, oncogenic Ras, via the PI3K and MAPK pathways, perturbs the state of the cellular redox, which signals to the ER stress-regulated apoptotic machinery for the induction of apoptosis.

2J_03_P

AMYLOID B-PROTEIN POTENTIATES TUNICAMYCIN-INDUCED NEURONAL DEATH IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES

Yoshihisa Ito, Toru Imai, Yasuhiro Kosuge, Kumiko Ishige

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Research Unit of Pharmacology, Department of Clinical Pharmacy, College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi-shi, Chiba 274-8555, Japan. E-mail: yoshiito@pha.nihon-u.ac.jp

We have assessed amyloid β-protein (Aβ)-induced neurotoxicity, with and without added tunicamycin (TM), an inhibitor of N-glycosylation in the endoplasmic reticulum (ER), in organotypic hippocampal slice cultures (OHCs). In the OHCs cultured for 3 weeks, there was little neurotoxicity after treatment with $A\beta_{25-35}$ (25 μM) alone for 48 h. However, with TM alone, concentration-dependent neuronal death was observed at concentrations between 20 and 80 μg/mL. When Aβ was combined with TM (Aβ+TM), cell death was more acute than with TM alone. Western blot analysis revealed that calpain activity and the active forms of caspase-12 and caspase-3 were increased after exposure to Aβ+TM as compared with exposure to TM alone. In contrast, the levels of GRP94, GRP78 and CHOP were not changed in the presence of Aβ. Aβ potentiation of TM neurotoxicity was reversibly blocked by S-allyl-L-cysteine (SAC), an organosulfur compound purified from aged garlic extract, and the L-type calcium channel blocker, nifedipine, in a restricted neuronal area of the OHCs. Simultaneously applied SAC also reversed the increases in calpain activity and the active forms of caspase-12 and caspase-3 by Aβ +TM with no change in the increased levels of GRP 94 and 78 and CHOP. These data indicate that Aβ facilitates the calpain-caspase-12-caspase-3 pathway, thus potentiating TM-induced neuronal death in the hippocampus.

2J_04_P

SIGNALING EVENTS INDUCED BY STRESS FACTORS IN CEREBRAL ENDOTHELIAL CELLs

I.A. Krizbai[1], A.E. Farkas[1], I. Wilhelm[1], P. Nagőszi[1], M. Széll[2], P.O. Couraud[3], B. Wechsler[3], I. A. Romero[4], Z. Bálint[1], G. Váró[1],

[1]Biological Research Centre, Szeged, Hungary; [2]University of Szeged, Szeged, Hungary, [3]Institut Cochin, Paris, France; [4]The Open University, Milton Keynes, UK

Being located at the interface of blood and brain cerebral endothelial cells (CECs) are primary targets of different environmental stimuli. In our study we made an attempt to reveal novel elements of signaling activated by hyperosmotic, oxidative and calcium depletion induced stress in CECs. Hyperosmotic stress elicited by mannitol has been successfully used to reversibily open the blood-brain barrier. We have shown that hyperosmotic conditions induce protein phosphorylation on both Ser/Thr and Tyr residues. Among the targets of protein tyrosine phosphorylation are beta-catenin and possibly the ezrin/moesin complex. Furthermore, our

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

results suggest that besides src the receptor tyrosine kinase Axl plays an important role in mediating the effect of hyperosmosis. Activation of Rho signaling my be involved in the stress response induced by calcium depletion. We have shown that besides changes in junctional protein expression and localization calcium removal induces significant changes in the morphological parameters of CECs as well as revealed by atomic force microscopy. These changes could be partially inhibited by the Rho-dependent kinase inhibitor Y27632 suggesting a role for ROCK in mediating the effect of low calcium concentration in CECs. Recent research has revealed that non-coding RNAs are able to fulfill a wide range of regulatory functions in eukaryotic cells. In our studies we have observed, that the non-coding RNA PRINS (Psoriasis Susceptibility-Related RNA Gene Induced by Stress) is rapidly upregulated severalfold by oxidative stress induced by DMNQ. Further studies are underway to elucidate the role of PRINS in the regulations of endothelial processes. Our results suggest that multiple signaling pathways are activated in by different stress factors in CECs.

2J_05_P

THE OXIDATION OF ASK1 AND ITS REDUCTION BY THIOREDOXIN-1 REGULATE THE INDUCTION OF JNK AND APOPTOSIS BY H_2O_2

Philippe. J. Nadeau, Steve. J. Charette and Jacques Landry

Centre de recherche en cancérologie de l'Université Laval, L'Hôtel-Dieu de Québec, Québec, Canada, G1R 2J6, jacques.landry@med.ulaval.ca

The oxidative stress response of lower organisms is mediated by "redox sensors". They consist in transcription factors or oxidases of transcription factors which, when activated by specific oxidation of cysteine residus, induce an antioxidant response. In mammals, the oxidative stress response involves the activation of signaling pathways that induce many biological responses including apoptosis. Apoptosis signal regulated kinase-1 (Ask1) lies upstream of a major redox-sensitive pathway involving Jun N-terminal kinase (JNK). We found that cell exposure to H_2O_2 caused the rapid oxidation of Ask1 leading to its multimerization through the formation of interchain disulfide bonds. Oxidized Ask1 was fully reduced within minutes after induction by H_2O_2. During this reduction, the thiol-disulphide oxidoreductase thioredoxin-1 (Trx1) became covalently associated with Ask1. Overexpression of Trx1 accelerated the reduction of Ask1 and a redox-inactive mutant of Trx1 (C35A) remained trapped with Ask1 blocking its reduction. Preventing the oxidation of Ask1 either by overexpressing Trx1 or using an Ask1 mutant in which the sensitive cysteines were mutated (Ask1-ΔCys) impaired the activation of JNK but not the phoshorylation of Ask1 by H_2O_2.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Furthermore, oxidation of Ask1 was required for H_2O_2-induced Ask1-mediated apoptosis. Those results indicate that oxidation of Ask1 is not required for its own phosphorylation/activation but is essential for its function upstream of the H_2O_2-induced JNK signaling pathway. We suggest that Ask1 acts as a "redox sensor" in mammalian cells and we propose a new model for its activation mechanism by H_2O_2.

2J_06_P

THE TYROSINE KINASE C-ABL MEDIATES HEAT SHOCK ACTIVATION OF THE P38 AND JNK PATHWAYS.

Sébastien Ian Nadeau, Herman Lambert and Jacques Landry.

Centre de recherche du CHUQ-L'Hôtel-Dieu de Québec, Université Laval, Québec, Canada, GIR 2J6, jacques.landry@med.ulaval.ca

Heat shock (HS) rapidly induces the activation of numerous signaling pathways. The triggering switches for the activation of these pathways and their functions in the HS response are still poorly understood. In this study, we show that their induction correlates with an increase in the total content of phosphotyrosines. Accordingly, the c-Src and c-Abl tyrosine kinases (TK) are activated by HS. To evaluate the possible involvement of these TK, we analyzed the HS induction of the p38 and JNK pathways after pretreatment with different TK inhibitors. Our results notably show that STI571, a TK drug which inhibits c-Abl and PDGFr, greatly reduces the HS activation of the p38 and JNK pathways, but has no effect on H_2O_2-mediated p38 activation. By using a specific inhibitor of PDGFr and siRNA for either PDGFr or c-Abl, we show that c-Abl and not PDGFr is involved. Moreover, the expression of a specific siRNA-resistant plasmid encoding for c-Abl rescues the HS activation. Since an important fraction of c-Abl is nuclear, we initially suggested that the HS signal might arise from the nucleus. This was further supported by the observation that HS activates ATM, a known upstream activator of c-Abl, and induces H2AX phosphorylation. However, cells deficient in ATM or in both DNA-PK and ATM were still proficient in HS-induced p38 activation, whereas they could not support HS-induced H2AX phosphorylation. From these last results, we infer that the HS signal instead comes from the cytoplasmic c-Abl. Indeed, the existence of an actin binding domain in c-Abl hints at a possible link between the early HS disruption of actin filaments and the activation of the p38 and JNK pathways.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2J_07_P

THE HSP40 CHAPERONE MDG1/ERDJ4 IS A NOVEL MODULATOR OF THE INTEGRATED STRESS RESPONSE

M El-Hadi, L Merkel, F Pröls

University of Freiburg. Institute of Anatomy and Cell Biology II, D-79104 Freiburg, Germany

The Hsp40 chaperone Mdg1/ERdj4 is localized in the ER compartment and is upregulated in cells subjected to various stresses like heat shock, hypoxia and ER stress. Recently it has been shown that expression of the Mdg1 hamster homolog is significantly decreased in a high metastatic cell line in comparison to a low metastatic cell line. Overexpression of Mdg1 in these cell lines led to a cell cycle arrest. In this work we show that Mdg1 is largely upregulated in a variety of tumors. As a target of the IRE1α-XBP1-pathway, Mdg1 is considered to play a role in the sustained Unfolded Protein Response (UPR), in which protein degradation, cell cycle arrest or even apoptosis are induced. The investigation of the UPR signaling cascade revealed CHOP, which is part of the Integrated Stress Response (ISR), as a downstream target of Mdg1. We further show that Mdg1 protein has the ability to interact with ATF4 protein indicating that CHOP transcription might be specifically induced by Mdg1-ATF4 heterodimers. Mdg1 protein thus links the signaling pathways during sustained UPR with the ISR. Together with the findings that ATF4, CHOP and Mdg1 are highly expressed in tumor cells we conclude that ATF4-Mdg1 heterodimers lead to cell cycle arrest via CHOP upregulation thereby reducing the risk of metastasis formation.

2J_08_P

TITLE: THE NEUTRAL SPHINGOMYELINASE IN THE HEAT-INDUCED APOPTOSIS

Takeshi Yabu and Michiaki Yamashita

National Research Institute of Fisheries Science, Japan

A neutral sphingomyelinase (SMase) is considered a major candidate for mediating stress inducible ceramide production. Here, we identified and characterized the neutral SMase that is activated and generated ceramide under stress conditions. The enzyme was found to be a regulator of apoptosis mediated by heat stress. A zebrafish cDNA clone responsible for Mg^{2+}-dependent neutral SMase activity by the expression cloning system

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

was isolated. The cDNA clone encoded a polypeptide of 420 amino acid residues (putative molecular mass: 46.9 kDa) containing predicted two transmembrane domains at the C-terminal region. The bacterially expressed recombinant neutral SMase hydrolyzed a [choline-mythyl-^{14}C]sphingomyelin optimally at pH 7.5 in dependent on Mg^{2+} ion. The loss of neutral SMase function with anti-sense phosphothiate oligonucleotides inhibited ceramide generation, caspase-3 activation and apoptotic cell death under the heat stress conditions, indicating that the neutral SMase induces a ceramide mediated pro-apoptotic signaling pathway that executes the heat-induced apoptosis.

2K_01_P

REGULATION OF FOX GENES IN T LYMPHOCYTES BY NOREPINEPHRINE AND CAMP

Daniel H. Davis, Lloyd Graf, Jr, Rhonna L. Cohen and Donald A. Chambers

University of Illinois College of Medicine, 1853 W. Polk St., Chicago IL, 60612, USA, donc@uic.edu

Catecholamines, e.g., norepinephrine (NE) have been identified as psychogenic stressors and associated with altered immune function. We have identified a norepinephrine/β2-adrenergic receptor/cAMP/PKA-dependent mRNA decay system in T lymphocytes. The Fox family of transcription factors has been implicated in regulating lymphocyte physiology. Foxm1b is reported to be a master gene related to cellular proliferation and Foxo1 has been associated with the suppression of proliferation and promotion of apoptosis. Therefore, we explored whether a PKA-selective cAMP analogue (8Br-cAMP) and NE could regulate BALB/c murine thymocyte cell lines, S49wt and S49kin- (PKA deficient), by affecting the genetic expression of Foxm1b and Foxo1. Real-time PCR studies revealed a 2-3 fold upregulation of Foxo1 mRNA in S49wt, but not kin- cells with 150uM NE or 600uM cAMP as early as 2 hrs after treatment. Foxm1b mRNA was downregulated in S49wt cells by greater than 2 fold by 8Br-cAMP after 6 hrs (at 24 hrs). Treatment with an Epac-selective cAMP (500uM) analogue resulted in no change in Foxo1 or Foxm1b expression in S49 wt or S49 kin- cells. These results associate PKA in modulating both increased Foxo1 mRNA expression and decreased Foxm1b expression and consequent fox gene-mediated regulation of lymphocyte physiology. Supported by NIH.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2K_02_P

EXPRESSION QUANTIFICATION OF TWELVE ABA-REGULATED GENES UNDER WATER-STRESS AND RECOVERY CONDITIONS IN *MEDICAGO TRUNCATULA*

Matilde Cordeiro[1], Cátia Nunes[2], Anabela Silva[2] and Pedro Fevereiro[1, 2]

[1]Plant Cell Biotechnology Laboratory, Instituto de Tecnologia Química e Biológica - Universidade Nova de Lisboa (ITQB-UNL), Oeiras, Portugal; [2]Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal.

Medicago truncatula is a model used to study legume related processes. The expected climate changes towards hottest and drier conditions raised the need to fully understand molecular and metabolic basis of legume response to abiotic stresses. Abscisic acid (ABA) is produced under drought conditions and plays important roles in plant stress response, namely signalling changes at the transcriptional level. The conserved ABA response element (ABRE) and the more degenerated coupling element (CE) are the necessary and sufficient *cis*-acting elements for ABA-regulated gene expression. Twelve stress-related genes, orthologs of reported ABA regulated genes, containing ABA specific *cis*-elements in their promoter regions, were identified *in silico*. *M. truncatula* plants were grow and collected under different water stress conditions. Plants were re-watered until they recovered from the different water-stress status and were collected. Physiological parameters were measured. The expression of the twelve genes will be analysed using qRT-PCR in dehydrated and re-hydrated *M. truncatula* plants. The transcription profile of the twelve genes will be correlated with the available genomic data. Gene expression is not enough to measure the importance of each gene, but together with the recognition of the gene product and identification of the *cis*-elements that are directly related with transcription regulation, is a strong tool to understand the molecular switches of stress-inducible genes and their role in stress response.

2K_03_P

STUDIES ON GLUTATHIONE S-TRANSFERASE ACTIVITIES AND GENE EXPRESSION LEVELS IN *TRITICUM AESTIVUM* CULTIVARS DURING POLYETHYLENE GLYCOL-INDUCED OSMOTIC STRESS

Ágnes Gallé[1], Jolán Csiszár[1], Mária Secenji[2], Irma Tari[1], Adrienn Guóth[1], Dénes Dudits[2], János Györgyey[2] and László Erdei[1]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[1] *Department of Plant Physiology, University of Szeged, PO Box 654, H-6701 Szeged, Hungary*
[2] *Institute of Plant Biology, Biological Research Center, Szeged, Hungary*
E-mail: gallea@bio.u-szeged.hu

We investigated the effect of osmotic stress on two drought tolerant wheat cultivars: *Triticum aestivum* cv. Kobomugi is a near isohydric Asian landrace, *T.a.* cv. Öthalom is an anisohydric, dehydation tolerating Hungarian genotype. Osmotic stress treatment was applied gradually by polyethylene glycol (PEG 6000) treatment reaching 400 mOsm (-0.976 MPa) on one-week-old plants under controlled conditions. Changes in glutathione S-transferase (GST) and glutathione peroxidase (GSPX) activities and in their expression levels were determined during a 2-week-period as function of time. GST isoenzymes represent a large and variable group of antioxidative enzymes, with several different activities and sequence patterns. Phylogenetic analysis of wheat *GSTs* was performed *in silico* and using the tentative consensus sequences (TC) a dendogram was constructed. According to the conserved sequences used for classification of GST proteins, we could identify four groups of wheat GSTs (*phi, zeta, theta* and *tau*). Real Time PCR analysis with two group-specific primer showed a significant increase in the amount of GST transcripts two days after plants were exposed to 400 mOsm PEG. The changes in the transcript levels were compared with the GST activities measured in the same times. According to our results the members of *phi* GSTs can be responsible for the fast response after the osmotic stress, while the *tau* GST group for the later enhancement of GST activity.
This work was supported by the National R&D program: NKFP 4/064/2004.

2K_04_P

DISSECTING CRHR1-MEDIATED PATHWAYS VIA MICROARRAY TECHNOLOGY

C. Graf, P. Weber, B. Pütz, F. Holsboer, W. Wurst, J.M. Deussing

Max-Planck-Institute of Psychiatry, Kraepelinstr. 2, 80804 München, Germany
Email: cgraf@mpipsykl.mpg.de

The corticotropin releasing hormone (CRH) system is involved in endocrine, autonomic and behavioural responses to stress. The biological actions of CRH-like neuropeptides are mediated by G-protein-coupled receptors, CRH receptor 1 (CRHR1) and CRHR2. CRHR1 is widely expressed in the mammalian brain (e.g. cerebral cortex, cerebellum, amygdala, hippocampus) and in the pituitary gland. Mice deficient for

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

CRHR1 display decreased anxiety-like behaviour and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. Ligand binding increases the affinity of the CRHR to G-proteins. Binding of a Gαs-protein will activate adenylate cyclase (AC) and protein kinase A (PKA) as well as other cyclic adenosine monophoshpate (cAMP) dependent pathways. The reported coupling of additional G-proteins to CRHR1 suggests that also other second messengers are involved in CRHR-signaling. In order to identify specific target genes of CRHR1-mediated signaling pathways we applied cDNA and oligonucleotide microarray technology using a mouse corticotroph cell line (AtT20) and pituitaries of CRHR1-deficient mice. 102 genes in vitro and more than 400 genes in vivo were found stress- and/or CRH- and CRHR1-dependently regulated. A subset of candidate genes was validated in independent material by quantitative real time PCR (qRT-PCR). These candidates are involved e.g. in cAMP, mitogen activated protein kinase (MAPK) or epidermal growth factor receptor (EGFR) signaling. In order to examine their functional role the effect of these genes on different known target genes downstream of CRHR1 signaling is analyzed using reporter assays.

2K_05_P

POST-TRANSCRIPTIONAL REGULATION OF ALTERED HEAT SHOCK RESPONSE IN LONG LIVED *C. ELEGANS*

Gawain McColl, Aric Rogers, Alan E. Hubbard, Christopher D. Link, Silvestre Alavez, Pankaj Kapahi and Gordon J. Lithgow

Buck Institute for Age Research, Novato, CA, USA,.
gmccoll@buckinstitute.org

The insulin-like signaling (ILS) pathway plays a major role in the modulation of lifespan in *C. elegans*. Altered stress responses, HSP expression and resistance to a wide range of stressors are also associated with changes in ILS. The causal relationship of these phenotypes with altered aging is yet to be fully determined. The transcription factors DAF-16 and HSF-1 have both been purported as key regulators for these effects. Here we present new data that demonstrates that increased resistance to heat shock, due to lowered ILS, is not dependent on a transcriptional response, but does still require active translation. Furthermore, we describe new genes under post-transcriptional regulation in long-lived ILS mutants following heat shock via a genome wide translational state array analysis (TSAA).

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2K_06_P

REGULATION OF THE NF-κB PATHWAY BY LOW LEVELS OF H_2O_2 AND IMPACT ON INFLAMMATION

Oliveira-Marques,V.[1*]; Cyrne, L.[1], Marinho, H.S.[1], Antunes, F.[1,2]

[1]Grupo de Bioquímica dos Oxidantes e Antioxidantes, Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Portugal
[2]Instituto Bento Da Rocha Cabral, Lisboa, Portugal
** email of corresponding author: vmmarques@fc.ul.pt*

During the inflammatory response, host cells are exposed to pro-inflammatory cytokines, such as TNF-α, and also hydrogen peroxide (H_2O_2). It is known that the cytokines liberated by neutrophils and macrophages induce the NF-κB transcription factor in the surrounding cells thus promoting an inflammatory response. MCF-7 and HeLa cells were exposed to moderate doses of H_2O_2 using the steady-state approach: $[H_2O_2]_{ss}$. It is a controlled and calibrated method that allows applying lower concentrations of H_2O_2 than those applied with the bolus approach, because H_2O_2 consumption by cells is compensated by the continuous production of H_2O_2 by glucose oxidase. TNF-α (0.37 ng/mL) and $[H_2O_2]_{ss}$ (25 μM) acted synergistically to induce NF-κB translocation to the nucleus, while an antagonism was observed when H_2O_2 was delivered as a bolus addition (1 mM). The differences in the results might be explained with the high oxidative level caused by the bolus addition, which is far from the physiological conditions and can disrupt cellular redox homeostasis. The synergistic translocation to the nucleus, with $[H_2O_2]_{ss}$ (12.5 μM) and TNF-α for 6 h, led to an up-regulation in the expression of some NF-κB-dependent genes with a pro-inflammatory role (e.g. IL-8, MCP-1, TNF-α), but also anti-inflammatory genes, such as Heme oxygenase-1 and IL-6. We propose a regulatory role for H_2O_2 during inflammation, through the up-regulation of the expression of pro-inflammatory genes, that stimulate the inflammatory response, and anti-inflammatory genes that prevent an excessive build up of a pro-inflammatory environment.

2K_07_P

PKCα -DEPENDENT PHOSPHORYLATION OF THE MRNA STABILIZING FACTOR HUR: IMPLICATIONS FOR POSTTRANSCRIPTIONAL REGULATION OF CYCLOOXYGENASE-2

Anke Doller[*], Andrea Huwiler* [†], Roswitha Müller[*], Heinfried H. Radeke[*], Wolfgang Eberhardt[*] and Josef Pfeilschifter[*]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

* *Pharmazentrum frankfurt/ZAFES, Klinikum der Johann Wolfgang Goethe-Universität, Frankfurt am Main, Germany*
† *Institut für Pharmakologie, Universität Bern, CH-3010 Bern, Switzerland*

In this study, we investigated the molecular mechanisms underlying the ATP analog adenosine 5´O-(thiotriphosphate) (ATPγS)-induced nucleo-cytoplasmic shuttling of the mRNA stabilizing factor HuR in human mesangial cells (hMC). Using synthetic protein kinase C (PKC) inhibitors and siRNA approaches we demonstrate that knock-down of PKCα efficiently blocked the ATP-dependent nuclear HuR export to the cytoplasm. The functional importance of PKCα in HuR shuttling is highlighted by the high cytosolic HuR content detected in hMC stably overexpressing PKCα when compared to mock-transfected cells. The ATP-induced recruitment of HuR to the cytoplasm is preceded by a direct interaction of PKCα with nuclear HuR and accompanied by increased Ser-phosphorylation as demonstrated by co-immunoprecipitation experiments. Mapping of putative PKC target sites identified serines 158 and 221 being indispensable for HuR-phosphorylation by PKCα . RNA pull-down assay and RNA EMSA demonstrated that the HuR shuttling by ATP is accompanied by an increased HuR-binding to COX-2 mRNA. Physiologically, the ATP-dependent increase in RNA binding is linked with an augmentation in COX-2 mRNA stability and subsequent increase in PGE_2 synthesis. Regulation of HuR via PKCα-dependent phosphorylation emphasizes the importance of posttranslational modification for stimulus-dependent HuR shuttling.

2K_08_P

ZBP1 REGULATES MRNA STABILITY DURING CELLULAR STRESS

Nadine Stöhr and Stefan Hüttelmaier

NBL3 Research Group, Zentrum für Angewandte Medizinische und Humanbiologische Forschung, Department of Medicine, Martin-Luther-University, 06120 Halle, Germany

An essential constituent of the integrated stress response (ISR) is a reversible translational suppression. This mRNA silencing occurs in distinct cytoplasmic foci called stress granules (SGs) that transiently associate with processing bodies (PBs) typically serving as mRNA decay centers. How mRNAs are protected from degradation in these structures and if SGs serve a physiological significant role remains largely elusive. In recent studies we identified that the Zipcode-binding protein 1 (ZBP1) regulates the cytoplasmic fate of specific mRNAs in non-stressed cells, but also is a key regulator of mRNA turnover during the ISR. The association of ZBP1 with target mRNAs in SGs was not essential for mRNA-targeting to SGs.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

However, ZBP1 knock down induced a selective destabilization of target mRNAs during the ISR, while forced expression increased mRNA stability. Our results indicate that although targeting of mRNAs to SGs is nonspecific, the stabilization of mRNAs during cellular stress requires specific protein-mRNA interactions. These retain mRNAs in SGs and prevent premature decay in PBs or by the exosome. Hence, RNA-binding proteins (RBPs) are essential for translational adaptation during cellular stress by modulating mRNA turnover.
Based on our resent observations we establish a new stress-based screening procedure for RNA ligands and thereby identify molecular networks facilitating ZBP1 function in SGs. Moreover, we are interested in the physiological role of SGs intending to set the stage for the identification of SGs in cancer cells and primary tumors.

2K_09_P

GENE EXPRESSION AND REGULATION OF HEAT SHOCK PROTEINS IN *TRYPANOSOMA CRUZI*

D. R. de Carvalho, R. A. Campos, M. Fernandes, R. Silva, E. Rondinelli, T.P. Ürményi.

Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, CCS, Bl. G, Cidade Universitária, Rio de Janeiro, RJ 21941-902, Brazil. Email: turmenyi@biof.ufrj.br

The understanding of gene regulation of molecular chaperones will shed light on the mechanisms of post-transcriptional regulation in trypanosomatids. The gene organization and gene expression pattern of HSP70, HSP60 and HSP10 genes of *Trypanosoma cruzi* have been previously characterized by our group and others, and characterization of gene structure and expression of ClpA/HSP100 are currently under way. Our aim is to study the mechanisms of gene regulation of these proteins, and to that end the presence and function of heat shock-responsive elements in the corresponding mRNAs are being investigated. Plasmids containing the chloranfenicol acetyltransferase (CAT) reporter gene under the control of the 18S rRNA promoter were constructed in which the CAT gene is flanked by segments of intergenic regions containing either the 5' or 3' UTR of the HSP70 and Rab7 mRNAs. CAT assays of transiently transfected *T. cruzi* cells show that heat shock-responsive elements are present in both the 5’ and 3’ UTRs of HSP70 mRNA. A similar series of reporter plasmids are being constructed with HSP10 and HSP60 sequences. CAT mRNA levels in transfected cells are being determined to assess the contribution of mRNA stability and its translation to the induction of the CAT enzyme. We are also determining the half-life of the endogenous HSP10, HSP60, HSP70 and ClpA/HSP100 mRNAs under

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

stressing and non-stressing conditions to further characterize gene regulation mechanisms of these proteins. Supported by CNPq and FAPERJ.

2K_10_P

GENOME-WIDE TRANSCRIPTIONAL CHANGES IN RESPONSE TO OXIDATIVE STRESS IN *DROSOPHILA*

Chris G. Mahon[1], Tony L. Parkes[2], and J. Tim Westwood[1]

[1]Dept. of Cell and Systems Biology, University of Toronto, Mississauga, ON and [2]Dept. of Biology, North Bay, ON, Canada. Email: t.westwood@utoronto.ca

Aerobic organisms have evolved a series of metabolic defenses whose role it is to contain and/or repair the deleterious effects of reactive oxygen species (ROS). One of the most prominent of these is Cu-Zn or cytosolic superoxide dismutase (cSOD1) which converts superoxide anion radicals to hydrogen peroxide and oxygen. Previous genetic studies investigating the phenotypic consequences of a null mutation for cSOD in *Drosophila melanogaster* have resulted in the characterization of a series of phenotypes that include: increased superoxide concentration in cells; dramatic reduction in longevity; male sterility and female semi-sterility; and locomotory and behavioural defects. It has been speculated that increases in superoxide anions in cells triggers a number of defense responses. In this study, we used $cSODn^{108}$ null flies as well as flies in which cSOD activity was restored via transgenic rescue. Flies were collected at 0-24 and 48-72 hours post eclosion and total RNA extracted. Transcriptional profiles comparing cSOD null vs. transgenic rescue were obtained using long oligonucleotide DNA microarrays from the Canadian *Drosophila* Microarray Centre (CDMC, www.flyarrays.com). Transcripts from more than 200 genes decreased in the cSOD null flies including genes involved in reproduction, carbohydrate metabolism, lipid biosynthesis and proteases. More than 80 genes showed increased transcript levels including ones involved in lipid catabolism, DNA damage response, apoptosis, and cell adhesion. Unexpectedly, heat shock genes were not induced. (This work was supported by NSERC grants to JTW, TLP and the CDMC).

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2K_11_P

FUNCTIONAL STUDIES OF UBIQUITIN CARBOXY-TERMINAL HYDROLASE L1 NOVEL VARIANT USING PROTEOMICS AND FUNCTIONAL GENOMICS

Sun Kim, Hyun Jung Kim, Hee-Jung Kim, and Kong-Joo Lee

The Center for Cell Signaling & Drug Discovery Research, College of Pharmacy and Division of Life & Pharmaceutical Sciences, Ewha Womans University, Seoul 120-750, Korea. E-mail: kjl@ewha.ac.kr

Ubiquitin Carboxy-terminal Hydrolase isozyme L1(UCH-L1) is a thiol protease that recognizes and hydrolyzes a peptide bond at the carboxy-terminal of ubiquitin, and is involved in the processing of ubiquitin precursors and ubiquitinated proteins. It is normally expressed exclusively in neurons and testis, and abnormal expression of UCH-L1 has been shown to correlate with several forms of cancer, such as cancer of the lung, colon, and pancreas. Mutations in the UCH-L1 gene have been reported to be linked to susceptibility to and protection from Parkinson's disease. Although the correlation between UCH-L1 and disease is quite reported, the molecular mechanism of biological function of UCH-L1 is poorly understood. To find out the biological function of UCH-L1, Proteomics combining 2D-gel electrophoresis and ESI-q-TOF tandem MS were employed and four variants of UCH-L1 were identified. Subsequently, differentially regulated genes or proteins in cells overexpressing one of the four variants were identified by proteomics and cDNA microarray, and confirmed by western blot analysis and real-time quantitative RT-PCR. The results demonstrate that UCH-L1 has heterogeneous populations and each variant plays significantly different biological roles. [Supported by KOSEF NCRC for CCS & DDR and FPR05A2-480. S Kim, HJ Kim and HJ Kim were supported by BK21]

2K_12_P

COMPARATIVE GENOMICS RESPONSE TO HEAVY METAL STRESS IN THE CILIATE *TETRAHYMENA THERMOPHILA*.

Virginia Campos, Ana Martín-González and Juan C. Gutiérrez

Dpto. Microbiología-III. Facultad de Biología. Universidad Complutense (UCM). Madrid 28040. Spain. E-mail: juancar@bio.ucm.es

Gene expression libraries from the ciliate protozoa *Tetrahymena thermophila*, were obtained after exposition to Cd (5 mg/L, 3h or 24h) or

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

arsenate (As^{+5}) (7.5 mg/L, 1h). All isolated cDNA molecules presented a polyA tail, confirming the mRNA nature of all inserts. From the Cd-gene expression library 109 clones were analysed, and 112 clones from the As-gene library. Gene expression results show important differences between both heavy metal (Cd / As) cell resistance mechanisms, and also between both Cd gene libraries after different treatment times (3h or 24h). From the different gene libraries we can point out the following identified protein groups; transporters proteins (ABC, ATPases, etc.) 4.6% in Cd3h, 2.4% in Cd24h and 2.6% in As1h library, protein-kinases (5.5% Cd3h, 4.8% Cd24h, 3.5% As1h), enzymes involved in protein degradation (6.4% Cd3h, 4.8% Cd24h, 6.3% As1h), oxidative stress protein (2.7% Cd3h, 7.3% Cd24h, 18.7% As1h), DNA binding proteins (H4 histone, NgoA) 6.4% Cd3h, 26.8% Cd24h, 7.1 As1h). About this last group, the NgoA protein (exclusive of this ciliate) is the most abundant (2 clones in Cd3h, 11 clones in Cd24h and 7 clones in As1h), especially after Cd long exposure. Metallothioneins are also induced (1.8% Cd3h, 14.6% Cd24h, 0.9% As1h), and among the 3 CdMTs reported in this ciliate (Díaz et al., 2007) the MTT5 is the most induced, which agrees with the quantitative RT-PCR results (MTT5 >> MTT1 > MTT3). Several of these genes might be selected to elaborate, in the next future, DNA macro- or micro-arrays to detect the presence of these environmental pollutants, or a general cellular stress, from soil or aquatic samples. Supported by project: CGL2005-00548BOS.

2K_13_P

ANALYSIS OF GENE EXPRESSION AND REGULATION OF A NOVEL, STRESS-INDUCIBLE TRANSCRIPT IN THE MOUSE

C. Liebl, M. V. Schmidt, J. Schülke, D. Trümbach, K. Ganea, V. Sterlemann, T. Rein, M. B. Müller

Max Planck Institute of Psychiatry, Munich, Germany
liebl@mpipsykl.mpg.de

We studied the effects of 24 hours maternal deprivation on gene expression in the paraventricular nucleus (PVN) of 9 day-old mice using microarray technology. Among the strongest regulated genes we discovered a novel transcript (interim name MPIP-101), which showed a pronounced upregulation in the maternal deprived group. This gene codes for a short hypothetical protein of unknown function that has also a human ortholog. The aim of the current study is the functional analysis of the expression and regulation of this gene in neonatal and adult mouse brain. In the neonate , GR-antagonist treatment prevented an maternal deprivation induced upregulation of MPIP101 mRNA in the PVN, indicating a GR dependant gene regulation. This finding is in line with a promoter

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

analysis of this gene, which revealed several functional GRE sites. In conditional CRHR1 knockout mice the maternal deprivation induced upregulation of MPIP-101 was significantly reduced, suggesting an additional regulatory mechanism via CRH mediated pathways. In adult mice MPIP-101 is mostly expressed in the CA3 region of the hippocampus, in the cortex and the cerebellum, with very little expression in the PVN under basal conditions. Dexamethasone treatment, restraint stress and food deprivation result in a profound induction of gene expression in the PVN, confirming the data of the neonate. In ongoing studies we analyze the cellular localization of MPIP-101, its interaction with other proteins and co-localization with central stress markers.

2L_01_P

OPTICAL TWEEZERS COMBINED WITH A MICROFLUIDIC DEVICE FOR STUDIES OF STRESS RESPONSE IN SINGLE CELLS

Emma Eriksson[a], Elzbieta Petelenz[b], Stefan Hohmann[b] and Mattias Goksör[a]

[a]*Department of Physics, Göteborg University, SE-41296 Göteborg, Sweden, emma.eriksson@physics.gu.se*
[b]*Department of Cell and Molecular Biology, Göteborg University, Sweden*

Traditionally biological experiments are performed on large populations of cells. However, such experiments cannot reveal behaviours on a subcellular level. Optical tweezers have proven to be a useful tool for conducting single cell studies. By combining optical tweezers and a microfluidic device, we have developed a platform where we rapidly can change the environment of a single cell. In our microfluidic device different media, flowing in separate channels, are combined into a single channel. In such small geometries the flow is laminar, which means that different media only mix due to diffusion. Consequently a sharp concentration gradient of a substance can be established. By moving a cell across the gradient, using the optical tweezers, we are able to change the environment of a single cell in a fraction of a second. During the experiment the cell is monitored using fluorescence microscopy. Our method is universal, enabling experiments on many types of cells with different stress media. The system can be expanded to include holographic optical tweezers, thus enabling several cells to be studied and analyzed simultaneously. The experimental platform is demonstrated on yeast cells (*Saccharomyces cerevisiae*). We have monitored the diameter of yeast cells that are moved between a flow of pure growth medium and a sodium chloride containing flow. In addition we have also studied yeast cells where GFP is fused to a protein involved in the induced stress response. In this way the spatial localization of both the Hog1 protein and the Yap1p protein were followed during osmotic and oxidative stress,

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

respectively.

2L_02_P

PHYSIOLOGICAL CHARACTERIZATION OF CPR1 ENCODING PEPTIDYL-PROYL *CIS-TRANS* ISOMERASE IN THE YEAST *SACCHAROMYCES CEREVISIAE* KNU5377 UNDER THE MENADIONE-INDUCED OXIDATIVE STRESS

Il-Sup Kim[a], Sunhye Kwak[a], Sang-O Kwon[a], Hyunsook Lee[a,b], Ilsoo Moon [b], Ingnyol Jin[a*]

[a]*Department of Microbiology, Kyungpook National University, Daegu 702-701, Korea*
[b]*Department of Anatomy, Dongguk University Medical School, Kyongju 780-714, Korea*

Abstract
Cpr1p coding cytosolic cyclophilin (Cyp)-related peptidyl-proyl *cis-trans* isomerase was strongly induced during ethanol fermentation at 40ºC in *Saccharomyces cerevisiae* KNU5377. The *cpr1Δ* mutant in *S. cerevisiae* KNU5377Y, not a laboratory strain such as *S. cerevisiae* BY4741, showed the stress sensitivity to manadione (MD). Cell viability in the *cpr1Δ* mutant decreased up to 20% under 0.4 mM concentration of MD. Under the stress condition of 0.4 mM MD, markers of oxidative damage such as the levels of hydroperoxide, carbonyl content and malondialdehyde was higher in the *cpr1Δ* mutant than in the wild type of KNU5377. But there was no difference between the wild type and the *cpr1Δ* mutant in a laboratory strain of *S. cerevisiae* BY4741 even in the 0.2 mM MD stress. This *cpr1Δ* mutant KNU5377 in these stress conditions repressed the expression of various antioxidant enzymes including Cu/Zn superoxide dismutase (Sod1p), thioredoxin 2 (Trx2p), thioredoxin reductase (Tlr1p), thioredoxin peroxidase (Tsa1p), glucose-6-phosphate dehydrogenase (G6PDH), cytosolic isocitrate dehydrogenase (cIDH), alcohol dehydrogenase (Adhp), and Hsp104. Interestingly, the expression of Sod1 was reduced in two *cpr1Δ* mutants of KNU5377 and BY4741. The down-regulation of G6PDH and cIDH in the *cpr1Δ* mutant of KNU5377 caused a reduction in the cellular NADPH level. These repressed proteins in the *cpr1Δ* mutant of KNU5377 were recovered after treatment of such inhibitor as PMSF under the MD stress. In addition, the expression of Cpr1p in KNU5377 was regulated by HSF1 under the MD stress. These data indicates that the CPR1 in KNU5377 has an effect on the expression of antioxidant enzymes and heat shock proteins during the MD stress and is regulated by HSF1..

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2L_03_P

CHANGES OF PROTEOME AND CAROTENOID PRODUCTION IN STRESSED RED YEASTS

Marova I. [1], Halienova A. [1], Carnecka M. [1], Hanusova V. [2], Hezinova V.[1]

[1]Faculty of Chemistry, BUT, Brno, Czech Republic, e-mail: marova@fch.vutbr.cz
[2]Faculty of Chemical and Food Technology, STU, Bratislava, Slovak Republic

Environmental stress surrounding yeast cells evokes various changes in their behaviour in order to survive. In this work, protein and metabolic profiles of some carotenogenic yeasts grown in optimal and stress conditions were compared. Different 11 carotenogenic yeast strains were cultivated under osmotic, oxidative and metal stress. Produced carotenoids and ergosterol were measured using HPLC//MS. Glycerol was analysed using Boehringer kit. Proteins were analyzed by PAGE-SDS, 1D microfluidic system and by 2D electrophoresis. Under all stress conditions expression of some protein fractions was changed. Osmotic stress led to overexpression of some specific protein fractions in most of studied yeast strains. These proteomic profiles were different from protein profiles obtained at oxidative as well as metal stress. Presence of exogenous stress led to important overproduction of pigments as well as of lipidic substances (ergosterol, glycerol). Production of carotenoids by R. *glutinis* cells was about 5-6x higher under oxidative stress, while production of ergosterol increased more than 10-x. Salt and metal stress led also to slight increase of carotenoid production. Combination of stress factors in cultivation media induced significant increase of beta-carotene formation mainly in *S. roseus* (230mg/g in medium with 2% NaCl and 5mM H_2O_2) and in salt stressed *R. glutinis* (200mg/g). Production of carotenoids under exogenous stress changed simultaneously with ergosterol production, but inversely to glycerol production.
Acknowledgements: This work was supported by projects MSM 0021630501 of the Czech Ministry of Education and IAA400310506 of Grant Agency of the Academy of Sciences CZ.

2L_04_P

BIPHASIC MODULATION OF THE CELLULAR STRESS RESPONSE BY HISTAMINE H_3R AND H_4R RECEPTOR ANTAGONISTS IN EUKARYOTIC CELLS

Papamichael K[1], Delitheos B[1], Thurmond RL[2], Tiligada E[1]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[1]*Dept. of Pharmacology, Medical School University of Athens, Greece;*
[2]*Johnson & Johnson Pharmaceutical Research & Development, L.L.C., San Diego, CA, USA*

The highly conserved inducible cellular stress response (CSR) to adverse environmental/microenvironmental conditions, like heat and drugs, is partly coupled to heat shock protein (hsps) expression. Amongst others, histamine (HI), a key inflammatory mediator, interacts with diverse components of the CSR. This study aimed at exploring the role of H_3R and H_4R antagonists, thioperamide (THIO) and JNJ7777120, respectively, on the CSR in yeast, an established experimental model, which lacks known HR but possesses HI metabolizing systems. The response was evaluated by determining microscopically the viability of post-logarithmic phase grown cell cultures after heat shock (HS) at 53°C for 30min. The effects of THIO and JNJ7777120 were investigated following administration through to the post-logarithmic phase or for 2h (preconditioning) prior to HS. Induction of thermotolerance after thermal preconditioning at 37°C for 2h served as positive control. The expression of hsps was determined by western blotting. Similarly to HI, administration of either THIO or JNJ7777120 induced biphasic effects on cell viability, with significant increases at 0.03-0.3mM and decreases at 0.3-3mM. The presence of cycloheximide, a *de novo* protein synthesis inhibitor, reversed the effect of THIO or JNJ7777120 preconditioning. Preliminary investigation of hsp104, hsp70 and hsp60 expression showed a comparable pattern. The ability of the HR antagonists to induce a biphasic CSR in yeast, along with the *de novo* protein synthesis contribution in preconditioning with the agents and the alterations in hsp expression point to the need for elucidating the potential clinical implications of this phenotype.

2L_05_P

TRANSCRIPTION FACTORS SPT3P AND MED3P IN RECOVERY OF YEAST CELLS AFTER THERMAL INSULT

M. Simola, A.-L. Hänninen, L. Seppä, P. Auvinen and M. Makarow

Institute of Biotechnology, P.O.Box 56, FIN-00014 University of Helsinki, Finland, mari.j.simola@helsinki.fi

Upon subjection to heat shock temperature 37°C, *S. cerevisiae* cells acquire thermotolerance, which enables survival after exposure to 50°C. We found earlier that when cells grown at 24°C, adapted at 37°C and treated for 20 min at 50°C are shifted back to 24°C, expression of chaperones Hsp104 (cytosol), Kar2p/BiP and Lhs1p (ER) and Hsp78 (mitochondria) is up-regulated after 2-4 h of recovery at 24°C. We designated this novel mechanism delayed up-regulation (DUR), and found

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

it to be mediated by Hsf1p *via* heat shock elements. Analysing global gene expression we found here that production of transcription factors Spt3p and Med3p (Pgd1p/Hrs1p), essential for thermotolerance, also was subject to DUR. Spt3p is a member of the SAGA complex (Spt-Ada-Gcn5-acetyltransferase complex) and Med3p is a subunit of the Mediator complex. *HAC1*^{u} (uninduced) mRNA is transcribed under normal conditions. When unfolded proteins accumulate in the ER, *HAC1*^{u} is spliced by the ER-membrane kinase Ire1p to *HAC1*^{i} (induced) mRNA, which is translated into the transcription factor Hac1p, causing increased expression of ER chaperones. We found that expression of *HAC*1 mRNA is subject to DUR. Production of *HAC1*^{i} was strongly up-regulated during recovery at 24°C, due to increased transcription and splicing of *HAC1*^{u} mRNA. In the absence of Spt3p or Med3p, DUR of Hac1p production was diminished and delayed. Consequently, a heat-denatured cytosolic reporter enzyme failed to be effectively reactivated, and refolding of heat-denatured secretory proteins in the ER to secretion-competent conformations was delayed. Moreover, in the *spt3*Δ *med3*Δ mutant the cell shape and bud formation were strongly affected.

2M_01_P

ARSENITE-ELICITED SKP2 PROTEOLYSIS REQUIRES LEUPEPTIN-SENSITIVE PROTEASES AND APC/C^{CDH1} BUT NOT 26S PROTEASOME

Yen-An Tang, Ju-Pi Li, and Jia-Ling Yang[1]

Molecular Carcinogenesis Laboratory, Institute of Biotechnology & Department of Life Sciences, National Tsing Hua University, Hsinchu 30013, Taiwan
[1]To whom correspondence should be addressed. E-mail: jlyang@life.nthu.edu.tw

Skp2, an F-box protein of SCF E3 ubiquitin ligase, targets key cell cycle regulators for degradation including p27 and p21 to timely control the progression from late G_1 until mitotic entry, while its protein level is keep low via the APC/C^{Cdh1} and 26S proteasome mediated ubiquitination-degradation pathway at G_0-G_1. Here we show a novel mechanism for the Skp2 destruction under arsenite stress. Arsenite decreased cellular Skp2 protein and mRNA levels in a dose- and time-dependent manner. Under protein synthesis inhibition, arsenite markedly induced Skp2 ubiquitination and degradation. Depletion of Cdh1 using small interfering RNA lowered the arsenite-induced Skp2 ubiquitination and degradation; however, co-treatment with 26S proteasome inhibitors MG132, ALLN, or ALLM did not avoid the Skp2 degradation under arsenite. Intriguingly, co-treatment with leupeptin, an inhibitor of lysosomal proteases, rescued the arsenite-

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

induced Skp2 destruction. The Skp2 down-regulation was parallel to increases in p21 and p27 levels, causing a marked delay of S entry in arsenite-treated G_1 cells. These results suggest that the arsenite-elicited ubiquitinated-Skp2 generated by APC^{Cdh1} is not a target of 26S proteasome, while Skp2 proteolysis under this stress requires lysosomal proteases. This study also suggests that Skp2 down-regulation may be a key contributor to the G_1 arrests caused by arsenite.

2M_02_P

MICROELASTIC GRADIENT GELS TO INDUCE CELLULAR MECHANOTAXIS

Satoru Kidoaki[1*] and Takehisa Matsuda[2]

1: Division of Biomolecular Chemistry, Institute for Materials Science and Engineering, Kyushu University, Fukuoka 812-8581, Japan, e-mail: kidoaki@ms.ifoc.kyushu-u.ac.jp
2: Genome Biotechnology Laboratory, Kanazawa Institute of Technology, Ishikawa 924-0838, Japan

The understanding and realization of directional cell movement towards a harder region of a cell culture substrate surface, so-called *mechanotaxis*, might provide a solid basis for a functional artificial extracellular matrix, enabling manipulation and elucidation of cell motility. The photolithographic surface microelasticity patterning method was developed for fabricating a cell-adhesive hydrogel with a microelasticity-gradient (MEG) surface using photocurable styrenated gelatin to investigate the condition of surface elasticity to induce mechanotaxis as a basis for such substrate-elasticity-dependent control of cell motility. Patterned MEG gels consisting of different absolute surface elasticities and elasticity jumps were prepared. Surface elasticity and its two-dimensional distribution were characterized by microindentation tests using atomic force microscopy (AFM). From analyses of trajectories of 3T3 cell movement on each prepared MEG gel, two critical criteria of the elasticity jump and the absolute elasticity to induce mechanotaxis were identified: 1) a high elasticity ratio between the hard region and the soft one, and 2) elasticity of the softer region to provide medium motility. Design of these conditions was found to be necessary for fabricating an artificial extracellular matrix to control or manipulate cell motility.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2M_03_P

APOPTOSIS OF BLOOD''S CELLS IN FAMILIES OF PATIENT WITH NEUROTIC, STRESS-RELATED AND SOMATOFORM DISORDERS

Lyudmila Ryadovaya

Objective: To establish processes of apoptosis in families of patients with mental disorders. Methods: 55 families of patients with dissociative (conversion) disorders and 36 families of patients with adjustment disorders. We estimate of processes of apoptosis at receptor and cell-like levels for the patients and relatives of the first degree of relationship. Results: We have observed statistically significant increase of expression of a receptor CD95 in patients with dissociative (conversion) disorders in comparison with control (14,73±0,85% and 11,79±0,44%, accordingly, p<0,05). The level of spontaneous apoptosis of neutrophils for the patients and their relatives statistically did not differ from values of control (0,86±0,21%; 0,70±0,48% and 0,25±0,12%, accordingly). Such neutrophils had the smaller size, sometimes in cytoplasm a little large vacuoles were allocated on one pole. Its nucleus had the smaller size with condensation and granulation chromatin on perimeter of its border. For persons with dissociative (conversion) disorders and their relatives is characteristic the tendency to increase of level of spontaneous apoptosis of lymphocytes in comparison with control (1,27±0,28%; 0,99±0,44% and 0,97±0,35 %, accordingly). The statistically significant increase of expression of a receptor CD95 is characteristic for patients with adjustment disorders and their relatives in comparison with control (15,84±0,91%; 14,67±2,56% and 11,79±0,44%, accordingly, p<0,05). The level of spontaneous apoptosis of neutrophils for the patients and their relatives differed from values of control (1,83±0,39%; 1,41±0,85% and 0,25±0,12%, accordingly, p<0,05). The tendency to increase of level of spontaneous apoptosis of lymphocytes is characteristic for the persons with adjustment disorders and their relatives (0,92±0,3%; 1,39±0,73% and 0,97±0,35%, accordingly). Conclusions: the amplification of processes of apoptosis at receptor and cell-like levels is characteristic for the patients with dissociative (conversion) disorders and adjustment disorders in comparison with the mentally healthy people, the tendency to an amplification of this process is characteristic for their relatives of the first degree of relationship. This attribute can be used as a probable marker of mental disorders.

2M_04_P

CDC20 DEGRADATION REQUIRES P38 MAPK AND CDH1-INDEPENDENT APC/C ACTIVITIES DURING CADMIUM-INDUCED MITOTIC ARREST

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Ai-Hsin Yen and Jia-Ling Yang[1]

Molecular Carcinogenesis Laboratory, Institute of Biotechnology & Department of Life Sciences, National Tsing Hua University, Hsinchu 30013, Taiwan
[1]To whom correspondence should be addressed
E mail : jlyang@life.nthu.edu.tw

Cdc20, an activator of the anaphase promoting complex/Cyclosome (APC/C) E3 ligase (APC/C^{Cdc20}), timely targets key mitotic regulators for destruction via ubiquitination, while its destruction triggered by Cdh1-dependent APC/C (APC/C^{Cdh1}) is essential for mitotic exit. We report here the mechanism involved in unscheduled Cdc20 destruction during cadmium stress. Cadmium dose- and time-dependently decreased the Cdc20 levels in CL3 human non-small-cell lung carcinoma cells at exponential growth and early G2 phase. This Cdc20 down-regulation was due mainly to a decrease in protein half-life mediated via the ubiquitin-proteasome pathway. Cdc20 destruction in cadmium-treated early G2 cells was correlated to mitotic arrest, which was rescued by blockage of p38 MAPK activation using SB202190; whereas, JNK or ERK activation were dispensable. The cadmium-elicited Cdc20 degradation was also suppressed in cells stably expressed a dominant negative form of p38 MAPK or knockdown of p38□ via siRNA. Forced expression of a constitutive active MKK6 and p38 markedly induced Cdc20 destruction. Intriguingly, Cdc27 (an APC/C subunit) but not Cdh1 (an APC/C activator) depletion markedly suppressed the Cdc20 destruction under cadmium or overexpressing p38 signaling. Together, these results suggest that stress-induced Cdc20 ubiquitination and proteolysis requires the p38 MAPK signaling and a Cdh1-independent APC/C activity. Such a regulation is necessary for preventing the progression from metaphase to anaphase after cadmium stress.

2M_05_P

MECHANISMS OF TRANSLATION REGULATION DURING COLD-SHOCK IN *ESCHERICHIA COLI*.

Anna Maria Giuliodori, Paolo Marchi, Mara Giangrossi, Anna Brandi, Claudio O. Gualerzi and Cynthia L. Pon.

Laboratory of Genetics, Dept. of Biology MCA, University of Camerino, 62032 Camerino (MC), Italy.
e-mail: annamaria.giuliodori@unicam.it

Cold-shock (cs) translational bias, namely the condition which favors

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

translation at low temperature of cs mRNAs, is one of the main mechanisms by which Escherichia coli cells ensure the selective expression of its cs genes after cold stress. The bias is partly due to intrinsic features of cs mRNAs, which make them prone to translation at low temperature, and to a cold stress-induced transient increase of the Initiation Factors (IFs)/ribosome ratio. In this study we have undertaken the task of: i) identifying the mechanism generating the stoichiometric imbalance of the IFs/ ribosome ratio; ii) unraveling the role of the IFs in the translation bias; iii) elucidating the secondary structure of the paradigm cs mRNA, namely the E. coli cspA mRNA and iv) detecting possible temperature-dependent variations of its structure.
The results obtained indicate that: i) transcription and translation of infA and infC which encode IF1 and IF3, respectively, are activated de novo by cs while ribosomal subunits assembly is slowed down; ii) at low temperature IF3 stimulates the rate of "30S initiation complex" formation with cs mRNAs while inducing the formation of non-productive 70S initiation complexes with non-cs mRNAs; iii) the increased level of IF1 and IF3 during cs is essential to provide a sufficient pool of dissociated 30S ribosomal subunits capable of "70S initiation complex" formation and iv) the structure of cspA mRNA, as determined by chemical and enzymatic probing, changes upon temperature down-shift exposing the translation initiation region.

2M_06_P

EFFECTS OF SOME STRESS FACTORS ON THE BLOOD ANTIOXIDANT DEFENSE SYSTEM IN C57BL/6 MICE

Mikhaylov I.V., Baizhumanov A.A., Akhalaya M.Ya.

Laboratory of Radiation Biophysics, Department of Biophysics, Biology Faculty, M.V. Lomonosov Moscow State University, Moscow, Russia
Corresponding author: Mikhaylov I.V., e-mail: evanmih@gmail.com

Intracellular factors determining the resistance to adverse environmental events probably play a role in the adaptive response. Any moderate adverse event activates defense reserves of the organism via stimulation of oxidation processes, which in turn activate antioxidant system and improve general organism's resistance to stress factors. We studied the effect of short-term swimming in cold water (13°C) on parameters of the blood antioxidant system (SOD, catalase, ceruloplasmin (CP), and nonprotein thiols). The test parameters of antioxidant protection increased 1 h after cold exposure and remained high 1 day after treatment. These changes were accompanied by an increase in the adaptive capacity. After swimming in cold water the resistance of animals to another stress factor (administration of epinephrine) was higher compared to controls. The

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

similar changes of blood antioxidant systems were observed after investigation of another stress exposure (immobilization of animals during 30 min.). But the nonspecific reaction of the organism to environmental factors may be accompanied by the appearance of stress markers which can evaluate role of different stress factors on adaptive response. It is well known the immunosuppressive action of ultraviolet B irradiation (UVB) on living systems. In our experiments *in vivo* we showed the different modulation action of UVB-irradiation on the immobilization stress-induced changes of SOD and CP in blood plasma of animals. The combined exposure of immobilization and UVB provided to stimulation of SOD activity as compared to only action of immobilization, but abolished the stress-induced increase in plasma CP level. The different sensitivity of SOD and CP to UVB-irradiation was observed in *in vitro* experiments: the dose-dependent decrease of CP was shown whereas SOD activity did not changed. We conclude that plasma CP level may be used as marker of UVB-induced immunosuppressive action.

2M_07_P

EVOLUTION OF DESICCATION TOLERANCE: GENOMIC ASPECTS

Dorothea Bartels

Institute of Molecular Physiology and Biotechnology of Plants (IMBIO), University of Bonn, Germany
dbartels@uni-bonn.de

The Scrophulariaceae are one of the most diverse plant families and have been shown to be polyphyletic. Several Scrophulariaceae which belong to the genera Craterostigma and Lindernia desiccation tolerant and are therefore of particular interest. Craterostigma plantagineum has been used extensively to identify molecular mechanisms, which are involved in the acquisition of desiccation tolerance. These studies revealed a large number of genes which are up-regulated in response to dehydration. The genes can approximately be divided into two groups. One group encodes proteins which are directly involved in protection of cellular structures. The other group of genes encodes genes which are involved in regulating the response to dehydration. The dehydration/rehydration cycle is also characterized by a massive sugar conversion from octulose to sucrose.
C. plantagineum belongs to the Linderniae which comprises desiccation tolerant and non-desiccation tolerant plants. Using molecular markers a phylogenetic tree was constructed to determine the molecular relationships of the different plant species. It appears that desiccation tolerant plants cluster together. Comparative studies of these plant species identified conserved genomic structures and very recently evolved mobile signals in stress-relevant genes.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

2M_08_P

MOLECULAR CHAPERONES IN DIAPAUSE EMBRYOS OF THE EXTREMOPHILE *ARTEMIA FRANCISCANA*

Svetla Bojikova-Fournier, Yan Hu, Zhijun Qiu, Tania S. Villeneuve, Yu Sun, Tao Chen, Reinout Amons and Thomas H. MacRae

Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1, Canada

Diapause destined embryos of the extremophile crustacean, *Artemia franciscana*, encyst as gastrulae (cysts), exhibiting profound reduction of metabolic activity and extreme stress tolerance. Resistance to stress is thought to depend partly on the developmentally regulated synthesis of three small heat shock proteins (sHSPs), only one of which is stress inducible. The sHSPs form poly-disperse oligomers and function as molecular chaperones. The best characterized *Artemia* sHSP, termed p26, confers thermal tolerance on transfected mammalian cells and inhibits apoptosis. Molecular analyses by site-directed mutagenesis revealed the importance of individual p26 amino acid residues, including a highly conserved arginine, in chaperone activity, and suggested that β-strand 7 has an important role in oligomerization. Artemin, a ferritin homologue, is also abundantly up-regulated in diapause destined *Artemia* embryos. Artemin lacks the di-iron ferroxidase center characteristic of ferritin and is enriched in cysteines that may promote protein stability by disulphide bond formation. Interestingly, artemin and ferritin both function as molecular chaperones *in vitro* and artemin confers thermal and oxidative tolerance on transfected mammalian cells. Artemin appears to have evolved from ferritin, losing the ability to bind iron, but retaining chaperone activity. As a consequence of substantial accumulation in diapause embryos, artemin has the potential to contribute significantly to stress resistance. The available evidence collectively favors the hypothesis that molecular chaperones play critical roles during diapause in *A. franciscana*, and by extrapolation, in other organisms.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Module 3 – Oral lectures:

3A_01_S000

AN INTEGRATED VIEW OF THE STRESS RESPONSE AND STRESS-RELATED BEHAVIORAL AND /OR SOMATIC DISORDERS

George P. Chrousos

Athens University Medical School, Athens, Greece

Stress activates the central and peripheral components of the stress system, i.e., the hypothalamic-pituitary-adrenal (HPA) axis and the arousal/sympathetic system. The principal effectors of the stress system are corticotropin-releasing hormone (CRH), arginine vasopressin, the proopiomelanocortin-derived peptides alpha -melanocyte- stimulating hormone and beta-endorphin, the glucocorticoids, and the catecholamines norepinephrine and epinephrine. The developing brain undergoes rapid growth and is characterized by high turnover of neuronal connections during the prenatal and early extrauterine life. These processes and, hence, brain plasticity, slow down during childhood and puberty and plateau in young adulthood. Hormonal actions in early life, and to a much lesser extent later, can be organizational, i.e., can have effects that last for long periods of time, frequently for the entire life of the individual. Hormones of the stress system and sex steroids have such effects, which influence the behavior and certain physiologic functions of individuals for life. Exposure of the developing brain to severe and/or prolonged stress may result in hyperactivity/hyperreactivity of the stress system, with resultant amygdala hyperfunction (fear reaction), decreased activity of the hippocampus (defective glucocorticoid negative feedback, cognition) and the mesocorticolimbic dopaminergic system (dysthymia, novelty seeking, addictive behaviors), hyperactivation of the HPA axis (hypercortisolism), suppression of reproductive, growth, thyroid and immune functions, and changes in pain and fatigue perception. These changes may be accompanied by disturbed childhood, adolescent and adult behaviors, including excessive fear ("inhibited child syndrome") and addictive behaviors, dysthymia and/or depression and gradual development of components of the metabolic syndrome X, including visceral obesity, diabetes mellitus type 2 and essential hypertension. Prenatal stress exerted during the period of sexual differentiation may be accompanied by impairment of this process, with behavioral and/or somatic sequelae. The vulnerability of individuals to develop varying degrees and/or components of the above life-long syndrome is defined by as yet unidentified genetic factors, which account for up to 60% of the variance. CRH has marked kindling and glucocorticoids have strong consolidating properties, hence both of these hormones are crucial in the development of, and can each alone produce, the above syndrome. CRH and glucocorticoids may act in synergy, as in acoustic startle, while glucocorticoids may suppress or

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

stimulate CRH, as in the hypothalamus and amygdala respectively. A CRH receptor type 1 antagonist antalarmin inhibits both the development and expression of conditioned fear in rats and has anxiolytic properties in monkeys. Profound stressors, such as those from sexual abuse, rape, etc., may elicit components of post-stress the syndrome in older children, adolescents and adults as well. Most frequently, chronic dysthymia and/or depression may ensue, associated with gastrointestinal complaints and/or the premenstrual tension syndrome. A lesser proportion of individuals may develop the classic posttraumatic stress disorder characterized by hypocortisolism and intrusive and avoidance symptoms; in younger individuals it may present as dissociative personality disorder.

3A_02_S

STRESS AND ADAPTATION: CHANGES IN THE SYMPATHOADRENAL SYSTEM ACTIVITY

Kvetnansky, R.

Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia

According to Hans Selye stress is an unspecific response of the organism to any demand made upon it. However, the specific activation of two components of the sympathoadrenal system (adrenomedullary and sympathoneural) by various stressors has been shown.
The aim of the present work was to investigate changes in enzymes involved in norepinephrine and epinephrine (EPI) synthesis - tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) – their gene expression, immunoprotein levels and activities in the adrenal medulla (AM), sympathetic ganglia, hearts and brains of rats and mice after a single or repeated exposure to various stressors. Immobilization for 2h (IMO), cold 4ºC (COLD), administration of insulin 5IU (INS) or 2-deoxyglucose 500 mg/kg (2DG) were used. A single exposure to IMO, COLD, INS or 2DG was found to induce increases in TH, DBH and PNMT mRNA levels in the studied organs. Increased transcription rate is responsible for stress-induced TH and PNMT gene expression. Repeated exposure to these stressors elevated besides mRNA also activity and immunoprotein levels of the enzymes. Various stressors regulate TH, DBH and PNMT gene expression by different transcriptional mechanisms. PNMT gene expression is mainly regulated by HPA axis and in corticoliberine gene knock-out mice is basically reduced especially after stress exposure. One day cold exposure elevated TH mRNA levels in AM, however, 28 day cold exposure did not show any changes. Cold-adapted rats, however, responded to

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

heterotypic novel stressors (IMO, INS, or 2DG) by exaggerated responses. Thus, our data suggest an adaptation of TH and PNMT gene expression during long-term exposure to stressors. An exposure of adapted rats to novel stressors induces exaggerated responses. It is the readiness of the long-term stressed organism to overrespond, already at the level of expression of genes, to the changed quality of stressor, that we consider as an important adaptive phenomenon of the sympathoadrenal system.
Supported by Slovak APVV Grant 0148-06 and VEGA Grant 2-5125.

3A_04_S

CHOLINESTERASE MODULATIONS OF MAMMALIAN STRESS AND ANXIETY REACTIONS

Hermona Soreq

The Hebrew University of Jerusalem; Safra Campus-Givat Ram; Jerusalem 91904; Israel

Stress and anxiety disorders present a major mental health problem, but their putative involvement in the initiation and/or progression of neurodegenerative diseases is being debated. Recent research in the lab focuses on the molecular mechanism(s) underlying anxiety-induced changes in cholinergic neurotransmission. These mechanisms modulate the motor control over movement, regulate working memory, and activate brain-to-body communication through the neuron-immune interface modifying blood cells composition and platelet production. Importantly, stress-associated changes were found in the expression pattern of the acetylcholinesterase *ACHE* gene, which encodes the acetylcholine hydrolyzing enzyme AChE. AChE is not one, but a combinatorial series of proteins having indistinguishable enzymatic activity yet with variant N- and C-termini due to alternate promoter usage and 3'-alternative splicing. Differentially induced under stress, they show distinct non-hydrolytic properties, interact with variant-specific protein partners and induce inverse signaling cascades. Surprisingly, transcriptional and post-transcriptional regulation of AChE pre-mRNA not only protects blood and nerve cells from acute dangers, but may also entail long-term advantages. Specifically, causal involvement of both AChE and its closely related enzyme butyrylcholinesterase (BChE) in the progression of Alzheimer's and Parkinson's diseases, anticipates future therapeutic needs for drugs targeting specific cholinesterases or the corresponding RNA transcripts.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Module 3 – Poster lectures:

3A_01_P

SEVERE STRESS AND ITS IMPLICATIONS: THE NEED TO RECOGNISE CONTRA-VITAL PATHOPHYSIOLOGICAL RESPONSES

C. P. Arun

Kellogg College, University of Oxford, Oxford, UK
Email: arunpeter@yahoo.com

INTRODUCTION For animals in the wild, predation, *not* old age is the most common mode of death. Nature appears to provide for the almost inevitable event of death from some form of trauma by allowing a quick final exit. REVIEW OF LITERATURE Our previous work (Arun, 2004) presented a contrarian view of pathophysiology, asserting that the catecholamine response (that lasts for only 24 hours) in acute stress plays a deciding role in the survival of the animal exposed to severe stress and could actually hasten death if the situation is hopeless. HYPOTHESIS We propose the use of the term 'contra-vital'(= deleterious to life) to qualify actions taken by an organism's organ systems to accelerate its own death. Our view is at variance with contemporary scientific thinking that reflexes exist only to promote the organism's survival. From the point of view of the individual animal, initiation of the contra-vital response is beneficial when death is inevitable since it would diminish suffering. From a sociobiology standpoint, a quick death of a severely injured member is advantageous to the herd since rather than a futile wait for an irrecoverably injured animal to recover, the herd can move on, accepting the animal's death as a fact. DISCUSSION The systemic response to sepsis appears to incorporate a contra-vital reflex and this will be elaborated in this paper. The recent worldwide initiative 'Surviving Sepsis' campaign is a tacit recognition of the existence of contra-vital responses. CONCLUSIONS Physiological systems appear to have a self-destruct mechanism available to accept defeat in the face of insuperable stress. There is a need for the scientific community to mature in its thinking, fight the taboo of studying death and recognize that contra-vital reflexes are a reality in severe stress.

3A_02_P

THE EFFECT OF AGEING ON THE STRESS PROTEINS OF THE CYTOSOL AND ENDOPLASMIC RETICULUM.

Jordan L. Holtzman

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Departments of Pharmacology and Medicine, and Division of Environmental Health Sciences University of Minnesota, Minneapolis, MN, USA

The cytosol, the endoplasmic reticulum (ER) and the mitochondria contain a number of stress proteins which are essential for life. A major group of these proteins are the heat shock proteins (Hsps). Members of this family are found in all three of these compartments. The cytosolic forms were initially identified because they show significant increased levels when cells are exposed to mild hyperthermia (42° C). This response is mediated through the heat shock factors (HSFs). These are transcription factors which when activated by heat stress form trimers which bind to the promoter regions of the Hsp DNAs. In *ex vivo* studies of human leukocytes and rat heptocytes, cells from older subjects showed a decrease in the Hsp70 response to heat stress, while the levels of the constitutive form, Hsc70 were unaffected. In some studies this decreased response was attributed to an age dependent loss of the HSFs; while others have suggested that their levels do not decline, but that the trimers become functionally inactive. Hence there is a decline in the transcription of the mRNA for Hsp70, even though the HSFs form trimers. Other studies have suggested that the level of Hsp70 has a major impact on longevity. For example, in humans it has been reported that longer lived individuals have higher levels of Hsp70 than that is seen in those with shorter life spans. In particular, centenarians have a mutation in the promoter region of HSP70-1 which increases its level. Similarly, the *dauer* mutation of the IGF2 receptor in *C. elegans*, which doubles longevity, is also associated with increased levels of Hsp70 and Hsp90. Finally, in *Drosophila* high levels of the mitochondrial chaperone, Hsp22, increases longevity by 30%. One treatment which has repeatedly been demonstrated to increase longevity by as much as 30% is caloric restriction. This increased longevity is associated with an increase in the cellular levels of the cytosolic Hsps. The Hsp70 level is also modulated by both plasma membrane and cytosolic hormone receptors. For example, stimulation of the α_1 receptor of vascular smooth muscle leads to increased hsp70 as does estrogen treatment of neurons. Both responses marked decline with age. There are a number of stress proteins found in the ER. Yet, unlike the cytosolic Hsps, these do not appear to respond to heat stress, even though some, such as Bip (GRP78) and GRP94 are members of the Hsp family. Similarly, unlike the cytosolic Hsps, Bip is not affected by caloric restriction. Finally, studies from our laboratory have indicated that in rat, hepatic microsomes the constitutive levels of some of the ER stress proteins decline with age. These included BiP, Erp55 (PDI), Erp57, Erp72 and calnexin; while a sixth, calreticulin, was unaffected. The most surprising observation was that, even though these animals were maintained from weaning to death in a colony with a constant environment, three of these proteins, Bip, Erp55 and Erp57, showed a circsemiannual rhythm with peaks in January and July. This rhythm would appear to have been imprinted in the genome of their wild ancestors and

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

has remained stable for over 200-400 generations. In conclusion, there is a large body of data indicating that declines in stress proteins with age may be a major factor in the reduced capacity of elderly animals to respond to a variety of stresses.
These decreased responses would also appear to have a major effect on longevity.

3B_01_P

PSYCHOPHYSIOLOGICAL EFFECTS OF NEUREXAN® ON STRESS-INDUCED ELECTROPSYCHOGRAMS. A DOUBLE BLIND, RANDOMIZED, PLACEBO-CONTROLLED STUDY IN HUMAN VOLUNTEERS

Wilfried Dimpfel

Justus Liebig University Giessen c/o NeuroCode AG, D 35578 Wetzlar, Sportparkstr. 9, Germany, w.dimpfel@neurocode-ag.com

Objective. There is evidence for the effectiveness of natural drugs in treatment of anxiety. However, there is a need for further prospective, double blind, randomized, placebo controlled clinical studies which demonstrate efficacy.
Methods. A preparation containing (amongst others) low dose extracts of passiflora incarnata and avena sativa (Neurexan®) was tested in 30 healthy human volunteers in a randomised, placebo controlled cross-over trial. Eligible subjects aged 30 – 60 years were assessed under conditions of relaxation and under experimental stress-induction (calculation of mathematical tasks with financial reward or punishment according to performance). After recording of the electropsychogram (electrical activity in the presence of emotional provocations) frequency analysis using Fast Fourier Transformation and analysis of source density was performed 1 to 4 hours after administration of 4 tablets verum or placebo. Task dependent increase of beta 2 power was used as a surrogate parameter of a stress induced anxiety. The same experimental procedure was repeated for each subject on the 'crossed-over' medication after an interval of at least one week.
Results. In the presence of verum, circadian enhancement of alpha 2 waves was blunted. Beta 2 power was statistically significantly lower during the second and third hour after administration in comparison to placebo. Values returned to baseline values 5 hours after administration. The preparation was very well tolerated.
Conclusion. Recording of electrical activity during a stress-related emotional situation revealed a smaller increase of beta 2 power under verum compared to placebo. This is indicative of better coping with task related stress whilst on Neurexan®.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

3B_02_P

GLYCOBIOLOGY OF STRESS

Olga Gornik and Gordan Lauc

[1]*University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia. glauc@pharma.hr*

Psychological stress is associated with numerous diseases, but molecular mechanisms linking stress to the development of disease are only starting to be understood. Stress alert is conveyed by hormonal signals throughout the body, yet a particular cell response to a hormonal signal is not determined by the signal itself, but by the molecular composition, energy content, and by the physiological role and current status of the target cell. Stress induced changes in glycoconjugate structures and expression of their receptors lectins appear to be an important molecular consequences of stress experience. At the moment only several fragments of the glycobiological mechanisms involved in the physiological response to psychological stress are known, but the complete picture is slowly emerging. Corticosteroids affect activity of at least one glycosyltransferase both in vitro and in vivo. Altered activity of glycosyltransferases results in different carbohydrate structures attached to glycoproteins, and these changes have been demonstrated both in humans and in experimental animals. A change in the carbohydrate structures attached to a glycoprotein is a well-established way to change its structural and functional properties, and recently this was shown to be one of the mechanisms that control activity of membrane receptors. Although this type of glycosylation-mediated receptor modulation in stress still has to be proven, it is a very interesting hypothesis. On the other hand, new glycoconjugate structures could also represent novel signals on the cell surface that could alter interaction of the cell with neighboring cells. Stress is also known to be associated with the appearance of novel lectins that could be receptors for either novel, or also "normal" glycoconjugate structures, translating their structures into molecular functions. Although most of this is still largely speculative, hopefully more will be known soon about the molecular role of glycoconjugates, their lectin receptors, and glycosyltransferases in the physiological response to psychological stress.

3B_03_P

SECOND-HAND STRESS: NEUROBIOLOGICAL EVIDENCE FOR A HUMAN ALARM PHEROMONE

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

LR Mujica-Parodi, Helmut H Strey, Blaise Frederick, Robert Savoy, David Cox, Bosky Ravindranath, Yevgeny Botanov

Laboratory for the Study of Emotion and Cognition, Departments of Biomedical Engineering and Psychiatry, Stony Brook University School of Medicine, Health Sciences Center T18, Stony Brook, NY 11794-8181 USA

Pheromones function as airborne chemical signals released by an individual into the environment that, even in the absence of conscious perception, affect the physiology or behavior of other members of the same species. While almost all clinical research in the area has concentrated on human *reproductive* pheromones, non-human mammals are known to also possess *alarm* pheromones, which rapidly transmit warning of danger to others of the same species via olfaction. The existence of alarm pheromones is well-established in mammals, with animals exposed to odors secreted by acutely stressed conspecifics expressing physiological and behavioral changes that are indistinguishable from their reactions to predators, including increased neural activity in the amygdala, analgesia, increased hiding and avoidance, freezing and rearing, hyperthermia, and air-sampling. Using functional MRI, we demonstrate here the first direct evidence for a human alarm pheromone, with humans showing brain activation of the amygdala and hypothalamus—the primary brain regions responsible for the fear response—during inhalation of sweat taken from a sample of first-time skydivers, with exercise sweat as a control. The fMRI subjects were unable to identify the samples, and rated the odors for both conditions as mild and non-aversive. The results were found for breathing the fear sweat, but not sniffing it, most likely reflecting the fact that the neural circuitry associated with sniffing is explicitly tied to conscious odor detection, while pheromones are hypothesized to operate unconsciously. Both males and females showed equivalent neural activation, thereby ruling out the influence of sexual pheromones in accounting for our results. Our findings indicate that there may be a hidden biological component to human social dynamics, in which emotional stress is, quite literally, "contagious."

3B_04_P

OVER-EXPRESSION OF THE ESTROGEN RECEPTOR BETA ASSOCIATED PROTEIN HSP27 IS ATHEROPROTECTIVE

Edward R. O'Brien, Xiaoling Zhoa, Katey Rayner, Yong-Xiang Chen

University of Ottawa Heart Institute, 40 Ruskin Street, Ottawa, ON K1Y 4W7 CANADA, eobrien@ottawaheart.ca

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Recently, we discovered that HSP27 is an estrogen receptor beta associated protein that represses estrogen mediated transcription (Miller et al, ATVB 2005). Moreover, in human coronary arteries the expression of HSP27 decreases with the progression of atherosclerotic disease stage and serum levels are >3-fold higher in normal controls compared to patients with angiographic evidence of coronary artery disease. Purpose: To determine if HSP27 is protective against the development of atherosclerosis. Methods: Mice over-expressing human HSP27 (hHSP27) were crossbred with atherosclerosis-prone apoE null mice (apoE) to yield hHSP27apoE mice (n=9 female, 5 male) and compared to apoE mice (n=7 female, 6 male) for aortic lesion development. All mice were fed a cholesterol supplemented diet for 4 weeks and euthanized at age 10 weeks. Results: All mice were viable, of similar body weight and had equally elevated cholesterol levels (approximately 1100 mg/dL). Quantitative histomorphological studies of en face aortic specimens revealed that the percentage aortic area with overt atherosclerosis was similar in both groups of male mice. However, amongst female mice there was a 41% reduction in atherosclerotic lesion area in the hHSP27apoE vs. apoE mice ($p<0.001$). Moreover, there was a decrease in the degree of vessel wall lipid deposition and inflammation in the hHSP27apoE vs. apoE mice. Conclusions: Over-expression of HSP27 is associated with attenuated atherogenesis but appears to occur only in female mice – thereby suggesting that ovarian hormones may be instrumental in mediating this effect. Studies focusing on the precise mechanisms of the HSP27 atheroprotective effect including the interplay with estrogen receptor modulation are ongoing.

3B_05_P

CHARACTERIZATION OF AUTONOMIC NERVOUS SYSTEM (ANS) STABILITY AND VARIATIONS BY THE ANS SPOT

Markku Paloheimo, MD, PhD

Eye Hospital, Helsinki University Hospital, Box 220, 00029 HUS, Finland, and GE Medical, Research, Helsinki, Finland, markku.paloheimo@hus.fi

The autonomic nervous system enables all of our body systems to operate in an external environment that is both physically and emotionally challenging. A Google search using words "stress autonomic nervous system" yielded 43,500 hits. Among the tests of parasympathetic cardiovagal regulation include heart rate analysis, heart rate variation with deep breathing, variation of beat-to-beat blood pressure and heart rate (HR), and the Valsalva ratio. Tests of sympathetic adrenergic vascular regulation include blood pressure analysis in various situations, the

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Valsalva manoeuvre, sustained handgrip, mental stress, and cold water immersion. Tests of sympathetic cholinergic sudomotor function include the sympathetic skin response, quantitative sudomotor axon reflex test, sweat box testing, and quantification of sweat imprints.

We have developed a method to display neural or hormonal β-adrenergic responses of **heart rate** and peripheral cholinergic neural vascular responses of **finger pulse amplitude** to characterize the autonomic state and variations during general anaesthesia and sedation. This was done by displaying pulse-to-pulse intervals (in ms) on the X-axis and subsequent plethysmographic pulse wave amplitudes on the Y-axis (see Figures). The data is obtained from Datex-Ohmeda monitors *via* serial interface and the source of the plethysmographic signal is a common pulse oximetry sensor on the subject's finger. The number of traced observations can be set (Number of points). Six most recent data is shown as red dots and the older ones in white, which forms a trend. Low sympathetic activity is characterized by high pulse amplitudes and low heart rates (long intervals). Changes in autonomic activity are shown as a movement of the spot; vagal activation is seen as pulse rate slowing and increase in sympathetic activity is heralded by a decrease in pulse plethysmographic amplitude and increase in heart rate. During anticholinergic and β-blocking medication, heart rate variability is decreased, but these drugs do not affect pulse amplitude variability.

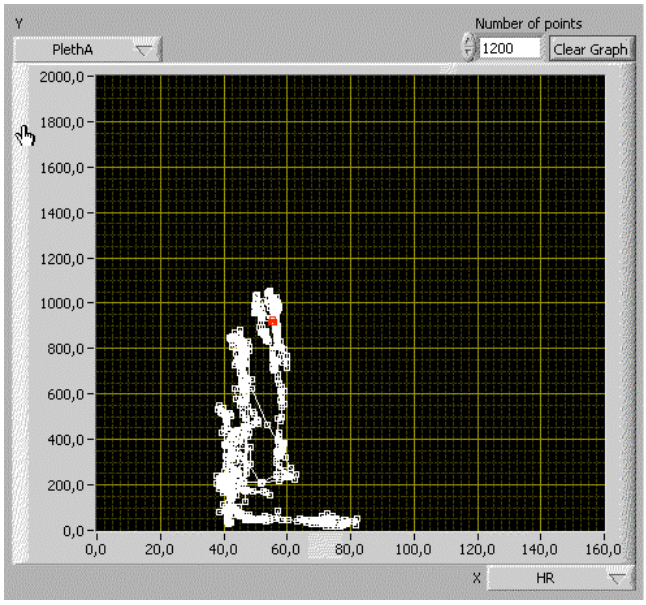

Fig 1. ANS spot after induction of anesthesia, intubation and start of operation.

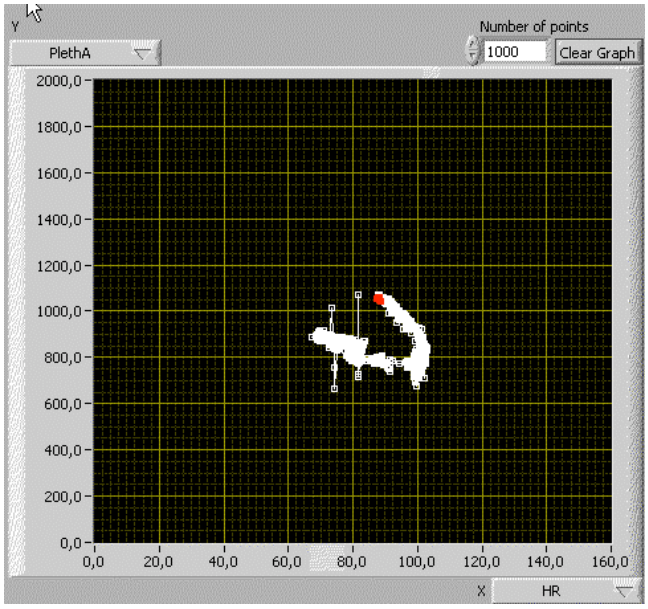

Fig 2. Response to painful stimulus and fentanyl

This noninvasive method has been used to follow autonomic state and responses during general anaesthesia, conscious sedation, intensive care and psychological testing. A Surgical Stress Index (see Google) displayed on a scale of 0 -100 is under development.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

3B_06_P

A SYSTEMS GENETICS ANALYSIS OF THE GENERAL STRESS RESPONSE

David Threadgill

Department of Genetics, University of North Carolina, Chapel Hill, NC 27599, USA dwt@med.unc.edu

A new multidisciplinary research effort called the Interdisciplinary Consortium on the Genetics and Co-Morbidity of Stress (ICOGS) has been formed to investigate the genetic variability among individuals in the molecular and physiological consequences of chronic activation of the general stress response. This international effort is exploiting systems genetics to understand the biological consequences of chronic activation of the general stress responses—a growing problem in modern societies affecting millions of people. The ICOGS will provide a common platform for data integration to support a better understanding of the interactions between genetics and environmental stress that leads to increased disease susceptibility. The integrating platform is a novel and highly innovative, diverse genetic reference population of mice called the Collaborative Cross (CC). The CC is a population-level mouse model to facilitate integration of multiple data types using fixed, reproducible genomes representative of the genetic complexity existing in humans. The beauty of the CC model is its extensibility, supporting data integration over space and time and at all levels - from molecules, to cells, physiological systems, and environments. The current application by the ICOGS is linking genetic polymorphisms within the CC population with variable levels of transcripts, metabolites and physiological characteristics in response to emotional and metabolic stress. Variability in these intermediate biomolecules is being linked to differential susceptibility to behavioral, cardiovascular, immunological and metabolic diseases. The platform developed by the ICOGS is open to the larger stress research community. The current status of the ICOGS program and routes of access will be presented.

3B_07_P

RAPID EFFECTS OF ESTRADIOL (E_2) REVEAL THAT CORTICOTROPIN-RELEASING FACTOR (CRF) IS AN IMMEDIATE EARLY GENE (IEG)

Rosalie M. Uht* and Avin S. Lalmansingh

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

University of Virginia, School of Medicine, Departments of Pathology and Biochemistry and Molecular Genetics (RMU) and the Neuroscience Graduate Program (RMU and ASL)
**corresponding author; ruht@virginia.edu*

Depression is a widespread psychiatric disorder that affects 19 million people a year in the United States alone. Although its etiology is poorly understood, one feature is clear: it involves dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. Additionally, an epidemiologic feature is that depression is 2-3 times as prevalent in women than in men. Thus, understanding the molecular pathogenesis of the disease involves elucidating mechanisms by which E_2 influences HPA activation. E_2 exerts many of its effects by binding to estrogen receptor -alpha or -beta (ERα or β). A subpopulation of hypothalamic neurons expresses CRF and ERβ. Also, there is evidence for E_2 regulation of CRF *(crf)* gene expression through both receptors. Thus, we postulated that E_2 regulates *crf* expression through ERs. To test this hypothesis, we used a neuronal cell line that expresses CRF. Treatment with E_2 led to an increase in CRF mRNA levels within 1-3 mins. This increase was unaffected by treatment with cyclohexamide. Thus, *crf* meets the standard criteria of an IEG. Additionally, the increase in mRNA levels correlated tightly with *crf* promoter occupancy by ERα an ERβ and an altered state of histone 3 and 4 acetylation. Taken together the data suggest that, like a cellular stress response (e.g. rapid expression of c-*fos*), a systemic stress response involves IEG activation. Furthermore, it does so *via* an epigenetic mechanism. Support: NIH R01 NS39951, and an NARSAD Young Investigator Award (RMU), and NIH T32 NS044851, a Temporal Biology Training grant for Minorities (ASL).

3B_08_P

"KEEPING ALL TOGETHER IN A FRAGMENTED WORLD: THE EVIDENCE BASE OF THE SALUTOGENIC RESEARCH

Monica Eriksson and Bengt Lindström

Folkhälsan Research Center, Health Promotion Research Program, Paasikivigatan 4, FIN-00250 Helsinki, Finland

This conference focuses on linking knowledge from different areas of stress research particularly exploring the system level. Thus trying to make sense of our fragmented world. Social trends point to a major upset of the traditional social structures such as the rupture of local and intimate networks because of migration into urban areas, changed function and

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

structure of family networks, changes in the patterns of working life and communication and information technology. At the same time there are concerns regarding increasing violence, alienation, a decreasing physical and mental health. All this makes it difficult to find and run a coherent life. Besides the research of Selye there are other theories and frameworks contributing to stress research. One of them is the salutogenic theory developed by Aaron Antonovsky in the late 1970s. This presentation focuses on the salutogenic approach such as Aaron Antonovsky formulated his salutogenic theory "Sense of Coherence" (SOC) as a global life orientation to view the world as comprehensible, manageable and meaningful. He claimed that the way people view their life has a positive influence on their health.[1, 2] The salutogenesis has become an established concept and considered as a paradigm shift from the pathogenic focus on risk factors for disease to the salutogenic focus on the strengths and determinants for health. How do people manage the lack of control of the life? The answer was formulated in terms of SOC and General Resistance Resources. The SOC is a resource that enables people to manage tension, to reflect about their external and internal resources (GRRs), to identify and mobilize them, to promote effective coping by finding solutions and resolve tension in a health promoting manner.

The findings from an ongoing review of the salutogenic research 1992-2003 by the authors is presented.[3-7] The review consists of 458 scientific articles and 13 doctoral theses. To date the SOC questionnaire has been used in at least 33 languages in 32 countries all over the World. The SOC scale seems to be a reliable, valid, and cross culturally applicable instrument measuring health. SOC tends to increase with age. SOC is strongly related to perceived good health, especially mental health, and QoL. The stronger the SOC the better the perceived health in general. SOC seems to have a main, moderating or mediating role in the explanation of health. It seems to be able to predict health and quality of life. The applicability of the SOC concept in practice on both an individual, a group and on a societal level is discussed. With the evidence base of the salutogenic research on hands the conclusion is that the Salutogenesis is a valuable approach for individuals and groups in order to build capacity and coherence and healthy societies.

1. Antonovsky A. Health, Stress and Coping. San Francisco: Jossey-Bass; 1979.
2. Antonovsky A. Unraveling the Mystery of Health. How people manage stress and stay well. San Francisco: Jossey-Bass; 1987.
3. Eriksson M. Unravelling the Mystery of Salutogenesis. The evidence base of the salutogenic research as measured by Antonovsky's Sense of Coherence Scale. Doctoral thesis, submitted. Turku: Åbo Akademi University; 2007.
4. Eriksson M, Lindström B. Validity of Antonovsky's Sense of Coherence Scale - a systematic review. J Epidemiol Community Health 2005;59(6):460-466.
5. Eriksson M, Lindström B. Antonovsky's Sense of Coherence Scale

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

and the relation with health - a systematic review. J Epidemiol Community Health 2006;60:376-381.
6. Eriksson M, Lindström B. Antonovsky's Sense of Coherence Scale and It's relation with quality of life: A systematic review. J Epidemiol Community Health 2006;Accepted January 2007.
7. Eriksson M, Lindström B. A Sense of Coherence and health. Salutogenesis in a societal context: Åland - a special case? J Epidemiol Community, Health In press 2007.

3B_09_P

O-GLCNAC, A NEW PARADIGM FOR REGULATING STRESS-SIGNALING NETWORKS

Natasha E. Zachara[1], Henrik Molina[1,2], Akilesh Pandey[1,2], Gerald W. Hart[1].

1. Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21218.
2. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD 21287.
nzachara@jhmi.edu

In response to numerous forms of cellular stress, levels of the O-GlcNAc protein modification are elevated rapidly and dynamically on myriad nucleocytoplasmic proteins. Increased O-GlcNAc levels are linked to stress tolerance in both cell culture and animal models, suggesting that this phenomenon is a survival response of cells. Elevated levels of O-GlcNAc have been linked to changes in Hsp70 and Hsp40 protein levels, as well as altered capacitive calcium entry, suggesting at least two mechanisms by which O-GlcNAc may protect cells. These, and other data, *suggest a new paradigm in the regulation of stress mediated signal transduction pathways, further investigation of which will elucidate new roles for O-GlcNAc in diverse clinical and cellular settings such as Ischemia-reperfusion injury, neurodegenerative diseases and aging.* In order to better understand the mechanism(s) by which O-GlcNAc modulates the cellular response to stress, we have determined which proteins are dynamically O-GlcNAc modified in response to cellular stress using Stable Isotope Labeling with Amino Acids in Cell Culture. We have identified 27 O-GlcNAc modified proteins, of which 19 exhibited an increase in O-GlcNAc protein modification in response to heat shock. These proteins are predominantly involved in signal transduction, transcription and vesicle transport. Currently, we are examining the role of glycosylation on these proteins, and determining how this contributes to O-GlcNAc mediated stress tolerance. Under a licensing agreement between Covance and Johns Hopkins University, GWH receives a share of royalties on sales of CTD110.6.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

3C_01_P

THE IMPACT OF THE YEAST RAC-SSB CO-TRANSLATIONAL SYSTEM OF CHAPERONES ON THE CELLULAR ACTIVITY OF MUTATED PROTEINS

Katarzyna Tomala, Ryszard Korona

Jagiellonian University, Institute of Environmental Sciences, address: Gronostajowa 7, 30-387 Kraków, Poland; email: tomala@eko.uj.edu.pl

A large fraction of spontaneous mutations lead to destabilization of protein structure. This results in exposure of hydrophobic patches on the protein surface. It is thus very likely that mutant proteins are more frequently bound by the molecular chaperones. The subsequent processing of excessively unstable proteins is poorly known. The more frequent binding by the chaperones may help to overcome the effect of mutation and secure functioning of the protein in the cell. Alternatively, the excessive activity of the chaperones concentrated on a defective polypeptide may constitute a signal that it has to be degraded. Our research was focused on the RAC-Ssb system of co-translational chaperones in yeast. We asked whether its role is to uphold folding efficiency of mutated proteins or rather sort them out and exclude from the cellular metabolism. We prepared a library of mutagenized *ADE2* gene whose inactivity or malfunctioning results in red pigmentation of the yeast colonies. We then screened for mutations producing red color in strains in which RAC-Ssb was inactivated by gene deletion. No such synthetic phenotypes were found indicating that mutational destabilization of Ade2p is not aggravated in the absence of the chaperone activity. In a second screen, we first identified an array of mutationally destabilized variants of Ade2p proteins. This was marked by their thermosensitivity. In case of all mutants, their growth on media lacking adenine was better when the RAC-Ssb was inactivated. This indicates that the activity of chaperones leads to a decline of a cellular pool of active Ade2(ts) molecules. Current experiments show that the defective protein is present in the cell in form of aggregates. We suggest that the RAC-Ssb can have a role in sequestering defective proteins from the cytosol and restricting their uncontrolled interactions with other elements of the cellular metabolism.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

3C_02_P

MOLECULAR STUDY OF ACID SHOCK PROTEINS IN A DIARRHEAGENIC ENTEROAGGREGATIVE *ESCHERICHIA COLI* (EAEC)

Puneet Badesha

EAEC is an emerging pathotype for persistent pediatric diarrhea characterized by an aggregative adherence (AA) pattern to HEp-2 cells. Its pathogenesis is poorly understood. Like other enterics, it faces acidic conditions in stomach and small intestine. Therefore, the adaptive strategies like acid tolerance responses (ATRs) are induced to ensure its survival during the disease progression. Outer membrane proteins (OMPs) are the key molecules that interface cell with the environment and are important in adhesion, invasion and intracellular survival of pathogens in the host. Most notable pH-response regulators like Fur, PhoP, OmpR and RpoS are reported and the *rpoS*, an alternative sigma factor is well known for the induction of ATRs in *S. typhimurium* and EHEC.
ATR in EAEC was studied by growing cells at variable pH range (2.0-7.4). Cell survival was observed till pH 4.0 but the preadapted cells (adapted at mild acidic pH 5.0 for 2 hours), survived two fold better than that of the non-adapted cells (pH 4.0). Phenotypic characterization under acid stress conditions was checked *in vitro*. At pH 4.0, clump formation was totally abolished. The biofilm score was <+1.0 (partial honeycomb formation) even at 12h of growth at pH 4.0 whereas in control it was +3 (three dimensional mounds with visible substratum). Diffusely adherent (DA) pattern with HEp-2 cells was observed at pH 4.0 in contrast to typical aggregative adherence (AA) in control. The overlapping of 2-D gels of OMPs (pH 7.4 vs. 4.0) and digitized PDQuest bioinformatic software analysis confirmed the six differentially expressed proteins at pH 4.0. The intensity of the main porins was lower at pH 4.0. The spots of interest (pH 4.0) were digested with trypsin and were analyzed by MALDI-TOF/MS, displayed a "peptide fingerprint" of the protein. Western blot analysis with stress specific antibodies further revealed ASPs in EAEC are differentially expressed, which might be the reason for variation in phenotypic characteristics. The expression of ASPs in *rpoS* mutant was absent (pH 4.0). Therefore, the putative ASPs associated with an acid-tolerant phenotype under *rpoS*, provides a new information regarding EAEC survival at low pH. Further, characterization of *rpoS* may reveal more specific targets aimed for the disease treatment.

3C_03_P

SYSTEMIC ANALYSIS OF HEAT SHOCK AND PROTEASOME INHIBITION USING PROTEOMICS AND FUNCTIONAL GENOMICS

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Hee-Jung Kim, Yung Hee Kim, Jaeho Jeong, Kong-Joo Lee

Center for Cell Signaling and Drug Discovery Research, College of Pharmacy and Division of Life & Pharmaceutical Sciences, Ewha Womans University, Seoul 120-750, South Korea
kjl@ewha.ac.kr

Heat shock response is well conserved adaptation phenomenon characterizing drastic repression of total cellular protein synthesis and induction of heat shock proteins (Hsps). Various extracellular stresses as well as heat shock induce heat shock response and render cells cross resistance to lethal stresses. Recently, denatured proteins have been suggested as the sensor of extracellular physical stresses. Cells have two clearance systems of the misfolded proteins, proteasomal and autophagic degradation. For the proteasomal degradation, misfolded protein are tagged by polyubiquitin and guided by proteasome shuttle chaperone CDC48/p97. MG132 is the inhibitor of proteasomal function, resulting in accumulation of the denatured proteins in cells. In accordance with the suggested possibility above, MG132 activates JNK2 mediated transcriptional activity of heat shock factor 1. In this study, we compared differentially expressed mRNAs and proteins between heat shock and MG132 treatment using DNA chip, fluorescence 2-D difference gel electrophoresis (DIGE), and ^{35}S labeling proteomics. These systemic analyses provide the information on the common and differential signaling pathways between proteasome inhibition and heat shock response. [Supported by KOSEF NCRC for CCS & DDR and FPR05A2-480. HJ Kim is supported by BK21]

3C_04_P

LOCAL AND SYSTEMIC CHANGES IN PROTEIN EXPRESSION FOLLOWING BIOTIC STRESS IN EUROPEAN BEECH (*FAGUS SYLVATICA*)

Cristina-Maria Valcu and Katja Schlink

Technical University of Munich, Section of Forest Genetics, Am Hochanger 13, D-85354 Freising, email: valcu@wzw.tum.de

Pathogen and herbivore attack induced changes in protein expression patterns were investigated at proteome level in European beech (*Fagus sylvatica*) saplings. Two model systems were employed: (1) infection with the root pathogen *Phytophthora citricola* which induces root necrosis and plant wilting and (2) wounding as elicitor of the response to herbivore attack. The plant defence response was investigated at both local and

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

systemic level. Protein expression patterns were characterised for root and leaf samples by means of two-dimensional electrophoresis at three hours after wounding and at different stages of root infection. Infection experiments were performed in two experimental setups involving *in vitro* infection of saplings as well as infections in soil. Within the first system we investigated both local and systemic response to pathogen infection while the latter experiments were used for the study of systemic response in conditions close to the natural ones. Wounding experiments were carried out in similar conditions in order to enable the direct comparison of plant reaction to the two types of stress. The local response to wounding was characterised both for roots and leaves of plants grown in liquid system, while the systemic response was studied in leaf samples from plants grown in liquid culture or in soil. Protein spots up-regulated or down-regulated in response to infection or wounding were further identified by means of mass spectrometry. We were able to identify several proteins specifically regulated in response to pathogen or herbivore attack. Additionally, a significant overlap of the two defence pathways known to exist in plants was confirmed.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Module 4 – Oral lectures:

4A_01_S

EVOLUTION OF DESICCATION TOLERANCE: GENOMIC ASPECTS

Dorothea Bartels

Institute for Molecular Physiology and Biotechnology of Plants (IMBIO), University of Bonn, Germany
dbartels@uni-bonn.de

The Scrophulariaceae are one of the most diverse plant families and have been shown to be polyphyletic. Several Scrophulariaceae which belong to the genera Craterostigma and Lindernia desiccation tolerant and are therefore of particular interest. Craterostigma plantagineum has been used extensively to identify molecular mechanisms, which are involved in the acquisition of desiccation tolerance. These studies revealed a large number of genes which are up-regulated in response to dehydration. The genes can approximately be divided into two groups. One group encodes proteins which are directly involved in protection of cellular structures. The other group of genes encodes genes which are involved in regulating the response to dehydration. The dehydration/rehydration cycle is also characterized by a massive sugar conversion from octulose to sucrose.
C. plantagineum belongs to the Linderniae which comprises desiccation tolerant and non-desiccation tolerant plants. Using molecular markers a phylogenetic tree was constructed to determine the molecular relationships of the different plant species. It appears that desiccation tolerant plants cluster together. Comparative studies of these plant species identified conserved genomic structures and very recently evolved mobile signals in stress-relevant genes.

4A_02_S

FUNCTIONS OF TOCOPHEROLS AND OTHER LIPID-SOLUBLE ANTIOXIDANTS IN PLANTS UNDER LIGHT- OR METAL-INDUCED OXIDATIVE STRESS

Michel Havaux[1], Valérie Collin[1], Pascal Rey[1], Christian Triantaphylidès[1], Michel Matringe[2]

[1]CEA/Cadarache, IBEB/SBVME, Laboratoire d'Ecophysiologie Moléculaire des Plantes, F-13108 Saint-Paul-lez-Durance, France
michel.havaux@cea.fr
[2]CEA/Grenoble, iRTSV, Laboratoire de Physiologie Cellulaire Végétale, F-38054 Grenoble, France

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

The term 'vitamin E' describes the beneficial biological activity of a group of structurally related compounds, the tocochromanols, in animals and humans. Those compounds are composed of a chromanol head group and a prenyl side chain. Natural vitamin E includes four tocopherols and four tocotrienols, which are synthesized exclusively by oxygenic photosynthetic organisms. In leaves of vascular plants, α-tocopherol is the predominant form of vitamin E. A detailed analysis of tocochromanol distribution in chloroplasts isolated from young tobacco leaves showed that α-tocopherol is predominantly located in the thylakoid membranes. The protective role of vitamin E and the functional interactions between vitamin E and other plastid antioxidants (e.g. xanthophyll carotenoids) were studied using Arabidopsis and tobacco mutant/transgenic plants that lack or over-accumulate vitamin E constituents and/or carotenoids. This genetic approach was combined with the use of new biochemical and biophysical methods that allow characterization, quantification and imaging of lipid peroxidation in vivo. Both tocopherols and tocotrienols were found to protect thylakoid membranes against photooxidative stress. We also found that vitamin E and the xanthophyll zeaxanthin have overlapping functions, with lack of vitamin E being compensated by an increased level of zeaxanthin and vice versa. Lack of both compounds resulted in a very photosensitive phenotype. Vitamin E was also found to be essential for tolerance of Arabidopsis towards oxidative stress induced by stress conditions different from high light, such as heavy metals.

4A_03_S

MOLECULAR MECHANISMS OF PHOTOINHIBITION OF PHOTOSYSTEM II

Imre Vass, Krisztián Cser and Otilia Cheregi

Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, 6726 Szeged, Temesvári krt. 62, Hungary
Corresponding author: Imre Vass. Phone: +36-62-599-700, Fax: +36-62-433-434, Email: imre@brc.hu

Light-induced decline of photosynthetic activity, generally called as photoinhibition, is a general phenomenon in all oxygenic photosynthetic organisms under conditions when the metabolic processes can not keep up with the electron flow produced by the primary photoreactions. Although light-induced damage occurs in all pigmented photosynthetic complexes the main site of photodamage is Photosystem II. The main factors, which are responsible for the light sensitivity of Photosystem II are excited pigment molecules, oxygen, manganese, as well as electron donors with high oxidizing potential. Photosystem II can be efficiently

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

protected from photodamage by the combination of harmless dissipation of absorbed light energy, non-radiative charge recombination and repair of damaged reaction center complexes making possible the safe utilization of light, the highly energetic substrate of photosynthesis. The lecture will cover the principles and basic mechanisms of photodamage of photosynthesis, and its repair.

4B_01_S

USE OF GENE SILENCING AND METABOLOMICS TO CHARACTERIZE INTERACTIVE STRESS AND DEFENSE PATHWAYS IN SOYBEAN

T.L. Graham, M.Y. Graham, S Subramanian, O Yu

Ohio State University, Columbus, OH, USA 43210; corresponding author: graham.1@osu.edu

Induced resistance to pathogens can be specific to a given race of a pathogen or effective against a range of pathogens. The former type of resistance involves a form of programmed cell death called the hypersensitive response (HR). The latter type of resistance, called general or basal resistance, is often induced in plants by elicitors from the pathogen called pathogen associated molecular patterns (PAMPs). This form of resistance has some mechanistic similarities to innate immunity in animals. In the work described here we have used RNAi gene silencing and metabolic profiling to study the molecular bases of soybean resistance to the pathogen, *Phytophthora sojae*. Resistance responses were examined in the context of one of the most highly characterized PAMPs, the cell wall glucan elicitor (WGE) from *P. sojae*. Silencing either of two biosynthetic enzymes for soybean isoflavonoids (isoflavone synthase or chalcone reductase) led to a complete loss of race-specific resistance and HR cell death in response to *P. sojae*. Consistent with this, WGE, the major pathogen elicitor of the isoflavonoids in soybean, induced an HR-like cell death in root tissues. Silencing of the endoglucanase thought to release active elicitor fragments from WGE abolished both HR cell death and WGE-induced cell death. Silencing of a unique metallothionein gene (MMT) led to greatly enhanced WGE-induced cell death, suggesting a role of MMT in preventing runaway cell death. Finally, silencing of a pathogenesis-related protein, PR-1a, led to loss of expression of MMT and general resistance. Thus, our studies led to the revelation of very interesting cross-talk between the cell death and general resistance pathways in soybean.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

4B_02_S

ENGAGEMENT AND MODIFICATION OF THE PLANT HOST STRESS MACHINERY: A VIRULENCE STRATEGY OF THE PLANT PATHOGEN *PSEUDOMONAS SYRINGAE*.

Jean T. Greenberg

The University of Chicago, Chicago, Il 60637, USA
jgreenbe@midway.uchicago.edu

Pseudomonas syringae cause disease on a broad range of plant hosts. To have a successful infection, these bacteria utilize a specialized secretion apparatus called the type III secretion system (T3SS). *P. syringae* secrete a large group (20-40+) of effectors through the T3SS directly into plant cells. There is tremendous diversity in the repertoire of secreted effectors between different *P. syringae* strains. However, a few effectors are common to all *P. syringae* strains. We have selected one such common effector and one rare effector to functionally analyze in detail, including their localization in plants and their interaction with potential host targets. We have found that these effectors can modify host functions and alter their stress responses. This points to an interesting intersection between bacterial virulence mechanisms and host stress-coping mechanisms.

4B_03_S

THE NDR1-ACTIN CONNECTION: LINKING GENE-FOR-GENE RESISTANCE AND THE ACTIN CYTOSKELETON.

Brad Day, Miaoying Tian, Marina Varbanova and Caleb Knepper.

Michigan State Universit, Department of Plant Pathology, East Lansing, MI, USA
bday@msu.edu

Disease resistance in plants involves a molecular surveillance mechanism capable of responding to a myriad of plant derived elicitors. Recent research in this area has revealed a complex genetic and biochemical network required for the regulation of innate immune responses in plants. In total, the coordinated interactions of bacterial effector proteins, plant chaperones and resistance (R)-proteins contribute to the molecular and biochemical events which dictate host susceptibility and resistance. Research in our laboratory focuses on the identification and characterization of protein-protein interactions which not only function in the activation of resistance, but also in the negative regulation of effector-triggered immunity in *Arabidopsis thaliana*. Using NDR1, a protein

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

required for the activation of R-protein mediated resistance in *Arabidopsis*, as a molecular and biochemical model for the activation of resistance, we are working towards the elucidation of the dynamic linkages between effector-triggered immunity and the host actin cytoskeleton. To this end, we have begun characterizing several proteins required for reorganization of the actin cytoskeleton in plants, and have identified a genetic interaction between bacterial effector action and the dynamics of the host cytoskeleton. Similarities between the activity of *Yersinia pestis* effector proteins and *P. syringae* effectors will be discussed. Our working model suggests that the actin cytoskeleton of plants may be a virulence target for pathogens.

4C_01_S

REGURATORY GENE NETWORK IN DROUGHT STRESS RESPONSE

Kazuo Shinozaki[1] and Kazuko Yamaguchi-Shinozaki [2, 3]

[1]RIKEN Plant Science Center, Suehiro-cho, Tsurumi-ku, Yokohama, Japan,
[2]Laboratory of Molecular Physiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo, Japan,
[3]Biological Resources Division, Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan
sinozaki@rtc.riken.jp

Drought stress induces a variety of genes of which products function in drought stress tolerance and response in plants. Many stress-inducible genes have been used to improve stress tolerance by gene transfer. In this congress, we present our recent studies on molecular mechanism in drought stress response and tolerance. We have identified complex regulatory systems in stress-responsive gene expression: ABA-dependent and ABA-independent systems. In one of the ABA-independent pathways, a cis-acting element (DRE/CRT) and its binding proteins, DREB2s, are important cis- and trans-acting elements in drought-responsive gene expression, respectively. DREB2 is also involved in heat stress response. In the ABA-dependent pathways, bZIP transcription factors (AREB/ABF) are involved in the major process. Protein phosphorylation is important for the activation of AREB proteins. The MYB/MYC and NAC transcription factors are involved in ABA-responsive gene expression and jasmonic acid response In the ABA-dependent pathway, stress-inducible NCED3 is mainly involved in the ABA biosynthesis during drought stress. We analyzed metabolic profiles regulated by ABA using T-DNA tagged mutant and with GC-MS and LC-MS. We analyzed the function of CYP707A3 in the regulation of ABA metabolism during stress responses. We also report the functions of SnRK2 protein kinases in drought and ABA responses using mutants and transgenic overexpressors.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Umezawa et al. Curr Opin Biotech 17:113-122 (2006)
Yamaguchi-Shinozaki and Shinozaki. Ann Rev Plant Bio 57:781-803 (2006)

4C_02_S

STRESS-REGULATED SIRNAS AND MIRNAS

Jian-Kang Zhu

Institute for Integrative Genome Biology, Department of Botany and Plant Sciences, University of California, Riverside

It is often presumed that RNA interference (RNAi) evolved as a cellular surveillance mechanism to silence "foreign" double stranded RNAs (dsRNAs) so that cells can defend against viral infection or transposons. However, recent work suggests a widespread occurrence of dsRNAs unrelated to viral replication or transposons. A significant portion of cellular dsRNAs can be formed by natural antisense transcripts (NATs). Approximately 10-20% of genes in plant and animal genomes encode cis-NATs, i.e. these genes overlap but are on the opposite strands of DNA. In Arabidopsis, dsRNAs generated from cis-NATs can be processed by Dicer-like enzymes into 21-24 nt nat-siRNAs which then direct the cleavage of complementary mRNAs. Evidence indicates that many cis-NAT genes are regulated by biotic and abiotic stresses, and they generate nat-siRNAs only under specific stress conditions. The stress-induced nat-siRNAs are important components in the regulatory circuits leading to stress acclimation. These results suggest that RNAi is not only critical for cellular surveillance but it is also an important cellular gene regulatory mechanism. In this presentation, I will talk about abiotic stress-regulated nat-siRNAs and other endogenous siRNAs in Arabidopsis. In addition, I will discuss the role of miRNAs in plant responses to abiotic stresses.

4C_03_S

FROM MUTANTS TO GENES, PATHWAYS AND NETWORKS IN PLANT STRESS SIGNALING

Hans J. Bohnert

University of Illinois, Department of Plant Biology, Urbana, IL, USA
bohnerth@life.uiuc.edu

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Arabidopsis thaliana transcript profiles can report the effects of abiotic and biotic stresses on tissue- and cell-specific gene expression. Organizing these datasets could reveal the structure and mechanisms of responses and cross-talk between pathways, and in which cells the plants perceive, signal, respond to and integrate environmental inputs.

We have clustered *Arabidopsis* transcript profiles for >30 treatments, comprising abiotic, biotic and chemical stresses. Ubiquitous stress responses in *Arabidopsis*, similar to those of fungal and animal cells, employ genes in pathways related to MAP kinases, Snf1-related kinase, vesicle transport, mitochondrial functions, and the fundamental transcription machinery. The induced response to various stresses can be attributed to genes whose promoters are characterized by a small number of common regulatory motifs, while secondary motifs have also been identified. Most genes that are down-regulated by stress show distinct tissue-specific expression patterns and appear to be under strict developmental regulation. The ABA-dependent transcriptome is delineated in the cluster structure, while functions dependent on reactive oxygen species are widely distributed, possibly indicating evolutionary pressures conferring distinction to different stresses in time and space. Cell lineages in the root express stress-responsive genes at different levels. The intersection of stress-responsive and cell-specific profiles identified cell lineages affected by abiotic stress.

In an extension of these studies, the Graphical Gaussian Model (GGM) was used to assemble a gene network for the Arabidopsis transcriptome. Based on partial correlation (pcor), GGM infers co-regulation patterns between gene pairs conditional on the behavior of other genes. We used 'regularized' GGM, coupled with iterative random samplings, to expand the network to cover the whole Arabidopsis genome (22,266 genes). This resulted in a network of ~18,000 interactions (edges) among ~7,000 genes (nodes) with high confidence ($p < 2.2E-19$), where the connections represented ~0.01% of all possible edges.

When querying for selected genes, locally coherent sub-networks emerged that were predominantly related to metabolic functions and stress responses. Sub-networks for sulfate, phosphate, nitrogen, carbohydrate, tryptophan, cell wall metabolism, and the cold stress response were analyzed in detail. GGM recovered interactions with biological significance that escaped capture by Pearson correlation networks, while eliminating ambiguous interactions inherent in the latter. GGM displayed many known co-regulation pathways as sub-networks and added novel components to known edges. Finally, the network reconciled individual sub-networks in a topology joined at the whole genome level, and provided a general framework that can instruct future studies on plant metabolism and stress responses.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

4C_04_S

REGULATORY ROLES OF AMP-ACTIVATED PROTEIN KINASES AND PRL1-CDC5 SPLICEOSOME ASSEMBLY COMPLEX IN PLANT STRESS SIGNALLING

Csaba Koncz, Dóra Szakonyi, Gergely Molnár, Mihály Horváth, and Zsuzsa Koncz

Max-Planck Institut für Züchtunsgsforshung, Carl-von-Linne-Weg 10¸ 50829 Köln, Germany
(Corresponding author: koncz@mpiz-koeln.mpg.de)

AMP-activated kinases (AMPKs) control essential metabolic and signaling pathways in response to stress stimuli. Trimeric AMPKs, called Snf1-related kinases (SnRK1s) in *Arabidopsis*, are formed by combinatorial assembly of 3 catalytic alpha (AKIN10, 11 and 12), 3 substrate targeting beta (AKINβ1, 2 and3) and one AMP-binding gamma (AKINβγ/SNF4) subunits. SnRK1s undergo self-activation by autophosphorylation, their genes show different regulation, and the stability of subunits is controlled by proteasomal degradation. SnRK1α kinases are found in complex with the α7 subunit of proteasome and SKP1 subunit of SCF ubiquitin ligases suggesting a role in ubiquitination-dependent protein degradation. Genetic dissection of SnRK1 signaling is hampered by the lack of *akin10* and *akin11* insertion mutations and deficient male transmission of *snrk1γ* mutants. Inducible overexpression of SnRK1α *AKIN10* results in hypersensitivity to the stress hormone abscisic acid (ABA). AKIN10 phosphorylates and thereby stabilizes the bZIP transcription factor ABI5, a key regulator of germination response to ABA. SnRK1α subunits interact with the nuclear WD-40 repeat protein PRL1 (Pleiotropic Regulator Locus 1) that functions of SnRK1-inhibitor *in vitro*. In addition to other defects, *prl1* mutants display ABA hypersensitivity, and hyperphosphorylation and stabilization of ABI5. Stability of PRL1 is controlled by proteasomal degradation. PRL1 is found in complex with the Myb3R factor CDC5 that immunoprecipitates the proteasome, CULLIN1, and several unknown ubiquitinated proteins. PRL1 and CDC5 are conserved subunits of Ntc (nineteen-complex) spliceosome-activating complex. Inactivation of *CDC5* results in *prl1*-like phenotype suggesting functional interdependence. Both *prl1* and *cdc5* mutations cause early flowering and changes in petal and stamen development. Steady-state mRNA level of *FLC*, a key repressor of flowering, is reduced and *FLC* pre-mRNA shows defective splicing in *prl1*. Floral defects of *prl1* reflect aberrant splicing of pre-mRNAs of floral homeotic genes *AP1*, *AP3*, *AG* and *PI*. Alterations in the levels of microRNAs controlling ABA response, leaf development and flowering time in *prl1* suggest implication of spliceosome activating complex and SnRK1s in the control of pre-mRNA splicing and biogenesis of small inhibitory RNAs.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

4C_05_S

DEAD-BOX HELICASES IN PLANT STRESS TOLERANCE

Narendra Tuteja

Plant Molecular Biology Group, International Centre for Genetic Engineering and Biotechnology (ICGEB), Aruna Asaf Ali Marg, New Delhi – 110 067, India. Email: narendra@icgeb.res.in, Tel.: +91-11-26189358; Fax: +91-11-26162316

Abiotic stress is an increasing threat in reducing agricultural productivity worldwide. Among abiotic stresses, the high salinity stress is most severe environmental stress, which impairs crop production on at least 20% of irrigated land worldwide. In saline soils, high levels of sodium ions lead to plant growth inhibition and even death. As salinity stress affects the cellular gene-expression machinery, it is evident that molecules involved in nucleic acid processing including helicases, are likely to be affected as well. Helicases are one of the smallest molecular motors of biological system, which harness the chemical free energy of ATP hydrolysis to catalyze the opening of energetically stable duplex DNA (called DNA helicases) or unfold the secondary structures in RNA (called RNA helicases) and thereby are involved in almost all aspect of nucleic acid metabolism including replication, repair, recombination, transcription, translation, and ribosome biogenesis. Mostly all the helicases contain some conserved signature motifs including DEAD-box, which act as an engine to power DNA unwinding. In plants the role helicases in abiotic stress is just begening to emerge. Here we present the isolation of two pea DNA helicases (PDH45 and PDH47) and their role in salinity stress tolerance.

PDH45: It contains both the DNA and RNA unwinding activities and is homologous to translation initiation factor 4A (eIF4A). Antibodies against the PDH45 inhibit *in vitro* translation, confirming its role in translation initiation. The enzyme is localized in the nucleus and cytosol and unwinds DNA in the 3′ to 5′ direction. We found that *PDH45* mRNA is induced in pea seedlings in response to high salt and its over-expression in tobacco plants confers salinity tolerance, thus suggesting a new pathway for manipulating stress tolerance in crop plants. The T1 transgenics were able to grow to maturity and set normal viable seeds under continuous salinity stress, without any reduction in plant yield. Measurement of Na^+ in different parts of the plant showed higher accumulation in the old leaves and negligible in seeds of T1 transgenic lines as compared with the WT plants.

PDH47: It is also a DNA and RNA helicase both and is homologous to eIF4A. It is unique bipolar helicase that contains both the 3′ to 5′ and 5′ to 3′ directional helicase activities. The transcript of *PDH47* was induced

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

under salinity stress. ABA treatment did not alter its expression in shoot but induced its mRNA in root indicating the role of PDH47 in both the ABA-independent and-dependent pathways in abiotic stress. This is also localized in the nucleus and cytosol.
The discovery of salinity stress-induced helicases should make an important contribution to our better understanding of DNA and RNA metabolisms and stress signaling in plants. This study suggests a new pathway to engineer to restore crop yield in sub-optimal conditions. The role of some other new helicases in stress tolerance and the possible mechanism of salinity stress tolerance will be discussed.

4D_01_S

WILL FOREST ECOSYSTEMS CONTINUE TO REMOVE CO_2 FROM THE ATMOSPHERE AS CLIMATE CHANGES?

P.G. Jarvis* and S. Linder

University of Edinburgh, UK and Swedish University of Agricultural Sciences, Uppsala, Sweden
**margaretsjarvis@aol.com*

In almost every forest we look today we find that there is a net removal of CO_2 from the atmosphere and carbon is accumulating in the soil and in the trees. A *net* gain of carbon by forest ecosystems implies a lack of balance between the processes removing CO_2 from the atmosphere and the processes returning CO_2 to the atmosphere. Ecosystem *net primary production* (NPP) is the difference between the *gross photosynthetic production* (GPP) and the losses of carbon resulting from the *autotrophic respiration* (R_A), i.e., NPP = GPP – R_A. The *net ecosystem production* (NEP) is the difference between the *net primary production* (NPP) and the *heterotrophic respiration* (R_H) associated with mineralisation of organic matter in the soil, i.e., NEP = NPP - R_H. For a forest at equilibrium, we would expect NEP to be zero (i.e., NPP = R_H). Conversely, when we measure NEP > 0, NPP must exceed R_H. This world-wide *disequilibrium* in forests today is the reason why forests are a large global carbon sink, removing from the atmosphere close to 40% of CO_2 emissions. It is commonly assumed, and has been shown by some models, that as atmospheric [$C0_2$] and surface temperature increase in the future, this current disequilibrium will reverse (i.e., RH > NPP). A key question is whether this is likely?
Whilst including a carbon cycle in GCMs and other models has been a major step forward, a carbon cycle without an associated nitrogen cycle is unrealistic. Firstly, correlation across sites shows that NPP is stimulated by the concurrent deposition of atmospheric N. Secondly soil warming experiments show that N released by decomposition of litter and soil

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

organic matter leads to increases in tree leaf area, uptake of CO_2 and tree growth. Thirdly, after an initial enhancement on warming, R_H settles down to the rate prior to the increase in temperature. For these reasons, recent projections by models linking the carbon and nitrogen cycles show NPP of forests increasing over the next 100 years *in parallel* with R_H, as atmospheric [CO_2] and surface temperature increase. Thus we may expect the ongoing removal of [CO_2] from the atmosphere by forests (NPP > RH) to be maintained.

4D_02_S

STRESS TO PLANTS AND THE ECOSYSTEM UNDER ELEVATED CO_2

Hartmut K. Lichtenthaler

Botanical Institute, University of Karlsruhe, Kaiserstr. 12, D-76133 Karlsruhe, Germany
(Email: hartmut.lichtenthaler@bio.uka.de)

The original stress concept of Hans Selye has also been extended to plants and is today well defined to describe the action of unfavorable environmental constraints and stressors on plants as well as the plants' response via stress avoiding and stress coping mechanisms including special short-term acclimations and long-term adaptations. Several examples of stress coping mechanisms are presented. Elevated CO_2 is a new challenge for plants and the ecosystem. It may not be a real stress, but it is a continuous strain. This requires particular acclimation and adaptation responses some of which are known. In fact, the plants make use of their general stress coping mechanisms either directly applied or in a modified form to avoid or compensate possible negative effects of elevated CO_2. One has to consider that on top of this all the classical abiotic, biotic and anthropogenic stressors are threatening plant growth and development also under elevated CO_2. Thus, the knowledge of the general stress coping and stress avoiding responses and tolerance mechanisms is needed to understand the regulation of the plants' metabolism. Emission of volatile isoprenoids, such as isoprene or methylbutenol or the accumulation of mono- or diterpenes, are now understood as a possibility to regulate the internal cell metabolism and to protect the plants against photoinibition and photooxidation.
Measurements of chlorophyll fluorescence and particularly the imaging of chlorophyll fluorescence and the plants' blue-green fluorescence provide excellent means to early detect and describe stress symptoms of plants and the ecosystem. Several examples are given.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

4D_03_S

TWO-FACE CHARACTER OF THE ELEVATED CARBON DIOXIDE IMPACTS ON THE PLANT METABOLISM

Michal V. Marek, Otmar Urban

Laboratory of Plants ecological Physiology, Institute of Systems Biology and Ecology, v.v.i. , Academy of Sciences of the Czech Republic, Porici 3b, CZ-6030 00 Brno, Czech republic
emarek@usbe.cas.cz

The rising atmospheric CO_2 concentration (EC) is an important ecophysiological topic. Because of the important CO_2 functions (effects on carboxylation, RUBISCO activation, stomatal response) on assimilation, it is possible to expect wide range of plants responses to EC. Thus, positive stimulation of photosynthesis or the assimilation depression - photosynthetic adjustment were observed and may be explained by (*1*) a decrease of RuBPCO amount and/or activity, (*2*) dilution/redistribution of nitrogen and phosphorus mineral status of assimilatory apparatus, (*3*) starch, (*4*) decrease of photosynthetically active pigments content and diminution of light-harvesting complexes, and (5) differences in the new sinks-source status of the plant. All mentioned responses were observed on the example of a dense spruce stand exposed for the long-term affected EC. The pronounced vertical profile of the photosynthetically active radiation led to the differentiation of the photosynthetic apparatus between the shaded (S) and sun needles (E). The prolonged exposure to EC was responsible for the apparent assimilatory activity stimulation observed mainly in deeply S needles. In E needles some signals on a manifestation of the acclimation depression of the assimilation were found. The long-term effect of EC was responsible for the decrease of nitrogen content. Moreover, the prolonged exposure to EC did not cause any stimulation of electron transport rate (ETR) for the E-needles but a strongly positive effect of EC on ETR was observed for the S-needles. The analysis shows that the depression of photosynthetic activity by long-term impact of EC is mainly caused by decreased RUBISCO carboxylation rate. All mentioned modifications to photosynthetic assimilation depend on time during the growing season. The strong depression of assimilation observed in the autumn months was the result of insufficient carbon sink capacity.

4D_04_S

GRASSLAND ECOLOGY UNDER ELEVATED AIR CO_2 CONCENTRATIONS

Zoltán Tuba[1,2], Zoltán Nagy[2]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[1]*Plant Ecological Research Group of Hungarian Academy of Sciences*
[2]*Department of Botany and Plant Physiology, Faculty of Agricultural and Environmental Sciences, Szent István University, Gödöllő, H-2103 Gödöllő, Páter K. u. 1, Hungary, tuba.zoltan@mkk.szie.hu*

Global climate change appears to be the greatest ecological problem of the future. For example the CO_2 emitted into the atmosphere will have a long-term effect and this is one of the most influential ecological factor at global scale. Its increasing concentration affects the plants directly, causing changes in their ecophysiological processes. Consequently, the tolerance, reproduction, distribution and abundance of plant species will be altered. Species composition and diversity of plant communities, the vegetation dynamics are all expected to be changed, too. Considering terrestrial ecosystems, grasslands are the second largest vegetation formation after the forests and their area is further increasing with the land use changes (clearance of the forests, urbanisation, extensive agricultural practices and abandonment of agricultural land). On a global scale grassllands cover 24% of the Earth's vegetated area. They occur over a broad range of climatic and soil conditions and vary from intensively managed sown pastures to natural grassland communities. Despite their importance, the potential effects of climate change on grasslands have received much less attention than effects on other ecosystems such as forests. The main objective of the proposed talk is to discuss the effects of elevated air CO_2 concenrations on the structure (botanical, species composition, floral diversity, canopy and below ground/root architecture) and function (CO_2,and H_2O exchange carbon cycling, dry matter production responses, etc.) of grasslands and to quantify the carbon storage in grassland ecosystems.

Module 4 – Poster lectures:

4A_01_P

CYTOCHROMES B561, NEW PLAYERS IN PLANT IRON METABOLISM AND OXIDATIVE STRESS METABOLISM

Asard Han, Bérczi Alajos, Van Beek Kim, Griesen Daniel

University of Antwerp, Antwerp, Belgium; Biological Research Center, Szeged, Hungary.
han.asard@ua.ac.be

Cytochromes b561 (Cyt b561) are a newly identified class of trans-membrane proteins, using **ascorbate** as an electron donor. These

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

proteins have been demonstrated to transfer electrons across the membrane in which they are embedded, but their physiological role remains unclear. We have identified four Cyt b561 isoforms (AtCytb1-4) in Arabidopsis and are characterizing their mechanism of action and using biochemical, molecular biological and physiological approaches. Several lines of evidence suggest that the plant Cyts b561 are involved in **iron metabolism and in oxidative stress responses**: 1) A knock-out in one of the four Cyts b561 identified from Arabidopsis demonstrates a particular phenotype under iron deficiency. 2) The recombinant AtCytb1 protein can be oxidized by iron chelates. 3) The AtCytb1 gene appears upregulated under iron-deficiency conditions. And 4) In vivo experiments with AtCytb1 expressed in yeast demonstrate its ferric-reductase capability. Recent experiments however also demonstrate that the AtCytb1 knock-out plants show a particular phenotype under oxidative stress conditions. Strongly reduced root development is observed in the mutant plants when treated with paraquat. These results suggest that Cyts b561 may provide a link between plant iron metabolism and oxidative stress phenomena, using ascorbate as the electron donor.

References: Bérczi et al. (2007) An Arabidopsis cytochrome b561 with trans-membrane ferrireductase capability. FEBS Lett. 581: 1505-08. Griesen et al. (2004) Localization of an ascorbate-reducible cytochrome b561 in the plant tonoplast. Plant Physiol. 134:726-34.

Acknowledgements: this work was supported by grants from the Belgian Fund for Scientific Research (FWO) and the Hungarian Scientific Research Fund (OTKA T-034488)

4A_02_P

SELENIUM-INDUCED OXIDATIVE STRESS IN COFFEE CELL SUSPENSION CULTURES

Ricardo A. Azevedo[1], Priscila L. Gratão[1], Paulo Mazzafera[2], Peter J. Lea[3], Rui A. Gomes-Junior[4]

[1]ESALQ, Universidade de São Paulo, Brazil; [2]Universidade Estadual de Campinas, Brazil, [3]University of Lancaster, U.K., [4]Instituto Agronômico do Paraná, Brazil. E-mail: raazeved@esalq.usp.br.

Selenium (Se) is an essential element for humans and animals that is required for key antioxidant reactions, but can be toxic at high concentrations. We have investigated the effect of Se in the form of selenite on coffee cell suspension cultures over a 12 day period. The antioxidant defence systems were induced in coffee cells grown in the presence of 0.05 and 0.5 mM sodium selenite (Na_2SeO_3). Lipid

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

peroxidation and alterations in antioxidant enzymes were the main responses observed, including a severe reduction in ascorbate peroxidase activity, even at 0.05 mM sodium selenite. Ten superoxide dismutase (SOD) isoenzymes were detected and the two major Mn-SOD isoenzymes (bands V and VI) responded more to 0.05 mM selenite. SOD band V exhibited a general decrease in activity after 12 h of treatment with 0.05 mM selenite, whereas band VI exhibited the opposite behavior and increased in activity. An extra isoenzyme of glutathione reductase (GR) was induced in the presence of selenite, which confirmed our previous reports obtained with Cd (Gomes-Junior *et al*.: Chemosphere 65, 1330-1337, 2006) and Ni (Gomes-Junior *et al*.: Plant Physiology and Biochemistry 44, 420-429, 2006) indicating that this GR isoenzyme may have the potential to be a marker for oxidative stress in coffee. Funding by FAPESP and CNPq, Brazil.

4A_03_P

RESPONSE OF BRAZILIAN CULTIVARS OF SOYBEAN (*GLYCINE MAX*) EXPOSE TO OZONE UNDER CONTROLLED CONDITIONS

Patrícia Bulbovas[a*], Silvia Ribeiro de Souza[b], Marisa Domingos[b] and Ricardo Antunes Azevedo[a]

[a] *Departamento de Genética, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba CEP 13400-970, SP, Brasil*
[b] *Instituto de Botânica, São Paulo, CEP 01061-970, SP, Brasil*
** e-mail: patibul@usp.br*

Ozone (O_3) can enter the leaves and react with water forming reactive oxygen species (ROS). ROS is able to cause physiological and biochemical damage to plants. This study was carried out to investigate the O_3 effects on two distinct Brazilian cultivars of soybean (*Glycine max*): 'Tracajá' and 'Sambaíba'. Plants were grown in two chambers, one with filtered air (FA) and the other with filtered air plus 40 ppb of ozone (FA+O_3) for five days. Components of the antioxidative defense system such as ascorbic acid (AA), glutathione reductase (GR), ascorbate peroxidase (APX), guayacol peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD) were analyzed and the relative growth rate (RGR) and biomass production were also determined. APX and GPX in 'Sambaíba' and AA and GPX in 'Tracajá' decreased under FA+O_3. AA and CAT in 'Sambaíba' and CAT in 'Tracajá' exhibited similar levels under both treatments. GR in 'Sambaíba' and GR and APX increased in 'Tracajá' exposed to FA+O_3. CAT and SOD activity staining by non-denaturing PAGE revealed the same isoforms numbers for both cultivars, but different isoforms of GR. 'Tracajá'exhibited variations of some CAT and SOD isoforms in FA+O_3. The root/shoot ratio in 'Tracajá' and root/shoot ratio, leaf RGR and root biomass production in 'Sambaíba'

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

were lower under FA+O_3. O_3 treatment induced distinct antioxidative responses by the distinct antioxidant systems in response to O_3 (AF+O_3). The antioxidant defense system variations compensated the lower O_3 interference on growth parameters and biomass production.

4A_04_P

REACTIVE CARBONYL DETOXIFICATION AND STRESS RESISTANCE IN PLANTS

Gábor V. Horváth, Zoltán Turóczy, Petra Kis, Katalin Török, Éva Hideg, László Sass and Dénes Dudits

Institute of Plant Biology, Biological Research Center HAS, Szeged, Hungary. E-mail: hvg@brc.hu

Productivity of plants is greatly affected by environmental stresses, therefore there is a continuous need for the genetic improvement of stress tolerance in the agriculture. During oxidative stress rapid accumulation of reactive oxygen species (ROS) and reactive carbonyl species (RCS, carbonyl stress) significantly contributes to the damage of crop plants. Improvement of intracellular scavenging capacity of such compounds provenly lead to increased stress tolerance. Diverse enzymes can detoxify the reactive carbonyl species (eg. 4-hydoxy-nonenal or methylglyoxal). Their importance is clearly demonstrated by the fact that overproduction of alkenal reductases, aldehyde dehydrogenases or the members of the glyoxalase enzyme family in transgenic plants led to improved tolarance to wide range of stress conditions. We have isolated the *MsALR* aldose reductase homolog gene from *Medicago sativa* and showed the accumulation of the transcript at higher levels in response to different stress treatments. Transgenic tobacco plants ectopically expressing the *MsALR* cDNA were more tolerant to dehydration stress and recovered better from damages caused by water deficit than the untransformed wild type plants and were more tolerant to heavy metal, salt, dehydration and UV-B stress. Results of enzyme activity measurements and i*n vivo* assays for protection against methylglyoxal toxicity strengthened our hypothesis that one important function of aldo/keto reductase proteins is also the elimination of reactive aldehydes and the reduction of the consequences of carbonyl stress in plants.

This work was supported by NKFP Grant No. 4-064-2004, Gábor V. Horváth is grateful for the support of the "János Bólyai" Research Fellowship.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

4A_05_P

MAMMALIAN BAX INITIATES PLANT CELL DEATH THROUGH ROS PRODUCTION AND ORGANELLE DESTRUCTION

Maki Kawai-Yamada, Keiko Yoshinaga and Hirofumi Uchimiya

Institute of Molecular and Cellular Biosciences, The University of Tokyo, Japan, mkawai@iam.u-tokyo.ac.jp

Relatively few endogenous plant genes that share sequence homology with the mammalian apoptotic genes have been identified to date. Nonetheless, similarities in PCD exist in plants and animals. Mammalian proapoptotic gene Bax is known to cause cell death when expressed in yeast and plants. We examined transgenic plants expressing both Bax and organelle-targeted green fluorescent protein (GFP) to analyze the cellular event that occur during Bax-induced plant cell death. The results indicated that Bax induced temporal and special cell death events at the organelle level. Such events included ion leakage, DNA fragmentation and cell shrinkage. The mitochondria changed morphologically from being bacilli-shaped to being round. The chloroplasts lost membrane function and their contents leaked out, followed by the disruption of the vacuole. Light was not essential for Bax-induced ion leakage or organelle disruption, but for chlorosis. Furthermore, ROS production was involved in triggering cell death. To compare Bax-induced cell death and other ROS-mediated plant cell death, *Arabidopsis* leaves expressing mitochondrial-targeted GFP were treated with ROS-inducing chemicals, such as hydrogen peroxide, paraquat and menadione. After 24h treatment, mitochondria showed morphological changes from a bacillus-like to a round shape. The size of mitochondria decreased by half compared with controls. Such cellular events may cause energy depletion, and resulted in plant cell death.

4A_06_P

GLUTATHIONE HALF-CELL REDUCTION POTENTIAL: A UNIVERSAL STRESS MARKER FROM PLANTS TO HUMANS?

Ilse Kranner, Simona Birtić and Hugh W. Pritchard

Seed Conservation Department, Royal Botanic Gardens, Kew, Wakehurst Place, West Sussex RH 17 6TN, UK
E-mail address of corresponding author: i.kranner@kew.org

Ageing phenomena and programmed cell death (PCD) are typically studied in human or animal cells rather than in plants. However, the seeds of higher plants represent excellent models for the study of ageing because

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

viability loss can be easily induced experimentally. 'Orthodox' seeds are desiccation tolerant and 'recalcitrant' seeds are desiccation sensitive. Lethal damage, as judged by germination tests, can be induced by artificial ageing in orthodox, and by drying in recalcitrant seeds. We have investigated mechanisms of viability loss to find a reliable marker that quantifies 'stress'. Oxidative damage has previously been correlated with degenerative processes and death, but how exactly this contributes to viability loss is unknown. We show in four species subjected to ageing or desiccation, that seed viability decreased by 50% when the half-cell reduction potential of glutathione ($E_{GSSG/2GSH}$), a major cellular antioxidant and redox buffer, increased to a zone of -180 to -160 mV, in agreement with a model that has been suggested for human cells (1). In a meta-analysis of data representative of 13 plant and fungal orders we show that stress generally becomes lethal when $E_{GSSG/2GSH}$ exceeds -160 mV. We put forward that this change in $E_{GSSG/2GSH}$ is one of the 'death triggers' that initiate PCD, finally causing inter-nucleosomal DNA fragmentation in the final, or execution phase, of PCD. $E_{GSSG/2GSH}$ is therefore a universal marker of plant cell viability and allows us to predict whether a seed will live, germinate and produce a new plant, or if it will die.

1. Schafer, F.Q. & Buettner, G.R. (2001) *Free Radical Biol. Med.* **30**, 1191-1212.

4A_07_P

EXPRESSION ANALYSIS OF PHENYLALANINE AMMONIA LYASE IN RELATION TO OZONE STRESS IN YELLOW POPLAR PLANTS (*LIRIODENDRON TULIPIFERA*)

A. Francini, E. Pellegrini, G. Lorenzini, C. Nali*

*Department of Tree Science, Entomology and Plant Pathology "G. Scaramuzzi", University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy. *cristina.nali@agr.unipi.it*

Tropospheric ozone is a widespread phytotoxic pollutant that causes significant damage to plants, but relatively little is known about ozone stress in urban trees. The phenylpropanoid pathway is one of the most important pathways for the synthesis of natural products in plants such as phenols and lignin. In this study, the hypothesis that phenylalanine ammonia lyase (PAL) may be affected by ozone treatment (120 ppb, 5 h d^{-1} for 45 consecutive days) in *Liriodendron tulipifera* (yellow poplar) plants was examined. At the end of exposure, the material showed characteristic symptoms of injury in the form of severe minute roundish dark-blackish necrosis localised in the interveinal areas of the adaxial surface of leaves. Treated plants increase PAL activity after ozone

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

fumigation (+43%) and total phenols (+41%) compared to controls. Total leaf RNA protocol was optimised for yellow poplar. Degenerate primers were designed on highly conserved sequences available on GenBank, that encode for this enzyme. The primers yielded 679 bp cDNA fragments. Sequences obtained were translated in aminoacids and compared in the GenBank. Blast results showed high homology at aminoacids level with PAL, confirming that the cDNA fragments isolated effectively encode for this gene. In particular, the sequences showed high homologies with *Ulmus pumila* (93%) and with *Pyrus comunis* (96%). The northern analysis of PAL gene expression supports the view of ozone as an abiotic elicitor of defence responses in yellow poplar.

4A_08_P

INFLUENCE OF ANTIOXIDANTS ON SOME STAGES OF OXIDATIVE STRESS IN PLANT CELLS OF WHEAT AND BARLEY SEEDLINGS

N. Shkute, M. Savicka, N. Zatinajchenko, N. Rjabovola

Institute of research and management of biological resources, Daugavpils University, Vienības iela 13, Daugavpils, LV-5401, Latvia

The production of superoxide is crucial for normal morphogenesis at early stages and is causal of apoptosis and senescence at late stages of development of etiolated cereals seedlings (wheat, barley and others). It was shown, that cyclic change of rate of superoxide production is correlate with changes of nuclear DNA synthesis and apoptotic fragmentation in cells of some development and senescence separated organs (leaf, coleoptile) of this plants. It was found, that antioxidant defence systems: superoxide dismutase and peroxidase are activated in apoptotic cells of senescence organs. The influence of endogenic antioxidant (ascorbic acid) and synthetic antioxidant (α - Ionol) on superoxide production, on activation of antioxidant defence systems, on nuclear DNA synthesis and nuclear DNA apoptotic fragmentation and on structure of cellular organelles was investigated. It was found, that cyclosporin A, inhibitor of the mammalian permeability transition pore, inhibits of peroxide formation and nuclear DNA apoptotic fragmentation. Taking this date mitochondria are likely to be involved in plant programmed cell death, induced by oxidative stress, but the molecular mechanisms may be different from those found in animals.

4A_09_P

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

SEASONAL VARIATION IN THE ACTIVITY OF ANTIOXIDANT ENZYMES PEROXIDASE, SUPEROXIDE DISMUTASE AND CATALASE IN AN OPEN AND A SHADED POPULATION OF *IRIS PUMILAS*

Branka Tucić*, Ana Vuleta and Darka Šešlija

*Department of Evolurionary Biology, Institute for Biological Research „Siniša Stanković", Belgrade, Serbia, *btucic@ibiss.bg.ac.yu*

Seasonal variation in the activity of peroxidase (POD, EC 1.11.1.7), superoxide dismutase (SOD, EC 1.15.1.1), and catalase (CAT, EC 1.11.1.6) was determined in the leaves of two *Iris pumila* populations, one naturally growing at an open dune site and the other in the understory of a *Pinus* stand. A repeated-measures profile analysis revealed that the average level, as well as the mean change in the activity of these enzymes varied significantly between contrasting light habitats. POD activity was significantly greater at exposed dune site than under vegetation canopy, and reached its maximum in the summer but only in plants experiencing full sunlight. Conversely, in woodland understory, POD activity gradually increased from spring to autumn. The mean activity of SOD and CAT was consistently greater in plants inhabiting vegetation shade compared to those exposed to full irradiance. Throughout the growing season, the variation pattern of CAT activity was the same in both light habitats, while the response curve of SOD activity changed the shape with environmental conditions, particularly in the period from summer to autumn. At open dune site in the spring, the activity of POD appeared to be inversely related to SOD, suggesting that plants with lower SOD production up-regulate their POD level to compensate for SOD reduction under given environmental setup. The observed results imply that abiotic stress can disrupt the redox homeostasis in *I. pumila* plants, changing the balance between POD, SOD and CAT activities according to the extent of oxidative stress in cells.

4A_10_P

BATTLE AGAINST REACTIVE COMPOUNDS-AKRS AND STRESS

Zoltán Turóczy, Mátyás Cserháti, Petra Kis, Dénes Dudits, Gábor V. Horváth

Institute of Plant Biology, Biological Research Center HAS, Temesvári krt. 62, H-6726 Szeged, Hungary, E-mail: turoczy@brc.hu

Plants exposed to abiotic stress are subjected to oxidative damage. Reactive compounds produced under such conditions significantly increase

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

the cytotoxic effect of environmental stress factors. Aldo/keto reductases (AKRs) have been considered as effective enzymes for the detoxification of lipid peroxidation- and glycolysis-derived reactive aldehydes. The promoters of targeted rice AKR genes were screened for stress-related transcription factor binding sites (TFBS's) by a new, enumeration based method algorithm. QRT-PCR was used to determine the transcript profile for the selected genes in abscisic acid (ABA), H_2O_2, NaCl and mannitol treated rice cell suspensions. Among the genes, OsALR1 showed the highest inducibility and transcript level during the treatments, suggesting its important role in stress tolerance. Furthermore the Q-PCR experiments substantiated, that the number of motifs found in the promoters and the stress response of the genes were in close correlation. In addition, the photosynthetic parameters of the tobacco lines, overexpressing rice OsALR1 and OsALR4 were better then those of the wild type plants after paraquat and methylglyoxal (MG) treatments. The *in vitro* enzyme kinetic constants of the GST-OsALR1 fusion protein revealed a high reducing activity for toxic aldehydes like MG in the presence of NADPH. Results of i*n vivo* assays in *E. coli* for protection against MG toxicity have also made it clear, that one important function of these AKR proteins is the elimination of reactive aldehydes from plant cells expletively besides the glyoxalase system. *This work was supported by NKFP Grant No. 4-064-2004, Gábor V. Horváth is grateful for the support of the "János Bólyai" Research Fellowship.*

4A_11_P

INFLUENCE OF CADMIUM ON THE ANTIOXIDANT SYSTEM OF TOBACCO PLANTS

Cardoso, A.I.[(1)], Mourato, M.P. [*(1)], Martins, L.L. [(1)], Pinto, A.P. [(2)], Mota, A.M. [(3)], Gonçalves, M.L. [(3)], Varennes, A. [(1)]

[(1)] Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal
[(2)] Departamento de Química, Universidade de Évora. Colégio Luís António Verney 7000 Évora
[(3)] Instituto Superior Técnico, Technical University of Lisbon, Av. Rovisco Pais, 1049-001 Lisboa, Portugal
** Corresponding author: mmourato@isa.utl.pt*

In this work we studied the effect of cadmium (0, 0.010, 0.025, 0.050, 0.100 mM) on enzymatic and non-enzymatic parameters of tobacco plants grown in hydroponic culture, in order to get a further insight in the detoxification processes and cadmium absorption mechanisms of that plant. Tobacco has been chosen since it has been referred as a cadmium accumulator plant. Leaf dry weight percentage increased with cadmium

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

concentration indicating lower water absorption possibly due to affected root development. Chlorophyll a and b levels decreased markedly (down to 16 % of the control value for the highest cadmium concentration of 0.100 mM) showing that the photosynthetic system was affected by cadmium. Hydrogen peroxide measurements showed no significant variations, which could indicate that the antioxidant system of this plant is capable of quenching the possible excess of hydrogen peroxide induced by cadmium. In fact, both guaiacol peroxidase and ascorbate peroxidase activities were enhanced with increasing cadmium concentration in nutrient solution. Another enzyme usually involved in the antioxidant system of plants, superoxide dismutase, showed no difference in its activity levels compared to the control. A slight increase on malondialdehyde levels, both in leaves and roots, indicated that lipid peroxidation should occur as a result of increased ROS formation induced by cadmium toxicity.

4B_01_P

ELUCIDATION OF FUMONISIN B1-INDUCED CELL DEATH SIGNALLING IN *ARABIDOPSIS THALIANA*.

Daniel F. Tomé, John M. Hamilton, Stephen Chivasa, Keith Lindsey, and Antoni R. Slabas

Creative Gene Technology Ltd., Integrative Cell Biology Laboratory, University of Durham, Durham DH1 3LE, UK
E-mail: j.m.hamilton@durham.ac.uk

Programmed Cell Death (pcd) is a ubiquitous process in plants that is very similar to apoptosis in animals. It occurs in various tissues during plant development, such as death of the *tapetum* cell layer in mature anthers, the sheading of leaves in autumn in perennial plants, and in the differentiation of xylem. Pcd also occurs as a response to stress such as pathogen attack. However, the mechanism by which plants initiate pcd and the key proteins involved are poorly understood. Recently, we reported that removal of extracellular ATP (eATP) from plant tissues initiates pcd. We also reported that fumonisin B1 (FB1)-induced pcd is mediated via eATP depletion and can be blocked by addition of ATP. Rescue of cells from FB1-induced death by ATP is possible if ATP is supplied before the cells are irreversibly committed to death. Changes in gene expression at or around the time of commitment are of interest as these could be crucial players in cell death. We are now using 2-dimensional difference gel electrophoresis and DNA microarray technologies to identify the key components in this pathway, focusing on time points around commitment. We identified a number of differentially expressed genes and proteins associated with this death pathway.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Reverse genetic screening using homozygous knockout mutants has allowed us to identify candidates that could prove critical to the execution of pcd in plants. Moreover, the emerging data is beginning to give us insight into the mechanism by which eATP sustains cell viability in plants.

4B_02_P

TITLE: ROLE OF MAIZE PHENOLICS IN THE GENOTYPIC RESISTANCE TO *GIBBERELLA* STALK ROT (*FUSARIUM GRAMINEARUM* SCHWABE)

Rogelio Santiago, Lana M. Reid, John T. Arnason, German Sandoya, and Rosa A. Malvar.

Corresponding author address:
Rogelio Santiago, Consejo Superior de Investigaciones Científicas, Misión Biológica de Galicia.
Carballeira nº 8- Salcedo, Pontevedra. 36143, SPAIN
Telephone: +34 986 85 48 00
Fax: +34 986 84 13 72
Email: rsantiago@mbg.cesga.es

Six maize inbred lines known to represent a wide spectrum of susceptibility to *Gibberella* stalk rot were investigated for a relationship between their phenolic contents in the pith and their resistance to *Gibberella* stalk rot. The phenolic acid profiles were evaluated from silking to grain maturity. Four different fractions of phenolic compounds were extracted from inoculated and non-inoculated (control) pith tissues: insoluble cell wall-bound phenolics, free phenolics, soluble ester-bound phenolics, and soluble glycoside-bound phenolics. Analysis by HPLC revealed that *p*-coumaric and ferulic acid were the most abundant compounds in the soluble and cell wall bound fractions. The quantity of free, glycoside-bound and ester-bound phenolics in the pith was lower than the level required for inhibition to of *Fusarium* growth and/or mycotoxins production; however, significant negative correlations between diferulic acids contents in the cell walls and disease severity rating four days after inoculation were found. According to these results previous studies showed significant negative correlations between disease severity and diferulic acid contents in the maize grain. Special attention should be therefore given to levels of diferulic acids during the early infection process. Diferulates may play a role in genotypic resistance of maize to *Gibberella* stalk rot as barriers preformed prior to infection.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

4B_03_P

STRESS REACTIONS OCCURRING DURING BIRD CHERRY INFESTATION BY BIRD CHERRY-OAT APHID

Hubert Sytykiewicz, Iwona Łukasik, Bogumił Leszczyński

University of Podlasie, Department of Biochemistry and Molecular Biology, B. Prusa 12 St.,
08-110 Siedlce, Poland, e-mail: leszczb@ap.siedlce.pl

Quite often biochemical interactions between host-plants and herbivorous insect pests are related to stress reactions within infested plant tissues and feeding herbivores. In the present paper we report on such dual stress reactions within tissues of the bird cherry-oat aphid (*Rhopalosiphum padi* L.) fed on the bird cherry (*Prunus padus* L.).The carried out experiments showed that the aphid infestation had elucidated an increase in activity of leucylaminopeptidase and other proteinases isolated at pH 5 and pH 7 from the bird cherry leaves. It is important since these enzymes are involved in induction of the plant defensive mechanisms towards various pathogens. It has been recognized that the one of the earliest plant responses to herbivores involve generation of the reactive oxygen species and induction of oxidative stress within insect tissues. It appears to be real for the studied aphid species since depletion of total thiol groups and increase in lipid peroxidation was demonstrated within the bird cherry-oat aphid tissues exposured to plant phenolics. However, *R. padi* showed an ability to neutralize the free oxygen radicals with help of enzymatic and non-enzymatic antioxidants. For example, an induction of the aphid superoxide dismutase and catalase, neutralizing toxic oxygen forms was found during the spring migration period, when the highest level of the proteinases activity within the bird cherry leaves was noted. The role of the stress reactions in biochemical interactions between the bird cherry and the bird cherry-oat aphid is discussed.

4C_01_P

INDUCTION OF LECTIN EXPRESSION IN RICE AS A REPONSE TO STRESS TREATMENTS

Elke Fouquaert, Godelieve Gheysen and Els J.M. Van Damme

Department of Molecular Biotechnology, Ghent University, Coupure Links 653, 9000 Gent, Belgium, Elke.Fouquaert@Ugent.be

Numerous wild and cultivated plant species contain carbohydrate-binding

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

proteins called lectins. Most plant lectins are involved in the recognition and binding of glycans from foreign organisms, and accordingly are believed to play a role in plant defense. However, in recent years evidence has accumulated that plants synthesize well-defined carbohydrate binding proteins upon exposure to stresses like drought, high salt, wounding, treatment with some plant hormones or pathogen attack.
As early as 1990 Claes et al. (1) demonstrated that a protein called SalT is specifically induced when rice plants are subjected to salt stress. Later SalT was identified as a dimeric mannose-binding jacalin-related lectin composed of 15 kDa subunits (2). More recently we have identified two more lectins in rice, belonging to the class of OSR40 proteins and a Galanthus nivalis-related lectin, respectively. Expression of the OSR40 proteins is induced by salt stress or ABA treatment (3). Localization studies of the rice lectins demonstrated that they are exclusively located in the cytoplasm and the nucleus. The occurrence of the genes encoding the three different lectins in the rice genome was studied by screening different rice species and cultivars with a different genetic background. Lectin induction in response to different stress conditions was also analyzed. Since these lectins are synthesized only as a response to specific physical, chemical and biotic stress factors, it can be assumed that they play a specific physiological role in the plant.

(1) Claes et al., 1991. Plant Cell 2, 19-27.
(2) Zhang et al., 2000. Planta 210, 970-978.
(3) Moons et al., 1997. Planta 202, 443-454.

4C_02_P

EFFECT OF LOW-CONCENTRATION STRESSORS IN BARLEY SEEDLINGS

Erika Kovács[1,2], Péter Nyitrai[1], Pálma Czövek[1], Mihály Óvári[3], Áron Keresztes[2]

[1]Department of Plant Physiology and Molecular Plant Biology, Eötvös University, H-1518 Budapest, P.O.B. 120, Hungary
[2]Department of Plant Anatomy, Eötvös University, H-1518 Budapest, P.O.B. 120, Hungary
[3]Department of Organic Chemistry, Eötvös University, H-1518 Budapest, P.O.B. 112, Hungary
k-erika@elte.hu

The effects of some chemical stressors in high concentration have been thoroughly investigated in plants, but information about the same stressors in low concentration is very limited. In our experiments the effects of low-concentration Cd^{2+} ($5x10^{-8}$ M) and a herbicide, DCMU (10^{-7}

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

M) were investigated in barley seedlings. Chlorophyll accumulation indicated a stimulative effect of both stressors in treated plants. Our aim was to reveal the mechanism of this stimulation. The amount of active cytokinins increased in treated roots (prior to their transport to the leaves). This seems to be the key event in stimulation. In order to identify the signaling pathway involved in the increased cytokinin synthesis of roots, the PIP_2-IP_3/DAG and MAPK pathways were tested with different inhibitors added together with the stressors to the nutrient solution. It seems that the signal is transmitted through DAG and protein kinase C to the MAPK pathway. It is known that higher amounts of Cd^{2+} or DCMU cause oxidative stress in plants, upon which the activity of antioxidant enzymes (e.g. SOD) increases, and malonyl dialdehyde (MDA) might be finally produced by peroxidation of membrane lipids. After application of Cd^{2+} and DCMU, the amounts of SOD and MDA were measured to ascertain whether also low-concentration stressors may cause oxidative stress. We found a slight transient increase in the amount of SOD in roots.
This work was supported by grant T-047243 from OTKA.

4C_03_P

CALCIUM SENSORS ARE REGULATED BY DIFFERENT STRESS SIGNALS

Ana Lima, Edgar Cruz e Silva, Etelvina Figueira

Centre for Cell Biology, Department of Biology, University of Aveiro, Portugal
agusmaolima@gmail.com

In order to adapt to environmental stresses, plants use diverse signalling strategies. Calcium functions as a versatile messenger in mediating responses to biotic/abiotic stress signals and a variety of developmental cues in plants. The signal-specific Ca signature is readily decoded by an array of Ca-binding proteins or Ca sensor. However, little is known about the specificity of calcium signatures and how the signal is decoded. Several families of Ca^{2+} sensors have been identified in higher plants, such as calmodulin (CaM) and CBL (calcineurin B-like) proteins. Recent investigations have demonstrated that this protein variety may be are related to the specificity of stress signal perception and to the activation of the corresponding stress tolerance mechanisms. Therefore, the knowledge of which calcium sensors are triggered by specific stress signals is of major importance. Calcium-binding proteins were isolated from *Vitis vinifera* cells, after exposure to several stresses, with the aim to understand the specificity and regulation of each individual stress. *Vitis vinifera* cell cultures were exposed to salt, heavy metal, heat and cold stresses. Calcium-binding proteins were purified by hydrophobic

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

interaction chromatography. Results demonstrated that exposure to different stresses induced alterations in the proteins eluted. Protein profiles were separated by SDS-PAGE and Native PAGE in the presence and absence of calcium. The eluted proteins comprised not only calcium binding proteins, but also their targets, since only few proteins presented calcium-induced alterations in their conformations.Results provide new insights in stress signal transduction and corroborate the importance of calcium signaling in plant stress responses.

4C_04_P

ADAPTATION MECHANISMS IN RICE (*ORYZA SATIVA* L.) UNDER SALT STRESS

Md A. Kader and S. Lindberg

Department of Botany, Stockholm University, 106 91 Stockholm, Sweden. E-mail: Sylvia.Lindberg@botan.su.se

The project focuses on two important aspects of Na^+ toxicity in salt-tolerant rice cv. Pokkali and salt-sensitive cv. BRRI Dhan29, namely i) how Na^+ stress induces a change in cytosolic Ca^{2+}, $[Ca^{2+}]_{cyt}$, and pH, $[pH]_{cyt}$, and ii) how cells could maintain a low cytosolic Na^+ and/or Na^+/K^+ ratio. The salt-induced changes in $[Ca^{2+}]_{cyt}$ and $[pH]_{cyt}$ and their sources were monitored in single rice protoplasts by fluorescence microscopy. The expression of the transporter genes OsHKT1, OsHKT2 and OsVHA, which are thought to play a significant role in maintaining correct cytosolic Na^+ and or Na^+/K^+ ratio, were examined in both rice cultivars under salt stress condition by real time RT-PCR and *in situ* PCR. The results show that Na^+ must be sensed inside the cytosol, before any changes in $[Ca^{2+}]_{cyt}$ and $[pH]_{cyt}$ occur. Sensing of Na^+ induced different changes in $[Ca^{2+}]_{cyt}$ and $[pH]_{cyt}$ in the two rice cultivars with different sources for the changes. The $[pH]_{cyt}$ changes were coupled to different H^+-ATPases in the two cultivars. The expression analysis of OsHKT1, OsHKT2 and OsVHA showed variable and cell- specific induction in these cultivars under salt stress condition. The important mechanism for salt tolerance in cv. Pokkali was to keep cytosolic Na^+ at a low level, by reducing Na^+-influx (through down-regulation of OsHKT1) and compartmentalizing cytosolic Na^+ into the vacuole (through the induction of vacuolar H^+ATPase OsVHA, an energizer for the tonoplast Na^+/H^+ antiporter). Pokkali might also induce increased uptake of K^+ through the induction of OsHKT2, as evident in this study. Vacuolar compartmentalization of Na^+ is also present in salt-sensitive cv. BRRI Dhan29, but to a lesser extent and much later than in cv. Pokkali. The results suggest that the signaling and subsequent adaptive responses in the salt-tolerant rice cv. Pokkali are different from that in the salt-sensitive cv. BRRI Dhan29.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

4C_05_P

GLUTATHIONE-S-TRANSFERASE GENES OF THE CILIATE *TETRAHYMENA THERMOPHILA*: A LARGE FAMILY OF KEY DEFENCE ENZYMES AGAINST DIFFERENT STRESSES.

Ruth Ortega, Ana Martín-González and Juan C. Gutiérrez

Dpto. Microbiología-III. Facultad de Biología. Universidad Complutense (UCM). Madrid 28040. Spain. E-mail: anamarti@bio.ucm.es

Eukaryotic Glutathione-S-transferases (GSTs) comprise a large family of enzymes protecting cell from a wide range of biotic or abiotic stresses (xenobiotics, heavy metals, oxidative stress). After the complete macronuclear genome sequence of the ciliate *T. thermophila* becomes published, 63 putative ORFs encoding GSTs have been detected. Based on the amino acid sequence similarity these GST genes have been assigned to the following phylogenetic classes; 7 to the class Omega (91% identity), 5 to the class Theta (86 % identity), 2 to the class Zeta 47 (65% identity), to the class Mu (90% identity) and 2 have not been assigned to any known class. Theta and Omega classes are present in all eukaryotic organisms, while the Mu class is mammalians specific. So, ciliates (*T. thermophila* and *Paramecium tetraurelia*) are the only eukaryotic microorganisms with Mu GSTs, which have 30-41% identity with human Mu GSTs. The structural and chromosomal location characteristics of these 63 ciliate GSTs are described and discussed. Likewise, a phylogenetic analysis of all known ciliate GSTs is carried out. Several GST genes are clustered in the genome of *T. thermophila*, and this location is related with their expression capacity. The expression gene analysis, by RT-PCR, under different stress conditions (heavy metals, oxidative stress) and the screening of other EST databases (starvation, conjugation), have shown that; 1- about a 28% of Mu GSTs are expressed under the used stress conditions. 2- about 50% expressed GST genes, from the same class, are not clustered. 3- several GST genes are differentially over-expressed under a specific stress with regard control.

4C_06_P

ALTERNATIVE ELECTRON DONORS SUBSTITUTE THE INACTIVATED OXYGEN-EVOLVING COMPLEX IN DIFFERENT PLANT SPECIES

Szilvia Z. Tóth, Jos Thomas Puthur and Győző Garab

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, PO Box 521, H-6701 Szeged, Hungary
E-mail: Sztoth@brc.hu, jtputhur@rediffmail.com

Electron transport processes were investigated in different plant species in vivo under the conditions in which the oxygen-evolution was fully inhibited by a heat pulse (48-51 °C, 40 s in water bath). This treatment induces no visible symptoms and the plants recover in 1-2 days. Under these conditions, the K peak (~$F_{400\ \mu s}$) appears in the fast chl *a* fluorescence (OJIP) transient reflecting partial Q_A reduction, which is due to a stable charge separation resulting from the donation of one electron by tyrozine Z. Additional fluorescence increase occurs in the 0.2-2 s time range of the fluorescence kinetics indicating additional Q_A^- accumulation. Recently we have shown that this Q_A^- accumulation was due to naturally occurring alternative electron donors at the donor side of photosystem II. The donation probably originates from ascorbate, which provides electrons with a halftime of ~30 ms in barley (Tóth et al. 2007, Biochim Biophys Acta 1767: 295-305). In this study, we investigated several other plant species, including *Pisum sativum, Arabidopsis thaliana, Agropyron elongatum, Marchantia polymorpha*, as well as the cyanobacterium *Synechocystis* PCC 6803 and the green alga *Chlamydomonas reinhardtii.* We established the functioning of the alternative electron donors in all the investigated species. Preliminary data indicate that the inactivation temperatures of the oxygen-evolving complex and the halftimes of electron donation may vary from species to species, and may also depend on the growth conditions. We will discuss the physiological significance of this alternative electron donation to photosystem II in light and heat stress.

4C_07_P

DEAD-BOX HELICASES IN PLANT STRESS TOLERANCE

Narendra Tuteja

International Centre for Genetic Engineering & Biotechnology, New Delhi, India
narendra@icgeb.res.in

Abiotic stress is an increasing threat in reducing agricultural productivity worldwide. The high salinity stress impairs crop production on at least 20% of irrigated land. As salinity stress affects the cellular gene-expression machinery, it is evident that molecules involved in nucleic acid processing including helicases, are likely to be affected as well. Helicases are one of the smallest molecular motors of biological system, which harness the chemical free energy of ATP hydrolysis to catalyze the

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

opening of energetically stable duplex DNA (called DNA helicases) or unfold the secondary structures in RNA (called RNA helicases) and thereby are involved in almost all aspect of nucleic acid metabolism. Mostly all the helicases contain some conserved signature motifs including DEAD-box, which act as an engine to power DNA unwinding. In plants the role helicases in abiotic stress is just begening to emerge. Here we present the isolation of two pea DNA helicases (PDH45 and PDH47) and their role in salinity stress tolerance. PDH45 and PDH47 both are homologous to eIF4A, involved in translation initiation, localized in nucleus and cytosol, and contain multiple activities including DNA and RNA unwinding, ATP-binding and ATPase. The transcript of both the genes were found to upregulated under salinity stress. However, both the proteins contain only 56% identity and differ in following properties: PDH45 is unipolar, active at only basic pH, expressed more in root than shoot and not regulated by ABA treatment; while PDH47 is a bipolar enzyme, active at both acidic and basic pH, expressed more in shoot than root and upregulated by the ABA treatment. The over-expression PDH45 in tobacco plants confers high salinity tolerance without yield loss, thus suggesting a new pathway for manipulating stress tolerance in crop plants.

4C_08_P

CHARACTERIZATION OF BIOCHEMICAL PROPERTIES OF A RICE DEHYDRATION INDUCIBLE SUCROSE NONFERMENTING1 (SNF1)-RELATED PROTEIN KINASE 2 (SNRK2) FAMILY

Sun Joo Kim [1], Myung Hee Nam[2], Dool Yi Kim1, Yeon Hee Lee1, Seok-Cheol Suh[1] and In-Sun Yoon[1]*

1 Cell and Genetics Division, National Institute of Agricultural Biotechnology, Suwon 441-707, Republic of Korea. 2 Metabolome Analysis Team, Korea Basic Science Institute,
** Corresponding author; isyoon@rda.go.kr*

OSRK1 is a rice SnRK2 protein kinase activated by hyperosmotic stress and ABA. In the present study, we investigated regulatory mechanism and down stream targets of OSRK1. GST-fused recombinant OSRK1 showed strong substrate preference for rice bZIP transcription factors and uncommon cofactor requirement for Mn^{2+} over Mg^{2+}. We observed positive relationship between autophosphorylation level and kinase activity. Moreover, OSRK1 could transphosphorylate itself. By deletion of C-terminus 73 amino acids or mutations of Ser-158 and Thr-159 to aspartic acids (Asp) in the activation loop, the activity of OSRK1 was dramatically decreased. Our data suggests that inter-molecular (auto)phosphorylation of catalytic domain of OSRK1 is important for enzyme activation. In an attempt to investigate OSRK1 signaling components, we identified two

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

putative calcium binding proteins by yeast two hybrid screening. In vitro OSRK1 activity was stimulated by those CBPs. By in gel kinase assays, we also showed that ca. 52 kDa and 61 kDa protein kinase activities were highly stimulated in response to salt or ABA in roots of transgenic rice over-expressing OSRK1. They are likely to be downstream target kinases for OSRK1 signaling pathway. This work was supported by On-Site Cooperative Agriculture Research Project, RDA, Republic of Korea.

4D_01_P

GRASSLAND ECOLOGY UNDER ELEVATED AIR CO_2 CONCENTRATIONS

Zoltán Tuba[1,2], Zoltán Nagy[2]

[1]Plant Ecological Research Group of Hungarian Academy of Sciences
[2]Department of Botany and Plant Physiology, Faculty of Agricultural and Environmental Sciences, Szent István University, Gödöllő, H-2103 Gödöllő, PáterK. u. 1, Hungary, tuba.zoltan@mkk.szie.hu

Global climate change appears to be the greatest ecological problem of the future. For example the CO_2 emitted into the atmosphere will have a long-term effect and this is one of the most influential ecological factor at global scale. Its increasing concentration affects the plants directly, causing changes in their ecophysiological processes. Consequently, the tolerance, reproduction, distribution and abundance of plant species will be altered. Species composition and diversity of plant communities, the vegetation dynamics are all expected to be changed, too. Considering terrestrial ecosystems, grasslands are the second largest vegetation formation after the forests and their area is further increasing with the land use changes (clearance of the forests, urbanisation, extensive agricultural practices and abandonment of agricultural land). On a global scale grassllands cover 24% of the Earth's vegetated area. They occur over a broad range of climatic and soil conditions and vary from intensively managed sown pastures to natural grassland communities. Despite their importance, the potential effects of climate change on grasslands have received much less attention than effects on other ecosystems such as forests. The main objective of the proposed talk is to discuss the effects of elevated air CO_2 concenrations on the structure (botanical, species composition, floral diversity, canopy and below ground/root architecture) and function (CO_2,and H_2O exchange carbon cycling, dry matter production responses, etc.) of grasslands and to quantify the carbon storage in grassland ecosystems.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

4D_02_P

HOW DO PLANTS SURVIVE EXTREME CLIMATIC EVENTS?

Ivan Nijs

University of Antwerp, Department of Biology, Universiteitsplein 1, B-2610 Wilrijk, Belgium, ivan.nijs@ua.ac.be

Climatic extremes, such as heat waves and extreme droughts, are predicted to become more frequent and severe in a future atmosphere with more greenhouse gases. However, untill now nearly all experimental studies on the impact of climate warming on plants have concentrated on changes in mean temperature, and studies on extremes are only beginning to emerge. Here I report on six recent field studies concerning the ecophysiological effects of heat waves, including three studies on temperate grassland and three on arctic tundra. In all experiments, the heat waves were generated with computer-controlled infrared irradiation in the field. In some cases we allowed natural precipitation, in others we excluded rainfall completely. We investigated the following questions: (1) is resistance to extremes in individuals plants coupled to specific plant traits? (2) does resistance vary with the complexity of the community? (3) do climatic extremes exert physiological after-effects long after the event has passed, e.g. on stomatal functioning, photosynthetic efficiency, chlorophyll synthesis/breakdown, etc.? (4) does tundra react differently than temperate grasslands? The main findings can be summarized as follows. In grassland species, morphological and ecophysiological indicators that best explained plant resistance to extreme drought, were different from known indicators of resistance to moderate stress. Plant survival in complex grassland communities was different from survival in monocultures, which can be explained by differences in resource use (water). In arctic communities, heat waves alleviated stress during exposure, but stress symptoms aggravated after the heat waves had ended. Because plant responses were species-specific in most communities, future shifts in species composition when current regimes of extreme events change, can be anticipated.

4E_01_P

STRUCTURE-FUNCTION STUDIES OF TOMATO ASR1, A WATER STRESS- AND SALT STRESS-REGULATED PLANT-SPECIFIC PROTEIN

Dudy Bar-Zvi[1], Zvia Konrad[1], Slava Rom[1], Doron Shkolnik[1], Natalia Shadrin[2], Rodolfo Ghirlando[3], and Yehuda Goldgur[2]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Departments of Life Sciences[1], and Chemistry[2], Ben-Gurion University, Beer-Sheva 84105, Israel. [3]Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, USA. E-mail of corresponding author: barzvi@bgu.ac.il

ASR1 (abscisic acid stress ripening) is a low molecular weight plant specific protein encoded by an abiotic-stress regulated gene. The ASR1 protein possesses a zinc-dependent DNA-binding activity. The DNA binding site was suggested to reside in the central part of the polypeptide by using truncated forms of the protein. Two additional zinc-binding sites were shown to be localized at the amino terminus of the polypeptide. ASR1 protein is presumed to be a natively unfolded protein using a number of prediction algorithms. The degree of order of ASR1 was determined experimentally using non-tagged recombinant protein expressed in *E. coli* and purified to homogeneity. Purified ASR1 was shown to be unfolded using dynamic light scattering, gel filtration, microcalorimetry, circular dichroism and Fourier transform infrared (FTIR) spectrometry. The protein was shown to be monomeric by analytical ultracentrifugation. Addition of zinc ions resulted in a global change in the ASR1 structure, from monomer to homodimer. Upon binding of zinc ions, the protein becomes ordered as shown by FTIR and microcalorimetry, concomitant with dimerization. Cytosolic ASR1 is unfolded whereas nuclear ASR1 is ordered. The effect of zinc binding on ASR1 folding and dimerization, and activity of cytosolic and nuclear ASR1 protein in is discussed.

4E_02_P

AUTOMATIC PLANT LEAF TEMPERATURE MONITORING FOR STRESS DETECTION

Laury Chaerle[1,*] Ilkka Leinonen[2,3] Hamlyn G. Jones[2] and Dominique Van Der Straeten[1,*]

[1]Unit Plant Hormone Signalling and Bio-imaging, Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium
[2]Plant Research Unit, Division of Environmental and Applied Biology, University of Dundee at
SCRI, Invergowrie, Dundee DD2 5DA, Scotland, UK
laury.chaerle@ugent.be, dominique.vanderstraeten@ugent.be

Transpiration and photosynthesis are the key parameters of plant development. Stress situations inevitably alter the kinetics of these key plant physiological processes. Measuring these parameters to compare and characterize agricultural performance of plant cultivars is routinely done with portable gas analysis equipment, directly monitoring water loss

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

and CO_2 use. Since repeated measurements are needed, this procedure is tedious and consequently few leaves per plant can be measured. Thermal imaging reveals leaf temperature in a non-contact way, and can be used effectively for monitoring transpiration as a function of time. To achieve single-plant resolution in Arabidopsis, a plant growth-chamber based robotised imaging system was used. A number of Arabidopsis ecotypes selected on maximum genetic diversity was ranked on average rosette temperature. Due to both natural variation in leaf temperature and the fact that plant leaf temperature changes during a day, rosettes cannot always be discerned from the background of well-watered substrate. Therefore leaf area detection was based on co-located chlorophyll fluorescence images. In comparison with direct transpiration measurements, thermal imaging coupled to automated plant selection detected more significant temperature differences. This study proves the applicability of thermography under controlled conditions to compare the transpirational responses of different plant cultivars, with applications in drought tolerance and disease resistance screening.

4E_03_P

T22, A BIOCONTROL *TRICHODERMA HARZIANUM* STRAIN ENHANCED DROUGHT STRESS RESISTANCE OF PEACH ROOTSTOCK 'PEMA' (*PRUNUS PERSICA* X *PRUNUS AMYGDALUS*)

P. Czövek[1], I. Király[1], I. Balla[2] and E. Szűcs[2]

[1]Eötvös University Institute of Biology, Department of Plant Physiology and Molecular Plant Biology
H-1117, Budapest Pázmány sétány 1/C Hungary, E-mail: ikiraly@ludens.elte.hu
[2]Research Institute for Fruitgrowing and Ornamentals H-1223 Budapest, Park u. 2.

Biocontrol is an environmentally sound method used for the prevention and control of plant diseases. Advisedly selected fungal and bacterial strains are marketed worldwide promoting to spread of this inexpensive and effective technology. The effect of biocontrol species is composed of (1) direct interaction with potential plant pathogens, (2) eliciting host-mediated defense reactions, (3) other beneficial effects as enhanced root growth. *Trichoderma harzianum* is a soil fungi living in the close vicinity of plant roots. As a hyper parasite it kills many pathogenic fungi generally by hydrolyzing mycelia walls. The T22 *T. harzianum* strain has also a strong chitinase activity and many beneficial effects on the host plant, including enhanced root growth. We used the commercially available T22 strain to defend fruit trees against drought stress profiting its effect on the plant defense reactions. The changes of the superoxide dismutase activity and

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

the raise of malondialdehyde level as a consequence of far gone lipid peroxidation were monitored during the stress treatment. The physiological state of the control and stressed fruit trees was characterized also by the chlorophyll content, the fresh weight-dry weight ratio and the stomatal conductancy. The results obtained clearly demonstrate that T22 treated fruit trees resisted well even the vigorous drought stress applied. The enhanced resistance correlated with the changing of the activity of the reactive oxygen scavenging system. This work was supported by GVOP-3.1.1-2004-05-0061/3.0.

4E_04_P

EFFECTS OF POTASSIUM ON SALT TOLERANCE OF SEEDLING IRANIAN PISTACHIO

Majid Fekri

Vali-asr University, Rafsanjan, Iran, mjdfekri@yahoo.com

The effects of two K levels (50 and 100 mg/kg K2SO4) and six treatment of irrigation with solution of NaCl (1, 2, 4, 8, 16 and 20 ds/m) on the growth , Chemical composition and plant water relationships seeding of Iranian pistachio (Pistachio vera L .) were studied in an arid calcareous soil in a glasshouse experiment. Tolerant to salt was less in the low K level than high K level, probably because of higher uptake and /or transport of Cl and Na ions, lesser osmoregulatory ability, and greater reductions in the top and root dry weights. The 50% reduction the top and root dry weights were obtained, respectively, at an electrical conductivity of the saturation extract (ECe) of 12 and 13 ds/m for low K level and 16 and 17.5 for high k level. Higher levels of K fertilization may be beneficial for Pistachio to tolerate to salt stress conditions.

4E_05_P

ROLE OF PHOTOSYNTHETIC PERFORMANCE IN SALT STRESS ACCLIMATION OF TOMATO AFTER SALICYLIC ACID PRE-TREATMENT

Katalin Gémes, Ágnes Szepesi, Adrienn Guóth, Irma Tari

Department of Plant Physiology, University of Szeged, 6701 Szeged POB 654, Hungary
pigsy17@freemail.hu, tari@bio.u-szeged.hu

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Imposition of salt stress reduced the net CO_2 assimilation rate, chlorophyll (Chl) and carotenoid contents, stomatal conductance and biomass production of tomato (*Lycopersicon esculentum* Mill. L. cv. Rio Fuego). Pre-treatments of plants with 10^{-4} M, but not with 10^{-7} M salicylic acid (SA), could partially restore the CO_2 fixation rate, Chl a/b ratio, carotenoid levels under 100 mM NaCl exposure. Accumulation of soluble sugars, a biochemical marker of salinity tolerance of tomato, could be detected in pre-treated plants. After SA application, concentrations of hexoses (glucose and fructose) remained high in the leaves and that of sucrose in the roots during salt stress. Both SA and salt stress increased the H_2O_2 production of tissues. Activities of superoxide dismutase and catalase (CAT), a H_2O_2 generating and scavenging enzymes, respectively, decreased significantly under salt stress, but in 10^{-4} M SA pre-treated plants, CAT activity was significantly induced both in the root and shoot tissues. The improved photosynthetic performance, the accumulation of soluble sugars as compatible osmolytes and the effective elimination of H_2O_2 by CAT contributed to the successful acclimation of 10^{-4} M SA pre-treated tomato plants to high salinity. – This work was supported by OTKA T038392.

4E_06_P

COMPARISON OF CHANGES IN PHOTOSYNTHESIS, CHLOROPHYLL FLUORESCENCE PARAMETERS AND ABSCISIC ACID LEVELS IN WHEAT CULTIVARS UNDER DROUGHT STRESS DURING GRAIN FILLING AND IN SEEDLINGS UNDER OSMOTIC STRESS

A. Guóth[1], I. Tari[1], Á. Gallé[1], J. Csiszár[1], L. Cseuz[2], L. Erdei[1]

[1]*Department of Plant Physiology, University of Szeged, 6701 Szeged, POB 654 Hungary*
[2]*Cereal Research Non-Profit Company, 6701 Szeged, POB 391 Hungary*
guotha@bio.u-szeged.hu

We investigated the effect of water deficit on the photosynthetic parameters and the abscisic acid (ABA) levels in wheat. A comparison was made between the changes of the parameters mentioned above, in seedling stage under osmotic stress and in reproductive growth phase under soil drought in two Hungarian wheat cultivars *Triticum aestivum* L. cv. MV Emese (resistant) and GK Élet (sensitive). The water status parameters, CO_2 assimilation, chlorophyll *a* (chl*a*) fluorescence, pigment content and ABA levels were determined as a function of the development of water deficit. The tolerant genotype cv. Emese maintained higher water potential in upper leaves under osmotic or drought stress than the sensitive cv. Élet. In spite of this, the tolerant line exhibited an earlier ABA accumulation in the grains (DPA 9) than the sensitive line (DPA 24). The

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

characteristics of the CO_2 assimilation and chl*a* fluorescence parameters measured in flag leaf (Yield, qP, NPQ) did not differ between the two varieties and compared to the control, the Yield changed similarly to the CO_2 fixation. But in seedling stage under osmotic stress the CO_2 assimilation declined significantly and in contrast to the flag leaf the qP decreased and the NPQ increased significantly in both varieties, and the tendencies were the same in both genotypes. The chl*a* fluorescence parameters and the efficiency of photosynthesis measured on the seedlings did not correspond to those measured on the flag leaf in the reproductive growth phase.-*This work was supported by Grant No. NKFP 4/064/2004*

4E_07_P

EVALUATION OF ABIOTIC STRESS TOLERANCE OF *NICOTIANA TABACUM* PLANTS BEARING AN ANTISENSE SUPPRESSOR OF THE PROLINE DEHYDROGENASE GENE

A.V. Kochetov, Ya.S. Kolodyazhnaya, S.E. Titov, M.L. Komarova, A.V. Romanova, V.S. Koval, E.A. Trifonova, V.K. Shumny

Institute of Cytology and Genetics, Novosibirsk 630090, Russia; ak@bionet.nsc.ru

Investigation of proline metabolism is important both to understand stress response mechanisms and to improve crops. Earlier evaluations of stress resistance of transgenic arabidopsis plants bearing antisense suppressor of proline dehydrogenase resulted in some controversial observations (Nanjo et al., 1999; Mani et al., 2002; Ribarits, 2007). We obtained tobacco plants bearing a fragment of arabidopsis proline dehydrogenase gene in antisense orientation. The usage of heterologous antisense suppressor resulted in a mild increase in proline content in non-stressed plants (by a factor of 2 to 3 in different lines). Under the stress conditions (200 mM of NaCl) the content of proline was increased to similar levels in both transgenic and control plants. According to the results obtained transgenic plants were characterized by a mild increase in general (non-specific) stress tolerance: (1) they survive better in the presence of NaCl or PEG6000 in growth media and contain a smaller quantity of MDA; (2) they tolerate higher concentrations of various heavy metals (Pb, Cd, Ni, Hg); (3) the detached leaves of transgenic plants lose water at a lower rate; (4) the electrolyte leakage experiments showed lower membrane permeability of cells of transgenic plants; (5) after the exposition at high temperature, seeds of transgenic plants were characterized by a higher germination rate. It may be assumed that an increased level of proline under normal conditions could partially alleviate the damage at stress onset and facilitate rapid induction of stress response mechanisms.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Transgenic plants were characterized by normal phenotype and development.

4E_08_P

RESOURCE SHARING AMONG INTERCONNECTED RAMETS ENHANCES STRESS TOLERANCE IN *IRIS PUMILA*

Sanja Manitašević[1], Ana Vuleta*, Gordana Matić, Jadranka Dunđerski and Branka Tucić*

*Department of Biochemistry and *Department of Evolutionary Biology, Institute for Biological Research „Siniša Stanković", 11060 Belgrade, Serbia, [1]manitas@ibiss.bg.ac.yu*

Physically connected ramets of clonal plants are able to reciprocally exchange essential resources (i.e., water, assimilates and nutrients) if they are unevenly distributed over space. We tested the hypothesis that translocation of resources between source and sink ramets increases stress tolerance of the whole clone growing in a patchy environment. This study was conducted in an exposed population of *Iris pumila* growing in the wild. In early spring, circle-shaped clonal genotypes were cut into two equal parts with different integration status: C - with physically connected rhizome segments and NC - with ceased connections. One half of each clone was shaded with a neutral screen in such a way that both C and NC parts consisted of exposed (high-light stressed) and shaded (up to 50% of ambient irradiance) quarters. During the summer, the leaf tissues were sampled from each quarter within every clone. Variation pattern of the following traits was examined: the level of stress proteins Hsp70 and Hsp90 (Hsp90a and Hsp90b isoforms), as well as relative water content (RWC) and specific leaf area (SLA). While the value of SLA decreased with irradiance, Hsp90a expression and RWC increased, regardless of their integration status. Conversely, the amount of Hsp70 and Hsp90b dramatically elevated exclusively in the exposed quarter interconnected with shaded one. These results suggest that resource-sharing among interconnected ramets could provide a powerful strategy in *I. pumila* to enhance the tolerance to stressful abiotic heterogeneity commonly occurring within its natural habitats.

4E_09_P

ACCLIMATION AND TOLERANCE TO HEAT AND HIGH ILLUMINATION IN *BRASSICA*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

María José Quiles, Milagros Díaz, Virginia de Haro, Romualdo Muñoz

Department of Plant Biology. University of Murcia. 30100 Espinardo, Murcia, Spain
Corresponding author: mjquiles@um.es

Two species of *Brassica* were used to study their acclimation to heat and high illumination during the first stages of the development. One, *B. fruticulosa*, is a wild species from south-east Spain and is adapted to both heat and high light intensity in its natural habitat, while the other, *B. oleracea*, is an agricultural species that is widely cultivated throughout the wold. The *B. fruticulosa* plants grown under high illumination and heat showed a greater reduction of the foliar area and biomass than the *B. oleracea* plants in relation with the respective control plants,. The quantum yield of the PS II, the capacity of photosynthetic electron transport and photochemical quenching, decreased in *B. oleracea* plants when grown under stress conditions, indicating the inhibition of PS II. However, in *B. fruticulosa* the values of these parameters were similar to control plants. PS I was more stable than PS II, probably because it plays a protective role though cyclic electron flow. The PS I activity increased in *B*. *oleracea* and *B. fruticulosa* plants exposed to heat and high illumination. The activities of plastidial NADH deshydrogenase complex and terminal oxidase were much higher in *B. fruticulosa* than in *B. oleracea*, and both were stimulated in plants grown in stress conditions. Acclimation and tolerance to high illumination and heat of the photosystem activities was higher in the wild species than in the agricultural species, indicating that plant adaptation to these stresses in natural conditions favours subsequent acclimation, and that, the chlororespiration process is probably involved in both adaptation and acclimation to heat and high illumination. This work was supported by the Spanish MEC (BFU2005-09243-C02-01).

4E_10_P

EFFECT OF SALINITY AND SOIL NITROGEN APPLICATION ON BIOCHEMICAL INDICES OF PISTACHIO

A.Razavi Nasab, A.Tajabadi Pour and H.Shirani

Soil Science Department, Agriculture College, Vali-Asr University, Rafsanjan, Iran. azamrazavinasab@yahoo.com

Soil salinity is an important growth limiting factor for most plants. Salinity inhibits plant growth by osmotic stress, nutritional imbalance and some specific toxicity and it is being progressively exacerbated by fertilization, especially in arid regions. In other hands, nitrogen (N) is an essential

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

element for plants. It has a fundamental role in amino acids, proteins, nucleoic acids and lots of enzyme structure. The proper use of N fertilizers in all soils is important, but particularly so in saline soils, where N might reduce the adverse effects of salinity. In high salinity levels, the antagonistic relationships are attributed to the negative membrance potential of root cells and the negative charge of both nitrate and chloride. Most plants respond to salinity stress in their environment by osmoregulation of their cellular content. Synthesis and accumulation of organic solute of carbohydrate and N compounds (e.g. proline) are used for this purpose. The present study, therefore, was initiated to evaluate the effects of N and salinity on proline and reducing sugars content in pistachio. Pistachio seedlings were grown in potted soils exposed to four salinity levels (0, 800, 1600, and 2400 mg NaCl kg^{-1} soil) and four N levels (0, 60, 120, and 180 mg kg^{-1} soil as urea). As the salinity levels increased, the leaf proline content significantly increased while concentration of reducing sugars decreased. A significant increase in proline and reducing sugars was observed with N application. The evidence presented in this study suggests that a function of proline under salt stress is that of osmoregulation. Also, the results show that the provision of the optimal nutritional regimes, therefore, has an important contribution to the determination of pistachio reaction to soil salinity.

4E_11_P

EFFECT OF SALINITY AND SOIL ZINC AND PHOSPHOROUS APPLICATION ON BIOCHEMICAL INDICES OF PISTACHIO.

R. Shahriaripour, A.Tajabadipour and V.Mozaffari

Department of Soil Science, College of Agriculture, Rafsanjan University, Rafsanjan, Iran. Roghayeh_shahriari2371@yahoo.com

Salinity is a world-wide problem which adversely affects the growth of many plants through a series of interacting factors including some specific ion toxicities, ion imbalance and suppression of water potential. Most plants respond to changing osmotic potentials in their external environment by osmoregulation of their cellular content. Synthesis and accumulation of organic solutes (e.g proline) are utilized for this purpose. The amount of proline accumulation correlates well with the degree of stress in plants subjected to different salts. Soil fertilization appears to be beneficial in reducing the plant growth depression due to soluble salts. The present study, therefore, was initiated to evaluate the main and interactive effects of zinc, phosphorous and salinity levels on proline and reducing sugars content in pistachio. Pistachio plants were grown in potted soils exposed to four salinity levels (0, 1000, 2000, 3000 mg NaCl Kg^{-1} soil), four phosphorous levels (0, 60, 120 and 180 mg P Kg^{-1} soil as $Ca(H_2PO_4)_2$) and

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

three zinc (Zn) levels (0, 5, 10 mg Zn Kg^{-1} soil as $ZnSo_4.7H_2O$). As the salinity levels increased, the leaf proline content significantly increased. However, Zn application declined the proline content but the application of phosphorous had not a significant effect on proline and reducing sugars content. The reducing sugars concentration was affected by salinity levels and Zn application. A significant decrease in reducing sugars was observed with increasing salinity levels and decreasing Zn levels. The evidence presented in this study suggests that a function of proline in pistachio under salt stress is that of osmoregulation. It seems that proline is not directly involved in osmoregulation, but it plays an indirect role by increasing hydration of protoplasm. The results of this study show that the provision of the optimal nutritional regimes, therefore, has an important contribution to the determination of pistachio reaction to soil salinity.

4E_12_P

INFLUENCE OF A NOVEL MITOCHONDRIAL PPR DOMAIN PROTEIN ON ABIOTIC STRESS RESPONSES OF *ARABIDOPSIS THALIANA*.

Laura Zsigmond, Gyöngyi Székely, Csaba Koncz, László Szabados.

Institute of Plant Biology, Biological Research Center, 6726-Szeged, Temesvári krt. 62, Hungary. Corresponding author: szabados@brc.hu

Abiotic stress can activate the expression of numerous genes through several abscisic acid (ABA) dependent and independent pathways. ABA plays important roles in dormancy, dessication control, and osmotic stress responses. We have identified a novel T-DNA tagged *Arabidopsis* mutant, which is characterized by hypersensitivity to high salinity, abscisic acid (ABA) and sugar treatments. The mutant was further characterized by enhanced proline accumulation, increased tolerance to methylglyoxalate, and altered expression of numerous stress-responsive and other genes. The T-DNA insertion disrupted the gene *At3g16890,* encoding an unknown mitochondrial protein, composed of 14 pentatricopeptide (PPR) repeats. Overexpression of the full length cDNA in the tagged mutant complemented the stress hypersensitivity and lead to increased salt tolerance in several transgenic lines. The encoded protein was localized in the mitochondria and was associated with complex III. in the mitochondrial electron transport system. Characterization of the mutant and the corresponding gene suggest a possible link between mitochondrial electron transport, stress responses and ABA regulation in higher plants.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

4E_13_P

EFFECT OF S-METHYLMETHIONINE ON MAIZE EXPOSED TO COLD STRESS CONDITIONS

D. Szegő[1], T. Visnovitz[1], E. Páldi[2], D. Lasztity[1], Z. Szigeti[1], I. Rácz[1]

[1]Eötvös Univ., Dep. of Plant Phys. and Mol. Plant Biol., Pázmány P. s. 1/C, H-1117 Bp
[2]Agr. Res. Inst. of the Hun. Academy of Sci., Brunszvik s. 2., H-2462Martonvásár
raczil@ludens.elte.hu

To improve stress- and disease-tolerance of cultivated plants are a crucial problem in the agriculture. Utilization of biologically active substances, possessing an effect on metabolism and directing it towards the required pathways and thus ensuring the favourable property, could be a possible way. The natural intermediate compound S-methylmethionine (SMM), which is synthesised from methionine by adenosyl-methionine-methionine-S-transferase, also belongs to biologically active substances. SMM has an important role in the sulphur metabolism, participating in methylation reactions, in the biosynthesis of osmoprotectant sulphopropionates and through this, in the stress- and disease-tolerance of plants. Physiological effect of SMM is similar to that of polyamines which is probably due to the fact that SMM can take part in reaction pathways leading to the biosynthesis of polyamines. In this work we studied the effect of exogenously added SMM in maize (*Zea mays* L., cultivar Norma) under cold stress conditions. Time course of changes in polyamine levels (e.g.: putrescine, spermidine) were followed under cold, SMM and the combined treatments. To understand the molecular background of changes of polyamine level under these circumtances, expression of arginine decarboxylase, spermidine synthase and SAM-decarboxylase genes, which take part in the polyamine synthesis, were also investigated. Changes in expression levels of CBF1, which is an early induced transcription factor under cold stress conditions and causes switching on several structural-genes, were also measured, so that we could get broader knowledge for stress avoiding role of the SMM.

4E_14_P

THE EFFECT OF SOIL ZINC APPLICATION ON LIPID PEROXIDATION OF CELL MEMBRANE, PHENOLIC COMPOUNDS AND FLAVONOIDS IN PISTACHIO (*PISTACIA VERA* CV. GHAZVINI) UNDER SALINITY STRESS.

M. Talebi, V. Mozaffari and A. Tajabadipour.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Soil Sciences Department, Vali-Asr University, Rafsanjan, Iran.
msctalebi@yahoo.com

Salt stress is considered as one of the most important abiotic factors limiting plant growth and yield in many areas of the world. One of the main criteria for salt tolerance is cell membrane stability under stress. Reactive oxygen species (ROS) and lipid peroxidation are considered to be destructive to cell membrane under salt stress. Phenolic compounds and flavonoids in plants decrease negative effects of reactive oxygen species and increase stability of cell membrane. The objective of this study was to evaluate the effect of zinc and salinity on some physiological and biochemical properties of pistachio (cv. Ghazvini). A factorial greenhouse experiment was carried out as completely randomized design with three replications. Treatments were 4 levels of Zn (0, 5, 10 and 15 mg kg^{-1} soil) and 5 levels of salinity (0, 800, 1600, 2400 and 3200 mg NaCl kg^{-1} soil). The results indicated that by increasing salinity, lipid peroxidation in leaves increased and levels of MDA (as an indicator of lipid peroxidation) increased significantly in response to NaCl. MDA concentrations increased with soil salinity levels up to 1600 mg NaCl kg^{-1} soil, but decreased at higher salinity. Simultaneously, concentration of other aldehyde increased with decreasing MDA. Application of 10 mg kg^{-1} zinc decreased over 33% MDA concentration in leaves. Also, phenolic content and flavonoids increased with increasing salinity. Application of 15 mg kg^{-1} of Zn significantly increased these compounds in comparison to control. In other words application of Zn increased phenolic compounds by 24% and flavonoids by 28%. The results presented in this paper suggest that zinc can increase the growth rate of salinity stress in pistachio in increase of phenolic compounds and flavonoids as an antistress compounds.

4E_15_P

ROLE OF PHOTOSYNTHETIC PERFORMANCE IN SALT STRESS ACCLIMATION OF TOMATO AFTER SALICYLIC ACID PRE-TREATMENT

Katalin Gémes, Ágnes Szepesi, Adrienn Guóth, Irma Tari

Department of Plant Physiology, University of Szeged, 6701 Szeged POB 654, Hungary
pigsy17@freemail.hu, tari@bio.u-szeged.hu

Imposition of salt stress reduced the net CO_2 assimilation rate, chlorophyll (Chl) and carotenoid contents, stomatal conductance and biomass production of tomato (*Lycopersicon esculentum* Mill. L. cv. Rio Fuego). Pre-treatments of plants with 10^{-4} M, but not with 10^{-7} M salicylic acid

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

(SA), could partially restore the CO_2 fixation rate, Chl a/b ratio, carotenoid levels under 100 mM NaCl exposure. Accumulation of soluble sugars, a biochemical marker of salinity tolerance of tomato, could be detected in pre-treated plants. After SA application, concentrations of hexoses (glucose and fructose) remained high in the leaves and that of sucrose in the roots during salt stress. Both SA and salt stress increased the H_2O_2 production of tissues. Activities of superoxide dismutase and catalase (CAT), a H_2O_2 generating and scavenging enzymes, respectively, decreased significantly under salt stress, but in 10^{-4} M SA pre-treated plants, CAT activity was significantly induced both in the root and shoot tissues. The improved photosynthetic performance, the accumulation of soluble sugars as compatible osmolytes and the effective elimination of H_2O_2 by CAT contributed to the successful acclimation of 10^{-4} M SA pre-treated tomato plants to high salinity. – This work was supported by OTKA T038392.

4E_16_P

PROTEIN MARKERS FOR REGENERATION CAPACITY FROM SALT STRESSED EMBRYOGENIC SUSPENSION CULTURES OF DACTYLIS GLOMERATA L.

Lyuben Zagorchev, Ivanka Tzacheva, Mariela Odjakova*

Dept. of Biochemistry, Faculty of Biology, Sofia University
8 Dragan Tzankov Blvd., Sofia, Bulgaria
*modjakova@biofac.uni-sofia.bg

Embryogenic suspension cultures of Dactylis glomerata were treated with different salt concentrations - 85 mM, 170 mM and 255 mM NaCl. While 85 mM NaCl enhances the level of somatic embryogenesis, higher salt concentrations affect negatively the regeneration potential. The protein patterns of cell wall-associated and culture media fractions showed significant alterations. Proteins, abundant in the cell wall at 85 mM NaCl and absent at higher salt concentrations are potential markers for the regeneration capacity. Many of these protein fractions appear in the culture media at 170 mM NaCl. A protein, with pI 9.2-9.4 and molecular weight 73 kD was chosen, as an antigen for screening the human semisynthetic single-chain Fv (scFv) phage library (Griffin.1) by 2D phage panning for selection of specific monoclonal antibodies. Eight phage clones were selected in four rounds of screening. All scFvs were positive on Western blot analysis. Selected recombinant antibodies were obtained in soluble form by expression in Escherichia coli HB2151. Western blot analysis against a 2D PAGE sample of extracellular proteins from cultures, treated with 170 mM NaCl, showed two fractions: the 73 kD protein, pI 9.2, used as antigen, plus a 33 kD, pI 8.3 protein. The 73 kD protein is

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

found in the cell wall at 85 mM NaCl and absent at 170 mM. To our opinion, the selected antibodies can be used as potential markers for the regeneration capacity of Dactylis glomerata cell cultures.
The research was funded by grant ВУ-Б-203/06 of the Ministry of Education and Science of Bulgaria.

4E_17_P

MEDICAGO TRUNCATULA PLANTS EXPRESSING THE ELIP-LIKE DSP22 SHOWED IMPROVED CAPACITY TO RECOVER FROM A SEVERE WATER DEFICIT.

Araújo SS[1a)], Duque AS[1], Marques da Silva J[2,3], Santos D[1], Bernardes da Silva A [2,3] and Fevereiro P[1,2]

[1] - Plant Cell Biotechnology Laboratory, ITQB, Universidade Nova de Lisboa, Apt 127, 2781-901 Oeiras, Portugal; [2] - Departamento de Biologia Vegetal, FCUL, Campo Grande, 1749-016 Lisboa, Portugal; [3] - Centro de Engenharia Biológica, FCUL, Campo Grande, 1749-016 Lisboa, Portugal.
a) Corresponding author: saraujo@itqb.unl.pt

Medicago truncatula cv Jemalong plants expressing the DSP22 showed improved capacity to recover from a severe water deficit episode after rewatering, when compared to the non transformed line. DSP22, an ELIP-like protein from *Craterostigma plantagineum*, is thought to protect the chloroplast against photo-oxidative damage during the dehydration and rehydration process so characteristic of this resurrection plant. Transgenic plants showed higher Φ_{PSII} values under moderate water deprivation and during rewatering when compared to the wild type. They also showed higher amounts of chlorophylls and lower Chl *a*.Chl b^{-1} ratio in both water regimes. Such results suggest that the protective roles of the ELIP-like DSP22 against water stress or during rewatering may be associated with the transient binding to photosynthetic pigments and/or with the preservation of chloroplast protein complexes, such as the LHCII. Biomass accumulation in the transgenic line was less affected by water deficit than in the non transgenic lines. Transgenic *M. truncatula* plants showed evidences of a higher potential to withstand water stress than the non transgenic, an aspect that could explain the better chances to recover from temporary water stress episodes, which is an important issue when addressing plant improvement toward water stress.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

4E_18_P

ANALYSIS OF SALT AND DROUGHT TOLERANCE IN TRANSGENIC TOMATO OVEREXPRESSING THE *ARABIDOPSIS* TRANSCRIPTION FACTOR ATHB7

R. Iannacone, A. Petrozza, N. Armentano, Henrik Johannesson*, P. Engstrom* and F. Cellini

*Metapontum Agrobios, Metaponto (MT), Italy; *Department of Physiological Botany, Evolutionary Biology Centre, University of Uppsala, Villavagen 6, SE-752 36 Uppsala, Sweden. E-mail: riannacone@agrobios.it*

ATHB7 is a member of HD-zip protein and was demonstrated to be involved in drought response in plants. ATHB7 is transcriptionally induced by dehydration in post-germinative stages of the life cycle and is supposed to act as a negative regulator of growth (Olsonn et al. 2004). Constitutive expression of *ATHB7* in *Arabidopsis* led to a suppression of shoot elongation growth and retardation of bolting.
To assess the effect of this gene in an heterologous system we obtained transgenic tomato plants overexpressing *ATHB7* mRNA, by using a CaMV35S::ATHB7 construct. Transgenic T0 plants did not show any visible phenotypic alteration and selected lines showed high level of expression of *ATHB7* RNA. Drought and salt tolerance was evaluated in T2 tomato progeny of four independent transgenic lines. Six replicates for each line were grown in greenhouse under normal agronomic trial and under stress condition. Drought tolerance was evaluated using one third of irrigation water respect to the not-stressed plants while salt tolerance was evaluated using irrigation water containing 150 mM NaCl. Physiological and agronomical data were collected during the trial and differences in stress response were observed among transgenic lines and respect to the isogenic lines. No meaningful differences were observed between transgenic and non transgenic lines in the non-stressed trials. The experiment was repeated using T3 progeny on the two best performing transgenic lines and the non transgenic control.
Results on agronomic performance and physiological data of tested plants will be discussed.

(Olsson et al. 2004. Plant Mol. Biol. 55:663-677)

4F_01_P

RESPONSE OF ORNAMENTAL PERENNIALS TO DROUGHT STRESS IN URBAN CONDITIONS

Wojciech K. Chylinski, Aleksandra J. Lukaszewska

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Department of Ornamental Plants, Faculty of Horticulture and Landscape Architecture,
Warsaw Agricultural University
159 Nowoursynowska Str., 02-776 Warsaw, Poland
chylinski@mail.pt

In comparison to natural habitats (forests, grasslands, wetlands and riparian zones) cities and towns represent very young habitats (Wittig 2004). The presence of high anthropopressure and the extensive infrastructure of urban areas have a major impact on such environmental factors as climate, soil layer, hydrology, disturbance regime, and management practices (Sukopp and Heneke 1983; Sudnik-Wójcikowska and Galera 2005; Turner et al. 2005). The most exposed to drought, which is the most important abiotic stress in urban areas, are the herbaceous plants: perennials and annuals. In this trial a response to water stress of three commonly used garden species, avens (*Geum coccineum* Sm.), heartleaf saxifrage (*Bergenia cordifolia* (Haw.) Sternb.) and stonecrop (*Sedum spectabile* Bor.) was compared. The first avens is very herbaceous and prone to wilting, heartleaf saxifrage has thick rhizome and leathery, evergreen leaves and the last one is a succulent species adapted to drought. During two vegetative seasons (2005 and 2006) plants were subjected to water stress: drought was imposed by withhelding watering during 10-days cycles, separated by 10-days of normal watering. Ammonium content in leaves all the species increased significantly under stress but the range of increase was different. The reduction in the a+b chlorophyll concentration in leaves of avens was significantly time- and stress dependent while reaction of stonecrop and heartleaf saxifrage was less spectacular. The above results show that ammonium and chlorophyll a+b contents merit further attention as possible indicators of plant response to drought stress in ornamental plants but additional studies are needed before these parameters can be used to evaluate new plants for introduction into urban growing conditions, or as selection criteria in breeding for adaptation to demanding growing conditions.

4F_02_P

TRANSCRIPT PROFILES MAY REFLECT DIFFERENT ADAPTATION STRATEGIES DURING DROUGHT STRESS IN WHEAT

Maria Sečenji*, Ágnes Lendvai*, Pál Miskolczi*, Gábor Kocsy†, Nils Stein‡, Patrick Schweizer‡, Dénes Dudits*, János Györgyey*

**Biological Research Center of the H.A.S., Temesvári krt.62., 6726 Szeged, Hungary*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

†Agricultural Research Institute of the H.A.S., Brunszvik u. 2., 2462 Martonvásár, Hungary
‡Institut für Pflanzengenetik und Kulturpflanzenforschung, Correnstraße 3., 06466 Gatersleben, Germany
Corresponding author: arthur@brc.hu

Drought is one of the major abiotic factors that limits agricultural crop production. Nevertheless, water deficit usually coincides with other abiotic stresses, adversely effecting plant growth. Plants can survive these adverse conditions using different strategies. Plant resistance to water deficit may arise from escape, avoidance, or tolerance strategies. Plants usually apply a combination of these strategies under dry conditions. These distinct strategies are caused by underlying transcriptional alterations of a high number of genes. To follow the transcriptional changes under water deficit, cDNA macroarray was hybridized with root samples of two wheat genotypes, both displaying tolerance to water-deficit stress but following distinct strategies, derived from an experimental system based on reduced irrigation for simulating stress conditions. As a result, 8.0 % of the genes were up-regulated and 8.5 % were down-regulated in the adaptive cultivar. In the "escaper" genotype, these ratios were 5.1 % and 4.8 %, respectively. After functional classification of genes induced by water-deficit stress, three groups of genes displayed significant differences between the two genotypes: stress- and defense-related; signal transduction components; cell organization and cell wall biogenesis-related genes. These results suggest a hypothetical elucidation of the molecular genetic background of the different tolerance strategies of the two examined wheat genotypes.

4F_03_P

DEVELOPMENT OF EXPRESSION CASSETTES FOR STRESS-INDUCED AND ROOT-SPECIFIC EXPRESSION IN CEREALS

Zoltán Zombori, Antal Mai, Katalin Török, Dénes Dudits, János Györgyey

Biological Research Center of the H.A.S., Temesvári krt. 62. H-6726 Szeged, Hungary
Corresponding author: arthur@brc.hu

Abiotic stresses are very important factors that can reduce the crop production. Plant root is the primary organ for uptake of water and nutrients, therefore plays important role in tolerance to stresses like drought or salinity. Plants developing stronger and deeper roots suffer less from water deficit. Genes facilitating development of efficient root system during drought stress can increase the survival of the plant. Fusions of drought stress-related root-specific promoters to these genes may provide

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

environment friendly and efficient solution to improve roots of crop plants under stress conditions. The aim of our work is to develop expression cassettes for cereals for the above purpose. Two promoters were selected, the rice Catalase B and the *RSOsPR10* promoters. The CatB promoter is known to be root-specific, the expression of the *RSOsPR10* mRNA is high in salt and drought stress conditions in root. Both promoters have stress-related transcription factor binding sites and motifs (MYB, WRKY, DREB, LTRE, root motifs) in their sequence. The 1.6 kb CatB promoter and the 2 kb of the 5` flanking region of the *RSOsPR10* were cloned, and fusions of these promoters to reporter genes (GUS, eGFP, YFP) were constructed and transformed into rice *Nipponbare cv*. calli. On the regenerated T_0 rice plants, salt stress was performed that revealed the *RSOsPR10* promoter directing root-specific and stress-induced expression pattern of GFP in stress conditions. Tests on the CatB transformed calli, regeneration of their T_0 plants are in progress. Independent T_1 lines of *RSOsPR10* transgenic rice plants will be generated for detailed characterization of the expression cassette.

4G_01_P

GENOTOXIC AND HISTOLOGIC EFFECT OF CHROMIUM EXPOSURE IN *LACTUCA SATIVA*

Eleazar Rodriguez, André Ferreira, Mónica Almeida, Armando Costa, João Loureiro, Conceição Santos

CESAM & Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal
Corresponding author: eleazar@bio.ua.pt

Chromium is a highly toxic non-essential metal for microorganisms and plants. Due to its widespread industrial use, chromium (Cr) has become a serious pollutant in diverse environmental settings. Cr toxicity in plants is less studied than in animals, but it seems to depend on Cr valence state: Cr^{6+}, more soluble and mobile, is more toxic than Cr^{3+}. So, in this work we studied the genotoxic and histologic effects of this two Cr species in three *Lactuca sativa* L. cultivars (Póvoa, Teide and Twistil). For this, plantlets were grown on solid matrix and watered with ½ Hoagland solution containing final concentrations of 0, 50, 150 and 300 mg/L of Cr^{3+} and Cr^{6+} (supplied respectively as $CrCl_3.6H_2O$ and as K_2CrO_4). After 30 days, leaves and roots were sampled for histological and flow cytometric (FCM) analyses (0 and 300 mg/L). Nuclear DNA content and the G_2:G0/G_1 ratio were estimated, and changes in the full peak coefficient of variation (mesure of DNA damage) of the G_0/G_1 peak were recorded. While in roots, significant differences in nuclear DNA content were detected between control and exposed plants exposed to Cr^{3+} (all cultivars), for leaves this

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

was verified for plants exposed to Cr^{6+} (cv. Póvoa). As for the G_2:G0/G_1 ratio, a significant increase was observed in roots of plants exposed to Cr^{6+}, whereas in leaves this ratio decreased (all cultivars).

Work funded by FCT/POCTI/AGR/60672/04; FCT Fellowships: Eleazar Rodriguez: FCT/SFRH/BD/27467/06 and João Loureiro FCT/SFRH/BD/9003/02,

---

4G_02_P

HEAVY METAL STRESS AND STRESS RESPONSES IN *PISUM SATIVUM* L.

B. Bartha, L. Erdei

Department of Plant Physiology, University of Szeged, P.O.B. 654., 6701 Szeged, Hungary
barthab@bio.u-szeged.hu ; erdei@bio.u-szeged.hu

Several metals - such as copper – are essential microelements but in high concentrations they damage plants by causing oxidative stress. The effect of copper was investigated in 2 weeks-old *Pisum sativum*, after one-week treatment with different $CuSO_4$ concentrations (5, 10, 25 and 50 μM). Growth parameters (root-shoot length and mass), the capacity of the heavy metal uptake, the root-to-shoot translocation and the changes of the macro element distribution were measured. Catalase (CAT), guaiacol-peroxidase (GPX), glutathione reductase (GR) and glutathione-*S*-transferase (GST) activities were also determined and NO synthesis was detected in roots. Plants kept the metal ions in their roots, while a minimal translocation to the shoot was detected only in cases of 25 and 50 μM treatments. Cu^{2+} caused growth inhibition in roots; it means 30-50% decrease in root length and mass compared to control plants. GR activity decreased dramatically in root tissues while in shoot we observed increased enzyme activities. GST activities slightly decreased in both tissues. In plants treated with 25 μM Cu^{2+}, time dependence of enzyme activities was measured. GPX activity increased in both roots and leaves; GR and CAT activities increased in shoots but decreased in roots. NO production was measured after 100 μM heavy metal treatment from 1 to 48 hours. On the basis of the time course, the Cu^{2+}-induced NO production clearly showed a biphasic reaction, namely, a fast burst of NO release followed by a slow increase. It is concluded that the immediate stress response in the alarm phase is the decrease of defence capacity accompanied by the appearance of NO in the root, while in the shoot acclimatization processes are initiated. –*This work was supported by grant No. NKFP 3A/009/2004.*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

4G_03_P

CD-INDUCED CHANGES IN THE EXPRESSION OF CHLOROPHYLL-PROTEIN COMPLEXES IN *POPULUS GLAUCA*

Basa B, Gárdonyi M, Sárvári É*, Tamás L

Dept Plant Physiol & Mol Plant Biol, Eötvös Univ, Budapest, Hungary
**corresponding author: e_sarvari@ludens.elte.hu*

The molecular response of chlorophyll(Chl)-protein pattern to Cd treatment was studied in poplar plants grown in hydroponics. The relationship between Chl-protein level and their mRNA level was aimed to examine. Plants were treated with 10 μM $Cd(NO_3)_2$ starting at their four-leaf stage. Structural and functional photosynthetic parameters were measured and RNA samples were extracted from developing and mature leaves during the two-week treatment. Chl-protein pattern analyzed by Deriphate-PAGE revealed that the most effected complex was the photosystem I including its light-harvesting antenna, LHCI. To investigate whether the reduced amount of proteins was due to the change of the expression profile, the mRNA level of the *Lhca1-4* genes has been analyzed by quantitative real-time RT-PCR. In addition, their stress-enhanced relatives *Lhca5* and *Lil1* gene (ELIPs early light induced proteins) were also studied. Transcription of the rarely expressed *Lhca5* slightly enhanced in mature leaves at the beginning of the Cd treatment. However, in young developing leaves high expression level was detected, which decreased remarkably during leaf development. In Cd-treated plants a strong reduction was demonstrated. Similar results were observed in the case of *Lil1* gene, though its enhancement was stronger in mature leaves in early phase of the treatment. The abundantly expressed *Lhca* genes (1-4) displayed identical transcriptional behavior. In young expanding leaves Cd inhibited their transcription and mRNA levels decreased gradually, whereas in mature leaves Cd-induced inhibition was less pronounced. The results obtained at mRNA level were consistent with those at protein level. It is hypothesized that the reduction in the amount of LHCI antenna under Cd stress was triggered by transcriptional response.
This work was supported by grant T-043646 (OTKA).

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

4G_04_P

ANTIOXIDANT AND MORPHOLOGIC ANALYSIS OF *LYCOPERSICON ESCULENTUM* CV MICRO-TOM PLANTS SUBJECTED TO CADMIUM STRESS

[1]Gratão, P. L., [1]Monteiro C. C., [2]Rossi, M. L., [2]Rodriguez, A. P. M., [1]Peres, L. E. P., [1*]Azevedo, R. A.

[1]Lab. Genética Bioquímica de Plantas/Dep. Genética/ESALQ/USP/Piracicaba/SP/Brasil; [2]Lab. Biotecnologia Vegetal/CENA/USP/Piracicaba/SP/Brasil; [1]Pesquisador Dep. Genética - ESALQ/USP/Piracicaba/SP/Brasil, raazeved@esalq.usp.br*

The contamination of environments by heavy metal pollution mainly by cadmium (Cd), which is considered one of the more toxic, is generated by mining, industrial activities, sewage sludge and phosphated fertilizers used in agriculture. Cd can induce excessive production of reactive oxygen species (ROS) which can cause serious damage to cellular metabolism, leading to growth inhibition. A class of antioxidant enzymes acts in the scavenging of ROS and plants exposed to heavy metals may exhibit alteration in enzyme activity. In this study we observed alterations in CAT and peroxidases activities in tomato cv Micro-Tom plants. SOD isoenzymes activities were also shown to be altered. Besides, morphologic analysis allowed the evaluation of Cd-induced damage. The results suggest that in tomato cv Micro-Tom the main defence system to Cd stress is variable during the heavy metal exposure period and its concentration. The study of such responses may allow the evaluation of tolerance levels and specificity of the response to levels of pollution in the environment. These data may be useful in breeding programs or biotechnological alternatives to produce and/or select tolerant plants that may be used in phytoremediation in order to reduce the amount of heavy metals in contaminated areas. Another important aspect is the identification or selection of plants that accumulate very low amounts of Cd in the parts that are used for animal and human food. (Financial support by FAPESP and CNPq).

4G_05_P

DEHYDROASCORBATE UPTAKE IS IMPAIRED IN THE EARLY RESPONS OF *ARABIDOPSIS* PLANT CELL CULTURES TO CADMIUM.

Horemans Nele[*a], Raeymaekers Tine[*], Van Beek Kim[*], D'Haese David[*], Cuypers Ann[**] Guisez Yves[*]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

* *Plant Physiology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium, nele.horemans@ua.ac.be*
** *Centre for Environmental Sciences, Hasselt University, Agoralaan Building D, 3590 Diepenbeek, Belgium*

The balance between antioxidants such as ascorbate (ASC) and glutathione and oxidative reactive oxygen species (ROS) is known to play a pivotal role in the response of plant cells to abiotic stress. Here cell cultures of *Arabidopsis thaliana* were investigated on their response to elevated levels of aluminum, zinc or cadmium. For Zn and Al no significant H_2O_2-accumulation was detected. Cd, however, induced a rapid and concentration dependent and initially extracellular H_2O_2-accumulation that could be inhibited by DPI (20 µM). RT-PCR analysis of 3 *Rboh* genes showed an increased transcription of *Rboh F* after 15 min. No change in ASC concentration was observed during the first three hours after Cd-addition. In contrast glutathione levels completely diminished within one hour. This drop could be attributed to an increase in phytochelatin 4. At the plasma membrane, Cd further induced a significant decrease in dehydroascorbate uptake activity (up to 90% inhibition after four hours). This decrease is not present when cells are treated with $LaCl_3$, before exposure to $CdCl_2$. $LaCL_3$ is a typical inhibitor of Ca channels and is known to prevent Cd uptake and Cd induced ROS production in plants. Therefore these results seem to indicate Cd uptake is a prerequisite for the change in DHA transport activity. However DPI did not prevent the drop in DHA uptake activity indicating that this response seems to be independent of the H_2O_2-production. The possible function of the drop in DHA uptake in response to Cd stress will be discussed.

4G_06_P

KINETICS OF PHOTOSYNTHETIC RESPONSES AND DEVELOPMENT OF PROTECTIVE MECHANISMS DURING CD STRESS IN POPLAR

P Szegi*[1], Á Solti[1], L Gáspár[1], I Mészáros[2], É Sárvári[1]

[1]*Department of Plant Physiology & Molecular Plant Biology, Eötvös University, Budapest, Hungary*
[2]*Department of Botany, Debrecen University, Debrecen, Hungary*
**corresponding author: sheedy@mad.hu*

It is well known that Cd inhibits photosynthesis and induces oxidative stress. However, the time scale of appearance of symptoms and the onset of the protective mechanisms has been scarcely studied. To get information on the kinetics of processes depending on leaf maturity, Cd effects on both photosynthetically competent and developing leaves of

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

poplar plants (Populus alba v. Kopeczki, grown in hydroponics) were followed during the treatment (with 10 μM $Cd(NO_3)_2$) of plants being in four-leaf stage for a three-week period. In mature leaves, the actual quantum efficiency of photosystem(PS)II (ΔF/Fm') and non-photochemical quenching (NPQ) did not change. Reduction of CO_2 fixation and stomatal conductance were the earliest responding parameters starting to change from the day 2-3. Decrease in chlorophyll (Chl) content, Chl a/b ratio, and in the amount of PSII core was slight (starting from day 3). The activity of ascorbate peroxidase (APX) decreased, and the amount of malondialdehyde (MDA) slightly increased from day 4 compared to controls. In newly developed leaves most of the studied parameters were more strongly affected. Lowered Chl content, Chl a/b ratio (decrease in PSI>LHCII>PSII), and inhibited CO_2 fixation appeared early (from day 3), while reduction in ΔF/Fm', NPQ, APX activity and increase in MDA content were observed from day 4. While CO_2 fixation was the most influenced parameter in mature leaves, the stage of photosynthetic structures was equally important in developing leaves. A recovery phase started on the second week of treatment even in the presence of Cd.
This work was supported by grant T-043646 (OTKA).

4G_07_P

CD-INDUCED INHIBITION OF PHOTOSYNTHESIS CAN BE RECOVERED BY ELEVATED FE SUPPLY

Á Solti[1*], P Szegi[1], L Gáspár[1], L Lévai[2], Z Szigeti[1], É Sárvári[1]

[1]*Department of Plant Physiology & Molecular Plant Biology, Eötvös University, Budapest, Hungary*
[2]*Department of Botany & Crop Physiology, Debrecen University, Debrecen, Hungary*
**corresponding author: sadambio@elte.hu*

Cd causes Fe deficiency-like symptoms, and thus inhibits photosynthesis. Surplus Fe supplied together with the Cd diminished or even abolished the effects. However, it was due to the decreased Cd translocation into the shoots. To clarify how important is the Cd-induced Fe deficiency among the effects of Cd on photosynthesis we studied the recovery effect of surplus Fe supplied after the Cd symptoms had been developed. Hydroponically cultured poplar (*Populus glauca* v. Kopeczkii) plants were treated with 10 μM $Cd(NO_3)_2$ for a week, during which the Cd symptoms – including growth retardation, decreased chlorophyll (Chl) synthesis (increased red/far-red fluorescence ratio), reduced Chl *a*/*b* ratio and photosynthetic activity (CO_2 fixation, the effective photosystem II efficiency – ΔF/Fm'), increased blue-green fluorescence (BGF) – were

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

developed. Next week normal (10 μM) or fivefold amount of Fe-citrate was given together with or without Cd into the nutrient solution. None of the recovery treatments restored the growth rate or BGF to the control level. However, photosynthetic parameters were partially or totally recovered, though the Cd content of leaves did not decrease. The recovery of red/far-red fluorescence started from the vein region and then developed into the farther parts. Kinetically, the higher the Fe content, the faster the recovery was. Concerning the dynamics of regeneration, Chl *a/b* ratio and ΔF/Fm' were the most sensitive parameters. In conclusion, the Cd induced Fe deficiency plays a key role in Cd effects on photosynthesis, and the defects can be repaired by extra Fe supply.
This work was supported by grant T-043646 (OTKA).

4G_08_P

THE INFLUENCE OF *ELEUTHEROCOCCUS SENTICOSUS* (RUPR. ET MAXIM. EX MAXIM) EXTRACT TO THE TOXICITY OF CADMIUM

[1]Virgilijus Zitkevičius, Nijole Savickiene[1], Alina Smalinskiene[2], Vaiva Lesauskaite[2], Stanislovas Ryselis[3], Oleg Abdrakhmanov[3], Rima Kregzdyte[3], Ilona Sadauskiene[3], Leonid Ivanov[3], Arunas Savickas[4]

[1]Department of Pharmaceutical Chemistry and Pharmacognosy, Kaunas University of Medicine, [2]Institute of Cardiology, Kaunas University of Medicine, [3]Institute for Biomedical Research, Kaunas University of Medicine, [4]Department of Drug Technology and Pharmaceutical Management, Kaunas University of Medicine, Kaunas, Lithuania
E-mail : virgzit@takas.lt

Cadmium is known to be human carcinogen based on sufficient evidence of carcinogenicity in humans. It disturbs the activity of biochemical systems of cells. The sensitivity of cells or tissues to cadmium appears to be related, at least in part, to their ability to produce metallothionein (MT), a protective molecule that binds heavy metals, including cadmium. Activation of the MT formation in response to cadmium exposure results in production of metallothionein, which sequesters cadmium, thus limiting its genotoxic effects.
Eleutherococcus senticosus (Rupr. et Maxim. ex Maxim) can modify cadmium influence and it`s toxicity. The aim of the study was to evaluate the effect of *Eleutherococcus senticosus* (Rupr. et Maxim. ex Maxim) extract on the accumulation of cadmium in liver, kidney and blood and mitotic activity of liver cells after the chronic intoxication by cadmium. Research was supported by a Grant (No. G-71/06) of Lithuanian Foundation for Research and Studies.
Experiments were carried out on the white laboratory mice. Mice (n=57) were periodically *i.p.* injected for 6 weeks with $CdCl_2$ (0.05 LD_{50} Cd^{2+}) and

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Eleutherococcus senticosus (Rupr. et Maxim. ex Maxim) extract solutions of two different concentrations (0.05 LD_{50} and 0.1 LD_{50}) and their combinations. Control mice were injected with 0.9% saline. Cadmium concentration in blood and tissue specimens was determined by atomic absorption spectrophotometer Perkin-Elmer/Zeeman 3030. The number of mitotic liver cells was counted in 10 randomly selected reference areas (0.04 mm^2). Preparation of extract from roots of *Eleutherococcus senticosus* (Rupr. et Maxim. ex Maxim) was made in the factory "Valentis" (Lithuania).

Cd^{2+} concentration in blood of mice in group *Eleutherococcus senticosus* (Rupr. et Maxim. ex Maxim) (0.05)+Cd was 2,45-fold higher, therefore in group *Eleutherococcus senticosus* (Rupr. et Maxim. ex Maxim) (0.1)+Cd was 1,72-fold higher comparing to cadmium group, in liver that was 1,61-fold and 1,43-fold, respectively, in kidney 1,72-fold and 1.90-fold, respectively. The mitotic activity of liver cells induced by Cd^{2+} after injection of *Eleutherococcus senticosus* (Rupr. et Maxim. ex Maxim) extract was the same as in control group.

Long-term injections of extract of *Eleutherococcus senticosus* (Rupr. et Maxim. ex Maxim) (0.05 LD_{50} and 0.1 LD_{50}) combined with $CdCl_2$ (0.05 LD_{50}) leads to the significant increase of cadmium concentration in blood and investigated organs of experimental mice. *Eleutherococcus senticosus* (Rupr. et Maxim. ex Maxim) decreases the mitotic activity of liver cells induced by cadmium.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Module 5 – Oral lectures:

5A_01_S

LONGEVITY ASSURANCE MOLECULAR PATHWAYS IN HUMAN CELLS

Efstathios S. Gonos

National Hellenic Research Foundation, Athens, Greece
e-mail: sgonos@eie.gr

Ageing and longevity are two multifactorial biological phenomena whose knowledge at molecular level is still limited. We have developed a clonal senescence induced system and we have cloned several senescence associated genes. Analysis of the function of one of the isolated genes, encoding for Clusterin/Apolipoprotein J (CLU), suggests that it is a novel survival factor. CLU is found over-expressed in vitro under a variety of stress conditions and in vivo in samples from patients suffering from various age-related diseases as well as in primary tumours which have acquired chemotherapeutic drug resistance. In addition, it has been demonstrated that inhibition of endogenous CLU expression by RNA interference induces growth retardation, higher rates of endogenous cellular death and sensitizes human cells to stress (Cancer Res 64, 1834-1842, 2004). Recent findings indicate that effective and sustained CLU depletion by siRNA induces late morphological alterations, growth arrest at the G_1/S checkpoint and activation of the mitochondrial axis of apoptosis that engages caspase-9. Moreover, CLU knock-down resulted in down regulation of the BH pro-survival (bcl-2 and bcl-X_L) proteins and activation of p53 and its downstream targets, namely $p21^{WAF1/CIP1}$ and bax.
We have also attempted an overall molecular and biochemical approach regarding proteasome function in replicative senescence and cell survival. We have observed reduced levels of proteasomal peptidase activities coupled with increased levels of oxidized and ubiquitinated proteins in senescent cells. We have found the catalytic subunits of the 20S complex and subunits of the 19S regulatory complex to be down-regulated in senescent cells. This is accompanied by a decrease in the level of both 20S and 26S complexes (J Biol Chem 278, 28026-28037, 2003). In support, partial inhibition of proteasomes in young cells by specific inhibitors induced a senescence-like phenotype. Stable over-expression of β subunits or POMP in human cell lines resulted in enhanced proteasome assembly and activities and increased cell survival following treatments with various oxidants. Moreover, stable over-expression of β_5 subunit delayed senescence in human fibroblasts (J Biol Chem 280, 11840-11850, 2005). Finally in search of natural compounds that may activate proteasome, we have identified that the main constituent of olives, oleuropein, exerts stimulatory effects on proteasome. Importantly, continuous treatment of human fibroblasts cultures with oleuropein delays

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

senescence by approximately 15%.

5A_02_S

CHEMICAL STRESS RESPONSE MIMETICS EXTEND LIFESPAN

Gordon J. Lithgow

The Buck Institute, 8001 Redwood Blvd., Novato, CA 94945, USA e-mail: glithgow@buckinstitute.org

There are considerable mechanistic links between organismal stress resistance and aging[1]. We have shown previously that long-lived mutants of the nematode *C. elegans* are resistant to thermal stress and over-accumulate small heat shock proteins (shsps) which alone can extend lifespan[2-4]. A large number of genes determine normal aging rate in the nematode and mutations in these genes tend to be highly pleiotropic including effects on organismal stress resistance and stress gene expression. We undertook genetic screen for mutations that conferred increased resistance to multiple stresses and were surprised when we tagged a gene shown in other organisms to affect checkpoint functions. Checkpoints are evolutionarily conserved signal transduction pathways that arrest cell division in response to DNA damage or stalled replication forks. We demonstrated that CID-1, CHK-1 or CDC-25 checkpoint proteins also determine organismal stress resistance and lifespan. This pathway and other signaling pathways have remarkable effects on lifespan; we have observed some mutations extend lifespan 500%.

Each of these genetic modulators of aging is a potential target for chemical interventions. We will describe a series of screens of chemical compound libraries and show that chemical modulators of aging can be identified. These may open up new therapeutic avenues for age-related diseases.

References

1. Lithgow, G. J. Stress response and aging in *Caenorhabditis elegans. Results Probl. Cell Differ.* **29**, 131-148 (2000).
2. Lithgow, G. J., White, T. M., Melov, S. & Johnson, T. E. Thermotolerance and extended life span conferred by single-gene mutations and induced by thermal stress. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 7540-7544 (1995).
3. Walker, G. A. *et al.* Heat shock protein accumulation is upregulated in a long-lived mutant of *Caenorhabditis elegans*. *J. Gerontol. A Biol. Sci. Med. Sci.* **56**, B281-B287 (2001).
4. Walker, G. A. & Lithgow, G. J. Lifespan extension in *C. elegans*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

by a molecular chaperone dependent upon insulin-like signals. *Aging Cell* **2**, 131-139 (2003).

5A_03_S

AGE DEPENDENT COLLAPSE OF PROTEIN FOLDING HOMEOSTASIS

Anat Ben-Zvi and Richard I. Morimoto

Department of Biochemistry, Molecular Biology, and Cell Biology, Rice Institute for Biomedical Research, Northwestern University, Evanston, IL 60208, USA

The balance between protein folding and degradation forms the cellular protein-folding homeostasis, which can compensate for inherent, off pathway misfolding. During the life span of an organism there are changes in expression of cellular folding and degradation components as well as an accumulation of damage proteins, however, the impact of these changes on the cellular folding homeostasis is not known. Using meta-stable proteins in *C. elegans* to probe for changes in the folding capacity, we find that there is an age dependent collapse of the cellular folding environment. This drop in folding capacity is modified genetically, by the Hsf-1 and Daf-16 pathways promoting or postponing homeostasis decline. We find that both pathways contribute independently to protein folding homeostasis but down regulation of either pathway is sufficient to disturb folding homeostasis. We suggest that the balance between misfolding loads and folding and turnover machinery determines this age dependent folding homeostasis collapse.

5A_04_S

MILD STRESS-INDUCED HORMESIS AND ANTI-AGING EFFECTS ON HUMAN CELLS

Suresh Rattan

Laboratory of Cellular Ageing, Department of Molecular Biology, University of Aarhus, Denmark. (Email: rattan@mb.au.dk)

The phenomenon in which adaptive responses to low doses of otherwise harmful conditions improve the functional ability of cells and organisms is known as *hormesis*. Several physical, chemical and biological stressors

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

exhibit hormetic effects, including heavy metals, pesticides, antibiotics, chemotherapeutic agents, ethanol, aldehydes, chloroform, pro-oxidants, hypergravity and ionizing radiation. The key conceptual features of hormesis are the disruption of homeostasis, the modest overcompensation, and the re-establishment of homeodynmaics. A critical component of the homeodynamic property of living systems is their capacity to respond to stress. Thermoregulation, detoxification, cell proliferation/apoptosis, DNA repair, heat shock protein synthesis, protein turnover and antioxidative responses are some of the main homeostatic responses. Therefore, it is observed that if cells and organisms are exposed to brief periods of mild stress so that their stress response-induced gene expression is upregulated and the related pathways of maintenance and repair are stimulated, several anti-aging, health-improving and longevity-promoting hormetic effects occur. Extensive studies performed in our labs have shown that repeated mild heat stress has anti-aging hormetic effects on various cellular and biochemical characteristics of human skin fibroblasts undergoing aging. These effects include the maintenance of chaperone protein profiles, reduction in the accumulation of oxidatively and glycoxidatively damaged proteins, stimulation of the proteasomal activities for the degradation of abnormal proteins, improved cellular resistance to ethanol, hydrogenperoxide and ultraviolet-B rays, and enhanced levels of various antioxidant enzymes. Further research will elucidate the mechanisms and possibilities of human applications of physical and mental stress as a beneficial challenge .

5B_01_S

HEAT SHOCK PROTEINS AND PROTECTIVE EFFECTS IN THE NERVOUS SYSTEM

Ian R. Brown, Sheng Chen and Ari Chow

Center for the Neurobiology of Stress, University of Toronto at Scarborough, Toronto, Ontario, Canada M1C1A4

Hsp70 is a multi-gene family composed of stress-inducible members (Hsp70) and other members that are constitutively expressed (Hsc70). The heat shock proteins Hsp70 and Hsc70 exhibit similar molecular structure and biochemical functions. Constitutively expressed Hsc70 is enriched in the mammalian nervous system compared with non-neural tissues and present at high levels in neuronal cell bodies. After thermal stress, Hsc70 is translocated to synapse-enriched areas of the cerebral cortex where it associates with Hsp40 to form a complex that can refold denaturated proteins. These results suggest that the heat shock response in the nervous system involves not only the synthesis of stress-inducible Hsps but also translocation of constitutively expressed Hsc70 to synapse-

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

enriched areas where it could participate in neuroprotective mechanism that preserve synaptic function during times of stress. Neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and ALS have been termed 'protein misfolding disorders'. These diseases differ widely in frequency and impact different classes of neurons. Hsps provide a line of defense against misfolded, aggregation-prone proteins and are among the most potent suppressors of neurodegeneration in animal models. Analysis of constitutively expressed heat shock proteins revealed variable levels of Hsc70 and Hsp27 in different classes of neurons in the adult brain. The differing levels of these constitutively expressed heat shock proteins in neuronal cell populations may confer a variable buffering capacity against protein misfolding disorders that correlates with the relative frequencies of the previously mentioned neurodegenerative diseases. Upregulation of Hsps could offer a therapeutic strategy to counter conformational changes in neuronal proteins that trigger pathogenic cascades resulting in neurodegenerative diseases. Differentiated neurons in both *in vivo* and *in vitro* systems have been reported to be refractory to Hsp induction by conventional heat shock. We will report on a compound that induces Hsps in differentiated neurons with the interesting feature that it induces a wider set of Hsps in differentiated human neurons compared to differentiated rodent neurons.

5B_02_S

MOLECULAR CHAPERONES AND PROTECTION FROM CEREBRAL ISCHEMIA

Rona G. Giffard, Yibing Ouyang, LiJun Xu and Midori Yenari

Department of Anesthesia, Stanford University, Stanford, CA USA

Stroke is the third leading cause of death in many countries. Data from both animal stroke models and primary cultures have demonstrated that Hsp70 overexpression can reduce cerebral ischemic injury. We have used viral vectors and DNA transfection to overexpress Hsp70 in the brain and in brain cells. We have previously reported that overexpression of Hsp70 and Hsp40 reduce ischemic injury in in vitro models of ischemia, and Hsp70 overexpression is effective in rodent models of stroke. To delineate which domains within Hsp70 are important for protection from ischemia in the brain we compared two mutants to full length wild type Hsp70. One was a point mutant in the ATPase domain- K71E, the second was a deletion mutant encoding the carboxy terminal domain, amino acids 381-640. The constructs were injected intracerebroventricularly, resulting in transfection of both neurons and astrocytes. We observed that both the ATPase deficient mutant and the carboxyterminal domain alone were similar to the wild type HSP70 in ability to significantly reduce focal

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

ischemic injury in the rat. Additional effects of Hsp70 that could contribute to protection were studied in Hsp70 overexpressing mice subjected to focal ischemia. Here we found evidence of reduction of inflammation-reduced activation of NFkB, and decreased expression of iNOS, in the Hsp70 overexpressing mice. We also found previously that Hsp70 overexpression was associated with higher levels of the antiapoptotic protein Bcl-2. We have thus found at least three mechanisms contributing to protection of the brain from ischemia by Hsp70. These are binding of unfolded proteins, inhibition of inflammation and inhibition of apoptosis. Hsp70 is a multifaceted protein, and at least several of its effects are involved in protecting the brain from ischemia.

5B_03_S

MANIPULATING THE HEAT SHOCK RESPONSE AS A THERAPEUTIC STRATEGY IN ALS

Bernadett Kalmar and Linda Greensmith

Institute of Neurology, University College London, UK
l.greensmith@ion.ucl.ac.uk

Heat shock proteins (hsps) are know to play a role in neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS), in which motoneurons degenerate, resulting in muscle weakness, paralysis and finally death, usually within 2-5 years of diagnosis. Evidence suggests a relationship between the level of the heat shock response (HSR) and the specific vulnerability of motoneurons to degeneration in ALS. For example, motoneurons have an unusually high threshold for the activation of the HSR which may result in abnormal protein folding and trafficking and an increased susceptibility to apoptotic insults. We have recently up-regulated the HSR in a mouse model of ALS. Treatment of $SOD1^{G93A}$ mice with arimoclomol, induces the expression of hsp70 and hsp90, resulting in a delay in disease progression and a significant increase in survival. However, the effects of arimoclomol are unlikely to result only by increasing hsp levels in motoneurons since genetic manipulations that increase hsp70 in $SOD1^{G93A}$ mice fail to alter disease progression. It is not only the mechanism, but the site of action of arimoclomol and hsps in $SOD1^{G93A}$ mice that remains unclear. We are characterising the HSR in this model of ALS as a function of disease progression and testing the differential neuroprotective effects of a variety of manipulations that up-regulate expression of hsps. Our results show that not all agents that activate the HSR will necessarily have beneficial neuroprotective effects on motoneurons. Moreover, activation of the HSR in non-neuronal cells may have greater neuroprotective effects than targeting the HSR within motoneurons themselves. We hope that a better understanding of the role

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

of the hsps in ALS will help to optimize targeting of the HSR as an effective therapeutic strategy for this fatal disease.

5B_04_S

NEUROPROTECTIVE EFFECTS OF EXTRACELLULAR HSP70

Michael Tytell

Neurobiology & Anatomy, Wake Forest Univ. School of Medicine, Winston-Salem, NC 27157, tytellm@wfu.edu

When the cytoprotective function of Hsps was a relatively new concept in the 1980s, we and Hightower respectively observed that Hsp70 was passed from one cell to another and released into the extracellular fluid (Tytell & Lasek, Brain Res 324:223, 1984; Hightower & Guidon, J Cell Physiol 138:257, 1989). These were unexpected results because the Hsp70 family was thought to be exclusively intracellular proteins. In the two decades since, extracellular Hsp70 has become well-documented. Our 1984 observation that glial Hsp70 was transferred into the axon led to the novel hypothesis that, in the nervous system at least, the stress tolerance of neurons was not solely dependent on their own Hsp70, but could be supplemented by additional Hsp70 from adjacent glia. For neurons, this hypothesis has major implications because those with long axons (mm to meters in length) require newly synthesized proteins to be transported from the neuronal cell body to remain functional. If stress or injury occurs in axons at some distance from their cell bodies, hours to days are required for newly synthesized Hsp70 to reach the injury site, which is too long to be of use. Thus, supplying Hsp70 at the injury site would be more effective in maintaining neuronal and axonal function. We have tested this idea in a number of models. . In the injured sciatic nerve, local application of Hsp70 improved sensory and motor neuron survival. Similarly, in the light-damaged rat eye, Hsp70 injected into the vitreous chamber promoted survival of photoreceptors. In cultured glia and neurons, we found that glia released Hsp70 and that neurons took up Hsp70 from the medium, leading to greater resistance to apoptosis induced chemically or by lack of trophic factor. Therefore, Hsp70 has potential as a therapeutic agent in acute nervous system injury.

5C_01_S

METALLOTHIONEIN-MEDIATED IMMUNOMODULATION; NEW ROLES FOR AN OLD STRESS RESPONSE

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

*Lynes, M.A., *Yin, X., *Unfricht, D.W., *Marusov, G., **Lyons, B.L., [†]Emeny, R.T., and [†]Lawrence, D.A.

**University of Connecticut, Storrs, CT, USA; **Jackson Laboratory, Bar Harbor, ME, USA; [†]Wadsworth Center, NYSDH, Albany, NY, USA*

Metallothionein (MT) is an unusual stress response protein with a novel and intriguing structure and a set of distinct biological functions. The protein is remarkably cysteine rich, and many of its functions revolve around cysteine-associated thiols. This highly conserved protein is known to be a reservoir of essential divalent heavy metals, it sequesters toxicants and scavenges free radicals, and it can act to regulate transcription factor activity. In each of these instances, the principal site of action is thought to be in the nuclear and cytosolic compartments, and this perspective is consistent with the observation that MT does not have a signal sequence for export through the endoplasmic reticulum and golgi apparatus. Despite this signal sequence absence, there is strong evidence that MT can be released to extracellular spaces in significant amounts under stressful circumstances. We have found that MT in the extracellular environment can act as a chemotactic agent, and that this response may be mediated by interactions with G-coupled protein receptors. Our recent studies have focused on mechanisms of cadmium-mediated immunomodulation, and the effect of manipulations of MT gene dose on the progression of humoral immunity against T-dependent antigens during low dose cadmium or zinc exposure. At doses of metal that do not influence the humoral response in wild type mice, mice that carry a targeted disruption of the *Mt1* and *Mt2* genes show significant immunosuppression in the presence of cadmium, but not zinc. In contrast, both cadmium and zinc suppress the humoral response of mice that carry the *Mt1* transgene construct that drives over-expression of metallothionein. This suppression is associated with changes in the cytokine profile produced by stimulated splenocytes. *B*oth cadmium and zinc are inducers of MT mRNA and protein expression, but at sub-toxic concentrations of these metals only cadmium provokes a selective release of MT to the extracellular environment. We have also explored the impact of changes to the MT gene dose in mice infected with *Listeria monocytogenes*. Clearance of *L.m.* was accelerated in mice that carry both the targeted disruptions of *Mt1* and *Mt2*, as well as in mice that carry the *Mt1* transgene construct when compared to congenic wild type controls. We hypothesize that MT may represent an central regulator of immune functions that occur under stressful circumstances, and that manipulations of MT levels in the extracellular environment may provide an avenue for the management of inflammatory, infectious, autoimmune and neoplastic disease processes.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

5C_02_S

COPPER METABOLIC DISORDER IN MYOCARDIAL PATHOGENESIS

Y. James Kang

Department of Medicine and Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY, USA

The association of copper (Cu) with cardiomyopathy has been recognized for a long time, but its clinical significance has not been explored until recently. In diabetic patients and rat model, Cu chelation therapy using a Cu chelator trientine has been shown to ameliorate cardiac pathological changes and improve heart function. In contrary, we have observed that dietary supplementation with physiologically relevant levels of Cu can reverse established hypertrophic cardiomyopathy in aortic banding mouse model even in the presence of pressure overload. Both results have indications for Cu metabolic disorders in cardiomyopathy in humans. However, it is important to distinguish the fundamental differences in Cu metabolic disorder between diabetic and pressure overload cardiomyopathy. Both diabetes and pressure overload cause cardiac oxidative stress, mitochondrial damage, hypertrophy and dysfunction. However, we have observed that Cu levels slightly increase in the heart, but significantly decrease in the liver of streptozotocin (STZ)-induced diabetic mice. In contrary, Cu concentrations decrease in the heart, but do not change in the liver of pressure overload mice. It is possible that diabetic complications lead to systemic disorder of Cu metabolism and disruption of Cu detoxification in the liver, thus increasing the risk of Cu toxicity to organs including the heart, but in pressure overload the heart is the primary organ of Cu metabolic disorder leading to cardiac Cu deficiency but maintaining liver detoxification function. In this context, Cu chelation would be beneficial to patients with diabetic cardiomyopathy, but Cu supplementation would improve the condition of pressure overload cardiomyopathy. Both cases have been demonstrated in animal studies and it is important to apply the information generated from animal studies to human cardiomyopathy patients. These studies were supported in part by US-NIH grants HL59225 and HL63760.

5C_03_S

BRAIN INJURY AND OXIDATIVE STRESS: THINK ZINC!

Stefano Sensi[1,2]

Depts. of Neurology, and Basic and Applied Medical Sciences, [1]Center of Excellence on Aging / University

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

"G. d'Annunzio", Chieti-Pescara, Italy, 2University of California-Irivine, Irvine., USA, ssensi@uci.edu

Zn^{2+} is a potent mediator of neuronal injury both "in vitro"and "in vivo", and trans-synaptic movement of the cation from pre- to post-synaptic neurons has been shown to greatly contribute to a variety of neurological conditions including cerebral ischemia, brain trauma and epilepsy. Zn^{2+} can enter neurons through glutamate receptor-associated channels [NMDA and AMPA/kainate channels (Ca-A/K channels), voltage-sensitive calcium channels (VSCC), or Zn^{2+}-sensitive membrane transporters (like the Na^{+}/Ca^{2+} and Na^{+}/Zn^{2+} exchangers). Mechanisms by which Zn^{2+} exerts its potent neurotoxic effects include both mitochondrial and extra-mitochondrial pathways. Experiments in cortical neurons have shown that mitochondria play an important role in restoring Zn^{2+i} homeostasis but this Zn^{2+} uptake leads also to prolonged mitochondrial depolarization and free radicals generation. In addition to roles in acute injury, Zn^{2+} might play roles in the selective neurodegeneration associated with aging and Alzheimer's disease (AD). Indeed, cumulative effects of repeated Zn^{2+} exposures could contribute to the oxidative damage and mitochondrial dysfunction seen in AD. Interestingly, Zn^{2+} homeostasis is affected by oxidative stress, as reactive oxygen species are potent triggers for injurious cation release from Zn^{2+} binding proteins (metallothioneins). In this talk, we examine how Zn^{2+} dyshomeostasis and oxidative stress might act synergistically to promote degeneration in the context of several neurological conditions.

5C_04_S

CYTOPROTECTIVE METAL IONS

George A. Perdrizet [a] and Lawrence E. Hightower [b]

[a] *Dept. Trauma, Hartford Hospital and Univ. Connecticut, Hartford, CT 06102*
[b] *Dept Cell and Molecular Biology, Univ. Connecticut, Storrs, 06269*

The cellular stress response and stress protein expression form the biologic basis of stress conditioning protocols. Currently most information on inducible cytoprotection has been focused on the use of hyperthermia as the inducing agent. Heat shock preconditioning has amply demonstrated the proof of concept that stress conditioning protocols have the potential to provide beneficial cytoprotection in the setting of elective invasive medical and surgical procedures. While whole-body hyperthermia is a very effective agent, a pharmacologic agent would be more attractive as a clinical method to achieve a cytoprotected state in diseased humans. Metal ions have been shown to be potent inducers of the cellular stress

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

response, are anti-inflammatory and capable of providing clinically relevant cytoprotection. We have successfully used the chloride salts of tin and zinc to provide in vivo cytoprotection in animal models of: 1. Rodent renal artery occlusion, 2. rabbit spinal cord ischemia 3. acute pulmonary inflammation, rabbit fat embolism syndrome (intravenous administration of oleic acid), 4. acute inflammation within the rodent mesenteric blood vessels. In these models, evidence for induction of stress proteins and inhibition of acute inflammation will be presented as potential mechanisms for the observed cytoprotection. The nature and activities of tin and zinc metals will be compared and contrasted.

5D_01_S

MOLECULAR UNDERSTANDING OF HPA AXIS INVOLVEMENT IN STRESS/IMMUNE CIRCUITS

Eduardo Arzt

Laboratorio de Fisiologia y Biologia Molecular, Universtity of Buenos Aires, and CONICET, Argentina

The immune-neuroendocrine systems have an intimate cross communication making possible a satisfactory response to environmental changes and stress. The hypothalamic-pituitary-adrenal axis (HPA) has a key role in the interaction between the immune and neuroendocrine systems and in the stress response. Cytokines activate the HPA axis and induce a rise in glucocorticoid levels, which are instrumental in order to control immune-cytokine overreactions, acting on T lymphocytes and other ctyokine/target cells. The specificity of hormonal and cytokine signals on their target cells is crucial to provide specificity to their actions. The cross-talk between cytokine-induced transcription factors such as Tbet, GATA-3, NFκB, and AP-1 and steroid (i.e. glucocorticoid) receptors involve both genomic and non-genomic actions, and constitutes the mechanism for fine tuning both hormone and cytokine responses. Corticotrophin releasing hormone (CRH) is the key mediator of the HPA axis of the central nervous system response needed to adapt to stressful conditions. The final outcome of CRH/CRH1 signaling depends on the context of specific cells and ligands, cross-talk of signaling pathways and the effector actions of the pathways once they are activated. This specificity bears consequences at the CNS level where CRH activates through the same receptor (CRHR1) different signaling pathways depending on neuroanatomical context. All these molecular interactions represent a key step for understanding, at the cellular and genetic level, the specificity and ultimate response of physiological neuroendocrine-immune interactions during stress.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

5D_02_S

INTERLEUKIN-1 SIGNALING AT THE INTERSECTION OF NEURONAL, IMMUNE AND METABOLIC STRESS

Tamas Bartfai

The Harold L Dorris Neurological research Institute. Molecular and Integrative Neurosciences Department, The Scripps Research Institute , La Jolla, CA 92037, USA

Macrophages , adipocytes and macrophages in white adipose tissue, microglia and neurons can synthesize the proinflammatory cytokine and endogenous pyrogen ; IL-1 β in response to acute immune, mechanic, oxidative and hypoxic insult , and to excitatory neurotoxic events. IL-1 synthesis is also induced by chronic elevation of misfolded proteins or metabolic/endocrine stressors like elevated leptin levels. The IL-1 levels being highly inducible within short time serve as a common signal in neuronal immune and endocrine signaling.
The wide spread expression of IL-1type 1 receptor on neurons, pancreatic-β cells and macrophages ,T and B cells accounts for the global inflammatory effects f IL-1 .
The acute stress leading to rapid activation of the HPA axis, lowering seizure threshold and fever response by IL-1 utilizes the rapid transcription independent neuronal effects of IL-1 . The molecular steps of this signaling have been delineated in molecular detail showing that IL-1R1-MyD88-Neutral Spingomyelinase-ceramide cSrc – phosphoprotein steps mediate the early IL-1 response. After 45-60 min the classical Toll signaling pathway involving the NFKB mediated induction of COX2 and the subsequent production of the inflammatory mediator PGE2.
The chronic inflammatory stress presented by obesity and the role of IL-1 in the conversion from insulin resistance to type 2 diabetes will also be discussed.

5D_03_S

ROLE OF LIPIDS IN THE MODULATION OF HUMAN T CELL ACTIVATION

Fulop T[1], Brassard P[2], Larbi A[1], Frisch F[2], Fortin C[1], Carpentier A[2].

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[1]*Division of Geriatrics and the Program of Immunology,* [2]*Division of Endocrinology, Department of Medicine, University of Sherbrooke, Sherbrooke, QC, Canada*

Studies have shown suppressive effects of PUFA on T cell functions suggesting that lipids can have potent immunomodulatory effects. It is now accepted that the membrane of T cells is heterogeneous and contains microdomains called lipid rafts (LR) playing an important role in TCR signalling. Thus, variations in lipid levels and composition may determine their effects on immune response. Each time when lipids are ingested the immune system is submitted to a lipid stress. The precise mechanism for this effect has not been fully investigated *in vivo* in humans. Our aim was to determine whether there are differences in T cell functions and signalling depending on the way of lipid administration and composition. Peripheral T cells were isolated from healthy subjects before and after 2-hours of an intravenous infusion of heparin + Intralipid (HI) during a euglycemic hyperinsulinemic clamp to induce a 2.5-fold elevation in plasma linoleic acid concentration. Similar experimental setting was designed after an oral meal (OM).. HI and OM reduced peripheral T cell membrane fluidity and altered lipid raft organisation. Both associated with reduced T cell proliferation upon CD3 + CD28 co-stimulation. Tyrosine phosphorylation of LAT and activation of Akt in T cells were also impaired without a reduction in T cell receptor expression. The LR polarization was also altered. A selective increase in plasma linoleic acid concentration and in intravascular lipolysis therefore have a suppressive effect on peripheral T cell CD28-dependent activation and this effect associates with changes in plasma membrane properties. The lipid composition of nutritional therapy in patients at high risk of septic complications may be crucial and may also be of relevance for type 2 diabetes. Furthermore, oral nutrition rich in lipids constituting a chronic lipid stress, at long term, could contribute to the observed immunosenescence.

5D_04_S

NEUROENDOCRINE RESPONSE IN SUSCEPTIBILITY TO INFLAMMATORY, AUTOIMMUNE AND INFECTIOUS DISEASES

Esther M. Sternberg

NIMH/NIH, Bethesda, MD, USA

The central nervous system (CNS) plays an important role in regulating immunity and in susceptibility and resistance to autoimmune, inflammatory and infectious diseases. Cytokines released during inflammation mediate changes in brain function, inducing sickness behaviors, sleep, fever and activation of the hormonal stress response

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

(hypothalamic-pituitary-adrenal axis, HPA axis). In turn neural and neuroendocrine responses, including the HPA axis, sympathetic and parasympathetic responses, regulate immune responses, thus providing important extra-immune system feedback control of immunity. HPA axis activation inhibits inflammation through the generally anti-inflammatory action of the glucocorticoids. However, physiologic concentrations of glucocorticoids are immunomodulatory, shifting cytokine production from a TH1 to a TH2 response and enhancing delayed type hypersensitivity. Interruptions of the HPA axis at any level and through multiple mechanisms, whether genetic, surgical or pharmacological, render inflammatory resistant hosts susceptible to inflammatory disease. In contrast, over-activation of this axis, as in chronic stress, enhances severity of infections, through the immunosuppressive effects of the glucocorticoids. The association of a blunted HPA axis with autoimmune/inflammatory disease occurs across species, strains and diseases, and in a variety of human autoimmune/inflammatory illnesses. Tissue resistance to glucocorticoids resulting from polymorphisms, mutations or dysfunction of the glucocorticoid receptor (GR) is also associated with enhanced autoimmune/inflammatory disease expression. We have described a new mechanism for glucocorticoid resistance related to bacterial toxin repression of the GR and other nuclear hormone receptors. This suggests a potential new mechanism for shock and inflammatory sequelae of bacterial infections, and potential new approaches to developing therapies for these conditions.

References:

Sternberg EM. Neural regulation of innate immunity: a coordinate non-specific host response to pathogens. *Nature Reviews Immunology* 6(4):318-28, 2006.

Marques-Deak A, Cizza, G and Sternberg EM. Brain-immune interactions and disease susceptibility. *Molecular Psychiatry*, 2005, 10 (3), 239-250.

Webster JI, Tonelli L, Sternberg EM. Neuroendocrine Regulation of Immunity. *Annual Review of Immunology*. 2002. 20:125-163

The Balance Within. The Science Connecting Health and Emotions. by E.M. Sternberg, M.D., W.H. Freeman, New York. Hard cover 2000; paperback Holt, Times Imprint, 2001

5E_01_S

THE MECHANISMS BEHIND THE BRAIN'S RESPONSE TO STRESS

Marian Joëls

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

SILS-CNS, University of Amsterdam, The Netherlands

When an organism experiences a stressful situation, this is perceived through the brain and thus gives rise to the activation of the sympathetic nervous system and the hypothalamo-pituitary-adrenal axis. As a result brain cells, including those involved in the initial appreciation of the stressor, are exposed to elevated levels of monoamines like noradrenaline, to specific neuropeptides and to corticosteroids. We have examined the effects of these hormones on neuronal function in limbic regions, particularly the hippocampal CA1 area. In the initial phase after stress (<1 hr), rapid effects of noradrenaline, CRH and vasopressin will increase the excitability. Corticosterone, through a non-genomic pathway, plays a permissive role in the effects of the other stress hormones but can also by itself increase local excitability and facilitate the induction of long-term potentiation (LTP). Collectively, the excitatory effects of stress hormones in this phase may help to promote focused attention, vigilance and start the encoding of information about the event. At the same time, though, gene-mediated cascades are started via corticosterone binding to its nuclear receptors, which will alter transcriptional activity of responsive genes. These effects develop slowly (1-2 hrs), i.e. at a point in time that hormone concentrations are back to the pre-stress level; the effects can last for hours. The gene-mediated actions include an enhanced influx of calcium -hence more firing frequency accommodation-, reduction of noradrenergic responses and suppression of LTP induction. These actions will help to normalize the earlier aroused activity in the hippocampus, which can be regarded as a 'central' negative feedback. As information reaching the circuits at this time must be very salient to pass the threshold for LTP induction, earlier encoded information will be preserved.

5E_02_S

GENETIC MANIPULATIONS OF HORMONAL SIGNALING IN THE HIPPOCAMPUS

Daniela Kaufer

Department of Integrative Biology & Helen Wills Neuroscience Institute
University of California, Berkeley, CA

Glucocorticoids (GCs), the adrenal steroids released during stress, compromise the ability of neurons to survive neurological injury. In contrast, estrogen protects neurons against such injuries. We designed three genetic interventions to manipulate GCs actions, which reduced their deleterious effects in rat both *in vitro* and *in vivo*. The most effective was a chimeric receptor combining the ligand-binding domain of the glucocorticoid receptor (GR) and DNA-binding domain of the estrogen

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

receptor. Expression of this receptor reversed the outcome of the stress response by rendering GCs protective rather than destructive. Our findings elucidate principal steps in the neuronal stress response pathway, all of which are amenable to therapeutic intervention. GCs are also implicated in reducing adult hippocampal neurogenesis. There has been little evidence for the presence of type 1 GR or type 2 (mineralcorticoid) receptors in neuronal precursor cells (NPC), and therefore suggested that GCs must indirectly inhibit NPC proliferation, though the mechanism has remained obscure. We demonstrate that GR mRNA is transcribed and yields a cytoplasm-localized receptor in isolated NPC from the adult hippocampus. Treatment of NPC grown in vitro with GCs induces decreased proliferation index, and a down-regulation of Nestin, a protein marker that is down regulated as NPC stop dividing and differentiate. This response is blocked using the GR-specific antagonist indicating that the GCs response is mediated by the glucocorticoid receptor. We demonstrate decreased proliferation and a shift in cell fate choice in NPC following GCs treatment, which is blocked by the anti-GCs genetic manipulation. The apparent responsiveness of NPC to GCs suggests that neurogenesis is directly modulated via GR signalling pathways.

5E_04_S

STRESS AND TRANSCRIPTION: SPECIFIC INACTIVATION OF THE GLUCOCORTICOID RECEPTOR GENE IN THE DOPAMINERGIC SYSTEM: NEW INSIGHTS ON DRUG ADDICTION

Francois Tronche

CNRS UMR7148 « Molecular Genetics, Neurophysiology and Behavior », Collège de France, 75005 Paris France; francois.tronche@gmail.com

A dysfunction of the stress response is suspected in the etiology of metabolic and behavioral disorders (ie anxiety, depression, drug dependence). A major component of this response involves the release of adrenal glucocorticoids (GCs) and the activation of the glucocorticoid receptor (GR), a transcription factor.
To study GR function in vivo, we develop and analyze animal models lacking GR in given brain cell populations. Molecular, physiological, electrophysiological and behavioral studies of these animals should allow us to locate the cell types involved and to better define the mechanisms underlying GR function in brain physiology and in pathological situations. We previously showed that the selective inactivation of the glucocorticoid receptor (GR) gene in mice brains (GRNesCre) profoundly reduces motivation for cocaine. More recently, we showed that these behavioural effects are associated with a change in the impulse activity of midbrain dopamine neurones.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

To determine in which cell type the function of GR is required to modulate motivation for cocaine, we generated animal models in which GR is selectively inactivated in either pre-synaptic dopamine neurons (GRDATCre) or post-synaptic cells (GRD1Cre). Characterization of these models will be presented. To address the question of the interaction of GCs and serotonergic pathway, we generated a mouse transgenic line that allow Cre recombination in all 5-HT1A neurones and obtained conditional GR inactivation. Analysis of this animal model will be presented.

Module 5 – Poster lectures:

5A_01_P

AISA ("ANTI INFLAMMATORY SENESCENCE ACTIVES") A PLANT DERIVED APPROACH OF ANTI-STRESS

Jean-François Bisson, Chantal Menut, Marc Moutet and Patrizia d'Alessio

ETAP-54500 Vandoeuvre-les-Nancy ; Laboratoire de Chimie Bio-Moléculaire-UMR 5032 ENSCM, 34296 Montpellier ; EandC Biocitech France; AISA Therapeutics CHU Paul Brousse 12, ave Paul Vaillant-Couturier 94807 Villejuif France dalessio@vjf.inserm.fr

Psycho-social stress induces arterial vasomotor alterations such as vasospams and transitory hypertension. Moreover, stress has a profound impact on vascular endothelium, inducing a systemic inflammation. Starting from plant extracts, we have isolated through bio-guided selection, characterized and patented 4 mono-terpens, that we have named "AISA", Anti-Inflammatory Senescence Actives, targeting activated endothelium. In our *in vitro* platform, they inhibit the expression of adhesion molecules and the polymerization of actin fibers, following the activation by TNF-α. Inhibition is conserved at all replicative passages of vascular endothelial cells. In pre-clinical models, AISA 5203-L is well tolerated and has a good oral bio-availability. This molecule has shown convincing anti-inflammatory performance in a rat TNBS colitis model, comparable to those obtained with ibuprofen. Using AISA-5203-L in a stress model, a substantial anti-stress activity could be documented by a FOB (Functional Observation Battery) of 56 tests (patent pending).
We would like to propose that a new anti-inflammatory molecule of plant origin interfering with cell replicative senescence would be responsible of relevant anti-stress effects. This hypothesis would permit the positioning of AISA molecules as candidates therapeutic applications in multi-factorial diseases, such as hypertension, neuro-degenerative diseases, chronic pain

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

syndrome, obesity and depression.

5A_02_P

INTERACTION OF HSP-16.2 WITH HISTONES AFTER HEAT-SHOCK IN THE NEMATODE *C. ELEGANS*.

Alavez S. and Lithgow GJ.

The Buck Institute, 8001 Redwood Blvd. Novato, CA 94945 USA. salavez@buckinstitute.org

Stress response is a very significant determinate of the life history of *C. elegans*. As the first metazoan genome to be sequenced, *C. elegans* is a major model for molecular genetic studies of aging because of the availability of a range of single gene mutant lines with greatly extended lifespan. Interestingly, these long-lived variants are also stress tolerant. Among others, we demonstrated that at least one chaperone gene encoding heat shock protein -16 (*hsp-16*) works together with reduced insulin signaling to bring about lifespan extension. However, the mechanism by which hsp-16 increases lifespan or thermotolerance in this animal model remains unclear.
We have now obtained an stable transgenic line that over-express the fusion protein HSP-16.2::GFP under the control of hsp-16.2 promoter that presents a remarkable thermotolerance and a high expression of the fusion protein after heat-shock (HS) measured by westernblot. The lifespan of these over-expressing lines is extended (36%, $p<0.001$) as compared to control strain, N2. This strain also presents a widespread expression of this transgene in multiple tissues. We have found a relocalization of HSP-16.2::GFP to the nuclei of several neurons of the nerve ring and some intestinal cells after HS in this strain as revealed by DAPI and GFP colocalization. On line with this observation, we have identified Histone-3 and Histone-4 through MALDI-TOF and LC/MS mass spectrometry by coimmunoprecipitation with an HSP-16.2 specific antibody and posterior separation of the proteins via 1-dimensional gel electrophoresis after HS.
These results show that histones are targets of HSP-16.2 during stress conditions (HS) and suggest a nuclear role of chaperones to regulate thermotolerance and maybe other stressing conditions like aging.

5A_03_P

LIPOIC ACID: HOW AN INCREASE IN RESPIRATION CAN DECREASE STRESS AND INCREASE LIFESPAN IN *C. ELEGANS*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Olivier Descamps, Michael Benedetti, Amanda L. Foster, Maithili Vantipalli, James N. Sampayo, Matthew S. Gill, Anders Olsen, and Gordon J. Lithgow

The Buck Institute, 8001 Redwood Blvd. Novato, CA 94945, USA

Aging is the most important risk factor in developed countries. Consequently, there is considerable interest in the pharmacological manipulation of aging as a way to uncover new avenues for development of therapeutics. Genetic alterations can extend the lifespan of the nematode *C. elegans* as much as five-fold and in principle chemical compounds could do the same. Based on the mechanistic relationship between aging and stress response, we have screened for compounds that enhance stress resistance. Lipoic acid (LA), a cofactor of the Krebs cycle enzymes pyruvate and glyceraldehyde dehydrogenases, increases respiration and lifespan in the adult nematode worm *C. elegans*. This seems somewhat paradoxical in the context of the rate of living theory of aging in which lifespan is inversely proportional to the rates of metabolic processes. We investigated the effect of the LA treatment on the mitochondrial physiology of *C. elegans*. We find that lipoic acid decreases the mitochondrial membrane potential and see some evidence for an increase in the amount of mitochondria. This in turn results in lower oxygen radical production (steady state superoxide anion levels), which may explain the lifespan extension. The LA-treated worms are not overtly hyperactive and have the same levels of ATP and fat content as untreated controls. Our working hypothesis is that stimulation of the Krebs cycle, without changing the other metabolic parameters, increases the number of mitochondria and in consequence lowers the burden of activity in each of the mitochondria, resulting in less stress and longer lifespan.

5A_04_P

INFLUENCE OF AGE AND ETHANOL INTOXICATION ON HEME OXYGENASE 1 EXPRESSION IN RAT LIVER.

Anna Lisa Furfaro, Stefania Patriarca, Emanuela Balbis, Cinzia Domenicotti, Damiano Cottalasso, Umberto Maria Marinari, Maria Adelaide Pronzato, Nicola Traverso

Department of Experimental Medicine, Section of General Pathology, University of Genova, Via L.B. Alberti 2, 16132, Genova, Italy; annalisa.furfaro@virgilio.it

Many authors recognize that heme oxygenase 1 (HO-1) expression is a marker of cellular response to oxidative stress; since ageing is related to oxidative “wear and tear”, HO-1 may represent a candidate biomarker of

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

ageing. In this study, we evaluated the hepatic expression of HO-1 mRNA in 2.5-24 month-old rats; this expression was higher at 6 months than at 2.5 months of age, but thereafter increased no further. However, while 2.5 and 6-month-old rats responded to acute ethanol intoxication by displaying increased expression of liver HO-1 mRNA, 18 month-old rats did not show any response; this phenomenon suggests that during development and ageing the transcriptional response to oxidative stress is progressively impaired. This may be due to decreased transcriptional ability to respond to stress in older animals, rather than by a reduction in oxidative stress. Grants from the University of Genova, MIUR PRIN #2004063943_001 and #2004068552_002, and FIRB 2001 #RBAU01JBH8_003.

5A_05_P

EARLY STRESS RESPONSE IN FXTAS AN INHERITED NEURODEGENERATIVE DISORDER

D. Garcia-Arocena[1], C.K. Iwahashi[1], F. Tassone[1], J.B. Brower[2], E.M. Berry-Kravis[3], R. Willemsen[2], P.J. Hagerman[1]

[1] Dept. Biochemistry and Molecular Medicine, University of California Davis, School of Medicine, One Shields Ave, Davis, 95616, USA
[2] Dept. Clinical Genetics, Erasmus University, Rotterdam, The Netherlands
[3] Neurology and Biochemistry, Rush University Medical Center, Chicago, Illinois, USA

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder that appears to affect carriers of premutation alleles (55 to 200 CGG repeats) of the *FMR1* gene. The pathogenesis of FXTAS involves the direct toxic effect of the elevated levels of expanded CGG repeat *FMR1* mRNA. We have recently described neural cell models that are capable of recapitulating the formation of intranuclear inclusions, the pathologic hallmark of FXTAS. We have discovered that several proteins including lamin A/C and the stress response proteins αB-crystallin and Hsp27 genes are dysregulated in response to expression of the expanded CGG repeat. To quantify these observations we analyzed the expression of *FMR1*, αB-crystallin, Hsp27, and lamin A/C by real time quantitative PCR in cultured human cells derived from subjects with severe FXTAS and age-matched controls. We also studied cultured cells derived from mice with expanded CGG repeats and wt controls. Our results show that subjects with premutations have elevated levels of expression of stress response genes and cellular redistribution in cultured cells. Immunofluorescent studies of additional proteins and flow cytometry DNA analysis indicate cell cycle dysregulation in cells with

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

expanded CGG repeats consistent with accelerated aging in both mouse and human models. We discuss the implications of these results on neural plasticity.

5A_06_P

DIURNAL AND AGE CHANGES IN STRESS RESPONSIVENESS OF THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS AND ERYTHROCYTE ANTIOXIDANT ENZYMES

N. D. Goncharova[1], T. N. Bogatyrenko[2]

[1]Institute of Medical Primatology, Sochi-Adler, Veseloye 1, 354376, Russia; [2]Institute of Problems of Chemical Physics, Chernogolovka, Russia

The aim of the study was to examine chronobiological characteristics of the HPA axis functioning and the antioxidant enzyme activities in stress and aging. Female *Macaca mulatta* of 6-8 years (young mature) and 20-27 years (old) were subjected to acute psycho-emotional stress (two hours immobilization) at 9.00 or 15.00 h. Levels of ACTH, cortisol (F), dehydroepiandrosterone sulfate (DHEAS), and testosterone (T) in peripheral blood plasma were measured before the stress and 5, 15, 30, 60, 120, 240 min and 24 h after the challenge. In parallel, activities of superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase (GR), glutathione-S-transferase, and lipid peroxidation (LPO) were measured in hemolyzed erythrocytes. Young monkeys demonstrated much higher increase of ACTH, F, T, DHEAS levels and GR activity in response to the stress imposed at 15.00 than to identical stress imposed at 9.00. However, no such circadian differences in dynamics of the hormonal secretion and GR activity after the stress were found for old animals. Young monkeys demonstrated also much higher accretion of ACTH, F, T, DHEAS levels and GR activity in comparison with old monkeys in response to the afternoon stress. The changes in GR activity with stress and aging correlated well with the changes in the F, DHEAS, and T level. SOD activity in old monkeys was lower than in young ones before the stress and increased in response to the stress that was accompanied by light decrease of LPO. In contrast to old monkeys, young ones demonstrated decrease of SOD activity and some increase of LPO with stress. There was a high level of correlation between the stress changes in SOD activity and the changes in the F level in old monkeys but there was no correlation in young monkeys. The lack of correlation between stress dynamics of F level and SOD activity may be caused by marked activation of adrenal T secretion in young monkeys. These results suggest that the HPA axis plays an important role in regulation of antioxidant enzymes defense in stress conditions and that this regulation shows age differences.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Supported by the RFBR (grant 06-04-97616-r_yug_a)

5A_07_P

POTENTIAL USE OF PLANT ADAPTOGENS IN AGE-RELATED DISORDERS

Makarov V., Makarova M.N., Stoloschyck N.V.

Interregional Center "Adaptogen", Piskarevsky ave., 47/5, St-Petersburg, 195067, Russia

Wikman G., Panossian A.

Swedish Herbal Institute, Prinsgatan 12 5tr, SE-413 05 GÖTEBORG, Sweden

In traditional medicinal systems, members of a small group of higher plants were considered to possess restorative properties and were used as general tonics in the treatment of disease and in convalescence. Through a series of studies conducted in the early 1950s, Soviet scientists first established that several of these plants also possessed the capacity to enhance the "state of non-specific resistance" (SNSR) of an organism to stress. Plants that exhibit this "adaptogenic effect" are currently used to increase mental and physical work capacity and performance against a background of fatigue and stress, and also to improve the quality of life. Additionally, the efficacy of plant adaptogens in the relief of mild and moderate depression has recently been demonstrated. The aim of the present study was to investigate the effect of adaptogens, administered in the form of standardised extracts of *Rhodiola rosea*, *Schisandra chinensis* and *Eleutherococcus senticosus* in their fixed combinations (ADAPT-232, ADAPT-Plus and ADAPT-Extra-Plus), on the age-related deterioration of function of the innate defence, cardiovascular and central nervous systems in 2 year old rats. Drugs and placebo were administered daily over a period of 4 months, and alterations in adrenal weight, ECG parameters, motor activity, learning ability, duration of hexanal-induced sleep, formation of immobilisation stress-induced stomach ulcers, spontaneous promotion of tumours, apoptosis of spleen lymphocytes, and levels of 17- hydroxy-corticosteroids in the urine, and cholesterol, albumins and total proteins in the blood, were monitored. The results showed that repeated administration of adaptogens can diminish or prevent a range of age-related disorders including reduced liver detoxifying function, malfunction of the central nervous system (i.e. loss of memory and learning ability), development and progression of cardiac insufficiency and hypercholesterolemia, impaired protein synthesis,

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

reduced activity of the hormonal system, increased sensitivity to stress (hypodynamia-induced damage to the stomach and adrenals), impaired apoptosis, and spontaneous promotion of tumours. It is concluded that adaptogens have potential value in the treatment of age-related disorders of the stress system in the elderly and may be effective in maintaining the health status of such individuals at the normal level.

5A_08_P

MITOCHONDRIAL J HAPLOTYPE AND ANTI-OXIDANT STATUS AND IN THE BELFAST ELDERLY LONGITUDINAL FREE-LIVING AGEING STUDY (BELFAST)

Irene Maeve Rea[1], Owen A Ross[2, 5], G Pooler Archbold[3], Ian R Young[4], Martin Curran[2], Derek Middleton[2]

[1] Department of Geriatric Medicine, Queens University Belfast, [2]Northern Ireland Tissue Typing and Immunogenetics Laboratory, Belfast City Hospital, [3]Biochemistry Laboratory, Belfast City Hospital, [4]Nutrition and Metabolism Group, Queens University Belfast, [5]Mayo Clinic, Jacksonville, USA

Mitochondria are the chief source of both energy and oxidants inside the cell. This dual role can damage cells despite an array of antioxidants available to mop up endogenous oxidants including hydrogen peroxide, superoxide and hydroxyl radicals. The mitochondrial haplotype J seems related to 'successful' ageing in Italian, Finnish and Irish groups of nonagenarian/centenarians. In this study we questioned whether J haplotype octo/nonagenarians from the BELFAST study demonstrated enhanced anti-oxidant profile or other phenotype characteristics.
Methods: Briefly, community-living, mentally alert (Folstein >27/30) octo/nonagenarian subjects were enlisted as part of BELFAST study. DNA typing for mitochondrial haplotypes was collected and blood for serum antioxidants together with Blood pressure measurements. Serum uric acid, bilirubin, ceruloplasmin, glutathione, selenium and vitamins C, A, E and α-carotene were not significantly different between J and non J mitochondrial haplotypes but whole blood and serum gluthatione peroxidase (GSHpx) were lower in J haplotype octo/nonagenarians. Both systolic and diastolic blood pressure was significantly lower in J haplotype octo/nonagenarians.
Conclusion: BELFAST study octo/nonagenarian subjects, carrying the J haplotype, do not show enhanced antioxidant status for most of the major antioxidants, including uric acid and vitamin C, but show reduced whole blood and serum glutathione peroxidase status. Interestingly J mitochondrial haplotype octo/nonagenarians did have lower systolic and

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

diastolic blood pressure.

5A_09_P

TISSUE AND CELLULAR RESPONSES TO STRESSES OF HYPOXIA AND HYPEROXIA OF IMPORTANCE IN TISSUE ENGINEERING

George K. B. Sándor, Riitta Suuronen

Regea Institute for Regenerative Medicine, Tampere University, Tampere, Finland and The Hospital for Sick Children, University of Toronto, Toronto, Canada. (george.sandor@utoronto.ca)

Future reconstructive surgical procedures will have the possibility of using tissue engineered constructs to rebuild missing or defective body parts in humans and in veterinary medicine. Such constructs require three essential components: cells, a scaffold and signalling molecules. A great deal of attention has been paid to the harvesting of stem-cells both of fetal and autogenous adult origins which are to be expanded in tissue culture. Such expanded stem-cell derrived populations of cells are used to populate resorbable scaffolds. Much work has been done in understanding the potential sources and roles of key bioactive cell signalling molecules such as Bone Morphogenetic Protein (BMP). Cells that exist in isolation or as parts of complex organs and tissues have known responses to certain stressors. Cells and tissues that are grown *ex vivo* in extra corporeal bioreactors must be provided with environments that minimize cellular stress and promote maximal growth when cellular populations are to be expanded. These cellular constructs are sensitive to varying concentrations of ascorbic acid, dexamethasone and β-glycerophosphate. The reactions of cells and tissues to hypoxic and hyperoxic environments are also important. At the molecular level these reactions occur in part due to superoxide radicals while at the tissue level they are mediated by bioactive signalling proteins such as Vascular Endothelial Growth Factor (VEGF). There is evidence that both hypoxia and hyperoxic states may promote angiogenesis and further tissue growth. This presentation will review our understanding of these processes and mechanisms in the context of tissue engineering.

5A_10_P

CREB-INDUCING ACTION OF *HYPERICUM PERFORATUM* MAY BE RESPONSIBLE FOR ITS PREVENTIVE EFFECTS ON STRESS- AND AGING-RELATED MEMORY IMPAIRMENT.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Emil Trofimiuk, Adam Hołownia, Jan J. Braszko

Department of Clinical Pharmacology, Medical University of Bialystok, Waszyngtona 15A, 15-274 Bialystok, Poland.

Chronic stress impairs a number of aspects of cognition such as acquisition of memory, its consolidation and recall. Similar alterations were observed in aged rats. It is well known that stress causes acceleration of the brain aging. In stressed as well as in aged rats disturbances of hypothalamo-pituitary-adrenal axis and consecutive hypercortisolemia are seen. Excessive cortisolemia leads to a number of neurochemical and neuroanatomical changes in brain, especially in the hippocampus, which is particularly vulnerable because of high density of glucocorticoid receptors. As a result neurogenesis is disturbed in the hippocampal areas CA1 and CA3 and the neuronal atrophy occurs leading to the decrease of its volume and lowering total number of neurons and their ramifications.
We recently found, that dried crude herb of *H. perforatum* (350 mg kg^{-1} for 21 days orally) reversed negative effects of chronic stress on cognition (spatial reference and working memory, recognition memory, conditioned behaviour, learning, acquisition and recall of the avoidance behaviour), but still our knowledge about the mechanisms of this improvement is poor. It is known that *H. perforatum* normalizes the dopaminergic and noradrenergic transmission in medial prefrontal cortex, normalizes decreased by stress 5-HT1A and 5-HT2 receptors, balance exaggerated NMDA receptor function, and also restores normal cortisolemia.
This study assessed an association of the *H. perforatum* improvement of age-related memory impairments with the increase of CREB and phosphorylated CREB in hippocampus. Middle-aged rats (78-weeks old) displayed clear-out decline in the acquisition of spatial working memory in the Morris water maze similar that in the young rats (8-weeks old) after 21-day restraint stress. Chronic administration of *H. perforatum* effectively prevented these negative effects of aging and stress ($p<0.01$) that could be partially explained by the concomitant increase of CREB phosphorylation in the hippocampi ($p<0.01$) of aged rats.
This study seems to justify the conclusion that St John's wort in a crude form may have a considerable action relieving stress- and aging-related cognitive deficits by an activation of CREB regulated genes in hippocampus.

5A_11_P

EVALUATION OF PHOTO-STRESS OF SKIN USING CATHEPSIN L

Masaki Yamaguchi[1], Kazutaka Teramura[1], Yusuke Tahara[1], Teruhiko

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Makino[2], Tadamichi Shimizu[2], Akira Date[3]

[1]*Graduate School of Science and Engineering, University of Toyama, Japan, yamag@eng.u-toyama.ac.jp*
[2]*Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Japan*
[3] *Procter & Gamble Japan K.K., Japan*

Objective: A noninvasive determination of skin surface proteolytic activity may be important for medical purposes. The aim of this research is to explore the relationship between photo-stress of skin and cathepsin L activity in the epidermis of human. Materials and Methods: Nine healthy male outdoor workers were enrolled (36.1 ± 12.2 yr, mean ± SD). The study protocol was approved by the Ethical Committee of the Institutional Review Board of the University of Toyama. A tape stripping technique using D-SQUAME tape (Cuderm Co., TX) was used to collect the human stratum corneum from both the posterior (solar-exposed area) and anterior (solar-protected area) regions of the forearm. The cathepsin L activity was analysed by the azocasein assay using a spectrophotometer. Unused tape stripping was analysed as a negative control. The calibration curve for cathepsin L activity was measured using a standard sample (human liver, EMD Chemicals Inc., Germany). Results and Discussion: The relative absorbance of the solar-exposed areas ranged between 0.045 and 0.107 and that of the solar-protected areas ranged between 0.064 and 0.125. The mean relative absorbance of the negative control, solar-protected and solar UV-unexposed areas were 0.002, 0.084 and 0.101, respectively. The cathepsin L activities of the solar-exposed and solar-protected areas were equivalent to 5.6 and 6.8 U/l. Thus, the cathepsin L activity of the solar-exposed area was lower than the solar-protected area (paired Student's *t*-test, $P < 0.05$). Conclusion: Cathepsin L activity may be a biomarker of the photo-stress.

5B_01_P

MANIPULATING THE HEAT SHOCK RESPONSE AS A THERAPEUTIC STRATEGY IN ALS

Bernadett Kalmar and Linda Greensmith

Institute of Neurology, University College London, UK.
l.greensmith@ion.ucl.ac.uk

Heat shock proteins (hsps) are know to play a role in neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS), in which motoneurons degenerate, resulting in muscle weakness, paralysis and finally death, usually within 2-5 years of diagnosis. Evidence suggests a

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

relationship between the level of the heat shock response (HSR) and the specific vulnerability of motoneurons to degeneration in ALS. For example, motoneurons have an unusually high threshold for the activation of the HSR which may result in abnormal protein folding and trafficking and an increased susceptibility to apoptotic insults. We have recently up-regulated the HSR in a mouse model of ALS. Treatment of $SOD1^{G93A}$ mice with arimoclomol, induces the expression of hsp70 and hsp90, resulting in a delay in disease progression and a significant increase in survival. However, the effects of arimoclomol are unlikely to result only by increasing hsp levels in motoneurons since genetic manipulations that increase hsp70 in $SOD1^{G93A}$ mice fail to alter disease progression. It is not only the mechanism, but the site of action of arimoclomol and hsps in $SOD1^{G93A}$ mice that remains unclear. We are characterising the HSR in this model of ALS as a function of disease progression and testing the differential neuroprotective effects of a variety of manipulations that up-regulate expression of hsps. Our results show that not all agents that activate the HSR will necessarily have beneficial neuroprotective effects on motoneurons. Moreover, activation of the HSR in non-neuronal cells may have greater neuroprotective effects than targeting the HSR within motoneurons themselves. We hope that a better understanding of the role of the hsps in ALS will help to optimize targeting of the HSR as an effective therapeutic strategy for this fatal disease.

5B_02_P

ELECTRO-ACUPUNCTURE AND BRAIN PROTECTION FROM CEREBRAL ISCHEMIA: DIFFERENTIAL ROLES OF ACUPOINTS

F Zhou[1,2], JC Guo[2,1], JS Cheng[2,1], GC Wu[2,1], Y Xia[3,1]

[1]Shanghai Research Center for Acupuncture and Moxibustion, Shanghai, China; [2]Fudan University Shanghai Medical College, Shanghai, China; [3]Yale University School of Medicine, New Haven, CT, USA.
Ying.Xia@Yale.Edu.

We have shown that electro-acupuncture (EA) significantly reduces ischemic infarction in the rat model of cerebral ischemia (right middle cerebral artery occlusion, MCAO). Since there are multiple acupoints that may cause different effects on the body, we asked, in this work, whether the EA protection from cerebral ischemia varies with the acupoints stimulated. We observed that 1-hour MCAO greatly reduced cerebral blood flow and caused the brain infarction and EA with sparse-dense wave (5Hz/4s-20Hz/4s) at 1.0mA for 30 minutes differentially attenuated the ischemic infarction depending on the acupoints used. In the group of head acupoints, i.e., “Shuigou” (Du 26) and “Baihui” (Du 20), the cerebral

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

infarction was greatly reduced from 33.4%±3.1% to 4.9%±1.2% of the brain (n=30, P<0.01) with significant improvement of neurological deficit (from 6.0±1.0 to 1.0±1.0 in scales of 0-7). EA at "Quchi" (LI 11) and "Neiguan" (P 6) on the left anterior limbs reduced the infarct volume to 8.6%±3.8% (n=12, P<0.01) with the scales of neurological deficit being reduced to 2.0±1.0 (n=22, P<0.01). In sharp contrast, EA at "Quchi" (LI 11) "Neiguan" (P 6) on the right anterior limbs did not lead to any significant changes in the infarct volume (29%±6.3%, n=9) and neurological deficit (5.5±0.5, n=9). Also, EA at "Yanglingquan"(GB 34) and "Sanyinjiao"(SP 6) on the left posterior limb had no protective effect on the ischemic injury. These results suggest that the EA protection from cerebral ischemia is relatively acupoints-specific. *Supported by STCSM (3DZ19544-1-1), 973-Program (2005CB523306), NSFC (30672721) and NIH (HD34852).*

5B_03_P

NEUROPROTECTIVE EFFECTS OF EXTRACELLULAR HSP70

Michael Tytell

Neurobiology & Anatomy, Wake Forest Univ. School of Med., Winston-Salem, NC 27157, tytellm@wfu.edu

When the cytoprotective function of Hsps was a relatively new concept in the 1980s, we and Hightower respectively observed that Hsp70 was passed from one cell to another and released into the extracellular fluid (Tytell & Lasek, Brain Res 324:223, 1984; Hightower & Guidon, J Cell Physiol 138:257, 1989). These were unexpected results because the Hsp70 family was thought to be exclusively intracellular proteins. In the two decades since, extracellular Hsp70 has become well-documented. Our 1984 observation that glial Hsp70 was transferred into the axon led to the novel hypothesis that, in the nervous system at least, the stress tolerance of neurons was not solely dependent on their own Hsp70, but could be supplemented by additional Hsp70 from adjacent glia. For neurons, this hypothesis has major implications because those with long axons (mm to meters in length) require newly synthesized proteins to be transported from the neuronal cell body to remain functional. If stress or injury occurs in axons at some distance from their cell bodies, hours to days are required for newly synthesized Hsp70 to reach the injury site, which is too long to be of use. Thus, supplying Hsp70 at the injury site would be more effective in maintaining neuronal and axonal function. We have tested this idea in a number of models. . In the injured sciatic nerve, local application of Hsp70 improved sensory and motor neuron survival. Similarly, in the light-damaged rat eye, Hsp70 injected into the vitreous chamber

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

promoted survival of photoreceptors. In cultured glia and neurons, we found that glia released Hsp70 and that neurons took up Hsp70 from the medium, leading to greater resistance to apoptosis induced chemically or by lack of trophic factor. Therefore, Hsp70 has potential as a therapeutic agent in acute nervous system injury.

5B_04_P

ELECTRO-ACUPUNCTURE INCREASES CEREBRAL BLOOD FLOW AND PROTECTS THE RAT BRAIN FROM ISCHEMIC INJURY

F Zhou[1,2], JC Guo[2,1], R Yang[2], JS Cheng[2,1], GC Wu[2,1], Y Xia[3,1]

[1]Shanghai Research Center for Acupuncture and Moxibustion, Shanghai, China; [2]Fudan University Shanghai Medical College, Shanghai, China; [3]Yale University School of Medicine, New Haven, CT, USA.
Ying.Xia@Yale.Edu

Traditional Chinese medicine has advocated the use of acupuncture to treat stroke for a long history. However, its efficacy and mechanisms need scientific testing with modern techniques. This study was performed to address three critical questions: 1) how effective does electro-acupuncture (EA) reduce ischemic infarction in the brain? 2) how long time of EA is optimal for the maximal efficacy? and 3) is the EA efficacy dependent on the regulation of blood flow since the infarction is caused by cerebral ischemia? The experiments were carried out in the rat ischemia model with middle cerebral artery occlusion (MCAO). EA was delivered to "Shuigou" (Du 26) and "Baihui" (Du 20) with sparse-dense waves (5Hz/4s-20Hz/4s) at 1.0mA±0.1mA, starting from 5 mins after the onset of MCAO. The results showed that EA induced a significant increase in cerebral blood flow in the ischemic cortex. EA for 5-30 mins significantly reduced ischemic infarct volume and relieved neurological deficits. This benefit effect increased with EA length between 5 and 30 min with the infarct volume being reduced by >80% in the 30-min group (n=60, P<0.01). In sharp contrast, EA for 45min or more did not reduce the ischemic infarction with an increase in neurological deficits and death rate. Interestingly, the cerebral blood flow was also increased in this group during EA. These data suggest that 1) EA does protect the brain from cerebral ischemia; 2) the EA efficacy is dependent on appropriate EA duration; and 3) the increase in the blood flow may contribute to the EA protection, but it is not a sole factor responsible for the EA-induced protection from cerebral ischemia. *Supported by STCSM (3DZ19544-1-1), 973-Program (2005CB523306), NSFC (30672721) and NIH (HD34852).*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

5B_05_P

ELECTRO-ACUPUNCTURE INDUCED PROTECTION FROM CEREBRAL ISCHEMIA IS DEPENDENT ON STIMULATION INTENSITY AND FREQUENCY

F Zhou[1,2], JC Guo[2,1], JS Cheng[2,1], GC Wu[2,1], Y Xia[3,1]

[1]Shanghai Research Center for Acupuncture and Moxibustion, Shanghai, China; [2]Fudan University Shanghai Medical College, Shanghai, China; [3]Yale University School of Medicine, New Haven, CT, USA.
Ying.Xia@Yale.Edu

We have observed that electro-acupuncture (EA) protects the brain from ischemic injury in an acupoint-specific manner. Since EA effects vary with stimulation parameters, we wondered whether the EA-induced protection changes with the stimulation intensity and frequency when applying EA and tested it in this work. The experiments were carried out in the rat ischemia model (middle cerebral artery occlusion, MCAO) with EA being delivered to acupoints of "Shuigou" (Du 26) and "Baihui" (Du 20). Because the increase in cerebral blood flow (CBF) is a key index of the EA protection from cerebral ischemia, we systemically investigated the effects of EA, with different stimulation parameters, on CBF in the ischemic model. At 1.0 mA of EA stimulation for 30 mins, 5-20 Hz (sparse-dense wave) led to a significant increase in CBF and decrease in the cerebral infarction (-85%, $P<0.01$ vs. MCAO alone). The CBF could be increased by EA at up to 40 Hz with a major increase between 2 and 30 Hz. At 40 Hz, EA induced a marginal increase in the blood flow. EA with >40 Hz stimulation led to no/little change in the CBF. On the other hand, the EA-induced increase in the CBF was sensitive to the change in current intensity. The intensity less than 0.6 mA did not induce any significant change in the CBF. With a higher intensity, 0.8 mA caused a slight but significant increase, and 1.0-1.2 mA led to a major increase in the CBF. With "optimal" intensity and "non-optimal" frequency (e.g., 1.0 mA and 70 Hz) or "optimal" frequency intensity and "non-optimal" intensity (e.g., 5-20Hz and 0.4 mA), EA could not induce a significant increase in the CBF. We conclude that the EA-induced increase in CBF is largely dependent on EA conditions, especially the intensity and frequency. *Supported by STCSM (3DZ19544-1-1), 973-Program (2005CB523306), NSFC (30672721) and NIH (HD34852).*

5C_01_P

DIFFERENTIAL STRESS PROTEINS EXPRESSION IN NRK-52E CELLS EXPOSED TO HG(II) OR PB(II)

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

§Stacchiotti A, *Morandini F, *Bettoni F, §Foglio E, °Cadei M, §Rodella LF, °Grigolato P and *Aleo MF

*§Human Anatomy, °2nd Pathology and *Biochemistry Units, School of Medicine, University of Brescia, Viale Europa 11, 25123-Brescia, Italy. Corresponding author: aleo@med.unibs.it*

Mercury Hg(II) and lead Pb(II), are two hazardous environmental contaminants having nephrotoxic effects and whose toxic action appear to be associated to the cellular increase of reactive oxygen species (ROS). Cells respond to oxidative damage by synthesizing highly conserved stress proteins, heat shock (HSP) and glucose related proteins (GRP). These molecular chaperones protect other cellular proteins and organules from injuries induced by a variety of stressors, including heavy metals. So, the present *in vitro* study is undertaken to compare the expression of five stress proteins in rat proximal tubular cells exposed to subcytotoxic concentrations of Hg(II) or Pb(II) salts. Proliferating NRK-52E cells received for 24h culture media containing 20μM $HgCl_2$ or 60μM $PbCl_2$ then the presence and abundance of HSP25, HSP60, HSP70, GRP75, GRP78 were analysed by immunohistochemistry and Western blotting. Concomitantly, in order to check if stress proteins response might be really related to a renal oxidative damage, ROS and glutathione (GSH) levels were measured by flow cytometry and spettrophotometric analysis. Our data proved that in NRK-52E both $HgCl_2$ and $PbCl_2$ treatments enhance the expression of constitutive chaperones (HSP25, GRP75 and GRP78) and the cell GSH content, even if at different grade. Interestingly, only Hg(II) ions stimulate either the ROS formation or the inducible HSP70 protein. These results suggest that, in our *in vitro* system, Hg(II)-induced ROS production and expression of peculiar stress proteins could be a consequence of a different mechanism of action of this metal with respect to Pb(II).

5C_02_P

INFLUENCE OF MERCURY ON RAT RENAL GLUCOCORTICOID RECEPTOR ASSOCIATION WITH HSP90 AND HSP70

Jadranka Dundjerski, Jelena Brkljačić, Tatjana Perišić, and Gordana Matić

Department of Biochemistry, Institute for Biological Research »Siniša Stanković«, Belgrade, Serbia, email: jadund@ibiss.bg.ac.yu

Unliganded glucocorticoid receptor (GR) is located in cytoplasm in form of multiprotein heterocomplex with various proteins including Hsp90 and Hsp70. The structure, composition, assembly and functional significance of

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

this complex depends on cell type, physiological demands and environmental conditions. It is known that toxic effects of mercury could be reflected on structure and function of transcriptional factors such as GR. The influence of mercury on association of rat kidney GR with heat shock proteins Hsp90 and Hsp70 and on cytosolic levels of these Hsps was investigated. The GR heterocomplexes with Hsp90 and Hsp70 were immunopurified from renal cytosol of rats administered with different doses of mercury (1, 2 and 3 mg Hg/kg b.w.). A quantitative immunoblotting procedure was applied to determine the levels of GR, Hsp90 and two Hsp70 isoforms (constitutive Hsp73 and inducible Hsp72) in the renal cytosol, as well as the amounts of these proteins within GR heterocomplexes immunoprecipitated by anti-GR antibody. Mercury was found to stimulate GR association with all examined Hsps. The most prominent effect of the metal was stimulation of Hsp72 interaction with GR. On the other hand, the metal administration led to an increase of Hsp90 level in the cytosol, while the cytosolic levels of Hsp70 isoforms remained unaltered. These findings suggest that association of Hsps, at least Hsp70, with the GR might be ascribed to changes in the affinity of their interaction rather than to changes in Hsps availability in the cytosol. Therefore, GR heterocomplex assembly seems to be a controlled process enabling chaperoning and functioning of the GR in concert with physiological demands.

5C_03_P

BRAIN INJURY AND OXIDATIVE STRESS: THINK ZINC!

Stefano Sensi[1,2]

Depts. of Neurology, and Basic and Applied Medical Sciences, [1]Center of Excellence on Aging / University "G. d'Annunzio", Chieti-Pescara, Italy, [2]University of California-Irivine, Irvine., USA, ssensi@uci.edu

Zn^{2+} is a potent mediator of neuronal injury both "in vitro"and "in vivo", and trans-synaptic movement of the cation
from pre- to post-synaptic neurons has been shown to greatly contribute to a variety of neurological conditions
including cerebral ischemia, brain trauma and epilepsy. Zn^{2+} can enter neurons through glutamate receptor-associated channels [NMDA and AMPA/kainate channels (Ca-A/K channels), voltage-sensitive calcium channels (VSCC), or Zn^{2+}-sensitive membrane transporters (like the Na^{+}/Ca^{2+} and Na^{+}/Zn^{2+} exchangers). Mechanisms by which Zn^{2+} exerts its potent neurotoxic effects include both mitochondrial and extra-mitochondrial pathways. Experiments in cortical neurons have shown that mitochondria play an important role in restoring Zn^{2+i} homeostasis but this

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Zn^{2+} uptake leads also to prolonged mitochondrial depolarization and free radicals generation. In addition to roles in acute injury, Zn^{2+}might play roles in the selective neurodegeneration associated with aging and Alzheimer's disease (AD). Indeed, cumulative effects of repeated Zn^{2+} exposures could contribute to the oxidative damage and mitochondrial dysfunction seen in AD. Interestingly, Zn^{2+} homeostasis is affected by oxidative stress, as reactive oxygen species are potent triggers for injurious cation release from Zn^{2+} binding proteins (metallothioneins). In this talk, we examine how Zn^{2+} dyshomeostasis and oxidative stress might act synergistically to promote degeneration in the context of several neurological conditions.

5C_04_P

OXIDATIVE STRESS PRODUCED BY ENVIRONMENTAL AGENTS ON AMPHIBIAN EMBRYOS

Jorge Herkovits [1], Cristina S Pérez-Coll [1], Jose L D´Eramo [1] Luis A Castañaga [1], Carolina Aronzon[1] , Abelardo A. Sztrum[1], Juan C Stockert [1,2]

Instituto de Ciencias Ambientales y Salud, Fundación PROSAMA, Buenos Aires, Argentina. herkovit@mail.retina.ar
[1]Instituto de Ciencias Ambientales y Salud [2]Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, Madrid, España.

Abstract: The decline of amphibian populations could be related to physico chemical agents producing oxidative stress. Although Reactive Oxygen Species (ROS) participate in metabolic signalling pathways that regulate cell survival, proliferation and apoptosis, an excess of ROS induce oxidative stress and could result in damage and death. In this study conducted with *Chaunus arenarum* embryos we report the effects of different experimental conditions that show the possibility (i) to prevent, or at least, to mitigate the adverse effects of oxidative stress, and (ii) to exacerbate the toxicity by means of the synergic effect of agents inducing oxidative stress. In the first case the lethality induced by ultraviolet B light (UV-B) was prevented with Zn and Se in single or combined pre-treatments. Zn also protected against the lethality produced by Cu, Al, Cd, Ni, and Pb. Synergic effects were obtained by simultaneous treatments of photodynamic toxicity plus Zn, UV-B plus Ni and 2,4-D plus Cu. The results point out the complexity of the environmental scenarios related to oxidative stress effects on living organisms which could be of major relevance in the case of endangered species.

Keywords: Amphibian embryo**,** Oxidative stress**,** Copper, Aluminum, Lead, Nickel, Zinc, Cadmium, 2,4-D, Photodynamic effect, UV-B, Antagonism,

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Synergism

Acknowledgements: This research was financially supported by CONICET (PIP 2316, 2317,) and FONCYT (PICT 03/14375).

5D_01_P

HEAT SHOCK PROTEINS ARE RELEASED BY AN INTESTINAL EPITHELIAL CELL LINE INFECTED WITH ROTAVIRUS

Barreto, A.[1,2], Rodriguez, L.S.[1], Angel, J.[1], Franco, M.[1]

[1]Instituto de Genética Humana, Facultad de Medicina, [2]Departamento de Microbiología, Facultad de Ciencias, Pontificia Universidad Javeriana. Carrera 7 No. 43-82, Bogotá-Colombia. alfonso.barreto@javeriana.edu.co

HSPs modulate the immune response by signaling throughout receptors and transporting antigenic peptides to antigen presenting cells. Here we have studied if an intestinal epithelial cell line infected with rotavirus (RV) release constitutive and inducible heat shock proteins (Hsc70 and Hsp70, respectively). Caco-2 cells (a human colon adenocarcinoma cell line) were grown 14-21 days in a transwell system until they achieved polarization. Cells were considered to be polarized when the transepithelial cell resistance (TER) was above 300 Ohms/cm^2. Caco-2 cells were infected with the RV strain RRV with a multiplicity of infection of 5, and the release of Hsc70 and Hsp70 was evaluated at different time points after infection in both the apical and basal chamber by western blot and ELISA, respectively. 24 h post-infection both HSP were found in the apical chamber of infected, but not in control treated cells. At this time the TER was still above 300 Ohms/cm2, and we detected a higher number of infectious RRV particles in the apical side of the wells than in the basal side. At 48 h, we evidenced a fall of the TER due to monolayer disruption, and we could detect both infectious virus and HSPs in the apical and basal side of the cultures. We also determined that released HSPs are mainly in a soluble form, not precipitable by ultracentrifugation. The simultaneous release of HSPs and RV by infected intestinal epithelial cell lines suggests that HSPs may modulate the induction of the mucosal immune response against this pathogen.

5D_02_P

PERIPHERAL LEUKOCYTE APOPTOSIS IN ACUTE CORONARY SYNDROME

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Boutsikou M[1], Aggeli C[2], Tentolouris C[2], Bistola V[1] , Economou V[1], Doumba P[1], Vavuranakis E[2], Stefanadis C[2], Konstadoulakis MM[1]

[1] Lab of Surgical Research, 1st Dept of Propaedeutic Surgery, Athens University
[2] 1st Dept of Cardiology, Athens University
114 V. Sofias Ave,
115 27 Athens
labsures@med.uoa.gr

Purpose: To evaluate leukocyte apoptosis in patients with acute myocardial infarction (AMI) and with unstable angina pectoris (UAP). Methods: The study comprised of 41 consecutive patients with AMI (mean±SD age:57±11) and 29 with UAP (61±10), all admitted in the Coronary Care Unit (CCU) within 6 hours from the onset of symptoms. Ten patients without acute heart disease hospitalized in the cardiology department, served as Control group (C) (56±10). Blood samples were obtained on admission (0h), 24h, 48h and 72h after enrollment. Apoptosis in peripheral leukocytes was determined once in 19 healthy volunteers (H) (52±9). Leukocyte apoptosis was evaluated by flow cytometry. Early apoptotic intact cells ($FITC^+/PI^-$) were discriminated from late apoptotic cells lacking membrane integrity ($FITC^+/PI^+$) by double staining with Annexin V-FITC and Propidium Iodine (PI). Results: Early apoptosis of lymphocytes was considerably decreased at 0h in AMI when compared to C and H group ([mean % ±SD] 6.3±5.1 vs 11.4±8.3, p=0.048 and 6.4±3.7 vs 10.2±4.8, p=0.042 respectively). Similarly, early apoptosis of monocytes was decreased at 0h in AMI and UAP when compared to H group ([mean % ±SD] 42.3±26.4 vs 63.2±24.5, p=0.016 and 42.9±20.3 vs 63.2±24.5, p=0.033 respectively). In contrast, early apoptotic neutrophils were notably increased at 0h in AMI compared to UAP group (6.2±7.8 vs 3.1±3.1, p=0.042). Leukocyte apoptotic expression patterns were monitored during the first 72 hours after admission. Late apoptotic lymphocytes increased at 72 hours after admission in AMI and UAP group. In contrast, early apoptotic neutrophils were gradually decreased from admission (0h) in AMI by 3.39% (p=0.001) at 24h, 3.38% (p=0.026) at 48h and 4.43% (p<0.001) at 72h. Conclusions: Supportive evidence of prolonged lifespan of peripheral leucocytes was provided by the observed delay of apoptotic manifestations in lymphocytes and monocytes. Increased neutrophil apoptosis after the onset of AMI could compromise a possible clearance process of inflammatory cells.

5D_03_P

MRNA BINDING PROTEINS ASSOCIATED WITH NOREPINEPHRINE AND CAMP-MEDIATED MRNA DECAY

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Melissa Lajevic, Sujatha Kodavayur, Lloyd Graf, Rhonna L. Cohen and Donald A. Chambers

University of Illinois College of Medicine, 1853 W. Polk St., Chicago IL, 60612, USA, rcohen@uic.edu

Psychogenic stress is associated with norepinephrine (NE) release and immune dysfunction. We have shown that NE increases the rate of Thy-1 mRNA decay in S49 T lymphoma cells through a classical β_2AR/AC/cAMP/PKA pathway. The Thy-1 mRNA sequence contains an ARE in its 3'UTR, a sequence commonly associated with message stability. In this study we use a 116 base pair Thy-1 ARE probe to identify by photoaffinity binding or biotinylation mRNA binding proteins (bps), determine if NE alters binding, and investigate associations between PKA substrate phosphorylation and the decay mechanism. RNA-protein binding experiments using Thy-1 ARE and S49 protein extract revealed binding of at least 10 proteins including, AUF1, HuR, TIAR, KSRP, and Hsc70. NE treatment of wt or PKA mutant cells had no effect on the ability of these proteins to bind to the ARE. NE induced phosphorylation of PKA substrates in wt cytoplasmic extract identified by immunoblotting a variety of proteins of molecular weights of 35-10.kD. Thy-1 ARE binding proteins, AUF1, Hsc70, and HuR were detected in phosphoprotein isolated from wt whole cell extract by affinity chromatography and AUF1 may be a PKA substrate. In addition, a pull down assay using a biotinylated ARE rich Thy-1 probe and ^{32}Pi metabolically labeled protein isolated at least five phospho-binding proteins, two of which, 60 kD and 105 kD, increased in phosphate incorporation after NE treatment. These results characterize mRNA bps and target phosphoproteins that may relate to NE-mediated Thy-1 mRNA decay. Supported by NIH

5D_04_P

STUDYING QUINAZOLINONES DERIVATIVES TREATMENTS AS KIDNEY CELLS STRESS IN BALB/C NEW BORN MICE

Samar Etemad and Maryam Shams Lahijani

Department of Biology, Faculty of Science, Shahid Beheshti University, Tehran, Iran

Water insoluble hetrocylcic compounds, such as quinazolinones are reported to having pharmacological effects such as anti-inflammation, sedation, ,anti-depression, anti-bacterial, anti-allergic, anti- blood pressur and...Aspects of their properties on reducing blood fat, inhibiting some proteins and specifially preventing cell movements, are the newest ways

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

of treating cancer and HIV disease.
Previous studies at the Department of Biology, Faculty of Science,University of Shahid Behehsti,showed significant teratogenic effects of two new derivatives of quinazlonines on skeletal and morphological structures(exencephaly, exophthalmia ,microphthalmia, anophthalmia, disturbance in polarity of limbs and….). So,finding any effects at histological level would have been quite interesting results,regarding not observing any obvious disturbences in organs such as kindneys.Pregnant Balb/C mice(n=20) were divided into 3 groups of : control, receiving only distilled water, sham , receiving 0.5%(methylcelloluse 9the solvent) and experimental groups, receiving 100mg/kg/ body weight of 4(3H) quinazolinone-2-propyl-2-phenylethyl) and 4(3H)quinazlonones-2-ethyl-2-phenylethyl , by IP injections,on day 8th of gestation. Kidneys were fixed in formaldehyde after birth and stained with H & E. Confirming the pathological results , one- way ANOVA and Chi-Square tests were applied for quantitative and qualititative data ($P<0.05$). Pathological examinations indicated numerous amacits cells (often single nucleus) and hyperaemia around kidney tubules and in interstitial spaces. Hypercellularity in glumerulus , swellings and diminished lumen diameter ,especially in proximal tubules were also observed.
Statistical analysis showed no significant differences between average size of glumerulus and average size of cells of distal tubules and collecting ducts in control, sham and treated groups, but it was significant in proximal tubules(decreasing). In this case the average Hypercellular glumerulus appeared in two experimental groups.
Regarding inflammation of different parts of kidney and effective roles of toxic components as cellular stress in stimulating immune system ,creating kidney nephritis(interstitial nephritis and glumeronephrits) and necrosis
suggest that these components may have some nephrotoxic effects on kidneys of Balb/C mice and can be harm structure of cells so treating with these components can be considered as one kind of cellular stress which effect specially the tubular system of kidney and using during pregnancy is prohibited.

Key words: Quinzaloinones, pathological effects, Balb/C mice, kidneys cells, cell stress

---

5D_05_P

PESTICIDES INCREASE AMPHIBIAN VULNERABILITY TO DISEASE VIA INDUCTION OF A STRESS RESPONSE AND SUBSEQUENT IMMUNO-SUPPRESSION

Tyrone B. Hayes, Laura Meehan, and Young Kim-Parker

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Department of Integrative Biology, University of California, Berkeley, CA 94720 USA

The popular herbicide, atrazine, is a potent endocrine disruptor that de-masculinizes and feminizes exposed amphibians, by reducing androgens and increasing estrogen production. This effect is not unique to amphibians, but has been demonstrated in fish, reptiles, and mammals (including laboratory rodents and humans cell lines) as well. In addition, several independent studies in amphibians and laboratory rodents have demonstrated now that atrazine also increases stress hormone (glucocorticoid) synthesis and secretion as well. Furthermore, when combined with other commonly applied pesticides, the effects of atrazine are magnified. Exposed larvae suffer from retarded growth and development and immuno-suppression resulting in increased disease contraction and increased mortality. These effects have been demonstrated in both the laboratory and in the field: Tadpoles downstream of agriculture show retarded growth and development and suffer high mortality rates in response to pathogens relative to tadpoles upstream of agriculture. These effects indicate that pesticide contamination may play a critical role in amphibian declines even in localities and incidences where population declines appear to be due to other causes such as disease.

5D_06_P

OPPOSING EFFECTS OF β-ENDORPHIN AND RESTRAINT STRESS ON PAW INFLAMMATION REFLECT CHANGES IN THE FUNCTIONS OF INFLAMMATORY CELLS

Kuštrimović N., Mitić K., Stanojević S. and Dimitrijević M.

Immunology Research Center "Branislav Janković" at Institute of Virology, Vaccines and Sera "Torlak", Belgrade, Serbia

It is well established that stress- induced release of opioid peptides could affect development of inflammation via mechanisms involving changes in the activity of inflammatory cells both locally and systemically. To compare the effects of restraint stress (RS) and local injection of opioid peptide on Concanavalin A-induced paw inflammation, male Dark Agouti (DA) rats were either exposed to RS for 2h or intraplantarly treated with β -endorphin (β -End, 0.01-10 μg). While 0.01 μg of β -End increased paw edema, exposure of rats to 2h of RS significantly diminished it. In addition, peritoneal macrophages from intact and previously stressed rats were tested for adherence and production of reactive oxygen species

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

(ROS) in the presence or absence of 10^{-12}-10^{-8} M of β -End in vitro. Results showed that β -End suppressed macrophages adherence and ROI production, in contrast to RS that increased both macrophage functions. However, previous exposure to RS counteracted suppressive influence of β -End on macrophage adherence but did not change the suppressive effect of β-End on macrophage ROI production. Our results suggest two main conclusions. First, ROI produced by inflammatory cells more likely suppress development of tissue inflammation than promote tissue destruction and swelling. Second, exposure to RS can functionally alter macrophages and make them less sensitive to the in vitro treatment with β -End. (Supported by the Ministry of Science and Environmental Protection, Republic of Serbia, Belgrade, Grant No. 145049).

5D_07_P

LASER-INDUCED AND EXOGENOUSLY INTRODUCED HSP70 SERVE AS THE EFFICIENT ADJUVANTS FOR INFLUENZA VACCINE

Sergey Onikienko[1], Irina Guzhova[2], Boris Margulis[2], Alexander Zemlyanoi[3]

[1] *Military Medical Academy, St Petersburg, Russia, onik@nwgsm.ru*
[2] *Institute of Cytology RAS, St Petersburg, Russia*
[3] *Institute of Military Medicine, St Petersburg, Russia*

Hsp70 chaperone is known to possess high adjuvant activity; for instance Hsp70 of Mycobacterium tuberculosis constitutes the active part of BCG vaccine. In order to assess the adjuvant ability of Hsp70 we used laser irradiation of skin of internal surface of mouse ear followed by the injection of anti-influenza vaccine Vaxigrip into the same site. According to Western blotting data Hsp70 almost completely disappeared from the area of the exposure to laser in comparison with non-treated site; 48 hours later the Hsp70 content returned back to basal value. We suggest that the chaperone can be released from laser-treated cells as shown earlier for other Hsp70-inducing factors. In another experimental setting pure Hsp70/Hsc70 preparation from bovine muscle was injected along with influenza vaccine. In both cases, stimulation of endogenous Hsp70 release or exogenous chaperone delivery was sufficient to induce stable cellular and humoral response to vaccine antigen as proved by *in vitro* and *in vivo* tests. Additionally, bacterial lipopolysaccharide (LPS) from *Serratia marcescens* (prodigiosin) modified by electron beam or laser irradiation was also found to possess adjuvant activity to Vaxigrip vaccine, when employed alone or with the exogenous Hsp70. The adjuvant effect of prodigiosin was due to activation of TLR2/4 and trans-activation of receptors to alpha-fetoprotein, GM-CSF and IL-2. In summary these data

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

prove that the combinatory application of chaperones with modified LPS can be promising in the novel vaccine development.

5D_08_P

THE ROLE OF APOPTOSIS IN IMMUNE SYSTEM RESPONSES TO ACUTE STRESS: IMPACT OF GENDER AND MENSTRUAL CYCLE

Bilge Pehlivanoglu, Sibel Bayrak, Esin Ileri, Dicle Z. Balkanci

Hacettepe University, Faculty of Medicine, Department of Physiology Ankara Turkey pbilge@hacettepe.edu.tr

It is evident that there are differences in immune variables under acute stress between genders and menstrual cycle phases. Marked impact of gender causes women to be more prone to stress-induced diseases. Involvement of the apoptosis in stress response has been studied recently and has a potential to be one of the mechanisms in varying immune response. We aimed to investigate the effect of acute stress on apoptosis of immune cells in men and women and at different phases of the cycle. Healthy men (n=17) and women (n=16) volunteers (age 18-25) were subjected to Stroop color-word interference and cold pressor tests. Women tested both at follicular and luteal phases. Pre and post-test lymphocyte subsets and apoptotic lymphocytes were determined flowcytometrically. Menstrual phase was assured by plasma estrogen and progesterone levels. Stress response was evaluated by blood pressure and heart rate measurements and plasma cortisol levels. Nitric oxide (NO) was measured by chemiluminescence. All the data was evaluated statistically. Acute stress decreased CD4+ cells in all groups, CD4+/CD8+ ratio in men and women at follicular phase. CD19+ cells were reduced in women at follicular phase and increased in men, whereas CD56+ cells were increased in women and decreased in men after the stress. Annexin V+ helper T cell ratio was higher in all groups in post-test samples. Acute stress resulted in increased NO levels in men. Stress-related apoptosis of T cells can explain the depressed cellular immunity, diverse immune responses in men and women at follicular and luteal phases which may be modulated by NO. Further studies can enlighten the diversity of immune responses to stress between men and women via apoptosis.

5D_09_P

GALECTIN-1 REGULATES RADIATION-INDUCED FIBROBLAST DIFFERENTIATION

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Ji-Yeon Ahn, Sarah Park, Youngsoo Han, Ji-Young Shim, Yeon-Sook Yun and Jie-Young Song

Laboratory of Radiation Immunology, Korea Institute of Radiological and Medical Sciences, 215-4 gongneung-dong, nowon-ku, Seoul 139-706, Korea
(Correspondence; Jie-Young Song, immu@kcch.re.kr)

Radiation-induced lung fibrosis is one of severe side effects for radiotherapy in lung or breast cancer patients. We previously reported that Galectin-1 was significantly increased in radiation-induced fibrotic lungs by proteomic analysis *in vivo*. In this study, we show that Galectin-1 mediates radiation-induced differentiation from fibroblast into myofibroblast characterized by accumulation of smooth muscle alpha actin (α-SMA) and collagen type I. Morphological changes of WI38 human lung fibroblast cells were observed from day 2 after 4Gy irradiation and sustained up to 9 days. Galectin-1 expression was up-regulated by radiation, and knockdown of endogenous Galectin-1 by specific short hairpin RNA (shRNA) significantly reduced α-SMA synthesis as well as switched the phenotype of myofibroblast. Exposure to radiation on WI38 cells also activated TGF-β secretion, which is a major profibrotic cytokine and a crucial mediator of fibrosis in many different tissues, and stimulated its downstream signaling molecule Smad-2 and -3 activation. However, knockdown of Galectin-1 did not affect the levels of Smad-2, -3 or inhibitory Smad-7 expressions. These data taken together suggest that galectin-1 could regulate radiation-induced fibroblast differentiation via Smad-independent pathway and may provide an alternative approach to the treatment of lung fibrosis.

5D_10_P

PILOTING ANTIGEN TO CROSS-PRESENTATION PATHWAY BY HEAT SHOCK PROTEIN

Yasuaki Tamura, Goro Kutomi, Kenjirou Kamiguchi, Toshihiko Torigoe, Noriyuki Sato

Department of Pathology, Sapporo Medical University School of Medicine, S-1 W-17 Chuo-ku 060-8556, Japan

Recent evidences have been indicating that Heat shock proteins (HSPs) play an important role as a “danger signal” in the extracellular milieu on behalf of immune surveillance. Above all, Hsp70 and Hsp90 elicit intriguing efficient CTL responses by so called “cross-presentation” with yet entirely unknown mechanism. Here, we discuss that the immunologic

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

roles of HSPs, particularly Hsp90, in the MHC class I-restricted cross-presentation by bone marrow-derived dendritic cells (DCs). We show that Hsp90-peptide complex enter the endocytic pathway via putative Hsp90 receptor and associated peptide might be transferred onto endosomal MHC class I molecules. Moreover, we show that immunization with Hsp90-peptide complex efficiently elicits CTL responses and antitumor effect. Interestingly, this presentation is TAP-independent, but rather follows endocytic pathway. Meanwhile, when Hsp90-whole protein (OVA) antigen complex were pulsed to DCs, this protein antigen could enter at least in part via TAP-dependent pathway to the ER, and finally was presented to MHC class I molecules. However, OVA alone without Hsp90 could not enter into this pathway, but rather into MHC class II pathway.
Here we discuss novel insights into the immunologic role of Hsp90 in cross-presentation of antigens, efficient induction of MHC class I-restricted CTL responses, and application to peptide/protein antigen-based immunotherapy of cancers.

5D_11_P

STUDY ON THE ACTION MECHANISM OF "SHUGAN LIFEI" THERAPY ON EXPERIMENTAL RATS WITH ASTHMA AND UNDER STRESS

Tong Y [1*], Zhang NX [2], Liu Q[1]

1. *School of Chinese Medicine, the University of Hong Kong*
2. *Department of Rehabilitation, Pu Tuo Hospital, Shanghai University of Traditional Chinese Medicine*

Abstract:
Traditional Chinese medicine (TCM) has long realized that an unpleasant environment or mental stimulation is closely related to the occurrence and progression of disease. In TCM, therefore, it is viewed that "Gan" can regulate psychological status. In addition, numerous studies believe that the function of the "Gan" in TCM is closely related to the neuroendocrine-immune network of modern medicine. Therefore, this study discusses the influence of psychological factors on the occurrence of asthma and the action mechanism of "ShuGan" therapy in regulating stress to prevent and treat asthma. This is done through the use of "ShuGan" and "LiFei" (lung-regulating) therapy to treat rats with asthma and under stress.
Objective: To discuss the influence of psychological factors on asthma occurrence and the action mechanism of "ShuGan LiFei" prescription of TCM in regulating stress to prevent and treat asthma.
Results: "ShuGan LiFei" therapy could reduce plasma corticosterone, decrease CRH positive neurons in the paraventricular nucleus of hypothalamus, up-regulate the expression of GRmRNA in hippocampus CA3, and alleviate hippocampal neuronal lesion. Moreover, the therapy

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

could significantly alleviate pulmonary pathological changes in rats with asthma under the condition of restraint stress through increasing CD_4^+, CD_4^+/CD_8^+ in peripheral blood and plasma IFN-γ, and decreasing plasma IL-4.

Conclusions: "ShuGan LiFei" therapy may regulate the hyperfunction of HPA axis and the immune dysfunction of asthma in rats under the condition of restraint stress.

Key words: Restraint stress; Asthma; "ShuGan LiFei" therapy; Traditional Chinese medicine

*E-mail address: tongyao@hku.hk

5D_12_P

HSP70 IS AN ANTIGENIC CHAPERONE SERVING FOR BOTH INTRACELLULAR AND EXTRACELLULAR ANTIGEN PRESENTATION

Toshihiko Torigoe, Yasuaki Tamura, Kenjiro Kamiguchi, Atsushi Sugawara, Shin Ohshima, Takehiro Kurotaki, Noriyuki Sato

Department of Pathology, Sapporo Medical University School of Medicine, S-1 W-17 Chuo-ku 060-8556, Japan, torigoe@sapmed.ac.jp

It is speculated that Hsp70 serves as an antigenic chaperone in the cytosol for MHC class I antigen presentation. However, there have been only a few evidences that support the hypothesis. We have found that HSP70 was associated with TAP in cells. In the presence of ATP, HSP70 was dissociated from TAP, indicating that peptide-binding form of Hsp70 might be preferentially bound to TAP and peptide-free form might be detached from TAP. In addition, we found that Hsp70-binding polyamine compound deoxyspergualine (DSG) could inhibit the association of Hsp70 with TAP. TAP-mediated transfer of cytosolic peptides was inhibited in the presence of DSG and cell surface level of MHC class I molecules was decreased to almost the equal level of those on TAP-deficient cells. By using radio-labeled antigenic peptides, it was shown that cytosolic peptides with high affinity to Hsp70 could be transported into the ER by TAP more efficiently than those with low affinity. Taken together, our data strongly suggest that cytosolic Hsp70 serves as a chaperone for cytosolic peptides and facilitate the antigen presentation of the client peptides. Stimulation of dendritic cells (DC) with Hsp70 induced activation of DC, leading to TNF release. Therefore, extracellular Hsp70 has a cytokine-like function that activates innate immunity. When mice were immunized with Hsp70-binding peptides in combination with Hsp70, antigen-specific cytotoxic T cells (CTL) was efficiently induced. In contrast, immunization with non-binding peptides in combination with Hsp70 failed to elicit the CTL response. These data indicate that extracellular Hsp70 might serve as a chaperone for extracellular antigens.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Hsp70 might facilitate the cross-presentation of client peptides to MHC class I molecules in DC, thus activating adaptive immunity as well as innate immunity. Interestingly, the binding of Hsp70 to the cell surface Hsp-receptors on DC was inhibited in the presence of DSG. It is therefore suggested that immunosuppressive and anti-inflammatory action of DSG may be explained at least in part by the suppression of both intracellular and extracellular antigenic chaperone function of Hsp70. Important roles of Hsp70 as an antigenic chaperone in the immune system and host defense system would be highlighted in this presentation.

5D_13_P

HSF2 AS A REGULATOR OF CYTOKINE EXPRESSION IN SPERMATOGENESIS

Anniina Vihervaara, Malin Åkerfelt, Eva Henriksson and Lea Sistonen

Turku Centre for Biotechnology and Department of Biology, Åbo Akademi University, BioCity, Tykistökatu 6, 20520 Turku, Finland. Email: aviherva@btk.fi

Heat shock factor 2 (HSF2) belongs to the family of heat shock transcription factors (HSFs), the members of which orchestrate cellular stress responses and participate in processes of development and differentiation. While HSF1 is the major executor of the heat shock response, HSF2 is involved in germ cell differentiation and in cortical development. To investigate the targets of HSF2 in mouse spermatogenesis, a ChIP-on-chip screen on whole testis was carried out. Our results reveal HSF2 binding on various cytokine genes, and show the corresponding proteins to be differentially expressed in wt and *Hsf2*$^{-/-}$ mice. Since HSF2 cooperates with and is regulated by HSF1 during heat shock, we investigated the possible influence of HSF1 on the novel HSF2 targets. Whereas certain cytokine genes are occupied by both factors, we, interestingly, also discovered HSF2 targets that are not bound by HSF1. Accordingly, similar protein levels of HSF2-specific target genes were detected in wt and *Hsf1*$^{-/-}$ mice, which indicates gene-specific effects for HSF1 and HSF2. Diverse functions of HSF1 and HSF2 are further supported by their different expression patterns in seminiferous tubules where HSF2 is expressed in a stage-dependent manner and the levels of HSF1 remain relatively constant throughout spermatogenesis. In testis, cytokines are important for germ cell migration and for creating and maintaining the site of immune privilege. Furthermore, the detected cytokine environment in *Hsf2*$^{-/-}$ mice is associated with testicular inflammation and infertility. Thus, HSF2-regulated cytokine expression elucidates novel testicular and immunological functions, emphasizing the

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

multifaceted character of HSF2.

5D_14_P

EUKARYOTICALLY EXPRESSED, ENDOTOXIN-FREE RECOMBINANT HUMAN HSP70 ENHANCES UPTAKE OF ANTIGENIC PEPTIDES IN MACROPHAGES AND PRIMARY HUMAN EPITHELIAL CELLS

Dong Wang#, Ralf Dressel+, Rainer Blasczyk*, Thomas Werfel#, Britta Eiz-Vesper*, Miriam Wittmann#

Department of Dermatology# and Institute for Transfusion Medicine, Hannover Medical School, Germany*
Department of Cellular and Molecular Immunology+, University of Goettingen, Germany

Stress-inducible heat shock protein 70 (HSP70/HSP72) has been shown to enhance or enable induction of antigen-specific T cell responses. Recently, the ability of HSP70/peptide complexes to elicit CTL responses by cross-presentation of exogenous antigen via HLA class I has been coming into focus of immunotherapy. Little is known about differences in the function between pro- and eukaryotically expressed HSP70 used for the in vitro induction of specific CTLs. When prokaryotically expressed HSP70 is used, endotoxin contamination is always a major concern. Therefore, we expressed HSP70 eukaryotically to analyse the role of endotoxin-free HSP70 in the induction of specific CTLs. FITC-labelled HSP70 could be shown to be rapidly taken up by monocytes and macrophages of the peripheral blood as well as by primary human keratinocytes. In our endotoxin-free system uptake of Tamra-labelled nonapeptides of different specificity was significantly enhanced in peripheral blood monocytes as well as in keratinocytes in the presence of rhHSP70. rhHSP70 could be shown to enhance cross-presentation to CD8 T-cells in different experimental approaches. Preliminary results support the predominant role of CD91 as HSP70 receptor. Our results indicate that eukaryotically expressed HSP70/peptide complexes could be a useful tool to generate antigen specific CD8 T cell responses.

5E_01_P

INTERPLAY OF STRESS-RELATED HORMONES IN THE CONTROL OF INSECT FITNESS

N.E. Gruntenko, N.A. Chentsova, N.V. Adonyeva, E.K. Karpova, E.V. Bogomolova and I.Yu. Rauschenbach

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Institute of Cytology and Genetics SD RAS, Novosibirsk 630090, Russia;
nataly@bionet.nsc.ru

In insects, biogenic amines, dopamine (DA) and octopamine (OA), and gonadotropins, juvenile hormone (JH) and 20-hydroxyecdysone (20E), are the main components of neurohormonal stress-reaction. For the progress of oogenesis in *Drosophila* under normal and stress conditions a proper balance between JH and 20E is of a paramount importance: imbalance (a shift of the balance in the direction of JH or 20E) leads to dramatic changes in oogenesis and fecundity (Soller et al., 1999; Gruntenko et al., 2003, 2005; Rauschenbach et al., 2004). Here we demonstrate that (I) *D. virilis* and *D. melanogaster* females possess a mechanism of reciprocal regulation of JH and 20E which is essential to maintain the JH/20E balance: (a) a rise in JH titre leads to a rise in the activity of ecdysone 20-monooxygenase (which converts ecdysone to 20E) and their 20E titre; (b) a rise in 20E titre results in a dose-dependent rise in JH levels; (c) 20E regulates JH indirectly via DA metabolic system - a rise in 20E titre increases DA content in young females and decreases it in mature females, thus leading to a rise in JH levels in both; (d) there is a feedback in the regulation of JH by DA - a rise in JH titre leads to a decrease in DA in young females and its rise in mature females. (II) Under normal conditions, reproduction is regulated by genes that control DA metabolic pathways (indirectly via JH); under unfavorable conditions, reproduction is regulated by genes that control OA metabolism. (III) Individual stress-resistance depends on expression of genes controlling the background level of DA. *The study was supported by RFBR grants ## 07-04-00194, 06-04-48357.*

5E_02_P

FIRING INCREASES IN AUTONOMIC PERIPHERAL NERVES AND C-FOS EXPRESSION IN HYPOTHALAMUS AND MEDULLA DURING SEIZURES IN RATS

Kiyomi, Koizumi, Rena Orman, and Mark Stewart

SUNY Downstate Medical Center, Department of Physiology & Pharmacology, Brooklyn, NY, USA

Autonomic nervous system consequences of seizures range from mild (e.g. sweating, piloerection) to severe (Sudden Unexplained Death in EPilepsy, SUDEP), but the severity of autonomic disturbances are poorly correlated with seizure severity. We used urethane anesthetized rats to study the spread of kainic acid-induced limbic seizure activity into hypothalamic, medullary, and peripheral autonomic relays. Peripheral

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

nerve activity was recorded with a linear array electrode. ECG and blood pressure were continuously monitored.
Peripheral multi- and single-unit activity, recorded from right or left vagus nerve, cervical sympathetic ganglion, and renal sympathetic nerve was elevated during each recurrent seizure. Activity recorded from the splanchnic nerve was not changed substantially. Increases in firing were sustained during seizures for vagus nerve in particular, whereas short episodes of increased activity were most frequently observed in renal sympathetic nerve recordings. Baroreceptor reflex responses to phenylephrine or nitroprusside were in the right direction, but prolonged.
Increased expression of c-fos was detected in autonomic and neuroendocrine parts of paraventricular nucleus and in the supraoptic nucleus. Increased expression of c-fos was also detected in the rostral ventrolateral medulla and dorsal motor nucleus of the vagus. Increases in expression were comparable in the right and left hemispheres.
We conclude that autonomic disturbances that occur during seizures will be "balanced" if seizure activity is bilateral. We suggest that the most sever disruptions will occur when the seizure activity, or its pattern of spread, results in a transient unilateral over-activation of hypothalamic or medullary brain regions. On the other hand, we suggest that the neuroendocrine consequences of seizures will be correlated with seizure severity, and will likely result in significant gender differences in seizure patients.

5E_03_P

Endocrine stress response induced by hypobaric-hypoxia

S. Simeoni, P. Monnazzi, R. Biselli* and F.R. Patacchioli

*Department of Human Physiology and Pharmacology "V. Erspamer", University "La Sapienza" and * Experimental Flight Center, Dpt of Aviation and Space Medicine, Pratica di Mare, Rome, Italy.*
francesca.patacchioli@uniroma1.it

The measurement of hormones in saliva provides an interesting and reliable tool to assess the response of the neuroendocrine system to different stressors.
Hypoxia familiarization is an integral part of the aviators' education. For many aviators, hypoxia prevention begins in the hypobaric (altitude) chamber. In order to reach the experience of typical signs and symptoms of hypoxic hypoxia, twelve proficient paratroops were exposed to high altitude in a hypobaric chamber.
We monitored the hypothalamic-pituitary-adrenal axis response to hypobaric hypoxia collecting salivary samples each two hours in the day of the simulation in the hypobaric chamber, and also the day before and the

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

day after.
Altitude-induced hypoxia represents a strong environmental stressor as shown by the significant increase of cortisol released during the "hypobaric chamber day". Moreover, dhea-s levels were significantly increased in the day of the strenuous hypobaric chamber training: this result is consistent with the more recent literature suggesting a stress-buffering role of dhea-s. A comparison most of interest was that contrasting the cortisol to dhea-s ratio measured in the day of stress exposure with that one measured the day before: no difference was found between the two measures. What can be drawn from this result, together with the observation that this very stressful experience appears not to be associated with an impairment of cortisol and dheas circadian fluctuation, is that the group of paratroops we have studied presented a good level of stress resilience.

5F_01_P

EXERCISE TRAINING MODIFIES INTESTINAL CELLULAR STRESS RESPONSE IN YOUNG MICE.

I. Brenner and K. Breton-Honeyman

Dept. of Biology, Trent University, 1600 West Bank Dr., Peterborough, ON., Canada. K9J 7B8. ibrenner @trentu.ca

Regular exercise reduces the incidence of colon cancer and possibly inflammatory disorders of the intestine, however, the mechanisms underlying these exercise-induced adaptations are not well understood. This study examined the effects of an exhaustive bout of exercise on cellular stress proteins. Fifteen young (4 weeks) C57BL/6 mice were randomly assigned to one of three experimental groups: (1) sedentary (S), (2) treadmill training (TT) and (3) wheel running (WR). Sedentary mice maintained normal cage activity, TT mice ran 5 days per week, at approximately 75% $\dot{V}O_2$ max, on a motorized treadmill and WR mice had free access to running wheels. Following 12-14 weeks of training, all mice underwent an exhaustive bout of exercise on the treadmill and were euthanized 2 hours afterwards by cervical dislocation. Blood samples were obtained by cardiac puncture and the intestines were removed for processing. Total HSP 70 expression, in segments of the small and large intestine, and plasma IL-6 concentration were quantified by ELISAs. Levels of JNK and ERK½ in the small and large intestine were measured by Western Blot techniques. Both modes of exercise training (TT and WR) significantly increased HSP 70 expression to exhaustive exercise in the large intestine, whereas in the small intestine, HSP70 expression was significantly increased by exhaustive exercise in the TT mice only.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Preliminary results indicate that JNK and ERK1/2 expression are down-regulated in the TT mice. There were no significant differences in plasma IL-6 levels between the groups. The results of this study are useful for identifying the intestinal molecular pathways that may be modified by different forms of exercise training and by the stress of exhaustive exercise.

5F_02_P

THE RESPONSE OF INTERLEUKIN-6 AND ITS SOLUBLE RECEPTOR TO REPEATED EXERCISE AND HEAT STRESS.

Stuart R Gray, Helen C Marshall and Myra A Nimmo

SIPBS, University of Strathclyde, Glasgow, UK
Corresponding author: m.a.nimmo@strath.ac.uk

Interleukin-6 (IL-6) is a pleiotropic cytokine that increases up to 100 fold with exercise (Ostrowski *et al*, 1998). It has also been linked to immunosupression (Smith, 2003) and underperformance syndrome (UPS) (Robson, 2003). The aim of the present study was to investigate the IL-6 response when subjects are under considerable stress from the combination of exercise and heat. This exposure regimen is similar to that of athletes acclimating to the heat.

Seven male subjects (age 30 ± 4 yr, height 1.80 ± 0.08 m, body mass 73.8 ± 8.5 kg; means ± SD) completed 2 hr cycle exercise (44% $\dot{V}O_{2peak}$) in a hot, humid environment (38 ºC, 60% relative humidity) on 3 consecutive days. Blood samples, obtained at rest and immediately post exercise on days 1 and 3, were analysed for plasma IL-6 and soluble IL-6 receptor (sIL-6R) via ELISA. Statistical analyses were performed using two-way repeated measures ANOVA and post-hoc Bonferroni corrected paired t-tests where appropriate.

There were no differences in resting IL-6 or sIL-6R concentration between days. Exercise heat stress resulted in an increase ($P<0.05$) in IL-6 on days 1 and 3, with the increase being greater ($P<0.05$) on the first day (ΔIL-6, day 1: 4.4 ± 1.7 $pg.ml^{-1}$ versus day 3: 2.5 ± 1.6 $pg.ml^{-1}$). sIL-6R concentration was increased ($P<0.05$) with exercise from 37.8 ± 10.3 to 41.6 ± 11.7 $pg.ml^{-1}$ on day one but the increase was not significant on day three. There were no differences between days.

The present study has demonstrated that within 3 days subjects adapt to exercise and heat stress by attenuating the rise in IL-6 during exercise.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Reference
Ostrowski *et al* (1998). J Physiol; 513(3):889-894
Robson (2003). Sports Med; 33(10):771-81
Smith (2003) Sports Med 33(5):347-364

5F_03_P

RESPONSE OF OXIDATIVE STRESS MARKERS AND ANTIOXIDANT PARAMETERS TO A 8-WEEK AEROBIC PHYSICAL ACTIVITY PROGRAM IN HEALTHY ELDERLY WOMEN

Karolkiewicz J., Pilaczyńska-Szcześniak Ł., Nowak A., Michalak E., Pospieszna B.

Chair of Physiology, Biochemistry and Hygiene, University School of Physical Education in Poznań, Poland, ul. Królowej Jadwigi 27/39. E-mail: karolkiewicz@awf.poznan.pl

Aerobic physical activity is associated with a reduced risk of coronary heart disease, and may favorably modify the prooxidant – antioxidant balance. The aim of study was to assess the influence of a 8-week aerobic physical activity program on oxidative stress markers and antioxidant parameters in healthy, elderly women.
The study was performed on 41 female subjects – members of the University of Third Age. Participants had an average age of 65, declared good health condition. Subjects performed 8 weeks of the cycle ergometer physical workout. The training consisted of 40 minutes sessions of physical exercise (30 minutes with workload at the level of 70-80% of ventilatory threshold intensity) repeated three times per week. Before and after 4 weeks of the training program subjects underwent a ventilatory threshold estimation during physical test with increased intensity. Before and after 8-weeks of the training, in a fasting state (between 8 and 9 a.m.) the blood was taken from the ulnar vein for biochemical analysis. Total antioxidative status (TAS) and concentrations of thiobarbituric acid reactive substances (TBARS) were measured in plasma. In the serum samples, levels of antibodies against oxidative modification of LDL (oLAB), glucose, HDL-cholesterol, triglycerides and insulin were assessed. Atherogenic index of plasma (AIP), insulin resistance index ($HOMA_{IR}$) were calculated. Reduced glutathione (GSH) concentration and glutathione peroxidase activity (GPx) were determined in the red blood cells hemolysate. The 8-week aerobic physical activity program resulted in a significant decrease ($P < 0.01$) in serum concentrations of glucose, LDL-cholesterol, TBARS, as well as a significant decrease glutathione peroxidase activity in red cells ($P < 0.05$) and index $HOMA_{IR}$ ($P < 0.01$). TAS and GSH concentrations increased significantly in the subjects ($P < 0.01$). The obtained results show that 8-week aerobic training enhanced

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

insulin sensitivity, and the balance between oxidants and antioxidants in healthy, elderly women. These favorable changes may decrease the risk of atherosclerosis in physically active elderly subjects.

5F_04_P

HORMONAL STRESS RESPONSES TO AN IMMERSION IN A SIPHON PLACED ON THE BOTTOM OF AN ALPINE CAVE OF 700M DEPTH

Stenner E°, Bussani A^, Biasioli B*, Micheli W*, Delbello G°, Gianoli E*, Piccinini C

°School of Sports Medicine, Faculty of Medicine and Surgery, University of Trieste, Italy
^Department of Biological Oceanography, National Institute of Oceanography and Applied Geophisics
**Department of Laboratory Medicine, A.O.U, Ospedali Riuniti di Trieste, Italy*
elisabetta.stenner@libero.it

We studied the hypothalamus pituitary adrenocortical (cortisol) and the hypothalamus pituitary (growth hormone GH) system responses to an immersion done in an unexplored siphon placed in a cave, 700m under the surface. The combination of heavy exercise in a demanding environment before diving, the absolute darkness, the confinement, the cold temperatures and the emotion for the exploration as well as the awareness that if an accident happened the situation became drastically very critical represent a unique multiple stress model that may be helpful in understanding the endocrine expression of exercise and psychological stress. Owing to the high skill of the performance only one cave-diver was tested; however this subject repeated the same immersion twice. Two blood drawings were performed: (1) at the bottom of the cave, pre siphon, (2) at the end of the exploration of the siphon lasted about 45 minutes. Two blood drawings, as controls, were performed on the same potholers, at the same resting time envisaged for the day of the experiment to minimize the specimen processing time influences and any circadian fluctuation. Serum GH and cortisol were measured with a chemiluminescence assay (DXI 800 Beckman Coulter, Fullerton, California, 2004) within 24 hours. The marked rise of GH values during diving underlines the great intensity of cave diving effort probably due to the difficult route that the cave diver covered and the bulky technical equipment dressed while the rise of cortisol is likely due to the combination of exercise, emotional stress, cold temperature and darkness.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

5F_05_P

WOULD LITERATURE ON TAI CHI FOR STRESS BE SUFFICIENT FOR A META-ANALYSIS?

Chenchen Wang, Timothy McAlindon

Tufts-New England Medical Center, Boston, MA, USA

Purpose: To systematically review world literature for the effects of Tai Chi (TC) exercise on psychological stress and determine the sufficiency of data for a meta-analysis of their outcomes.

Methods: We searched Medline, PsycINFO, Sportdiscus, Social Sciences, Health Star, EMBASE, China Hospital Knowledge, China National Knowledge Infrastructure, Traditional Chinese Medicine Databases and article references until February 2007. Inclusion criteria were all types of human clinical studies that reported original data written in English or Chinese and which included at least one clinical outcome relevant to psychological health. We extracted data on the population characteristics, study setting, type and duration of TC, study design, psychological outcome, and primary results.

Results: We screened 1172 abstracts and identified 23 studies (10 randomized controlled trials, 9 nonrandomized controlled trials, 4 observational studies). These eligible studies were conducted in 4 countries (USA, Canada, China, Australia) involving a total of 2335 participants (age range is from 10 to 92). Of the 23 studies, 19 clinical trials with a total of 1153 subjects consistently reported that 4 weeks to 46 months of TC training resulted in higher levels of several indices of psychological well-being, including reduction of stress, anxiety and depression, and improved life satisfaction compared with the controls. Four observational studies further demonstrated that 6 months to over 20 years TC practice improved mood, and reduced stress and depression.

Conclusions: The heterogeneity of the study designs, settings, methods of comparisons, exercise background, TC style, and duration, precluded a formal meta-analysis. However, TC appears to be associated with improvements in psychological stress and well-being in both eastern and western populations.

5G_01_P

QUERCETIN, A DIETARY-DERIVED FLAVONOID, ACTS AS A FREE RADICAL SCAVENGER IN SWINE GRANULOSA CELL

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Giuseppina Basini, Sujen Eleonora Santini, Francesca Grasselli

Dipartimento di Produzioni Animali, Biotecnologie Veterinarie, Qualità e Sicurezza degli Alimenti, Sezione di Fisiologia Veterinaria, Università di Parma, 43100 Parma, Italy. E-mail: basini@unipr.it

Quercetin (3,3',4',5,7-penthydroxy flavone) is an important constituent of the flavonoid family present in many fruits and vegetables. Various pharmacological activities of quercetin have been demonstrated including suppression of tumor growth by inhibition of neovascularization. However, its mechanism of action has not been clarified yet. Ovarian follicle is an excellent model to study angiogenesis since it is an unique exception to the general quiescence of the adult vasculature. In previous studies, we demonstrated an involvement of reactive oxygen species (ROS) in angiogenesis signalling. Therefore, the aim of this research was to evaluate if the treatment with 5 or 50 µg/ml of quercetin modulates superoxide anion (O_2^-) generation and superoxide dismutase (SOD) activity in granulosa cells collected from swine follicles. Our results evidenced that the highest concentration af quercetin was able to significantly ($p<0.01$) inhibit O_2^- output, while the lowest dosage was ineffective. On the contrary, SOD activity was unmodified by both concentrations tested. Since we previously demonstrated that an increase in O_2^- generation is a critical event in promoting angiogenetic process, we may argue that the antiangiogenetic effect of quercetin could be mediated, at least in part, by the inhibitory effect on O_2^- levels; in addition, since SOD activity is not augmented, we conclude that, as evidenced in other systems, quercetin could act as a free radical scavenger.

This work was supported by FIL and MIUR-COFIN grants

5G_02_P

STRESS AND NIGHT EATING SYNDROME AMONG UNDERGRADUATE STUDENTS

Stephanie A. Bughi, Jatturong Wichianson, Selena Nguyen-Michel & Donna Spruijt-Metz.

University of Southern California, Institute for Health Promotion & Disease Prevention Research, Los Angeles, CA, USA

OBJECTIVE: Increased stress is a disturbing trend in college student health nationwide, especially since students often engage in unhealthy behaviors such as ATOD use and overeating to deal with stress. This study investigates the relationship between academic stress, and night eating

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

syndrome (NES) in university undergraduates. **METHODS:** Participants were students from a private university in Southern California. Stress was measured by using the General Well-Being Scale, with a score ranging from 0 to 110 (stress <72 and distress <60). The Night Eating Questionnaire was used to assess the students' eating patterns after dinner. **RESULTS:** Participants were 93 undergraduate students (63 females, 30 males, mean age 20.4+/-1.76 years). From this sample, 53.8% of students reported stress, and half of them reported distress. The stress score (SS) was not different between female and male students. However, females (12.6+/-5.3) experienced more anxiety than males (15.2+/-4.6; t =2.38, p=0.021). In contrast to juniors (73.4+/-17), senior students (61.79+/- 14.9) experienced the highest stress level (F=2.43, p=0.04). NES was present in 7.7 % of students (4 males, 3 females), while evening hyperphagia was present in 72.8% students (20 males, 47 females). Evening hyperphagia correlated with anxiety (r= -0.24, p=0.020). **CONCLUSIONS**: 1) Senior college students experienced significantly more stress than juniors. 2) Anxiety was more prevalent in female students. 3) Evening hyperphagia was common and was associated with anxiety. Stress in college students is prevalent and may lead to abnormal eating patterns, which may partially explain the contribution of stress to development of obesity.

5G_03_P

AMBULATORY ACTIVITY AND THE ACCUMBENS C-FOS EXPRESSION WERE DECREASED BY REPEATED ORAL CAPSAICIN IN RATS.

Xue Feng Gu[1], Jong-Ho Lee[1], Vitaly Ryu[1], Sang Bae Yoo[1], Young Wha Moon[2], Jeong Won Jahng[1]

1. Department of Oral and Maxillofacial Surgery, Dental research institute, Seoul National University School of Dentistry, Seoul, 110-744, Korea; 2. Department of Biology, The Catholic University of Korea College of Medicine, Seoul, 137-701, Korea

Capsaicin (8-methyl-N-vanillyl-6-nonenamide), the active component of chili peppers, is widely believed to stimulate appetite. The "capsaicin high" is a euphoric sensation caused by the consumption of large quantities of capsaicin from capsaicin-laden foods. We have previously found that gene expression of a sweet taste receptor T1R3 as well as capsaicin receptor VR1 was decreased in the circumvallate papilla of rats that had repeated exposure to oral capsaicin, and they showed preferences on sweet solutions, revealing a molecular basis underlying the hedonic effect of oral capsaicin. In this study, we examined ambulatory activity and c-Fos expression in the nucleus accumbens (Accb) in rats with repeated oral

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

capsaicin. Male SD rats received daily 1 ml of 0.02 % capsaicin or distilled water in their oral cavity with *ad libitum* access to rodent chow and water. On days 1, 5 and 10, rats were placed in the activity chamber 30 min after capsaicin exposure, and then the ambulatory activity was recorded for 30 min. Two days after the behavioral session without capsaicin, rats were transcardially perfused with 4 % PFA and the brain tissues were processed for c-Fos immunohistochemistry. Ambulatory counts decreased significantly in the capsaicin group compared with the control group on each test day. The ambulatory counts of capsaicin rats, but not of control rats, further decreased on the second test day compared to the first test day. The number of c-Fos-ir nuclei significantly decreased in the Accb and the cingulate cortex of capsaicin rats compared to the control group. Together with our previous finding, this result suggests that hedonic properties of oral capsaicin may be mediated by decreased c-Fos expression in the brain reward pathway. Supported by KMOST (JWJ).

Key Words: Behavior, Immunohistochemistry, Reward pathway, Sucrose intake, Taste receptor

---

5G_04_P

OXIDATIVE STRESS IN HUMAN BODY AND PROBIOTIC APPLICATION

Tiiu Kullisaar, Tiiu Vihalemm, Marika Mikelsaar and Mihkel Zilmer

Department of Biochemistry University of Tartu, Tartu, Estonia
Department of Microbiology University of Tartu, Tartu, Estonia

Human beings living in oxygen environment and using oxygen for their aerobic metabolisms have to deal with free radicals, which are normally, at very low levels, produced by several metabolic processes. When antioxidants/oxidants ratio is balanced, nothing will happen, but when this balance is disturbed the oxidative stress (OxS) will appear. High-grade OxS has an impact on development of inflammatory diseases and any kinds of approach, which have beneficial effects, are welcome.
Lactic acid bacteria (LAB) of our intestinal microflora play a significant role in the gut ecosystem having beneficial cross- talk with human organism. LAB of human origin helps to restore normal microbial function, stimulating immune system, and some of them carry remarkable antioxidative, anti-atherogenic and anti-cancerogenic properties. Viable LAB considered as good tools for designing health supporting functional food.
Our previous studies have shown that antioxidative probiotic *L. fermentum* ME-3 possesses substantial antioxidative and antimicrobial activity and has beneficial effects on human well being. Consumption of

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

probiotic ME-3 correct OxS status in clinically non-problematic subjects, suppresses high-grade OxS in diseased persons in the association with clinical outcome, which points to the possibility to gain the better quality of life as adjunct therapy.
Regular intake of dietary products, enriched with special probiotics, without necessity to change typical eating habits of person (patient) might be used easily accessible low cost large scale tool for population.

5G_05_P

SAGE DRINKING IMPROVES PLASMA LIPID PROFILE, ERYTHROCYTE ANTIOXIDANT DEFENCES AND INCREASES LYMPHOCYTE HSP70

Piairo PC, Sa CM, Abreu-Ramos A, Lima CF*, Azevedo MF, Almeida MJ, Coutinho OP, Pereira-Wilson C

Centre/Department of Biology, School of Sciences, University of Minho, Braga, Portugal;
** lima@bio.uminho.pt*

Salvia officinalis (common sage) is a medicinal plant to which antioxidant, anti-inflammatory and antimutagenic properties have been attributed. Recent results from our laboratory showed cellular and in vivo antioxidant effects of sage as well as metformin-like effects at the rat liver level, suggesting an antidiabetic potential for sage. In order to test these effects in humans, we performed a pilot trial with six healthy female volunteers. The trial was carried out in three phases, which includes two weeks of baseline, four weeks of sage treatment (drinking of a sage infusion twice a day) and two weeks of wash-out. Sage treatment positively affected the erythrocyte antioxidant status as shown by increased SOD and CAT activities. Cholesterol and LDL levels significantly decreased and HDL levels significantly increased after treatment, indicating benefits also in lipid metabolism. However, no changes in glucose clearance were observed in the oral glucose tolerance tests at the end of treatment period. In addition, a reduction of in vitro lymphocyte DNA damage induced by H_2O_2 was observed during the treatment period, which was maintained through the wash-out period. During the *S. officinalis* drinking period, lymphocyte Hsp70 protein expression was significantly increased (about 2.25 times) and decreased to baseline following the wash-out period. Overall these results confirm the health improving potential of sage infusion drinking.
This work was supported by FCT research grant POCI/AGR/62040/2004. CFL and MFA were supported by FCT grants SFRH/BPD/26316/2006 and SFR/BD/12527/2003, respectively. We also acknowledge Rui Silva for assisting with blood samples collection.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

5G_06_P

BIOACTIVE PEPTIDES FROM BUFFALO MOZZARELLA-CHEESE WASTE WHEY: ANTIOXIDANT AND DIFFERENTIATION EFFECTS ON COLON ADENOCARCINOMA CACO-2 CELLS.

P. Ferranti, D. Cassese, G. Picariello, A. Dicitore, C. De Simone, G. Giuberti, A. Abbruzzese and P. Stiuso.

Corresponding author:
Dott. Paola Stiuso, Department of Biochemistry and Biophysics, Seconda Università degli Studi of Naples, Italy.e-mail:paola.stiuso@unina2.it

The gastrointestinal mucosa is constantly exposed to luminal oxidants from ingested foods as reactive oxygen species (ROS) that elicit membrane destabilization, alterations in DNA, inactivation of proteolytic enzymes . The ingestion and/or occurrence of these reactive species, particularly in the long term, are responsible for injury to the intestinal mucosa in several disease states, as well as inflammatory bowel disease and intestinal cancer. Bovine milk proteins are a source of biologically active peptides that can be released during gastrointestinal digestion or food processing. They may act as regulatory compounds which exhibit a wide range of biological functions such as antimicrobial, antihypertensive, antioxidant and opioid activities. However no study have been carried out on peptides in buffalo milk and/or on peptides released in whey during technological transformation of buffalo milk. Our study focuses on characterizing peptide fraction of buffalo mozzarella-cheese waste whey by complementary mass spectrometric techniques. Moreover we have investigated a cytomodulatory and antioxidant effect of peptide fractions from buffalo mozzarella-cheese whey on hydrogen peroxide-induced oxidative damage in CaCo-2 cell lines. We have demonstrated that a specific peptide fraction (F3) after 12h of treatment, decreased ROS production (-42%O_2^-) and enhanced cell differentiation (27%Alkaline Phosphatase activity/min). Our results showed that peptide fraction (F3) derived from buffalo mozzarella-cheese waste whey is potential health enhancing nutriaceutical for food and pharmaceutical applications.

5G_07_P

EFFECT OF IRRIGATION INTERVALS AND POTASSIUM APPLICATION ON SOME BIOCHEMICAL INDICES OF PISTACHIO

A.Tajabadi Pour

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Department of Soil Science, College of Agriculture, Rafsanjan University, Rafsanjan, Iran. ahtajabadi@yahoo.com.au

Water deficit of soils is the principle factor limiting crop yield in large areas of world. Plants undergo significant morphological and metabolic changes in response to drought. Many of these changes are believed to adaptive responses by which plants cope with water stress. It is generally accepted that the first result of stress is an alteration in the structure and function of cell membranes. There are more than 420000 ha of nonbearing and bearing pistachio trees in Iran. Despite the economic importance of this crop, very little information is available on its moisture needs and nutritional requirements. Therefore, the effects of three irrigation frequencies (1, 3, and 7 days) and five potassium (K) levels (0, 75, 150, 225, and 300 mg K kg^{-1} soil as K_2SO_4) on the proline and reducing sugars content and activity of catalase and peroxidase of pistachio were studied in a glasshouse experiment. Water frequency decreased all of growth parameters (shoot and root dry weight, stem length and leaf area) but potassium application had no significant effects on the growth parameters. As the irrigation intervals and K application increased, the proline content significantly increased. In contrast to proline, with increasing irrigation frequencies and K application the reducing sugars concentration significantly decreased. A significant decrease in peroxidaes and catalase activity was observed with increased K levels. However, with increasing irrigation intervals, activity of these enzymes increased. In conclusion, the results of this study clearly demonstrate that although K application had no significant effects on some growth parameters, it significantly modified the biochemical indices and somewhat overcame the depressing effects of water stress.

5H_01_P

CASPASE-3-MEDIATED PROAPOPTOTIC SIGNALING IN INTERSEXUAL GONAD OF ZEBRAFISH

Nanami Mizusawa and Michiaki Yamashita

National Research Institute of Fisheries Science, Stress-induced apoptosis in gonadal masculinization, nkawa@fra.affrc.go.jp

Phenotypic sex in fish is influenced by environmental conditions in addition to genotype. Therefore, various stressors, such as temperature, hypoxia and pollution at sublethal levels, are assumed to influence the sex ratio. In order to evaluate the hypothesis, we designed an experiment to assess the effect of heat shock on sex ratio using zebrafish as the model. As the time of heat stress during development is critical, temperature sensitive

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

stage was selected for the study. The phenotypic sex ratio was examined until 40 days, using histological observation. The results were compared with a control without heat shock. Results indicated that in control a 50/50 male to female ratio was maintained. However, a significantly higher increase in male phenotype was observed. Our study also indicates that 14 to 16 days post fertilization is critical for sex differentiation of primitive gonad. TUNEL staining and immunohistochemical observation of the gonads showed the induction of caspase-3 mediated proapoptotic signaling in the gonadal cells after the heat shock. Therefore, proapoptotic signaling mechanism may regulate the sex differentiation in the gonads at this stage and may be markedly induced by stress conditions. We report a model of the evaluation effects on sex differentiation using zebrafish. We anticipate that this model will facilitate further study of the exposure to stressors by monitoring their sex ratio and gene expression patterns in the gonad.

5H_02_P

VALIDATION OF A FECAL GLUCOCORTICOID ASSAY FOR THE SYRIAN HAMSTER (*MESOCRICETUS AURATUS*)

Marie-Odile M. CHELINI[1], Priscila F. VIAU[2], Cláudio A. de OLIVEIRA[2], Emma OTTA[1]

[1]Institute of Psychology, Department of Experimental Psychology, University of São Paulo, São Paulo, Brazil; [2]Hormone Quantification Laboratory, Department of Animal Reproduction, Faculty of Veterinary Medicine, University of SãoPaulo, São Paulo, Brazil.

The measurement of fecal steroid metabolites has proved to be a reliable technique for monitoring endocrine status in a variety of mammalian species. We verified the biological relevance of quantifying the concentration of cortisol fecal metabolites to assess the physiologic stress response of the Syrian hamster. Two experiments were performed using a total of 10 adult female hamsters. To suppress adrenocortical activity, five females were injected with 200 µg/100 g body weight of dexamethasone, dissolved in 1 ml of sterile isotonic saline solution. Dexamethasone is an artificial steroid that mimics endogenous glucocorticoids and reduces circulating corticosteroid levels via the negative-feedback mechanism of the HPA axis. To investigate the effects of the injection procedure itself on the pattern of excreted fecal steroid metabolites, five females received an injection of 1 ml of sterile isotonic saline solution. All fecal samples voided by each group were collected and homogenized on a daily basis for 5 days before (to assess basal concentration) and thereafter for 4 days after the injections. Cortisol fecal metabolites were extracted with ethanol and quantified by radioimmunoassay. The basal concentration of cortisol fecal

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

metabolites was 3.95 ± 1.25 □g/g of feces in the control group and 5.78 ± 0.62 □g/g of feces in the group injected with dexamethasone. On the first day after injection, whereas a slight increase in cortisol concentration in the control group (4.81 □g/g of feces) (**p<0.05??? – very important!!!)** was observed, it decreased abruptly to 1.60 □g/g of feces (28% in relation to basal value) **p<0.05???** in the dexamethasone group. These data strongly suggest that the changes in fecal concentrations of cortisol metabolites reflect the variations in blood values. Therefore the quantification of cortisol metabolites in feces enables non-invasive monitoring of adrenocortical activity in the Syrian hamster.

Noninvasive techniques to monitor reproductive or stress hormones are now widely used in captive and free-ranging wildlife. These methods offer great advantages and deserve to be used also in laboratory rodents. However, we remain naive about factors that may influence the accuracy of these techniques. The aim of this study was to evaluate the adequacy of were ovariectomized, and. We determined per-gram fecal cortisol metabolite concentrations, total 24-h fecal output and total 24-h fecal cortisol metabolite production. Surgery considerably affected fecal output, and using per-gram versus total cortisol metabolites led to different conclusions: whereas concentrations increased significantly just after ovariectomy and decreased on subsequent days, the total excreted cortisol metabolites varied in a symmetrical pattern. Therefore, the relative per-gram measure of hormones may not reflect the total amount of circulating hormones, because these measures are comparable only if the volume of the material in which the hormone is contained is the same in the 2 groups.

5H_03_P

EFFECT OF PROLONGED HEAT STRESS ON BLOOD METABOLITES AND RECTAL TEMPERATURE IN CATTLE

JB Gaughan

The University of Queensland, Gatton, Queensland, Australia 4343; j.gaughan@uq.edu.au

Climate change predictions suggest that for some regions heat waves will become more severe and be of longer duration. The effects of prolonged heat stress both at the whole organism and at the cellular level needs to be investigated. Angus steers (n=24) were housed in controlled climate rooms and exposed to 4 d of thermoneutral conditions (TN) followed by 9 d of heat stress (HS). Feed and water was available at all times. Rectal temperature (RT) was recorded every 30 min. Blood was collected from

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

each animal 4 times a day and analysed for creatine kinase (CK), aspartate aminotransferase (ASK), lactate dehydrogenase (LDH) and glucose. Ambient temperature (TA) and relative humidity (RH) were recorded every 5 min. Climatic conditions (mean±s.e.) during TN were 21.6°C±1.9 and 62.3%±8.8 respectively for TA and RH. During HS the conditions were 32.7°C±2.4 and 41.4%±12.1. During HS feed intake was 38% lower and individual water intake increased from 20L/d to 41 L/d. Serum CK increased ($P<0.05$) from 1.66±0.13 U/L during TN to 2.03±0.13 U/L on day 9 of HS. For the duration of the study CK, LDH and AST remained within the normal range. Mean glucose fell ($P<0.05$) from 4.86 mmol under TN to 4.41 mmol on day 8 of HS. During TN RT_{MEAN} was 38.7 °C, and ranged between 38.6 and 38.9 °C. RT_{MAX} occurred close to 1300 h each day of TN. RT_{MIN} occurred at around 0600 h. The diurnal pattern was consistent during TN. During HS, RT varied ± 1.0°C each day. RT_{MEAN} was 39.8°C on day 1 and 40.1°C for days 2 – 4. The maximum RT_{MEAN} occurred on day 6 (40.4°C) and remained close to this level for the next 3 d. RT_{MAX} occurred between 2100 and 2200 on days 1 – 4 of HS. From day 5 – 9 the diurnal pattern was characterized by multiple peaks and troughs with no consistency. During this period RT_{MAX} occurred between 1500 and 1800.

5H_04_P

EFFECTS OF SEX AND GENETICS ON BEHAVIOR AND STRESS RESPONSE OF TURKEYS

G. R. Huff, W. E. Huff, N. C. Rath, A. M. Donoghue, N. B. Anthony, and K. E. Nestor

USDA, Agricultural Research Service, Fayetteville, AR, USA 72701.
grhuff@uark.edu

Stress can lead to changes in the immune response of turkeys resulting in decreased resistance to opportunistic bacterial pathogens. Three lines of turkeys were tested for response in T-maze and open field tests during the first 8 days after hatch and behavior was observed after catching, moving, and transport. They were also compared for corticosterone (Cort) levels and heterophil/lymphocyte ratios (H/L) at 15 wk of age, in response to an *Escherichia coli* challenge followed by transport stress. Large commercial line birds (Comm) were faster and more active in the T maze at day 2 than smaller Egg line birds. Male Comm line birds were faster than male Egg line birds when tested in an open field at day 8. Egg line birds had more sleeping behavior after moving to a new floor pen as compared to both an intermediate-sized line (F line) and the Comm line. Transport stress increased Cort levels in all 3 lines and the increase was greater in males compared to females. The Egg line had higher basal Cort

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

levels (P = 0.03) and higher levels after transport (P< 0.0001). The H/L ratios were affected by both transport stress and line but not by sex. The H/L ratio was lower in the Egg line as compared to both the F line and the Comm line (P< 0.0001), with the Comm line having the greatest increase in response to transport. Previous studies determined that Egg line birds were more resistant to the deleterious effects of challenge and the Comm line displayed the most adverse effects. These data suggest that differences in activity of fast-growing turkeys may be used to select birds that are less susceptible to inflammatory bacterial disease and that the H/L ratio may be more useful than serum Cort in evaluating the deleterious effects of stress.

5H_05_P

DIFFERENTIAL REGULATION OF GLUCOCORTICOID AND MINERALOCORTICOID RECEPTOR AND 11ß-HYDROXYSTEROID DEHYDROGENASE 1 AND 2 MRNAS BY ACUTE PSYCHOSOCIAL STRESS IN PORCINE BRAIN

Kanitz, E., Tuchscherer, M., Puppe, B., Viergutz, T., Tuchscherer, A.

Research Institute for the Biology of Farm Animals, Research Unit Behavioural Physiology, Dummerstorf, Germany; email: ekanitz@fbn-dummerstorf.de

Early life events can have short and long-term effects on neuroendocrine, autonomic, and behavioural responses to stress and appear to play an important role in the etiology of stress-related disorders. Glucocorticoids are key mediators of these brain-endocrine-immune connections. To gain information on molecular modifications caused by psychosocial stress in coincidence with weaning of piglets, we investigated the effects of social isolation on days 7, 21 and 35 of age on the expression of genes regulating glucocorticoid response in stress-related brain regions. Evaluation of glucocorticoid receptor (GR), mineralocorticoid receptor (MR) and 11ß-hydroxysteroid dehydrogenase 1 and 2 (11ß-HSD1 and 11ß-HSD2) mRNA expression by real-time RT-PCR revealed significant differences in quantity of these genes in hypothalamus, hippocampus and amygdala. A single isolation from mother and siblings for 4 hours caused a significant increase of ACTH and cortisol concentrations. The hypothalamic GR, MR and 11ß-HSD1 mRNA levels significantly increased in piglets exposed to isolation stress, whereas in the amygdala the MR mRNA expression significantly decreased. The 11ß-HSD2 mRNA levels were not influenced by social isolation in the brain regions. In conclusions, psychosocial stress in form of a short-term maternal deprivation and social isolation in piglets caused age-dependent and region-specific modifications in mRNA levels of stress-related genes in the brain.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Furthermore, the results emphasize that these glucocorticoid regulating genes are involved in mediating emotional experience in pigs.

5H_06_P

INFLUENCE OF ACUTE AND LONG-TERM STRESS ON SIGA- AND CORTISOL-CONCENTRATIONS IN SALIVA OF WORKING DOGS

Scheideler A, Schneider M, Erhard MH, Wilhelm S, Waldhauser C, Pauly S, Slotta-Bachmayr L, Ahrens F

Institute of Animal Welfare, Ethology and Animal Hygiene, Ludwig-Maximilians-University Munich, Germany

In humans salivary secretory immunoglobulin A (sIgA) has been proved an objective and sensitive marker for stress. In animals, however, stress is hard to determine, and reliable, non-invasive parameters are needed for the assessment of strain. Salivary cortisol is commonly used to detect stress responses in animals but interpretation is not always easy for various reasons. In the present work sIgA is established as a dependable indicator for both physical and psychological stress in working dogs. Hence, the results of three different studies will be presented: 1) 19 search-and-rescue-dogs underwent search exercises on five different days, with 2 searches per day and a 20 min break in between; 2) 20 search-and-rescue-dogs took part in a 3-days-lasting program with 4 rubble searches per day, strains had duration of 20 min each and a 60 min break in between; 3) 26 police dogs were examined during 4 weeks of basic training with protection exercise units in weeks 1 and 4, each included a strain phase of 3-minute protection exercise. Salivary samples were collected in intervals before and after the strain periods. Salivary sIgA and cortisol were analyzed by ELISA. Resulting data from the three studies showed significantly, that acute stress leads to a decrease in sIgA from mean concentration of 1 g/L down to 0.2 g/L, whereas salivary cortisol concentration increased. Long-term strain resulted in an increase in sIgA in search-and-rescue-dogs during the 3-days-lasting program, while police dogs showed a reduction after their 4-week-training.

5H_07_P

ENDOCRINE, HEMATOLOGICAL AND BEHAVIORAL STRESS RESPONSES TO TAIL DOCKING IN PIGS

M. A. Sutherland*, P. Bryer, N. Krebs, J. J. McGlone

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Pork Industry Institute, Texas Tech University, Lubbock, TX, USA
**Corresponding author: mhairi.sutherland@ttu.edu*

Tail docking of piglets is a routine procedure on farms to prevent tail biting behavior, however docking causes an acute stress response. The objectives of this research were to determine stress responses to tail docking in piglets and to compare two methods of tail docking; cautery iron (CAUT) and the more commonly used blunt trauma cutters (BT). At approximately 6 days of age, piglets were tail docked using CAUT (n = 20), BT (n = 20), or sham tail docked, but their tails were left intact (CON; n = 40). Blood samples were taken prior to tail docking and at 30, 60 and 90 min after tail docking. Behavior and vocalizations were recorded during the tail docking procedure to measure the immediate reaction to tail docking and in the home pen before and after tail docking. Behaviors measured included tail movement, butt movement, leg kicking and overall activity. Hematological measures did not differ ($P > 0.05$) among treatments. Cortisol concentrations were higher ($P < 0.05$) among BT compared with CON piglets 60 min after tail docking, however, cortisol concentrations did not differ ($P > 0.05$) among CAUT and CON piglets at this time. Furthermore, cortisol concentrations were higher ($P < 0.01$) among BT than CAUT piglets 60 min after tail docking. Behavioral activity and vocalizations were greater ($P < 0.01$) in docked piglets compared with CON, regardless of treatment. Furthermore, butt movement was greater ($P < 0.005$) in BT compared with CAUT piglets. The sharp pain of BT caused both a rise in cortisol concentrations and more behavioral activity compared with CON or CAUT piglets. Combining behavioral and physiological measures of stress provided a more complete picture of piglet welfare in a challenging farm situation.

5H_08_P

ANTIOXIDATIVE ACTIVITY OF PARAOXONASE-1 (PON1) AND MALONDIALDEHYDE (MDA) LEVEL THROUGHOUT PREGNANCY AND POSTPARTUM PERIOD IN DAIRY COWS

Romana Turk[a]*, Dubravka Juretić[b], Darko Gereš[a], Nenad Turk[a] Zlata Flegar-Meštrić[c], Ante Svetina[a]

[a]Faculty of Veterinary Medicine University of Zagreb, Croatia; [b]Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia; [c]Institute of Clinical Chemistry, Clinical Hospital "Merkur", Zagreb, Croatia;
**rturk@vef.hr*

Serum PON1 is HDL-associated enzyme that hydrolyzes oxidized phospholipids generated on LDL and HDL during oxidative stress and it is considered as an anti-oxidative/anti-inflammatory component of HDL. The

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

aim of this study was to investigate serum PON1 activity and MDA level as well as the relationship between PON1 activity and total cholesterol and HDL-C concentrations in dairy cows during pregnancy and postpartum period. A total of 133 dairy cows were distributed into six groups: pregnant cows in 1st trimester (P1, n=21), 2nd trimester (P2, n=17) and 3rd trimester of pregnancy (P3, n=10); lactating cows in the early puerperium (L1, 1-15 days postpartum, n=23), late puerperium (L2, 16-30 days postpartum, n=27) and mid-lactation (L3, 40-60 days postpartum, n=11). The median PON1 activity was significantly lower ($P<0.05$) in groups P3 (216 U/L), L1 (223 U/L) and L2 (189 U/L) compared to P1 (283 U/L), P2 (257 U/L) and L3 (329 U/L). MDA concentration was significantly higher ($P<0.05$) in P3 (2.43 mmol/L) than in P1 and P2 (1.78 mmol/L and 1.5 mmol/L, respectively). A significant correlations were found between PON1 activity and total cholesterol ($r=0.536$; $P<0.001$) and HDL-C ($r=0.52$; $P<0.001$). There was no significant correlation between PON1 activity and MDA level ($P>0.05$). Results demonstrate lower PON1 activity in late pregnancy and early postpartum and higher MDA level in late pregnancy indicating that reproductive stress in dairy cows could influence an imbalance of antioxidans/prooxidants equilibrium.

5H_09_P

INDIVIDUAL DIFFERENCES IN STRESS COPING STYLES IN POLICE DOGS EXPOSED TO THREATENING SITUATION

Zsuzsánna Horváth[a,*] , Botond-Zoltán Igyártó[b], Attila Magyar[b], Ádám Miklósi[a]

[a]Department of Ethology, Institute of Biology, Eötvös Loránd University Faculty of Science, Budapest, Hungary
[b]Department of Human Morphology and Developmental Biology, Faculty of Medicine, Semmelweis University, Budapest, Hungary
** horvath.zsuzsanna@gmail.com*

According to some researchers animals use certain coping strategies to deal with stressful situations. In the case of social carnivores social stress is a substantial part of the overall stress load. Previous research has established two extreme (proactive and reactive) coping styles in several animal species, but means of coping with social stress has not yet been investigated in the case of dogs. The aim of this study was to develop a testing procedure, where police dogs had to deal with social stress caused by a strange human approaching threateningly in the absence of their handler. We simultaneously measured changes of cortisol levels and described the behavior displayed in the course of the test. A factor analysis of the behavioral variables discriminated three factors (‘shyness’,

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

‘boldness’, ‘ambivalence’). Based on these three factors as secondary behavioral variables a combination of an cluster and a discriminant analysis established that in case of police dogs three types of coping strategies can be identified, namely ‘proactive’, ‘reactive’ and ‘passive’. Individuals in our ‘Proactive’ group are characterized by low HPA-axis reactivity, high level of activity and the short attack latency, while subjects in our ‘Reactive’ group show high HPA-axis reactivity and long attack latency. Our ‘Passive’ group consists of the least active individuals, they failed to show attack behavior and reacted to approaching human with passivity and submission, while their HPA-axis reactivity has been normal.

5I_01_P

WITHANIA SOMNIFERA INDUCES THE STRESS INDUCED MODULATION OF NEUROBEHAVIORAL PATTERNS VIA NITRIC OXIDE SYNTHASE

Zaved Ahmed Khan[1] and Iftikhar Aslam Tayubi[2]

[1,2]School of Biotechnology, Chemical and Biomedical Engineering,Vellore Institute of Technology University, Vellore TN-632014, India.

Withania somnifera (ashwagandha, Indian ginseng) is an immunostimulant herbal medicine used to improve overall health and prevent diseases, particularly in the elderly. We had found to be an effective stress modulator herbal medicine. However, the mechanisms underlying its anxiolytics effect is poorly understood. To elucidate the mechanism of Withania somnifera, we investigated the effect of a methanolic extract from the root and stem of Withania somnifera (WS) on nitric oxide (NO) production in wistar rats.We found that WS (1–256 μg/ml) produced a significant and concentration-dependent increase in NO production, an effect which was abolished by N-nitro-L-arginine methyl ester (L-NAME, 3–300 μM), a nonselective inhibitor of NO synthase (NOS).As it has been already shown about the involvement of nitric oxide (NO) in stress-induced neurobehavioral changes in rats .Moreover, elevated plus maze (EPM) and open field test (OFT) analysis showed that WS increased, in a concentration-dependent fashion modulates stress induced neurobehavioral patterns. Activity of NOS was checked using NOS detection system(Sigma FCANOS) in brain sample. These results demonstrate that WS may induce the synthesis of neuronal NOS expression likely by acting at transcriptional level. nNOS level is checked by western blot analysis. The increased NO production by brain could account, at least in part, for the anxiolytics properties of Withania somnifera.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Keywords: wistar rats; Nitric oxide synthase; L-NAME; Ashwagandha; Withania somnifera; stress

---

5I_02_P

ROOT DEVELOPMENT UNDER OSMOTIC STRESS AND IN THE PRESENCE OF EXOGENOUS AUXIN IN *PISUM SATIVUM* L.: THE ROLE OF NITRIC OXIDE

Zs. Kolbert, B. Bartha, L. Erdei

Department of Plant Physiology, University of Szeged, P.O.B. 654., 6701 Szeged, Hungary
kolzsu@bio.u-szeged.hu ; erdei@bio.u-szeged.hu

During this work the effects of exogenous auxin (indolebutyric acid, IBA) and osmotic stress on root morphology and nitric oxide (NO) generation in roots were compared in pea plants. Five-day old plants were treated with IBA at a concentration range of 10^{-9}- 10^{-3}M and osmotic stress was induced by 50-400 mOsm polyethylene glycol (PEG 6000) for another 5 days. NO generation was examined by *in situ* and *in vivo* fluorescence method. Both root characteristics and NO intensities were followed as the function of time. The increasing concentrations of IBA as well as PEG resulted in shortening of primary roots (PRs), enhancement of lateral root (LR) number and significant increase of NO generation. By virtual sectioning of PRs, it was determined that the accumulation of NO occurred at the basis of LRs during both treatments. Time-dependence investigations revealed that in the case of IBA treatments, the LR number increased in parallel with an intensified NO generation, while elongation of PRs was not followed by changes in NO levels. During osmotic stress, the time curve of LR development was similar to that of IBA-treated roots, but, significantly, it was preceded by a burst of NO. This early phase of NO generation under osmotic stress was clearly distinguishable from that which accompanies LR initiation. It is concluded that osmotic stress and the presence of exogenous auxin resulted in partly similar root architecture but different time courses of NO synthesis. We suppose that the early phase of NO generation may fulfil a role in the osmotic stress-induced signalization process leading to the modification of root morphology. *–This work was supported by grant No. OTKA T048436.*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

5I_03_P

EVALUATION OF *IN VITRO* LDL OXIDATION MEDIATED BY CUPPER AND NITRIC OXIDE PRODUCTION IN HUVEC IN PRESENCE OF HUMAN DYSLIPIDEMIC PLASMAS.

Lamperti, L., Binder, P., Nova, D., González, I., Searle, A., Aguilar, R. & Aguayo, C.

Clinic Biochemistry and Immunology Department, Pharmacy Faculty, POX-237, University of Concepción, Chile, llampert@udec.cl

Low-density lipoprotein (LDL) is involved in the pathogenesis of atherosclerotic lesions, through modifying processes such as oxidation. We examined the *in vitro* susceptibility to oxidation of LDL isolated from normal individuals in presence of hypercholesterolemic human plasma and we assayed the same plasmas to evaluate the effects on the nitric oxide (NO) production in human fetal endothelial cells (HUVEC). Plasma from 20 dyslipidemic patients and 10 normal individuals were analyzed for total cholesterol (TC), high density lipoprotein (HDL), LDL, triglycerides and the ratio TC/HDL was calculated. The LDL susceptibility to oxidation was measured and expressed in seconds (lag phase and maximal rate time (MRT) for conjugated dienes formation). The NO production was evidenced by florescence microscopy and flow cytometry (FC) using 4,5-Diaminofluorescein Diacetate (DAF-2 DA). The kinetic curve for isolated normal control LDL (50 ug/mL, 3 uM Cu) shown a lag phase of 3145s ±43 and MRT of 5030s ±80. Plasma of patients with high cholesterol (>200 mg/dl) exhibited a shorter MRT for conjugated dienes (-15% ±4 *vs* control MRT) when the ratio TC/HDL is > 5.0, but MRT is longer (+25% ±11 *vs* control MRT) when the hypercholesterolemic patients ratio TC/HDL is <5. The MRT for normal individual was +80% ±15 *vs* control MRT. The NO production in HUVEC stimulated with Acetylcholine (7,5 uM, I_F=100%) was lower when cells were incubated 24 hrs with plasma ratio TC/HDL >5 (62.2% ±3.8 *vs* 49.2% ±6.2 normal cholesterol plasma). The mayor susceptibility to LDL oxidation showed a correlation with a minor disponibility of NO in hypercholesterolemic patients.

5I_04_P

UNCOUPLING ENDOTHELIAL NITRIC OXIDE SYNTHASE CAUSES ENDOTHELIAL CELL STRESS BY SUPEROXIDE FORMATION DUE TO DIMINISHED SUPPLY OF THE COFACTOR TETRAHYDROBIOPTERIN.

Pavel Martasek[†], Jeannette Vasquez-Vivar[§], B. Kalyanaraman[‡]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[†]*Charles University School of Medicine I and Univ. General Hospital, Dpt of Pediatrics, 128 08 Prague, Czech Republic (pavel.martasek@gmail.com);*
[§]*Dpt of Biophysics, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA*

In blood vessels, superoxide anion radical ($O_2^{\bullet-}$) formation has been ascribed to different enzymes, among them endothelial nitric oxide synthase (eNOS). The improved methods for the detection of $O_2^{\bullet-}$ has greatly contributed to the identification of the mechanisms involved in the shift of eNOS activity from a NO to a $O_2^{\bullet-}$ synthase. This is of high importance because this shift in activity plays a significant role in the pathophysiological mechanisms of vascular disease. In this presentation, we show evidence for the role of the cofactor tetrahydrobiopterin (BH4) as the molecular switch that controls $O_2^{\bullet-}$ release from eNOS. BH4 effects were shown to be dependent on both its redox state and concentration. For the enzyme reaction to be coupled and form exclusively NO, however, requires optimal BH4 supply. In the absence of BH4, eNOS generates superoxide from the breakdown of the heme–dioxygen complex at the oxygenase domain of the enzyme. Asymmetrical-dimethyl-L-argine (ADMA) or L-NMMA did not alter $O_2^{\bullet-}$ release, and L-NAME caused a partial inhibition. Examining the role of eNOS protein interaction revealed that caveolin-1- scaffolding peptide inhibits $O_2^{\bullet-}$ production in a dose dependent manner. Overall, the data presented indicate that $O_2^{\bullet-}$ production from eNOS is increased by the diminished availability of the reduced BH4 cofactor but not by deficient levels of L-arginine or accumulation of methylated L-arginine in the endothelium. [Supported by grants GACR 305/05/0336, MSMT 0021620806 and 1M6837805002].

5I_05_P

THE EFFECT OF *BERBERIS VULGARIS* ON INFLAMMATION-INDUCED STRESS

Parvu Alina Elena[1], Parvu M.[2], Mogosan Cristina[3], Tamas M.[4]

University of Medicine and Pharmacy Iuliu Hatieganu Cluj-Napoca - [1]Pathophisiology, [3]Pharmacology, [4]Pharmaceutical Botany; [2] University Babes-Bolyai Cluj-Napoca - Biology Faculty
[1] Adress: Emil Isac Street 13, 400020 Cluj-Napoca, Romania, E-mail: parvualinaelena@yahoo.com

Excessive nitric oxide (NO) synthesis by nitric oxide synthase 2 (NOS2) has been implicated in the pathogenesis of inflammatory illness, including inflammation-induced stress. Novel therapeutic approaches have changed the trend towards pharmacologic modulation of exaggerated host

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

response, namely host modulatory therapy. That is why the present study evaluated *Berberis vulgaris* extract effect on NO synthesis in experimental inflammation models. There have been used 10 groups of Wistar-Bratislawa male rats: 2 groups with experimental inflammation (turpentine-induced acute inflamation, Freund's Adjuvant-induced arthritis), 4 groups with experimental inflammation treated with *Berberis vulgaris* extract (administrated i.p. in two dilutions: 0,2 %, respectively 0,02% expressed in berberine), 2 groups with experimental inflammation treated with a nonselective NOS inhibitor (L-NAME), and 2 groups with experimental inflammation treated with ibuprofen, as an NOS2 inhibitor. After 24 hours from turpentine administration, respectivelly after 4 weeks from Freund's Adjuvant administration, NO synthesis was appreciated in all groups by measuring serum nitrite/nitrates (Griess) and citrulline concentrations. Conclusions: 1. *Berberis vulgaris* extract significantly reduced NO synthesis in experimental inflammation models; 2. The higer dilution of *Berberis vulgaris* extract had a stronger inhibitory effect on NO synthesis; 3. The effect of *Berberis vulgaris* extract was smaller than that of L-NAME and ibuprofen.

5I_06_P

PRELIMINARY APPLICATION OF HIGH SPEED NO-METABOLITE-ASSAY IN HUMAN SALIVA FOR EXERCISE STRESS USING LAB-ON-A-CHIP TECHNOLOGY

Wakida Shin-Ichi, Miyado Takashi, Tanaka Yoshihide, Nagai Hidenori, Yoshida Yasukazu and Niki Etsuo

Human Stress Signal Research Center, National Institute of Advanced Industrial Science and Technology (AIST), Japan, Ikeda, Osaka 563-8577, Japan, s.wakida@aist.go.jp

Nitric oxide (NO) has been identified as a mediator in many physiological functions. Several research scientists have reported the possibility that acute exercise induced endothelium-dependent vasodilation is due mainly to an increase in NO release and also discussed NO metabolites change in saliva. In general, NO metabolites assay is used by the Griess reaction, however, the assay is time-consuming.

We have studied high speed NO assay in human saliva using Lab-on-a-Chip integrated with analytical procedures, such as on-chip pre-concentration, separation and simultaneous detection of NO metabolites, NO_2^- and NO_3^- within 1 minutes including off-line sample pre-treatment with 10-fold pure water dilution.

In this paper, we will introduce the high speed Lab-on-a-Chip assay based

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

on capillary zone electrophoresis with UV detection method using a novel running buffer and an advanced microfluidic control. We achieved complete separation assay of NO metabolites in 10-fold diluted saliva within 15 seconds. And we will also introduce preliminary application for exercise tolerance test using bicycle ergometer. The exercise tolerance level was changed stepwisely from 0 to 183 W for 15 minutes. Human saliva samples were collected before and after the test in high exercise intensity group, 6 healthy volunteer (5 female and 1 male aged from 40 to 66 years) and low exercise intensity group, 4 healthy volunteer (1 female and 3 male aged from 21 to 47 years). The preliminary results of the change of NO metabolites in saliva will be introduced and discussed in the congress.

5J_01_P

HYPERGRAVITY STRESS RESPONSE IN RAT HYPOTHALAMUS AND MODULATION BY VESTIBULAR BLOCKADE

Yasuhiro Kumei[1)]*, Jorge Zeredo[2)], Mari Kimoto[3)], Tohru Ikeda[2)], and Kazuo Toda[2)]

*[1)]Graduate School of Tokyo Medical and Dental University, Tokyo 113-8549. [2)]Graduate School of Nagasaki University, 852-8588 Nagasaki and [3)]Japan Women's University, Tokyo 112-8681, Japan. * To whom contact by kumei.bch@tmd.ac.jp*

Spaceflight influences vestibular function and causes motion sickness in astronauts. Stress response is induced by microgravity and other spaceflight conditions. However, little is known about the relationship between stress response and vestibular function in gravitational alteration. The purpose of this study was to examine the hypergravity effects on the neuronal activities of hypothalamic arcuate nucleus (ARC) in the presence and absence of vestibular function in rats. Young male Wistar rats were exposed to gradually increasing gravity-force in a light-blocked dark centrifuge. Electrophysiological data were obtained through chronically implanted electrodes by telemetrical recording of the unit activities at ARC with or without injection of xylocaine into both inner ears. The firing frequency in the ARC started to increase when the centrifugal force reached to 2.0G, 40 seconds after initiating centrifugation. By contrast, the firing frequency in the ARC of xylocaine-injected rat started to increase when the centrifugal force reached 1.4G, 15 seconds after starting centrifugation. Thus, the latent period for hypergravity-responsive ARC activity was significantly shortened by vestibular blockade. On the following day when xylocaine effects were expired, the ARC responsiveness to hypergravity resumed the basal level. Data suggest that the vestibular function may modulate the gravity-stress response in

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

rat hypothalamus. Supported by Grant-in-aid from the Ministry of Education, Science, and Culture, Japan.

5J_02_P

GRAVITATIONAL STRESS ON SYMPATHETIC NERVE ACTIVITY IN HUMANS

Tadaaki Mano and Satoshi Iwase

Gifu University of Medical Science, Seki, Gifu 501-3892, Japan and Department of Physiology, Aichi Medical University, Nagakute, Aichi 480-1195, Japan
(E-mail address: tadaaki.mano@nifty.com)

Gravity is an important environmental stress for human being who maintains upright posture. Neural and humoral mechanisms operate to maintain upright posture in humans against gravitational stress. One of the mechanisms is sympathetic neural control of blood pressure. Sympathetic neural traffic leading to skeletal muscles called muscle sympathetic nerve activity (MSNA) has been proved to play an essential role to maintain blood pressure homeostasis through baroreflexes. The present study aimed to clarify how gravitational stress influences MSNA in humans. Subjects were 20 healthy male subjects ranging in age between 18 and 24 years. MSNA was recorded and identified from the tibial nerve using microneurography (1), simultaneously with hemodynamic functions. In 10 subjects, gravitational stress was loaded by head-up tilt changing posture from supine to standing using a tilt table. We analyzed how gravitational stress from the head to the leg (+Gz) expressed as sine function of the tilt angle influences MSNA and cardiovascular functions. We also analyzed MSNA responses in subjects who complained of orthostatic hypotension during head-up tilt. In another 10 subjects, MSNA was measured during exposure to short- and long-term microgravity induced by parabolic flight and simulated by head-down bed rest for 14 days, respectively. We analyzed the influence of microgravity on MSNA and compared the results with changes in the same activity reported in microgravity in space. We conclude that +Gz stress is a strong activator of sympathetic neural traffic leading to skeletal muscles in humans to maintain blood pressure homeostasis during standing. Excessively low and high sympathetic responders to gravitational stress can cause orthostatic hypotension. Short-term microgravity suppresses sympathetic nerve activity to muscles but long-term exposure to simulated microgravity rather enhances the same activity as reported in spaceflight depending on complex mechanisms including plasma volume reduction.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1. *Tadaaki Mano, Satoshi Iwase and Shinobu Toma: Microneurography as a tool in clinical neurophysiology to investigate peripheral neural traffic in humans (Invited review). Clin. Neurophysiol., 117: 2357-2384, 2006.*

5J_03_P

HSP72 DETERMINES THE OUTCOME OF VIRAL INFECTION IN BRAIN

Michael Oglesbee and Thomas Carsillo

Department of Veterinary Biosciences, The Ohio State University, Columbus, OH; oglesbee.1@osu.edu

Fever is a potent inducer of 70 kDa heat shock proteins, particularly hsp72, and in vitro studies show that hsp72 supports replication and gene expression for multiple viral families. Despite the frequent occurrence of fever in viral infection, the biological (i.e., in vivo) significance of virus-hsp72 interaction is poorly defined. The present work addresses the in vivo significance of virus-hsp72 interaction using a mouse model of measles virus (MV) encephalitis. Hsp72 stimulates MV transcription, a response that is disrupted in an N protein amino acid substitution mutant (N522D). Neonatal mice receiving an intracranial inoculation of MV are afebrile and exhibit H-2 restricted differences in susceptibility to infection; H-2^d mice are resistant to infection due to robust adaptive antiviral immune responses whereas H-2^b are susceptible due to deficiencies in this response. Here we show that transgenic overexpression of hsp72 increases MV transcription within infected brains of H-2^b (susceptible) C57BL/6 mice, resulting in increased viral protein expression, CPE and virus-induced mortality. In contrast, overexpression of hsp72 in congenic C57BL/10 having the H-2^d resistance phenotype are completely protected against MV challenge, compared to > 30% mortality in non-transgenic mice. Protection reflects a viral transcriptional response to hsp72 based upon no significant differences in infections by the N522D variant in transgenic versus non-transgenic mice. Collectively, these findings suggest that hsp72-mediated stimulation of viral transcription (and thus antigenic burden) can facilitate adaptive immune response leading to viral clearance, but if the host is immune compromised, this host protective strategy back-fires, leading to enhanced viral virulence.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

5J_04_P

CHRONIC STRESS AFFECTS THE EXPRESSION OF NEUROTROPHINS IN THE RAT SALIVARY GLANDS

Juri Saruta[1*], Ko Nakajima[1], Lee Tae Ki[1], Masayoshi Shirasu[1], Takeshi Takahashi[1], Chikatoshi Sato[1], Keiichi Tsukinoki[2] and Sadao Sato[1]

*Department of [1]Craniofacial Growth and Development Dentistry, Division of Orthodontics, [2]Maxillofacial Diagnostic Science, Division of Pathology, Kanagawa Dental College, Yokosuka, Japan; *corresponding author, sarujuri@kdcnet.ac.jp*

Purpose of this experiment; In the previous study, we reported that BDNF was produced from salivary gland in the condition of acute immobilization stress. In addition, salivary glands were origin of plasma BDNF in that condition. Next, in chronic immobilization stress, we thought to examine whether there is a relationship between salivary gland and neurotrophins expression.
Materials & Methods; Chronic stress group was also performed against rats which were resected the bilateral major salivary glands (sialoadenoectomy). Chronic stress was induced by enclosing each animal in flexible wire mesh shaped to fit its body.
Results; Increased neurotrophins mRNA and protein expression were observed in duct cells as a result of chronic stress, as demonstrated by real-time PCR, immunohistochemistry and ELISA. Chronic stress significantly increased the level of plasma neurotrophins. There were significant differences between non-stress and chronic stress in the plasma neurotrophins level ($p>0.05$). Rat submandibular gland was identified as an organ which expresses neurotrophins. Furthermore, the results of this study suggest that increased salivary neurotrophins expression occurs following chronic stress. There were no significant differences between stressed rats and stressed sialoadenoectomy rats in the plasma neurotrophins level.
Conclusion; Our data, therefore, suggest an existence of a different between acute and chronic stress was suggested in source of plasma BDNF and NT-3. Future studies are needed to examine the origin of plasma BDNF and NT-3 in chronic stress.

5J_05_P

ACUTE IMMOBILIZATION STRESS AFFECTS THE EXPRESSION OF BDNF IN THE RAT SALIVARY GLANDS

Keiichi Tsukinoki[1], Juri Saruta[2], Lee Tae Ki[2], Sadao Sato[2]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

*Department of [1]Maxillofacial Diagnostic Science, Division of Pathology, [2]Craniofacial Growth and Development Dentistry, Division of Orthodontics, Kanagawa Dental College, Yokosuka, Japan; *corresponding author, ktsukino@kdcnet.ac.jp*

Purpose of this experiment; The present study examined the effect of immobilization stress on BDNF and TrkB expression in male rat submandibular glands.
Materials & Methods; Male Sprague-Dawley rats, 9 weeks of age were used in this study. All experiments were performed using four rats per group. The rats were exposed to immobilization stress for 30 min, 60 min, or 180 min.
Results; Increased BDNF mRNA and protein expression were observed in duct cells as a result of immobilization stress, as demonstrated by real-time PCR, western blot, immunohistochemistry, and analysis by microdissection. TrkB mRNA was not detected in salivary gland tissue, or oral or esophageal mucosa, by RT-PCR. Rat submandibular gland was thus identified as an organ which expresses BDNF. Acute immobilization stress for 60 min significantly increased the level of plasma BDNF. However, plasma BDNF elevation was markedly suppressed in bilaterally sialoadenectomized rats. There were no significant differences between stressed (60 min) and non-stressed rats with respect to the BDNF mRNA expression in the hippocampus, heart, lung, liver, pancreas, or spleen, as determined by real-time PCR.
Conclusion; Under acute immobilization stress, the rat submandibular gland has been shown to increase production of BDNF, thereby contributing to the elevation of plasma BDNF levels. We believe that salivary gland can be influenced the health of distant organs. It is necessary to determine the target organs of plasma BDNF in this stress model.

5J_06_P

DEVELOPMENTAL EXPRESSION OF HEAT SHOCK PROTEIN 70 IN RAT KIDNEY

Chul-Ho Lee, [1] Ji-Hyun Song,[2] Yong-Hwan Kim,[2] Si-Yun Ryu,[2] Jin Kim,[3] Ju-Young Jung[2]

[1]Genetic Resources Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Korea; [2]Department of Veterinary Medicine & Institutes of Veterinary Science, Chungnam National University, Daejeon 305764, Korea; [3]Department of Anatomy, College of Medicine, The Catholic University of Korea, Seoul 137701, Korea

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Heat shock protein 70 (HSP70) is a major molecular chaperone and plays an important role in protection of cells in renal medulla against high osmolality. As the urinary concentrating mechanism is being built up after birth, urine osmolality increases gradually form 300 mOsm/kg to 2,000 mOsm/kgH_2O; accordingly, cells in renal medulla are exposed to an elevated and variable interstitial osmolality. To understand the possible roles of HSP70 in the developing kidney, expression of HSP70 and the changes of HSP70 expression in the loop of Henle following infusion of furosemide were investigated. Immunohistochemistry analysis revealed that little immunoreactivity of HSP70 was detected in embryonic kidneys. On postnatal day 1, HSP70 was first detected in the inner medullary collecting duct (IMCD) of the papillary tip. The intensity of HSP70 immunostaining in the IMCD steeply increases ascending to the border between outer and inner medulla during the first three week after birth. At birth, all loop of Henle in rat renal papilla have the configuration of short loop and there is no ascending thin limb. During the first two week of life the cuboidal epithelium of the thick ascending limb is gradually transformed into the ascending thin limb by a process that start just before the bend of the loop and proceeds toward the outer medulla. From 4 to 14 days of age, HSP70 was detected in the cells after transformation in the ascending thin limb, beginning at the papillary tip and ascending to the border between the outer medulla and the inner medulla. Since then expression of HSP70 gradually increased, and by 21 days after birth, HSP70 was detected in IMCD and ATL in most part of inner medulla. In adult rat kidney, expression of HSP70 was highest in IMCD. The gradual increase in HSP70 is associated with an increase in its mRNA abundance. However, furosemide infusion resulted the significantly decrease HSP70 expression in the renal papilla. These data demonstrated that expression of HSP70 was closely correlated with the changes in interstitial osmolality during the development of kidney. We suggest that HSP70 protects tubular epithelial cells in inner medulla from the stress of high osmolality during the development of kidney.

Keywords; HSP70, Developing kidney, Osmotic stress

5J_07_P

PHYSIOLOGICAL AND MOLECULAR RESPONSES TO HEAT STRESS DURING AVIAN ONTOGENY: HOW DO THEY REFLECT EVOLUTIONARY CHANGES IN THERMAL RESISTANCE

Ariel Shabtay[1] and Zeev Arad[2]

[1] *Department of Cattle and Genetic Sciences, Institute of Animal Science, ARO, Newe Ya'ar Research Center, P. O. Box 1021, Ramat Yishay 30095, Israel*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[2] *Department of Biology, Technion – Israel Institute of Technology, Haifa 32000, Israel*

Ectothermy and endothermy constitute the major thermoregulatory strategies in living organisms. While ectotherms can be prone to changes in body temperature (T_b) in correlation with ambient temperature (T_a) variations, the internal physiological milieu of endotherms remains relatively stable despite external thermal fluctuations. The chicken is ideal for comparing ectothermic and endothermic 'thermal states', as during ontogeny its embryo undergoes a transition from ectothermy to endothermy. By using avian development as a model system for transition from ectothermy to endothermy, we show that, in contrast to the ectothermic state, in the endothermic state the organism is more resistant to heat but relies less on HSPs as a first-line thermoprotective mechanism. Moreover, intraspecific, real-time, *in vivo* measurements in genetically diverse fowl strains relate improvement of thermoresistance in endotherms to improved T_b regulation, with a concomitant delay in HSF activation and HSPs expression. The time course of this delay and the T_b at which it occurs imply that the ontogenetic and evolutionary pathways leading to improved thermoresistance may have followed two, apparently non-related, parallel routes: a cellular one, in which the acquisition of thermoresistance is not HSP-dependent and could result from altered mechanisms of thermal sensation, and a peripheral one, characterized by altered homeostatic mechanisms that lead to differential patterns of T_b regulation.

5J_08_P

ELECTROSTRESS AS A RISK FACTOR OF THE RAT POSTNATAL NEUROGENESIS: IMMUNOHISTOCHEMICAL AND QUANTITATIVE STUDY

Orendáčová J.[1], Orendáč M.[2], , Labun J.[3], Račeková E.[1], Saganová K.[1], Mojžiš M.[2], Abdiová H.[4]

[1]*Institute of Neurobiology, CE, SAS, Šoltésovej 4, Košice 040 01, Slovakia; orendac@saske.sk*
[2]*Faculty of Electrical Engineering and Informatics, TU Košice;* [3]*Faculty of Aeronautics, TU Košice;* [4]*Secondary School for Medical Assistants, Košice, Slovakia*

Findings of the last years put evidence about persisted neurogenesis during the whole postnatal age and not only during prenatal period. One of the most studied region of postnatal neurogenesis is the subventricular zone, which harbours steadily dividing stem cells and progenitors, which migrate along a distinct pathway the rostral migratory stream (RMS), to

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

populate the olfactory bulb and establish connections with their neuronal targets. This system represents a simple well-defined model allowing experimental observations of postnatal neurogenesis. We used BrdU immunohistochemistry to study the effect of EMR on postnatal neurogenesis within the RMS in the newborn, adult and aging rats. Quantitative analysis showed significant differences of postirradiation changes in dependency to the rat age, the EMR duration and postirradiation survival of animals. The EMR of 2-6.7 mW/cm^2 induced significant changes of proliferating cells number within the RMS and disrupts typical double peeks of proliferating cells number, characteristic for the first postnatal month. Three sets of the mentioned EMR doses (5 hr/day) induced significant decrease of proliferating cell number within the RMS of rats irradiated at P8 regardless postirradiation survival period. On the other hand the same doses of the EMR applied in two sets induced increase of proliferating cells numbers only in the acute postirradiation period. In the aging rats the same doses of EMR induced decrease of proliferationg cell number regardless postirradiation survival period.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Module 6 – Oral lectures:

6A_01_S

THE EXTENDED *CCT-HSP60* GENE FAMILY IN THE HUMAN GENOME

Dennis L. Maeder,[1] Luciano Brocchieri,[2] Alberto J. L. Macario,[1] Everly Conway de Macario[1]

[1]*Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD, USA*
[2]*University of Florida, Department of Molecular Genetics and Microbiology and UF Genetics Institute, Gainesville, FL, USA*

The chaperonins, a subset of molecular chaperones have been classified into groups I and II, and we are investigating their occurrence in the human genome. Here we report on human CCT, which belongs to group II. Extensive searches of databases and published literature were performed using bioinformatics and complementary methods. The nine canonical *cct* subunit genes (*cct1-cct8*, *cct6A* and *B* included) were characterized. In addition, at least 15 *cct*-related pseudogenes and 3 *cct*-related protein-encoding genes were identified. All 9 *cct* genes are multiexonic and several have more than one mRNA variant with multiple protein isoforms. The proteins encoded by these genes with their mRNA variants so far identified have 339 (*cct7*, isoform "b") to 556 amino acids (*cct1*, isoform "a"), with the great majority having over 530 amino acids. Most of the *cct* genes have related protein-encoding genes and/or pseudogenes. Our analysis thus far revealed that of the 15 pseudogenes three are related to *cct1*, one to *cct3*, three to *cct4*, two to *cct5*, three to *cct6A*, two to *cct7*, and one to *cct8*. Several chromosomes do not harbor *cct* genes or pseudogenes but others have them in various numbers, e.g., chromosome 7 has one *cct* subunit gene and six pseudogenes; chromosome 5 has one *cct* subunit gene, and four pseudogenes; other chromosomes have between one and three genes and/or pseudogenes. We investigate the evolutionary relationships of *cct*, *hsp60*, and related protein-encoding genes and pseudogenes, and the diversity of their proteins (origin, structure, distribution, function, pathology).

6A_02_S

MOLECULAR GENETICS AND BIOLOGY OF SMALL HEAT SHOCK PROTEINS CAUSING INHERITED PERIPHERAL NEUROPATHY

Joy Irobi[1], Ines Dierick[1], Berlinda Vanloo[2], Jan Gettemans[2], Ludo Van Den Bosch[3], Wim Robberecht[3], Jean-Pierre Timmermans[5], Peter De Jonghe[1,4] & Vincent Timmerman[1]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1. *Department of Molecular Genetics, VIB, University of Antwerp, Antwerpen, Belgium*
2. *Department of Medical Protein Research, VIB, Ghent University, Gent, Belgium*
3. *Laboratory of Neurobiology, Department of Experimental Neurology, University of Leuven, Leuven, Belgium*
4. *Division of Neurology, University Hospital Antwerp, Antwerpen, Belgium.*
5. *Laboratory of Cell Biology & Histology, Department of Veterinary Sciences, University of Antwerp, Antwerpen, Belgium*

Distal hereditary motor neuropathies (distal HMN) are characterized by a selective degeneration of the axons of motor neurons, while the sensory neurons are spared. The biological process of this selective degeneration of motor neurons is still unknown. In 2 distal HMN type II pedigrees linked to chromosome 12q24.3, we identified the same mutation (K141N) in the small heat shock 22kDa protein 8 (*HSPB8*). A second mutation (K141E) was found in 2 smaller families. Co-immunoprecipitation showed an increased binding of both HSPB8 mutants to the interacting partner heat shock protein27 (*HSPB1*). We previously reported a Russian family with autosomal dominant axonal Charcot-Marie-Tooth disease (CMT) and assigned the locus (CMT2F) to chromosome 7q11-q21. This locus contained *HSPB1* as one of the candidate genes. Mutation analysis of *HSPB1* revealed a (S135F) missense mutation segregating in the CMT2F family. Screening for *HSPB1* mutations in a large cohort of CMT2/distal HMN patients identified additional mutations. Expression of mutant *HSPB8* in COS-1 and N2a cells promoted formation of intracellular aggregates and a reduction of neuronal cell survival. *In vitro* chaperone activity assay showed a reduction on the cytoprotective function of mutant proteins. Early passages of the primary fibroblast cultures from the distal HMN patient's skin biopsy showed the formation of aggregate/aggresome complex, which sequestered several molecules including mitochondria. Measurement of mitochondria transmembrane potentials in these primary fibroblast cells evidenced that patients but not controls persons exhibited a depolarized mitochondrial potential.

6A_03_S

THE EFFECT OF AGEING ON THE STRESS PROTEINS OF THE CYTOSOL AND ENDOPLASMIC RETICULUM

Jordan L. Holtzman

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Departments of Pharmacology and Medicine, and Division of Environmental Health Sciences University of Minnesota, Minneapolis, Minnesota, USA.

The cytosol, the endoplasmic reticulum (ER) and the mitochondria contain a number of stress proteins which are essential for life. A major group of these proteins are the heat shock proteins (Hsps). Members of this family are found in all three of these compartments. The cytosolic forms were initially identified because they show significant increased levels when cells are exposed to mild hyperthermia (42° C). This response is mediated through the heat shock factors (HSFs). These are transcription factors which when activated by heat stress form trimers which bind to the promoter regions of the Hsp DNAs. In *ex vivo* studies of human leukocytes and rat heptocytes, cells from older subjects showed a decrease in the Hsp70 response to heat stress, while the levels of the constitutive form, Hsc70 were unaffected. In some studies this decreased response was attributed to an age dependent loss of the HSFs; while others have suggested that their levels do not decline, but that the trimers become functionally inactive. Hence there is a decline in the transcription of the mRNA for Hsp70, even though the HSFs form trimers. Other studies have suggested that the level of Hsp70 has a major impact on longevity. For example, in humans it has been reported that longer lived individuals have higher levels of Hsp70 than that is seen in those with shorter life spans. In particular, centenarians have a mutation in the promoter region of HSP70-1 which increases its level. Similarly, the *dauer* mutation of the IGF2 receptor in *C. elegans*, which doubles longevity, is also associated with increased levels of Hsp70 and Hsp90. Finally, in *Drosophila* high levels of the mitochondrial chaperone, Hsp22, increases longevity by 30%. One treatment which has repeatedly been demonstrated to increase longevity by as much as 30% is caloric restriction. This increased longevity is associated with an increase in the cellular levels of the cytosolic Hsps. The Hsp70 level is also modulated by both plasma membrane and cytosolic hormone receptors. For example, stimulation of the α_1 receptor of vascular smooth muscle leads to increased hsp70 as does estrogen treatment of neurons. Both responses marked decline with age. There are a number of stress proteins found in the ER. Yet, unlike the cytosolic Hsps, these do not appear to respond to heat stress, even though some, such as Bip (GRP78) and GRP94 are members of the Hsp family. Similarly, unlike the cytosolic Hsps, Bip is not affected by caloric restriction. Finally, studies from our laboratory have indicated that in rat, hepatic microsomes the constitutive levels of some of the ER stress proteins decline with age. These included BiP, Erp55 (PDI), Erp57, Erp72 and calnexin; while a sixth, calreticulin, was unaffected. The most surprising observation was that, even though these animals were maintained from weaning to death in a colony with a constant environment, three of these proteins, Bip, Erp55 and Erp57, showed a circsemiannual rhythm with peaks in January and July. This rhythm would appear to have been imprinted in the genome of their wild ancestors and

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

has remained stable for over 200-400 generations. In conclusion, there is a large body of data indicating that declines in stress proteins with age may be a major factor in the reduced capacity of elderly animals to respond to a variety of stresses.
These decreased responses would also appear to have a major effect on longevity.

6A_04_S

DEFECTIVE CHAPERONE NETWORKS

Péter Csermely, Tamás Korcsmáros, István A. Kovács and Máté S. Szalay

Department of Medical Chemistry, Semmelweis University, Puskin street 9., H-1088 Budapest, Hungary; Email: csermely@puskin.sote.hu

Molecular chaperones are not only fascinating molecular machines, but have a number of functions, which can be understood only by considering the emergent properties of protein-protein interaction, signalling and organelle networks – and that of chaperones as special constituents of cellular networks. Moreover, chaperones themselves are networks of amino acid side chains offering vulnerable points for damage. Why are chaperones special in the context of cellular networks? Chaperones (1) have weak links, i.e. low affinity, transient interactions with most of their partners; (2) connect hubs, i.e. act as 'masterminds' of the cell being close to several centre proteins with a lot of neighbours; (3) are in the overlaps of network modules, which confers them a special regulatory role. Chaperones may be inhibited by (1) mutations; (2) their damage e.g. after oxidation; (3) their overload, i.e. a growth in the need of chaperones and/or a decrease of their availability or (4) by pharmacological inhibitors. Inhibitory modes (1) through (3) may occur in various diseases and during the aging process. Chaperone inhibitors are efficient multi-target drugs in several diseases such as cancer. Defective chaperones may uncouple or quarantine modules of cellular networks, which increase protection and efficiency of the cell during stress. Moreover, after stress chaperones are essential to re-build inter-modular contacts by their low affinity, 'quasi-random' sampling of the potential interaction partners in different cellular modules. This opens the way to the chaperone-regulated disassembly, re-assembly, adaptation and modular evolution of cellular networks, and helps us to design novel therapeutic and anti-aging strategies.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

6B_02_S

αB-CRYSTALLIN: A NEW PLAYER IN CANCER

Vincent Cryns (Invited Speaker)

Cell Death Regulation Lab, Feinberg School of Medicine, Northwestern University, Chicago, IL USA
v-cryns@northwestern.edu

αB-crystallin, a member of the small heat shock protein family, is induced by cellular stress and functions to limit stress-induced damage by suppressing protein aggregation. We have demonstrated that αB-crystallin inhibits apoptosis induced many stimuli, at least in part, by disrupting activation of the cell death protease caspase-3. We have also shown that αB-crystallin is commonly expressed in poor prognosis basal-like breast cancer and likely contributes to the aggressive behavior of these tumors. This presentation will focus on new insights into the mechanisms by which αB-crystallin inhibits apoptosis and promotes tumor progression and its integral role in the cellular stress response.

6B_03_S

IMMUNOLOGICAL ROLE OF MEMBRANE-BOUND AND EXPORTED HEAT SHOCK PROTEIN 70 (HSP70)

Gabriele Multhoff[1], Mathias Gehrmann[1], Jürgen Radons[2]

[1]*Dpt. of Radiotherapy and Radiooncology, Technical University Munich, and GSF – Inst. of Pathology Munich, Germany*
[2]*Inst. of Medical Biochemistry and Molecular Biology, University of Greifswald, Germany*

Stress or heat shock proteins (HSPs) are remarkably conserved in all living organisms. Their synthesis is induced in response to a variety of physiological and environmental insults. In the cytosol HSPs play an essential role as molecular chaperones by assisting the correct folding of nascent and stress-accumulated misfolded proteins, preventing protein aggregation, transport of proteins, and supporting antigen processing and presentation. On the plasma membrane and in the extracellular milieu they act as danger signals for the adaptive and innate immune system. Either they act as carriers for immunogenic peptides, induce cytokine release or provide recognition sites for activated natural killer (NK) cells. Here we will discuss the problem why Hsp70, the major stress-inducible, cytosolic member of the HSP70 family is selectively found in the plasma

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

membrane of tumor cells but not on normal cells and elucidate the immunological consequences (1,2).

References

1. C. Gross, W. Koelch, A. DeMaio, N. Arispe, G. Multhoff, J. Biol. Chem., 278 (2003) 1173-41181.
2. P. J. Beresford, M. Jaju, R.S. Friedman, M.J. Yoon, J. Liebermann, J. Immunol., 161 (1998) 161-167.

6B_04_S

HSP72 AND HSP27 REGULATE THE P53 PATHWAY AND SUPPRESS THE SENESCENCE PROGRAM IN CANCER CELLS

Yaglom J., Gabai V.L., O'Callaghan C., Sherman M.Y.

Boston University School of Medicine, Boston, MA, USA

Many tumors have high levels of the major heat shock proteins Hsp72 and/or Hsp27, which correlates with aggressiveness of tumors, resistance to chemotherapy and poor prognosis. Originally, it was suggested that these Hsps facilitate tumorigenesis because they can suppress apoptosis of cancer cells caused by activation of oncogenes, like myc, or by the adverse factors of tumor microenvironment. Here we demonstrate that both Hsp72 and Hsp27 can suppress the p53 pathway and prevent senescence, another major break on cancer development. For example, over-expression of Hsp27 inhibited activation of the p53 pathway by doxorubicin, nutlin-3 or TGF-β, and suppressed development of senescence in response to these stimuli. In contrast, specific depletion of Hsp27 or Hsp72 in a variety of cancer cell lines led to inhibition of Hdm2, activation of p53 and induction of p21. As a result, about 30-40% of cell population became growth arrested and developed features of cell senescence, while the rest of the population also having activated p53 continued to divide slowly, but became sensitive to radiation, doxorubicin and other drugs. These data indicate that high levels of Hsp72 and/or Hsp27 allow cancer cells to avoid activation of an intrinsic senescence program by suppressing p53. At least in certain cancer lines, the intrinsic senescence program was associated with activation of oncogenes. In fact, in MCF-7 cells, which have constitutively active mutant of PI3 kinase (PIK3CA oncogene), depletion of Hsp72 or Hsp27 led to activation of p53 and senescence. However, inhibition of PIK3CA that down-regulates PIP3 prevented activation of p53 and development of senescence upon depletion of Hsps. Similar results were seen with down-regulation of PIP3 in cell lines transformed by PTEN oncogenic mutations. These data indicate that overexpression of Hsps plays a crucial role in supporting division of cells at early stage of transformation upon activation of

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

oncogenes that control PIP3 levels.

6C_01_S

DIVERGENT OUTCOMES OF HSP90 INHIBITION – PROBLEM OR OPPORTUNITY ?

Francis Burrows

Biogen IDEC, San Diego, CA, USA

Hsp90 is a ubiquitous molecular chaperone implicated in the pathophysiology of a range of diseases and so is a target of high interest for therapeutic intervention. Most drug targets eventually become resistant, e.g. by mutation, overexpression or anergy, but Hsp90 is uncommon in that it directly controls both cytotoxic and cytoprotective pathways through it's key role in regulating mitogenic and survival signaling as well as antiapoptotic stress responses. Recent work has indicated that the HSF-1-dependent heat shock response (HSR) decreases the cytotoxic potency of Hsp90 inhibitors in cancer cells *in vitro* by several mechanisms, but, since normal cells are thought to mount a more robust HSR than their transformed counterparts, it is unclear what impact inhibition of the HSR might have on the therapeutic index of these drugs *in vivo.* On the other hand, conditions characterized by excessive cell death, such as neurodegenerative diseases, stroke and peripheral neuropathy, could potentially be treated by pharmacological induction of the HSR in neurons. Biogen IDEC has identified potent Hsp90 modulators that induce client protein degradation and the HSR with equal potency, which are in clinical development for cancer. In addition, we are also exploring the potential of compounds that selectively induce client protein degradation or favour cytoprotective pathways for use in Alzheimer's Disease and Huntington's Disease in addition to oncology.

6C_02_S

ACETYLATION AS A DYNAMIC REGULATOR OF HSP90 FUNCTION: IMPLICATIONS FOR FURTHER DEVELOPMENT OF PHARMACOLOGIC HSP90 INHIBITORS

L. Neckers

Urologic Oncology Branch, National Cancer Institute, Bethesda, MD, USA

Heat shock protein 90 (Hsp90) chaperones a key subset of cellular

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

signaling proteins and is necessary for malignant transformation. However, many aspects of Hsp90 regulation remain unresolved. Hsp90 is subject to an array of post-translational modifications which affect its function, including acetylation. In the last few years, several investigators have reported that histone deacetylase (HDAC) inhibitors and knock-down of HDAC6 induce Hsp90 acetylation and inhibit its activity. While pharmacologic inhibition and RNA knockdown of HDACs have been very useful in identifying reversible acetylation as a potential regulator of Hsp90 activity, it is unclear how this process functions at the molecular level. Use of HDACi and/or HDAC knockdown techniques allow study of only the hyperacetylated (but not hypoacetylated) chaperone, and the importance of the acetylation state of individual residues of Hsp90 in the context of hyperacetylation of the proteome cannot be queried. Furthermore, contributory effects due to histone hyperacetylation cannot be discounted. Direct determination of the functional consequences of Hsp90 acetylation has awaited mapping of specific sites. These sites are now being identified. K294 (Hsp90alpha) was the first amino acid in Hsp90 to be formally identified as an acetylation site and this residue is highly conserved in eukaryotic Hsp90. Conservative mutational analysis of K294 revealed its acetylation status to be a strong determinant of client protein and co-chaperone binding to Hsp90 in mammalian cells. Interestingly, although acetylation status of K294 affects Hsp90 ATPase activity, ATP binding is not altered. In yeast, human Hsp90 mutants that cannot be acetylated at K294 have reduced ability to support viability, while an acetylation-mimicking mutation of K294 possesses the opposite property. Further, acetylation status of K294 may determine Hsp90 sensitivity to N-terminal pharmacologic inhibitors. These data suggest that controlled acetylation/deacetylation of K294 plays an important role in regulating the Hsp90 chaperone cycle.

6C_04_S

DRUGGING THE CANCER CHAPERONE: PRECLINICAL DISCOVERY AND CLINICAL DEVELOPMENT OF HSP90 INHIBITORS

Paul Workman

Cancer Research UK Centre for Cancer Therapeutics, The Institute for Cancer Research, Haddow Laboratories, Sutton, Surrey SM2 5NG

We are in a very exciting era of cancer drug discovery in which effective mechanism-based therapies are being designed and developed that act on the oncogenic proteins and pathways that are hijacked by pathological genetic and epigenetic changes to bring about the initiation of cancer and subsequent malignant progression. Therapeutic selectivity for tumor versus normal cells is achieved by taking advantage of various

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

'dependencies' that develop during the induction of cancers. Two major forms of cancer dependency are related to the molecular chaperone and heat shock protein 90 (Hsp90). Firstly, many mutant and overexpressed oncoproteins that are involved in oncogene dependence or addiction require Hsp90 for their stability and function. Secondly, cancer cells also require Hsp90 and other stress response proteins to help protect them against the adverse environmental conditions present in solid tumors. Moreover, since Hsp90 inhibition causes combinatorial degradation of many cancer-causing proteins, Hsp90 inhibitors are able to attack all of the hallmark phenotypic traits of cancer cells and should have a reduced liability to the development of resistance compared to more conventional drugs. In this presentation, I will provide an update on the preclinical discovery and clinical development of Hsp90 inhibitors. In particular, I will describe what we have learned from the clinical experience with the first-in-class Hsp90 inhibitor 17-AAG. This is a derivative of the natural product geldanamycin which has been a pathfinder Hsp90 drug, demonstrating evidence of target inhibition and therapeutic activity in various cancers, including melanoma, breast, prostate and multiple myeloma. I will also describe our discovery of the new synthetic small molecule class of Hsp90 inhibitors based on the resorcinylic pyrazole and isoxazole scaffold Hsp90 and discuss their optimization by structure-based design in collaboration with Vernalis Limited. Finally, I will describe our recent work identifying genes and proteins that are involved in sensitivity to Hsp90 inhibitors and that have potential to act as biomarkers for clinical use.

6D_01_S

STRESS PROTEINS ARE INDUCERS OF ANTI-INFLAMMATORY REGULATORY T CELLS

Willem van Eden

Dept of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Yalelaan 1, 3584 CL Utrecht, The Netherlands. Email: w.vaneden@uu.nl

Especially since the (re-)discovery of T cell subpopulations with specialized regulatory activities, mechanisms of anti-inflammatory T cell regulation are studied very actively and are expected to lead to the development of novel immunotherapeutic approaches, especially in chronic inflammatory diseases. HSP are possible targets for regulatory T cells due to their enhanced expression in inflamed (stressed) tissues and the evidence that HSP induce anti-inflammatory immuno-regulatory T cell responses.
Initial evidence for an immuno-regulatory role of heat shock proteins (HSP) in chronic inflammation was obtained through analysis of T cell

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

responses in the rat model of adjuvant arthritis and the findings that HSP immunisations protected against the induction of various forms of autoimmune arthritis in rat and mouse models. Since then, immune reactivity to HSP was found to result from inflammation in various disease models and human inflammatory conditions, such as RA, diabetes type 1 and atherosclerosis.
Now, also in the light of a growing interest in T cell regulation, it is of interest to further explore the mechanisms through which HSP can be utilised to trigger immuno-regulatory pathways, capable of suppressing such a wide and diversified spectrum of inflammatory diseases.

6D_02_S

HEAT SHOCK PROTEINS AS IMMUNOMODULATORS

Salvo Albani

University of California San Diego, La Jolla, CA, USA

The focus in clinical research in rheumatoid arthritis is increasingly shifting toward early aggressive intervention to achieve remission or a state of low disease and to prevent the development of erosions and functional limitations. These objectives have been achieved in general by continuing therapies that are costly and have long-term side effects. The question remains on whether we can induce lasting immune tolerance and, if so, what mechanisms should we target? Are the drugs currently available sufficient to meet this goal and are we using them properly? The fact that DMARD withdrawal leads to relapses of active diseases in many patients indicates that complementary approaches aimed at maintaining tolerance are needed. A translational itinerary from idea to end of Phase II trial epitope specific T cell immune therapy will be discussed as a potential complementary therapeutic tool to currently existing therapies for RA. This approach relies on a mechanism of modulation of autoimmune inflammation based on immune recognition of heat shock protein derived epitopes.

6D_03_S

WHAT MAKES ARTERIAL ENDOTHELIAL CELLS A TARGET FOR THE AUTOIMMUNE ATTACK IN THE EARLIEST STAGES OF ATHEROSCLEROSIS?

Georg Wick

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Division of Experimental Pathophysiology and Immunology, Biocenter, Innsbruck Medical University Fritz-Pregl-Str. 3/IV, 6020 Innsbruck, Austria

The autoimmune hypothesis of atherogenesis postulates that preexisting cellular and humoral immunity to either microbial heat shock protein 60 (HSP60) or *bona fide* autoimmunity to biochemically altered autologous HSP60 leads to an attack on stressed arterial endothelial cells (ECs). We have shown in various animal models with spontaneously occurring autoimmune diseases that two essential sets of genes have to be present for an autoimmune disease to develop, i.e. genes that code for autoimmune reactivity of the immune system and genes that are responsible for target organ susceptibility. In the case of atherosclerosis, the arterial ECs express HSP60 that is also transported to the cell surface after being subjected to classical atherosclerosis risk-factors. We have shown the HSP60-inducing effect of most of these risk-factors, including mechanical stress (hypertension), oxygen radicals, oxidized low-density lipoproteins (oxLDL), proinflammatory cytokines (TNFα), and cigarette smoke constituents. Exposure to these classical atherosclerosis risk-factors entail the simultaneous expression of HSP60 and various adhesion molecules (ICAM-1, VCAM-1, P-selectin). Due to their lifelong mechanical pre-stress by the arterial blood pressure, arterial ECs have a lower threshold for the HSP60 inducing effect of atherosclerosis risk-factors as compared to venous ECs. However, when veins are subjected to arterial blood flow conditions, e.g. after arterial-venous bypass operations, HSP60 expression and intimal infiltration with mononuclear cells with subsequent restenosis occurs similar to the pathogenetic events in classical atherosclerosis.

This work was funded by the European Union MOLSTROKE (Molecular basis of vascular events leading to thrombotic stroke) project (LSHM-CT-2004-005206) and the European Vascular Genomics Network (EVGN) (LSHM-CT-2003-503254)

6D_04_S

MRNA BINDING PROTEINS ASSOCIATED WITH NOREPINEPHRINE AND CAMP-MEDIATED MRNA DECAY

Melissa Lajevic, Sujatha Kodavayur, Lloyd Graf, Rhonna L. Cohen and Donald A. Chambers

University of Illinois College of Medicine, 1853 W. Polk St., Chicago IL, 60612, USA, rcohen@uic.edu

Psychogenic stress is associated with norepinephrine (NE) release and immune dysfunction. We have shown that NE increases the rate of Thy-1

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

mRNA decay in S49 T lymphoma cells through a classical β_2AR/AC/cAMP/PKA pathway. The Thy-1 mRNA sequence contains an ARE in its 3'UTR, a sequence commonly associated with message stability. In this study we use a 116 base pair Thy-1 ARE probe to identify by photoaffinity binding or biotinylation mRNA binding proteins (bps), determine if NE alters binding, and investigate associations between PKA substrate phosphorylation and the decay mechanism. RNA-protein binding experiments using Thy-1 ARE and S49 protein extract revealed binding of at least 10 proteins including, AUF1, HuR, TIAR, KSRP, and Hsc70. NE treatment of wt or PKA mutant cells had no effect on the ability of these proteins to bind to the ARE. NE induced phosphorylation of PKA substrates in wt cytoplasmic extract identified by immunoblotting a variety of proteins of molecular weights of 35-10.kD. Thy-1 ARE binding proteins, AUF1, Hsc70, and HuR were detected in phosphoprotein isolated from wt whole cell extract by affinity chromatography and AUF1 may be a PKA substrate. In addition, a pull down assay using a biotinylated ARE rich Thy-1 probe and ^{32}Pi metabolically labeled protein isolated at least five phospho-binding proteins, two of which, 60 kD and 105 kD, increased in phosphate incorporation after NE treatment. These results characterize mRNA bps and target phosphoproteins that may relate to NE-mediated Thy-1 mRNA decay. Supported by NIH

6E_01_S

PSYCHOSOCIAL FACTORS IN THE METABOLIC SYNDROME

Eric Brunner

Department of Epidemiology and Public Health, University College London, WC1E 6BT, UK
e.brunner@ucl.ac.uk

The cluster of cardiovascular risk factors labeled the metabolic syndrome is linked with low social status. Prevalence studies show a stepwise relationship - the lower the social position, the greater the probability the syndrome will be present. Systematic differences in diet and physical activity contribute to social patterning of the syndrome. In addition, psychosocial factors including chronic work stress are linked with its development. The Whitehall II study, set up in 1985 to study the psychosocial, behavioral and biological causes of health inequalities in an office-based cohort of 10,308 adults, obtained repeat measures of job stress using a standard self-report questionnaire (Karasek-Theorell job strain questionnaire). Components of the metabolic syndrome (waist circumference, BP, fasting glucose, serum HDL-cholesterol and triglycerides) were measured after 14 years of follow-up. A dose-response relation was found between exposure to job stress and risk of ATPIII

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

metabolic syndrome which remained after adjustment for age and socioeconomic status (OR=2.25 (95% CI 1.3-3.9)). A second prospective study showed a similar dose-response relation with general (BMI) obesity and central obesity. These findings add to other evidence that psychosocial stressors from everyday life are linked to coronary risk. Cross-sectional studies link the metabolic syndrome to adverse neuroendocrine and autonomic activity (increased urinary cortisol and noradrenaline metabolite outputs, and decreased heart rate variability). The metabolic syndrome is a valuable research concept for studying population health and social-biological translation.

6E_02_S

NEUROENDOCRINE MECHANISMS IN THE METABOLIC SYNDROME

Jan W Eriksson

Sahlgrenska University Hospital, Gothenburg and AstraZeneca R&D, Molndal, Sweden
E-mail address: jan.eriksson@medic.gu.se , jan.eriksson@astrazeneca.com

Abstract
Psychosocial stress is now considered to be an important risk factor for metabolic syndrome and type 2 diabetes, and development of insulin resistance can be a common denominator. Insulin resistance can be described as an impaired insulin action in target tissues, i.e. muscle, fat and liver. In muscle, insulin-stimulated transmembrane glucose transport and the first step in intracellar glucose metabolism, i.e. glucose-6-phosphorylation, appear to be rate-limiting defects. In adipose tissue, insulin resistance is manifested as impaired glucose uptake but also as an impaired suppression of lipolysis and, in addition, there may potentially be dysregulated production and secretion of adipokines and other adipose-derived biomolecules. In the liver, there is attenuated insulin action with respect to glucose uptake and storage as well as suppression of glucose production.
Type 2 diabetes is in most cases caused by a combination of beta cell dysfunction and insulin resistance. Physical inactivity, adiposity due to overeating, stress and smoking are risk factors that interact with susceptibility genes in the development of the disease. The metabolic syndrome is often used to define a cluster of risk markers that predict cardiovascular disease but also type 2 diabetes.
There is no consensus about a unifying underlying mechanism in insulin resistance. However, it is now generally accepted that central adiposity plays a key role in the metabolic syndrome and it is linked to insulin resistance and risk for type 2 diabetes as well as cardiovascular disease. Dysregulated neuroendocrine signalling in adipose tissue can contribute to

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

the development of insulin resistance and metabolic syndrome, and such pathways can mediate the metabolic effect of stress responses that are evoked in the CNS. An overview of the field will be given and recent results will be presented. Topics will include the links between neuroendocrine dysregulation and visceral fat accumulation, fat cell metabolism, alterations in fat cell recruitment, differentiation and growth as well as adipokine production.
Recent data will be presented with focus on the the interplay between different neuroendocrine sysems - the cortisol axis, sex hormones, the autonomic nervous system, catecholamines and the renin-angiotensin system. A hypothesis will be presented about a common 'final pathway' that might merge the insulin-antagonistic actions of hormones, inflammation, glucose and lipids, namely the generation of excess reactive oxygen species (ROS). Potential pharmacological interventions targeting the different signalling pathways, including oxidative stress, will also be discussed.

6E_03_S

ROLE OF STRESS RESPONSE GENES, INCLUDING HO-1, IN TYPE 1 DIABETES AND DIABETIC COMPLICATIONS

Nader G. Abraham

Director of Stem Cells and Gene Therapy, New York Medical College, Valhalla, New York 10595

Hyperglycemic episodes, which cause vascular complications as a result of oxidative stress and complicate even well controlled cases of diabetes, are closely associated with the development of vascular disease. Several antioxidants and stress response genes have been shown to partially protect the vascular system. However, the continuous generation of O_2^- via heme-dependent enzymes, including NADPH oxidase and mitochondrial cytochrome, has shown that such a strategy is difficult to achieve and problematic in diabetes. Heme oxygenase (HO-1) is the sole enzyme that degrades heme and subsequently regulates NADPH oxidase and the cytochrome oxidase system and O_2^- formation via increases in bilirubin generation and ferritin synthesis, which are anti-oxidants, and CO, which is anti-apoptotic. In an effort to overcome this impasse, we examined molecular (gene transfer) and pharmacological (cobalt protoporphyrin, CoPP) approaches to increased HO-1/HO-2 expression in providing cardiovascular protection in Type I diabetic animal models. Upregulation of HO-1 expression attenuates hyperglycemia-mediated increases in vascular dysfunction, circulating endothelial cells and fragmentation, decreases O_2^-, heme levels and iNOS, but increases EC-SOD. Translocation of HO-1 to the mitochondrial compartments enhances

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

cytochrome oxidase and anti-apoptotic molecules, and decreases cytochrome C release, suggesting that HO-1 may regulate mitochondrial pro-apoptotic and anti-apoptotic proteins. Using the loss-of-function, HO-2(-/-) and gain-of-function strategy, we will present data to document that HO-2 siRNA treatment decreased basal levels of EC-SOD and phosphorylated Akt proteins (serine-473 and threonine-308), although no change in Akt protein expression was observed. HO-2 siRNA was also associated with an increase in 3-nitrotyrosine (3-NT) and apoptotic signaling kinase-1 (ASK-1). Additionally, we will present data to show that an increase in HO-1/HO-2 levels, i.e., CO and bilirubin, has a salutary effect, modulating the vascular phenotype, as reflected by the increases in anti-apoptotic proteins, thus rendering endothelial cells resistant to oxidant stress hence preventing vascular dysfunction and the development of Type 1 diabetes.

6E_04_S

TYPE 2 DIABETES, HYPERGLYCEMIA, AND CHRONIC OXIDATIVE STRESS

R. Paul Robertson, The Pacific Northwest Research Institute, Seattle, WA, USA

The pathogenesis of type 2 diabetes, also known as adult-onset diabetes, is usually attributed to a combination of pancreatic islet beta cell dysfunction and resistance to the action of insulin in important targets, such as liver, muscle, and fat tissue. The cause of beta cell failure is polygenic in nature, whereas the cause of insulin resistance is at least partially explained by associated obesity. It is important to note, however, that many type 2 diabetic people are not obese, and that the majority of obese individuals do not develop type 2 diabetes. Thus, it appears that type 2 diabetes is primarily a genetic disease that can be made worse, but is not caused, by excessive body fat.

A major issue in the field of type 2 diabetes research is why this disease is often characterized by a continual and inexorable decline in glucose control despite optimal drug treatment. This decline in beta cell function is associated with chronically elevated blood glucose levels and has led to the notion of beta cell exhaustion because of continual stimulation by glucose, on the one hand, and the possibility that high concentrations of glucose are chemically toxic to the beta cell on the other. There is an intrinsic paradox at play in these considerations. Since glucose is considered to be a physiologic compound and supportive of beta cell function at many levels from insulin gene transcription through insulin secretion, shouldn’t high glucose levels stimulate insulin synthesis and increase insulin stores rather than lead to exhaustion of the beta cell?

One concept that has emerged is that of glucose toxicity, i.e. chronically

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

high glucose levels form metabolites that can be harmful. This has led to the idea that glucose toxicity of the beta cell might be attributable to formation of excess levels of reactive oxygen species. Glucose excess can promote increased generation of ROS through several metabolic pathways. One can envision that the normal route of glycolysis and oxidative phosphorylation might become oversaturated with glucose. This in turn might lead to shunting excess traffic of glucose molecules along any of several alternative routes, including methylglyoxal formation and glycation; enediol and □-ketoaldehyde formaton (glucoxidation); diacylglycerol formation and protein kinase C activation; glucosamine formation and hexosamine metabolism; and sorbitol metabolism (1). ROS are normally generated along all these pathways, including oxidative phosphorylation. One need only to imagine a flooding of all these pathways by glucose as a mechanism for excessively high concentrations of ROS in many tissues, including pancreatic beta cells.
This pathophysiologic construction suggests that ROS, which like glucose function as positive chemical mediators in physiologic processes, become negative forces when they are present in excess concentrations. Both ROS and glucose are seen as having good and evil sides, depending on whether their levels are normal or excessive. This general consideration has led to the term glucose toxicity with a major mechanism of action being chronic oxidative stress.

6E_05_S

HSP72 PROTECTS AGAINST OBESITY-INDUCED INSULIN RESISTANCE

Mark A. Febbraio

Baker Heart Research Institute, Melbourne, Australia. Email: ***mark.febbraio@baker.edu.au***

Patients with type 2 diabetes have reduced gene expression of Heat Shock Protein (HSP) 72 which correlates with reduced insulin sensitivity. Heat therapy, which activates HSP72, improves clinical parameters in these patients. Activation of several inflammatory signaling proteins such as c-jun amino terminal kinase (JNK), inhibitor of κB kinase and tumor necrosis factor-α can induce insulin resistance, but HSP 72 can block the induction of these molecules *in vitro*. Accordingly, we examined whether activation of HSP72 can protect against the development of insulin resistance. We first show that obese, insulin resistant humans have reduced HSP72 protein expression and increased JNK phosphorylation in skeletal muscle. We next utilized heat shock therapy, transgenic overexpression, and pharmacologic means to overexpress HSP72 either specifically in skeletal

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

muscle or globally in mice. Herein we show that regardless of the means used to achieve an elevation in HSP72 protein, protection against diet or obesity-induced hyperglycemia, hyperinsulinemia, glucose intolerance and insulin resistance was observed. This protection was tightly associated with the prevention of JNK phosphorylation. These findings identify an essential role for HSP72 in blocking inflammation and preventing insulin resistance in the context of genetic obesity or high fat feeding.

6F_01_S

LEUKOCYTE REPROGRAMMING AFTER ENDOTOXIN ENCOUNTER

Jean-Marc Cavaillon

Unit Cytokines & Inflammation, Institut Pasteur, Paris.
jmcavail@pasteur.fr

Stressfull situation associated with sepsis and non-infectious systemic inflammatory response syndrome (SIRS) such as trauma, surgery, haemorrhage, or ischemia/reperfusion, is associated with a profound alteration of immune status that could explain the increased sensisitivity of the patients to nosocomial infections. Endotoxin (lipopolysaccharide, LPS) is often found in plasma of sepsis patients, and endotoxin translocation from the gut frequently occurs in SIRS patients. During sepsis and SIRS, pro-inflammatory cytokines and inflammatory mediators contribute in synergy to tissue injury, organ dysfunction, and possibly to lethality. To dampen this overzealous process, a counter regulatory loop exists, involving anti-inflammatory cytokines and neuromediators that can also alter the immune status. In sepsis and SIRS patients, a reduced *ex vivo* pro-inflammatory cytokine production, particularly tumor necrosis factor (TNF), in response to LPS and other Toll-like receptor (TLR) agonists has been reported. However, cells remain responsive to whole bacteria, and their capacity to produce anti-inflammatory cytokine is maintained or even enhanced. Thus the terms "leukocyte reprogramming" well define the process. Modification of intracellular signaling are presently deciphered in patients' leukocytes and some aspects recall what is known about "endotoxin tolerance". The reduced capacity of circulating monocytes to produce TNF and other cytokines can be mimicked *in vitro* by a pre-treatment of monocytes or macrophages with lipolysaccharide (LPS). This is not a specific phenomenon and it can be induced by other agents or events. Cross-tolerance between LPS, TLR2, 4, 5, or 9 specific ligands, and TNF has been reported. It is possible that cross-tolerance is induced by microbial and endogenous (alarmins) signals of danger.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

6F_02_S

ACYLOXYACYL HYDROLASE SHORTENS THE DURATION OF POST-INFECTION IMMUNOSUPPRESSION

Mingfang Lu and Robert S. Munford

University of Texas Southwestern Medical Center, Dallas, Texas, USA 75390-9113 Mingfang.Lu@utsouthwestern.edu
Robert.munford@utsouthwestern.edu

Serious tissue infections may be followed by a period during which the host animal is much more susceptible to infection with the same or other microbes. One experimental model for this clinical phenomenon is endotoxin tolerance or reprogramming. For several days after receiving a low parenteral dose of Gram-negative bacteria or their cell wall lipopolysaccharide (LPS, endotoxin), many of an animals' reactions to LPS and other microbial agonists are "reprogrammed" so that proinflammatory responses are reduced while anti-inflammatory ones are maintained or increased. Whereas the adaptive function of this stress reaction may be to prevent harmful inflammation-induced damage to the host, it is often also immunosuppressive. We found that deacylation of LPS by a host lipase, acyloxyacyl hydrolase (AOAH), is required to terminate LPS-induced immunosuppression in mice. Whereas wildtype mice recover within 10 days after receiving 10 µg LPS intraperitoneally, mice that lack AOAH remain immunosuppressed for at least one month. The peritoneal cells in *Aoah*$^{-/-}$ mice retain intact LPS for much of this time, their proinflammatory responses to LPS (a TLR4 agonist) and *Micrococcus luteus* (a TLR2 agonist) are blunted, and their ability to kill Gram-negative bacteria is impaired. By extinguishing the LPS signal, AOAH seems to help the body "re-load" its defensive armamentarium to fight another infection.

6F_03_S

REPROGRAMMING OF MACROPHAGES BY ENDOTOXIN TOLERANCE

Schade, F. Ulrich, Butterbach, Katja, Plitzko, Daniela

Surgical Research - Trauma Surgery, University Hospital Essen, D-45122 Essen, Germany
ulrich.schade@uni-due.de

The term "Endotoxin Tolerance" (ET) has been introduced for a state of low responsiveness of experimental animals or humans treated with a low dose of lipopolysaccharide (LPS, endotoxin). ET has attracted a lot of

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

attention since it was shown that cross tolerance exists to other bacterial products such as lipoteichoic acid or lipopeptides. Furthermore, it turned out that ET protected against bacterial infections - Gram-negative, as well as Gram-positive - and ischemia-reperfusion injury. It, therefore, is of potential clinical interest to get insight into the cellular and molecular basis of ET. To this end, we studied different cellular components of the peritoneal cell populations and Kupffer cells of endotoxin tolerant and normal mice with regard to gene expression, intracellular signal transduction and regulation of cytokine production. Corresponding results will be presented. HSP 70 is among the genes that are significantly upregulated in peritoneal cells of ET mice compared to normal mice.

6F_04_S

IMMUNO-NEURO-ENDOCRINE ADAPTATION IN CRITICAL ILLNESS

I.Vermes, A.Beishuizen and A.B. J.Groeneveld

Medical Spectrum Twente, Enschede & Free University Medical Centre Amsterdam,The Netherlands (i.vermes@home.nl)

Critical illness is a severe stress stimulus that disturbs the milieu interior and induces homeostatic responses specific to the stimulus and generalized responses when the disturbances are prolonged and severe. The immune-neuroendocrine response during critical illness is a dynamic process, differing between the acute and chronic phases. This acute phase lasts typically from about a few hours to several days, depending on the severity of the illness and is characterized by an appropriate hormonal reaction: the mobilization of fuel stores of the organism, together with apparent restrains on their utilization. During the acute phase of critical illness the secretory activity of the pituitary gland is stimulated whereas anabolic target organ hormones are inactivated. Due to the development of intensive care medicine patients who would previously have died during the acute phase nowadays survive and enter the chronic or prolonged phase of the critical illness. This prolonged phase lasts for days and is characterized by an inappropriate hormonal response, resulting in a chronic increase in metabolic rate and a breakdown of body tissue. The secretory activity of the pituitary gland is uniformly inhibited in relation to reduced levels of target organ hormones. These hormonal changes suggest an imbalance between immunosuppressive and immunostimulatory hormones which might be the cause of the increased susceptibility to infectious complications during the chronic phase of severe illness. The suppressed pituitary activity allows the respective target organ hormones to decrease, resulting in a restored balance between the catabolic and anabolic hormonal responses. This recovery phase is characterized by restored sensitivity of the pituitary gland to

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

reduced feedback control. The distinction of acute and prolonged critical illness as different entities with regard to the immuno-neuroendocrine adaptation may be helpful in further understanding of the pathogenesis of critical illness and the targeting of therapeutic intervention.

6F_05_S

IN VIVO IMAGING OF THE EFFECT OF BACTERIAL ENDOTOXINS ON ARTERIAL ENDOTHELIAL CELLS: MOLECULAR IMAGING OF HEAT SHOCK PROTEIN 60 EXPRESSION

Marius C. Wick[1], Christina Mayerl[2], Georg Dobrozemsky[3], Roland Haubner[3], Hermann Dietrich[4], Werner Jaschke[1], and Georg Wick[2]

[1]Department of Radiology, [2]Division for Experimental Pathophysiology and Immunology, Biocenter, [3]Department of Nuclear Medicine, [4]Central Animal Laboratory Facilities,Innsbruck Medical University, Austria

Background. Bacterial endotoxins (LPS) are known to act as stress factors for endothelial cells.
Aims. As a proof of principle, the present project aimed at developing a novel radiotracer-based non-invasive molecular imaging method for *in vivo* visualization of early aortal HSP60-expression in a normocholesterolemic rabbit model after induction of endothelial stress.
Material and Methods. In 14 rabbits, endothelial stress was induced by i.v. injection of bacterial endotoxin (lipopolysaccharide, LPS at 10μg/kg) while 8 animals were untreated controls. Non-invasive *in vivo* molecular imaging was performed 24 hours after intravenous injection of I-124 radiolabelled monoclonal anti-HSP60 antibodies using computertomography (CT) and positron-emission-tomography (PET). *In vitro* correlation of *in vivo* imaging was done by *en face* immunohistochemistry and autoradiography of the aorta.
Results. Compared to control animals, quantitative analyses of *in vivo* non-invasive molecular PET-CT images of rabbit aortae revealed a significantly increased endothelial binding of I-124 anti-HSP60 antibodies upon application of LPS as an endothelial stressor, especially at sites of aortal branching. This was confirmed by *in vitro* anti-HSP60 immunohistochemistry and autoradiography data.
Conclusion. Our first results showed, as a proof of principle, that HSP60-expression in normocholesterolemic rabbits is significantly increased after the induction of endothelial stress and non-invasive *in vivo* molecular imaging of early aortal HSP60-expression using radiotracer labelled anti-HSP60 antibodies is possible.
Acknowledgements: *This work was supported by the Propter Homines Foundation, Vaduz, FL.*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

6G_01_S

STRESS AND PRIONS: LESSONS FROM THE YEAST MODEL

Yury O. Chernoff

School of Biology and Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Altlanta, Georgia 30332-0230, USA; yury.chernoff@biology.gatech.edu

Amyloids are fiber-like ordered aggregates generated via intermolecular cross-ß interactions. *In vivo* amyloid formation is a widespread phenomenon in eukaryotes. Self-perpetuating amyloids provide a basis for the transmissible protein isoforms (prions) that cause infectious neurodegenerative diseases in mammals (including humans) and manifest themselves as non-Mendelian heritable elements in yeast and other fungi. Propagation of the prion state in yeast is controlled by the concerted action of chaperone proteins. A crucial role in this process is played by the chaperone protein Hsp104, which promotes breakage of amyloids into smaller oligomeric seeds initiating new rounds of prion proliferation. Prion formation and propagation are also influenced by other stress-related chaperones (Hsp70 and Hsp40), and by alterations of the protein trafficking and degradation networks. Therefore, prion propagation employs enzymatic machinery which is normally supposed to protect cells from environmental stresses. Some stresses induce prion formation or loss. It is possible that prions arise as by-products of the reversible assembly of highly ordered complexes, protecting certain proteins from destruction during unfavorable conditions.

6G_02_S

INTRACELLULAR FATE OF MISFOLDED PROTEINS ASSOCIATED WITH RETINAL DEGENERATION

Shalesh Kaushal, Syed M. Noorwez

University of Florida, Departments of Ophthalmology and Cell Biology, Gainesville, Florida, USA, skaushal@ufl.edu

Misfolded proteins are found in many inherited diseases, which collectively are called protein conformational disorders. These misfolded proteins, typically containing one or more mutations, are retained intracellularly in

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

the endoplasmic reticulum or in cytoplasmic aggregates. Our previous studies clearly demonstrated that the clinically common P23H opsin mutant associated with autosomal dominant retinitis pigmentosa is misfolded and retained in the cell. Pharmacological induction of the heat shock response has emerged as an attractive strategy for modulating the cellular folding environment and treating protein conformational diseases. The induced ensemble of heat shock proteins act as molecular chaperones in protein folding and protect against the formation of misfolded protein aggregates. Using a tetracycline-inducible HEK293 cell lines, we have studied the effect of the heat shock response on P23H opsin and folded rhodopsin levels in the cell. The known chemical inducers of the heat shock response, geldanamycin (GA) and celastrol, increase the total opsin levels by 1.8- and 1.6-fold (n=3), respectively. Further, GA and celastrol reproducibly increase folded rhodopsin levels by 1.7- and 1.4-fold (n=3). The level of hsp70 in the treated cells was elevated by 4 fold by GA and 1.2-fold with celastrol. Both treatments appear to induce the heat shock response and stabilize the folded form of opsin i.e. the form capable of binding retinal. Further, the extent of hsp70 increase correlates with te yields of folded P23H opsin. Thus, heat shock induction clearly alters the cellular stability and fate of P23H opsin. Both compounds are attractive candidates for treating P23H opsin associated retinal degeneration.

6G_03_S

MISFOLDED PROTEINS IN AGING AND NEURODEGENERATIVE DISEASE

Cindy Voisine

Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL, USA
c-voisine@northwestern.edu

Misfolded and damaged proteins challenge homeostasis and the capacity of molecular chaperones and clearance machineries to restore the health of the cell. Whereas acute exposures to heat shock and other environment stress leads to the induction of cytoprotective responses, the chronic expression of misfolded and mutant proteins can result in irreversible toxicity as occurs in human neurodegenerative disease. We are interested in understanding how an organism and specific populations of cells detect and respond to diverse types of proteotoxic stress. We have established *C. elegans* models to identify the genes that regulate protein homeostasis in response to chronic expression of aggregation-prone proteins associated with neurodegenerative disease. These studies have identified an important molecular link between the insulin-signaling pathway that regulates longevity and HSF-1, the master regulator of protein folding

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

quality control and the heat shock response, revealing that longevity is closely linked to the cellular folding capacity. Using forward genetics, genome-wide RNAi screens and a candidate gene approach, we have identified a functionally diverse set of genes that sort into six networks involved in RNA metabolism, protein synthesis, protein folding, protein degradation, protein trafficking, and mitochondrial function and energy metabolism that influence protein homeostasis. Many of these genes are also important for the regulation of heat shock gene expression which suggests that genetic modifiers for protein quality control defines a protein-folding proteome that also functions to sense an imbalance in protein homeostasis and regulation of chaperone expression. Using our *C. elegans* models, we are characterizing key Hsp70 chaperone networks that coordinately function to protect cells during aging and in response to misfolded disease proteins.

6G_04_S

CHAPEROME SYSTEMS AND PROTEIN MISFOLDING STRESS IN TRAFFICKING DISEASE

William E. Balch[1,2,6], Atanas Koulov[1], Darren Hutt[1], Bogdan Tanasa[1], Wendy Kellner[1], Helen Plutner[1], Paul Lapointe[1], Scott Stagg[1], Clint S. Potter[1,5], Bridget Carragher[1,5], Jeffery W. Kelly[3,4] and John R. Yates III[1]

The Scripps Research Institute, Departments of Cell[1] and Molecular Biology[2],and Chemistry[3], The Skaggs Institute for Chemical Biology[4], The National Resource for Automated Molecular Microscopy[5], and The Institute for Childhood and Neglected Diseases[6], 10550 North Torrey Pines Road, La Jolla, CA 92037.

Protein folding in the eukaryotic cell is highly sensitive to the local environment of the cytoplasm or membrane trafficking compartments, and requires the assistance of multiple chaperones that define the folding buffer of the cell- the chaperome (Wang et al. (2006) Cell, 127:803). Different cell types exploit the variable composition of the chaperome environment to maintain protein folding homeostasis- reflecting the kinetic and thermodynamic properties of the protein fold. Perturbation of this relationship, as occurs in misfolding diseases such as cystic fibrosis (CFTR), Gaucher's (glucocerebrosidase), childhood emphysema (alpha-1-antitrypsin), type II diabetes and numerous amyloid diseases (Alzheimers (APP), Parkinsons (alpha-synuclein)), results in an imbalance between the protein fold and the folding environment leading to disruption of normal physiology. It is becoming increasingly apparent that folding pathways in aging and disease can be altered by the cell through stress sensitive pathways to maintain or reestablish the proper chaperome environment to maintain functionality. Emerging mechanisms by which these activities

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

work provides insight into the pathways that can be manipulated through biological and chemical approaches to adjust the flow of folded and functional protein through the exocytic and endocytic pathways.

6H_01_S

THE NEURAL BASES OF NEGATIVE AFFECT AND STRESS RESPONSES IN SCHIZOPHRENIA

Ute Habel

Department of Psychiatry and Psychotherapy, RWTH Aachen University, uhabel@ukaachen.de

Stress has a major impact on the course and psychopathology of schizophrenia, having significant influences especially on emotion but also cognitive processes. In schizophrenia, emotion dysfunctions are a hallmark with a special role of negative affect and reduced capacities to manage stressful situations and stimulations. Data acquired from different samples of healthy subjects and schizophrenia patients will be presented to clarify the neural basis of dysfunctional emotion processes applying fMRI. Emotion and cognition are mostly investigated as different entities, while practically both functions are inseparable and are interacting with each other continuously. However, interactions between emotion and cognition have not been investigated in greater detail in patients. Similarly, in healthy persons there are only a few and rather contradictory studies exploring the general effects of emotion on cognitive function. We developed and validated tasks investigating the interplay between emotions and cognition. Stress was induced in patients and comparison subjects by means of negative olfactory stimulation while subjects had to perform a combined 0-back/2-back task. A control condition with neutral room air stimulation was also applied. According to subjective ratings mood induction was successful. The impairing effect of the negative mood induction was visible during working memory performance (2-back) only, in which the mean number of correct target reactions was significantly lower during negative olfactory stimulation as compared to the neutral condition. The neuroimaging data reveal a complex dysfunctional interaction in patients, where especially frontal and cingular regions show aberrant activation patterns. Hence, regions of major importance in emotion regulation and integration are affected.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

6H_02_S

STRESS, DOPAMINE, BRAIN TISSUE VOLUMES AND VULNERABILITY TO PSYCHOSIS

Machteld Marcelis, E. Cavalier, John Suckling, Paul Hofman, Philippe Delespaul, Jim van Os

Dept of Psychiatry & Neuropsychology, POBox 616, 6200 MD, Maastricht University, The Netherlands m.marcelis@sp.unimaas.nl

The present study investigated whether individuals with schizophrenia, and those with an elevated genetic risk for schizophrenia, display an altered plasma HVA-response to metabolic stress. Besides, associations between the metabolic stress response and brain tissue volumes were examined. Patients with psychosis (n=50), non-psychotic first-degree relatives of patients with psychosis (n=51) and controls (n=50) underwent, in randomised order, double-blind administration of placebo and the glucose analog 2-deoxy-D-glucose (2DG), which induces a mild, transient clinical state of glucoprivation. Plasma HVA was assessed twice before the start of the 2DG/placebo infusion (baseline), as well as four times post infusion. MRI cerebral tissue volumes were derived from automated segmentation procedures. During the stress condition, significant increases in plasma HVA were found. The increase in plasma HVA level during the stress condition was significantly stronger in patients than in controls, whereas this was not the case in relatives v. controls. The HVA level increase in the stress condition was stronger in patients with lower grey and white matter volumes. In conclusion, patients with psychosis, but not their non-psychotic relatives, show an altered dopaminergic/noradrenergic mediated stress response, possibly reflecting acquired sensitization of catecholamine systems by repeated environmental stressors or repeated stimulation with agonistic drugs. HVA level increases were stronger in patients with lower grey and white matter volumes, supporting the hypothesis that alterations in cortico-subcortical connections affect psychosis susceptibility through an altered stress response.

6H_03_S

STRUCTURAL BRAIN CHANGES UNDERLYING VULNERABILITY TO SCHIZOPHRENIA

Michio Suzuki[1,2], Tsutomu Takahashi[1,2], Shi-Yu Zhou[1,2,3], Yasuhiro Kawasaki[1,2], Masayoshi Kurachi[1,2]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[1]Department of Neuropsychiatry, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan, [2]CREST, JST, Tokyo, Japan, [3]Department of Psychiatry and Medical Psychology, Dalian Medical University, Dalian, China

Morphologic changes in schizophrenia are thought to represent a complex and dynamic process in which multiple brain regions are differentially affected. Common neurobiological abnormalities among the schizophrenia-spectrum may be essential for the pathogenesis of schizophrenia, but some additional pathological changes may also be required for the development of full-blown schizophrenia. Clarifying the neurobiological similarities and differences between established schizophrenia and schizotypal (personality) disorder, a schizophrenia-spectrum disorder without manifestation of overt and sustained psychosis, would potentially discriminate the pathophysiological mechanisms underlying the vulnerability to schizophrenia from those associated with overt psychosis. Detailed volumetric comparisons using magnetic resonance imaging (MRI) revealed differential morphologic alterations in the brain between the patients with schizotypal disorder and those with schizophrenia. Volume reductions in the amygdala, hippocampus, superior temporal gyrus, and anterior parietal cortex common to both patient groups may represent the vulnerability to schizophrenia. Volume loss of the prefrontal cortex, posterior parietal cortex, cingulate, insula, and fusiform cortex preferentially observed in schizophrenia may be critical for overt manifestation of psychosis. On the other hand, preserved volume in these regions might have relevance to the protection factors from overt psychosis in schizotypal disorder.

6H_04_S

EARLY EMOTIONAL STRESS BY ANALYSIS OF STRESS LINE IN MOLAR AND SUSCEPTIBILITY TO SCHIZOPHRENIA

Kunio Yui [1, 2)], Koichi Watanabe[3)], Masayoshi Kobayashi[3)], Koichi Nishijima[4)]

[1)] Division of Human Science, Kansai University of International Studies
[2)] Department of Psychiatry and Neurology, Kobe University, Graduate School of Medicine
[3)] X-ray Microanalyzer section, Center for Instrumental Analysis, Niigata University
[4)] Department of Psychiatry, Jichi Medical School

[Introduction]: It is well known that early life events or urbanicity, that interacts with multiple genes, induces persistent sensitization to stress

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

possibly through an imbalance in interactions between dopaminergic and glutamatergic systems. This stress sensitization may be critical in the development or relapse of schizophrenia. Laboratory method for estimating early stress is therefore needed. Ameloblast activity in human molar's enamel is slowed during 1-2 days extreme stress, and the segment of enamel rods is smaller and often misshapen, making a particular dark line seen by microscopy, which we called Pathological Stress Line (PSL). We studied the nature of stress sensitization by analysis of PSL. According to animal and clinical studies, severe emotional stress induce changes of mineral density in bone. Thus, to clarify the type of stress related to PSL, we examine mineral changes in PSL portions.
[Methods]: We examined PSL in third molar in 35 chronic paranoid schizophrenics (25 males and 10 females, 41.9±13.5 yeas old) and 32 normal controls (5 males and 27 female, 28.3±9.1 years old). Changes of density in potassium (P), calcium (Ca) and magnesium (Mg) in PSL portion were examined by Scanning Microscope and Electron Probe Microanalyser (EPMA). Since the rate of enamel elongate is well known, PSL was assessed due to its length and definition at half yearly between 9 to 13 years old: 0, none; 1, slight; 2, mild; 3, moderate; 4, severe. Mineral changes were assessed in PSL portions rated as 2-4 due to extent of changes of mineral density in one area (1.5 X 1.5 mm): 0, none; 1, 1/4; 2, 1/3; 3, 1/2, 4, 2/3.
[Results]: PSL scores in the 35 schizophrenics were significantly higher than the 32 normal controls (4.8±5.1 vs. 2.0±2.3, $P<0.01$). The 35 schizophrenics exhibited PSL indicative of stressors experienced at age of 10.5-11.5 years old. Scores in P and Ca were significantly decreased and those of Mg were significantly increased in PSL portions.
[Conclusion]: The present findings suggest that stress sensitization may be induced at age of 10.5-11.5 years, which is comparable to previous studies indicating that stress exposed at age of 10-13 years may be related to the development of adulthood schizophrenia. Decreased density of P and Ca and increased density of Mg have been reported to be caused by severe emotional stress. The findings on EPMA suggest that severe emotional stress at age of 10.5-11.5 years may induce stress sensitization.

6H_05_S

PSYCHOSIS IN CORRELATION WITH CHRONIC STRESS AND VASOPRESSIN

Dóra Zelena

Institute of Experimental Medicine, Hungarian Academy of Sciences, 1083 Budapest, Szigony 43, Hungary, zelena@koki.hu

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

The role of stress in the development and maintenance of symptoms in major psychiatric disorders such as schizophrenia is well-established. The biological component of stress is mediated largely through the endocrine system, predominantly by the hypothalamo-pituitary-adrenal (HPA) axis. The two main hypothalamic secretagogues of the axis are CRH and vasopressin with more important role of the latter during chronic stress. Vasopressinergic dysfunction in schizophrenics was also indicated and the naturally vasopressin mutant Brattleboro rats was suggested to be a good model for schizophrenia. Therefore we addressed here the question if the lack of vasopressin in Brattleboro rats will lead to disturbances in chronic stress-induced HPA axis changes parallel with the development of schizophrenia. The classical somatic chronic stress symptoms (body weight reduction, thymus involution, adrenal gland hypertrophy) and also the signs of HPA axis hyperactivity (resting corticosterone elevation and POMC mRNA elevation in the anterior lobe of the pituitary) were present in all three studied situation (repeated restraint, streptozotocin-induced diabetes mellitus, repeated morphine injections) with similar extent in control and vasopressin deficient rats disclosing the involvement of V1b receptors in the process. On the other hand vasopressin as a neurotransmitter may act on other brain regions and there are many compensatory mechanisms in Brattleboro rats (e.g. oxytocin, CRH). Moreover both the adrenomedullary system and the sympathetic nerves are more active in vasopressin deficient rats. So our conclusion is that vasopressin may act on the development of schizophrenia through influencing other neurotransmitters in brain and not the HPA axis. Chronic stress may exacerbate the schizophrenia not through HPA axis changes but e.g. glucocorticoid toxicity in hippocampus.

6I_01_S

STRESS AND THE GUT: ROLE OF CORTICOTROPIN RELEASING FACTOR (CRF) SIGNALING PATHWAYS

Y. Taché

Center for Neurovisceral Sciences, Department of Medicine, University of California, Los Angeles, CA, USA

The characterization and distribution of corticotropin-releasing factor (CRF) family of peptides, CRF, urocortin 1, urocortin 2 and urocortin 3, and the two G-protein coupled receptors, CRF_1 and CRF_2, as well as the development of selective CRF_1 and CRF_2 receptor antagonists provided novel means to understand mechanisms involve in the stress response. The activation of brain CRF_1 signaling pathway plays a primary role in the endocrine (activation of pituitary adrenal axis), behavioral (anxiety,

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

depression), autonomic (sympathetic activation), and decrease immune responses to stress. Combined anatomical, pharmacologic and molecular approaches support a role of CRF receptor activation in both the brain and the gut as part of key mechanisms involved in stress-related alterations of gut propulsive function. Inhibition of gastric emptying and stimulation of colonic motor function are the commonly encountered patterns resulting from exposure to various stressors in experimental animals and humans. Activation of brain and peripheral CRF_2 receptors mediates stress-related inhibition gastric motor function while that of CRF_1 receptors are involved in the stimulation colonic secretory and motor functions. Clinical investigations also support the notion that stress contributes to visceral hypersensitivity of the gut observed in patients with irritable bowel syndrome (IBS) as established by their lowered threshold of pain to colorectal distension (CRD). Experimental models have been developed that recapture clinical features of IBS with regard to stress-related hyperalgesia to CRD, gender differences, comorbidity with anxiety/depression and altered bowel habit. Data obtained using pharmacologic approaches in rodents subjected to stress indicate that the activation of CRF_1 receptor contributes to the development of hyperalgesia to CRD while CRF_2 receptors display opposite effects. The mechanisms through which CRF_1 antagonists alleviate colonic responses involved the prevention of stress-related activation of sacral parasympathetic ouflow, colonic enteric cholinergic neurons and mast cells. The pre-clinical and clinical phase I data support that targeting of CRF_1 receptors may open new therapeutic venues for stress-related functional gastrointestinal disorders. Supported by NIHDDK and VA Merit grants.

6I_02_S

EFFECT OF CAPSAICIN ON THE ESOPHAGEAL MOTILITY OF PATIENTS WITH GASTRO-ESOPHAGEAL REFLUX DISEASE (GERD)

Agnes Király

3rd Dept. of Medicine, University of Pécs, Hungary. e-mail: agnes.kiraly@aok.pte.hu

Capsaicin-sensitive afferents play a role in the regulation of esophageal motility. Vanilloid receptor is activated by acidic pH which triggers pain and motor responses leading to esophageal emptying . The aim was to investigate the effect of topical capsaicin (Tabasco) suspension on esophageal sensation and motility in healthy controls (N=10), patients with non-erosive GERD (NERD) (N=10), erosive esophagitis (ERD) (N=10) and with Barrett's metaplasia (BM) (N=10). Visual analog scale was used to determine sensation, the esophageal body and lower esophageal

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

sphincter (LES) response were analyzed. Topical 1.5 x 10^{-4} M capsaicin significantly increased the amplitudes (68 +/- 3 to 88.5 +/- 4.7 ; 66+/-2 to 80+/-3; 70+/-1 to 96+/-4 mm Hg, $P < 0.05$) and propagation velocity (2.7 +/- 0.3 to 4.3 +/- 0.3 ; 3.1+/-0.3 to 4.2+/-0.1; 2.5+/-0.1 to 3.7+/-0.2 cm/s, $P < 0.05$) of esophageal pressure waves and LES pressure (15 +/- 1.4 to 27 +/- 2.6; 13+/-1 to 20+/-1; 14+/-1 to 29+/-3 mm Hg, $P < 0.05$) in controls, NERD and ERD patients respectively. None of the above responses were present in BM patients. Capsaicin enhanced esophageal bolus transit (3.3+/-0.1 to 2.7+/-0.1; 3.2+/- 0.1 to 2.5 +/- 0.1 s, $P < 0.05$) in controls and NERD patients. Unchanged bolus clearance was found in ERD and BM patients (4.0+/-0.1 vs. 4.1+/-0.1; 4.2+/- 0.2 vs. 3.9 +/-0.1 s NS). Significantly increased perception to capsaicin was found in NERD (66+/-7) and ERD (78+/-9) patients vs. to healthy controls (47+/-3) mm. Esophageal capsaicin induced a profound increase in emptying which could improve clearance of the esophagus. Chronic acid exposure produces allodynia in NERD and ERD patients which is related to their symptom development. Capsaicin-mediated esophageal clearance is disturbed in ERD and BM patients.

6I_03_S

STRESS AT THE CELLULAR LEVEL – NOVEL ROLES OF HEAT SHOCK PROTEINS IN GASTRIC MUCOSAL PROTECTION AND HEALING.

A. S. Tarnawski and H. Gergely

VA Long Beach Healthcare System and University of California, Irvine, CA, USA. atarnawski@yahoo.com

Cells respond to stress by activating heat shock protein (HSP) response, which constitutes an universal defense system, essential for maintaining cell and tissue homeostasis by stabilization of denatured proteins. In gastric mucosa, the surface epithelial cells are directly exposed to temperatures ranging from 4°C to 75°C, therefore HSP activation is very relevant. We examined expression of constitutive and inducible HSP70 (c and iHSP70) in gastric mucosa of rats at baseline and following treatment with cytoprotective drugs: sucralfate, rebamipide, and talcid. In normal gastric mucosa cHSP70 is expressed mainly in the surface epithelium. Cytoprotective drugs enhanced expression of cHSP70 in surface epithelial and progenitor cells induced iHSP70 in the same areas and significantly reduced ethanol-induced gastric mucosal injury. Ethanol-induced gastric injury is significantly increased in mice with KO gene for heat shock factor 1 (HSF-1) a transcription factor for HSP genes. This is due to increased apoptosis in response to ethanol injury in HSF-1 null mice. This study provides evidence that HSPs after their HSF-1-dependent upregulation

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

protect gastric mucosa against injury. Recent data indicate that HSPs are involved in healing of experimental gastric ulcers. HSP32 are elevated during early stage of healing; HSP47 are increased in granulation tissue and HSP70 is increased in the epithelial cell of the ulcer margin and progenitor cells where it co localized to EGF, IGF-1 and Cox2. **Conclusions:** 1) Gastric mucosa expressed HSP at baseline and their expresses is activated by cytoprotective drugs and in response to injury. 2) HSPs also play important roles in ulcer healing by local interactions with growth factors and by protection of regenerating cells.

6I_04_S

INCREASED EXPRESSION OF TRANSCRIPTION FACTOR EGR-1 IN STRESS-INDUCED GASTRIC ULCERATION

T. Beregova[1], V. Kucharskyij[1], O. Drobinska[1], S. Szabo[2]

[1]Taras Shevchenko Kyiv National University, Ukraine; [2]Department of Phatology and Pharmacology, University of California, Irvine, VA Long Beach Healthcare System, CA, USA
Revutskogo str., 44, apt.86, Kyiv, Ukraine 02140 tberegova@univ.kiev.ua

The transcription factor early growth response-1 (Egr-1) is activated by many environmental signals. Recently it was demonstrated the enhanced duodenal Egr-1 transcription activity and followed increased expression of target genes such as bFGF, PDGF and VEGF in cysteamine-induced duodenal ulceration in rats. However, the involvement of Egr-1 expression in stress-induced gastric ulceration was not determined. The aim of our study was to investigate the effects of stress on Egr-1 expression in rat gastric mucosa (GM) at the different time point. The rats were immobilized for 6, 12 and 24 hr. Egr-1 expression was determined by immunoblotting. It was established that 6 hr of stress exposure induced only single punctated hemorragies in GM. 12 hr of stress produced the multiply punctated hemorragies. 24 hr of stress exposure developed ulcers, errosions and massive hemorragies in rat stomach. The gastric injuries were 23,01±4,07 mm^2. Egr-1 expression was barely detectable in GM of untreated rats. 6 hours of stress increased the gastric Egr-1 expression by 8 fold ($p<0.05$) compared to control. At 12 hr of stress exposure Egr-1 expression in injured GM was enhanced only 2 fold in comparison to control. Egr-1 expression dropped to the basal level after 24 hr of stress exposure. Thus, the highest level of Egr-1 expression was observed in rat GM 6 hr after stress exposure in comparison with untreated rats and rats after 12 and 24 hr of stress exposure. So stress-induced gastric ulceration leads to increased Egr-1 expression in rat GM. The results highlight the role of Egr-1 in experimental ulceration.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

6I_05_S

NEW MOLECULAR MECHANISMS OF DUODENAL ULCERATION.

S. Szabo, T. Khomenko, X.M. Deng, G. Tolstanova , L Chen, K. Osapay, Zs. Sandor

VA Medical Center and University of California-Irvine, School of Medicine, Long Beach, CA 90822, USA

Stress is a major etiologic factor in the pathogenesis of gastric & duodenal ulceration (DU), as first described in rats by Hans Selye (Nature, 1936). In patients with "peptic ulcers" duodenal ulcers are more frequent than gastric ulcers (except in Japan). Thus, our research during the last 3 decades focused on the molecular mechanisms of DU in rodent models of chemically induced DU (Szabo & Selye, Arch. Pathol., 1972; Selye & Szabo, Nature, 1973). While acknowledging the role of VacA, CagA & other molecules linked to the "pathogenic island" in H. pylori genome, we focus on our 3 recent findings on the molecular mechanisms of DU: Endothelins (ET-1), the immediate early gene egr-1 & imbalance of angiogenic/anti-angiogenic molecules. Namely, we found an enhanced expression & release of ET-1 within 15-30 min after the administration of duodenal ulcerogen cysteamine, most likely resulting in local ischemia that triggers the expression of hypoxia-inducible factors (HIF-1α). Our gene expression studies also revealed an early (0.5-2 hr) increase in the expression of egr-1 that is followed (12-24 hr) by upregulation of angiogenic growth factors (e.g., VEGF, bFGF, PDGF). Surprisingly, this event is also associated with an enhanced production of angiostatin & endostatin that probably counteract the supposedly beneficial effect of angiogenic molecules. Thus, the initial injury to endothelial & epithelial cells in DU seems to be aggravated (& not initiated) by HCl & proteolytic enzymes. The resulting mucosal necrosis does not rapidly heal because of the imbalance of VEGF & angiostatin/endostatin, hence duodenal ulcers develop. The experimental ulcers Selye described morphologically are now characterized at the molecular & genome level, involving unexpected mediators like ET-1, egr-1 & angiogenesis-related molecules.

6I_06_S

NEW MOLECULAR MECHANISMS OF ULCERATIVE COLITIS/IBD: DOES STRESS MATTER?

Zs. Sandor, G. Tolstanova, X.M. Deng, L Chen, T. Khomenko, S. Szabo

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

VA Medical Center and University of California-Irvine, School of Medicine, Long Beach, CA 90822, USA

Recent experimental & clinical studies indicate that adverse life events, chronic stress, & depression increase the likelihood of relapse in patients with quiescent IBD. Phsycologic stress increases plasma corticosterone, mast cells degranulation, reduces colonic mucin secretion and enhances intestinal mucosal permeability, facilitating bacterial adherence & uptake, followed by the sensitization of T cells and initiation of inflammatory/immune reaction. Here we review our recent findings on the new molecular mechanisms of experimental IBD induced by chemicals in rats & IL-10-KO mice. Namely, we recently found a concomitantly increased expression of the angiogenic (VEGF/VPF) and anti-angiogenic (angiostatin, endostatin) factors in the colonic mucosa before the morphologic appearance of chemically-induced colonic lesions in rats. Furthermore, the elevated levels of endostatin correlated with the severity of chemically-induced ulcerative colitis (UC) and disease progression in IL-10-KO mice. This probably results in insufficient angiogenesis & poor healing (by interfering with the proliferative action of VEGF), while letting the VPF action of VEGF/VPF be manifested in the early stages of IBD. This new mechanism is probably supported by another recent finding demonstrating a beneficial effect of VEGF neutralization on the severity of chemically-induced UC in rats that was associated with attenuation of VEGF, PDGF & bFGF expression. Moreover, we found rapidly increased phosphorylation of Erk1/2 and expression of Egr-1, its DNA binding and interaction with other transcription factors (AP-2, PPAR, NF-kβ) in colon during chemically-induced UC. VEGF neutralization significantly inhibited expression of pErk1/2 and Egr-1. Conclusions: 1) The unexpected parallel increased expression of both VEGF/VPF & angiostatin/endostatin in UC may explain the poor healing & sustained inflammation. 2) Transcription factor Egr-1 may play a pivotal role in the VEGF actions in the pathogenesis of experimental UC. 3) Stress may play a role in the exacerbation but apparently not in the initiation of IBD.

Module 6 – Poster lectures:

6B_01_P

SURFACE AND INTRACELLULAR HSP70 EXPRESSION IN MALIGNANT BLOOD DISEASES

F. Leoni, N. C. Dempsey[1], C. Hoyle[2] C. Hunter-Lavin[1] & J. H.H. Williams[1]

[1]*University of Chester., UK*
[2]*Glan Clwyd Hospital. Bodelwyddan, UK*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

n.dempsey@chester.ac.uk

The expression of Hsp70 has been extensively studied in many cancer types, however there is limited literature on Hsp70 expression in Myelodysplasic Syndrome (MDS). MDS is a clonal disorder of haematopoietic stem cells characterised by apoptosis of progenitor stem cells leading to dysplastic haematopoiesis and variable cytopenias. Certain subtypes are associated with a high risk of progression to Acute Myeloid Leukemia (AML). Chronic Lymphocytic Leukemia (CLL) is a haematological malignancy which may progress from a stable form to acute disease requiring treatment. We compared blood samples from patients with MDS, AML, CLL and normal age matched controls by flow cytometry for intracellular and extracellular expression of Hsp70. CD3 (T cells), CD14 (Monocytes) and CD15 (Neutrophils) were used to detect differences in Hsp70 expression between the main leucocyte populations. CD34+ was used as a marker for AML blasts and CD5+/CD19+ as a marker for CLL cells. Preliminary data show different patterns of expression of surface and intracellular Hsp70 with different subtypes of MDS. In some patients it is possible to show two distinct sub-populations of neutrophils one expressing more surface Hsp70. The AML data show populations of blast cells which are both intracellular and surface Hsp70+ and this expression is higher than in the normal cells. These preliminary data would suggest correlation with disease severity. CLL samples show Hsp70 expression on CD5+/CD19+ cells. We are currently increasing sample numbers for each stages of the individual diseases. We will use these results to determine the involvement of Hsp70 in the progression of the diseases and whether manipulation of Hsp70 in these cells would be beneficial.

6B_02_P

THE STRESS-INDUCIBLE IMMUNOLOGICAL DANGER SIGNALS HSP70 AND MICA/B JOINTLY AUGMENT THE CYTOTOXIC ACTIVITY OF HUMAN NK CELLS AGAINST TUMOR CELLS

Leslie Elsner,[1] Perris F. Flügge,[1] Vijayakumar Muppala,[1] Jingky Lozano,[2] Dörthe Malzahn,[2] Heike Bickeböller,[2] Edgar Brunner,[3] Gabriele Multhoff,[4] Lutz Walter,[5] and Ralf Dressel[1*]

*[1]Dept. of Cellular and Molecular Immunology, [2]Dept. of Genetic Epidemiology, [3]Dept. of Medical Statistics, University of Göttingen, 37099 Göttingen, Germany; [4]Dept. of Radiotherapy and Radiooncology, Technical University Munich, 81675 Munich, Germany; [5]Dept. of Primate Genetics, German Primate Center, 37077 Göttingen, Germany; *e-mail: rdresse@gwdg.de*

The stress-inducible heat shock protein (HSP) 70 is known to function as

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

an endogenous danger signal which can increase the immunogenicity of tumors and induce cytotoxic T lymphocyte responses. We show here that HSP70 also activates human natural killer (NK) cells which recognize the stress-inducible MHC class I chain-related (MIC) A and B molecules on tumor cells. We observed that the stress-inducible HSP70, in contrast to the constitutively expressed HSC70, activated human peripheral blood mononuclear cells *in vitro* to kill MICA-transfected target cells. The HSP70-derived peptide TKD (Multhoff et al., Cell Stress Chaperon 6:337, 2001) was able to replace the full-length HSP70 in these assays. Cell separation experiments identified NK cells as the cytotoxic effector cells which were activated by HSP70 or TKD. When MICA/B expression was induced on human melanoma cells by pharmacological means and NK cells were activated by HSP70 or TKD, both treatments jointly improved the killing of the tumor cells. Thus, the synergistic activity of two stress-inducible immunological danger signals, HSP70 and MICA/B, leads to enhanced cytotoxicity of NK cells. This work was supported by grants from the Deutsche Forschungsgemeinschaft (DR 394/2, GRK 289, GRK 1034) and the European Union (MRTN-CT-2004-512253; TRANS-NET).

6B_03_P

RESISTANCE TO HSP90-TARGETED THERAPY IS MEDIATED THROUGH THE HEAT SHOCK RESPONSE

A. McCollum, C. Ten Eyck, B. Stensgard, D. Toft, C. Erlichman

Mayo Clinic College of Medicine, Rochester, MN 55905, USA
erlichman.charles@mayo.edu

HSP90 is critical in the proper folding, stabilization, and trafficking of many client proteins that have a major impact on proliferation and antiapoptotic signaling necessary for the malignant process. HSP90-targeted therapy has been introduced into clinical testing with the expectation that with degradation of many client proteins, a significant antitumor response would be observed. However, clinical trials to date have shown only limited and anecdotal activity of this multitargeted kinase approach, even though stress response proteins such as Hsp27 and Hsp70 are induced in patients after treatment with Hsp90-directed agents. We have undertaken studies to delineate factors that may contribute to the apparent resistance that has been observed in patients. A549 cells have been selected for resistance to geldanamycin (GA) after exposure to increasing concentrations of GA in a stepwise fashion. The cells are resistant to not only GA but also to the clinically relevant compounds 17-allylaminogeldanamycin (17AAG) and (dimethylaminoethylamino)-17-demethoxygeldanamycin (DMAG). These cells overexpress the ABC transporter p-glycoprotein (pgp) which has been implicated in GA

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

resistance previously. Treatment of these cells with verapamil or GF120918, inhibitors of pgp, does not reverse the resistance to GA. However, knockdown of HSP27 or HSP70 with siRNA reverses the resistance of the cells resulting in an IC_{50} similar to that of the parent cell line. These results suggest that selective targeting of HSP90 alone will have limited clinical efficacy. On the other hand, a combined approach that affects the stress response by targeting other stress-related proteins in addition to HSP90 may overcome this self-induced resistance. Supported in part by CA90390 and CA15083

6B_04_P

ANTAGONISM BETWEEN MAJOR STRESS PROTEIN HSP70 AND MYC ONCOPROTEINS IN THE EXECUTION OF APOPTOTIC PROGRAM

Irina Guzhova[1], Elena Afanasyeva[1], Elena Komarova[1], Lars-Gunnar Larsson[2], Boris Margulis[1].

[1] Institute of Cytology RAS, St Petersburg, Russia
[2] Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden. guzhova@mail.cytspb.rssi.ru

Two proteins contribute to the targeting of a cancer cell population towards apoptosis or survival, Myc oncogene known mainly as proapoptotic factor and Hsp70 stress protein possessing the ubiquitous protective activity. Based on our data indicating that Hsp70 can have especially high protective activity in heat shock-treated U-937-v-Myc cells we suggested that the key function of the former is the abrogation of apoptosis specifically mediated by Myc. To check this we used two models, in one of whose over-expression of Myc oncogene was continuous (v-Myc in U-937 cells) and in another Myc expression was controlled by estradiol (c-Myc in Rat1MycER cells). It was found that the stable expression of Hsp70 rendered U-937-v-Myc cells more resistant than the original U-937 cell clones when both having been induced to apoptosis with etoposide and camptothecin. Using anti-Hsp70 siRNA we also demonstrated that resistance of U-937-v-Myc cells to the cytotoxic effect of above anti-cancer drugs was fully related to Hsp70 level, whereas in U-937 cells such effect of Hsp70 depletion was not observed. In order to prove the data we employed another model system, Rat1MycERcells, with inducible expression of Hsp70 and of c-Myc. It was found that apoptosis induced by camptothecin and sodium butyrate and mediated by active MycER was efficiently and dose-dependently suppressed by Hsp70. Taken together, our results led us to conclusion that Hsp70 might act as a complement to Myc-driven oncogenesis.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

6B_05_P

THE LEVEL HSP72 AND HSP27 EXPRESSION AND APOPTOSIS INDUCTION IN NEUROBLASTOMA CELLS AND NEURONS AFTER QUERCETIN TREATMENT

Jakubowicz-Gil Joanna[1], Rzeski Wojciech[2], Zdzisinska Barbara[2] , Wejksza Katarzyna[2], Piersiak Tomasz[1], Gawron Antoni[1]

[1]Department of Comparative Anatomy & Anthropology and [2]Department of Virology & Immunology, Maria Curie-Sklodowska University, Akademicka 19, 20-033 Lublin, Poland
Corresponding author: Joanna Jakubowicz-Gil, asiajgil@o2.pl

We studied the effect of quercetin, natural flavonoid, on apoptosis and necrosis induction in neuroblastoma cell line and the culture of neurons. Quercetin induced apoptosis and necrosis in both studied cell lines, whereas neurons were much more sensitive to cell death upon quercetin treatment. The number of apoptotic, as well as necrotic cells was 3-6 times higher in neurons than in neuroblastoma cells.
We also investigated the effect of quercetin on the level of Hsp72 and Hsp27 expression in neuroblastoma cells and neurons. It is worth to note, that no Hsp72 expression was observed in neurons. Quercetin appeared to be a good inhibitor of Hsp27 expression in neuroblastoma cells and in neurons and Hsp72 in cancer cells. Inhibition of Hsp72 and Hsp27 expression by specific antisense oligonucleotides in neuroblastoma cells made the cells more vulnerable for apoptosis induction after quercetin treatment. The number of apoptotic cells was comparable with results observed in neurons after incubation with flavonoid.
Quercetin changed the localization of Hsp27 in cancer cells. Direct correlation between drug concentration and gradual migration of protein from cytoplasm to the nucleus was observed. Quercetin had no effect on localization of Hsp72 in neuroblastoma and Hsp27 in neurons, where proteins were observed in nuclei.
Our results indicate that sensitivity of neuroblastoma cells and neurons to quercetin induced apoptosis depends on the level of Hsp72 and Hsp27 expression.

6B_06_P

CASPASES-7 IS SPECIFICALLY ACTIVATED BY TAXOL IN OVARIAN CARCINOMA CELLS

Anna Luria, Marina Wolfson

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Laboratory of Molecular Biology of the Cell, Department of Microbiology and Immunology,Ben Gurion University of the Negev, Beer Sheva 84105, Israel. E-mail: wolfson@bgumail.bgu.ac.il

Despite years of study, ovarian cancer remains one of the leading causes of cancer death in women. Primary ovarian carcinomas exhibit mitochondrial dysfunction of apoptosis in ~70% cases. Taxol, the first-line ovarian therapy drug, induces apoptosis in SKOV3 cell line and primary human ovarian carcinoma cells. We reported previously that taxol-induced apoptosis in these cells does not include activation of caspase-9 and caspase-3 *(Ofir et al, 2002.Cell Death Diff 9,636-642)*. Recently, we showed that taxol-induced apoptosis is associated with procaspase-7 processing in ovarian cancer cells. Molecular chaperone Hsp90 could directly inhibit apoptosome formation and caspase-9 and -3 activation, and its elevated expression was found in ovarian cancers. Using the specific Hsp90 inhibitor Novobiocin in combination with Taxol on SKOV3 cells, we observed the processing of caspase-3, however, synergistic effect of drug combination on apoptosis rate was insignificant. Primary ovarian carcinoma cells isolated from ascites of patients exhibited different sensitivity to taxol. Processing of caspase-7 was observed in "taxol-sensitive" primary cultures, whereas no processed caspase-7 has been found in "taxol-resistant" primary cultures. In accordance to the lack of caspase-7 cleavage, viability of "resistant" cells was not affected by taxol. However, processing of caspase-7 and decreased cell viability were observed under Novobiocin treatment in "taxol-resistant" primary cultures. Altogether, our results demonstrate that an inhibition of Hsp90 sensitizes ovarian cancer cells to drug treatment, and that the processing of caspase-7 could be a marker for *in vitro* testing of susceptibility to chemotherapy.

6B_07_P

PHARMACOLOGICAL INHIBITION OF STRESS-ACTIVATED P38 AND EXTRACELLULAR-REGULATED P44/42 PROTEIN KINASES STIMULATE P-GLYCOPROTEIN AND DECREASE APOPTOSIS IN DOXORUBICIN-EXPOSED HEP3B CELLS.

Irena Manov, Yulia Bashenko and Theodore C. Iancu

Pediatric Research and Electron Microscopy Unit, Ruth and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel, irmanov@tx.technion.ac.il

Doxorubicin (DOX) is the anthracycline with the widest spectrum of antitumor activity, but its clinical effectiveness is limited by the development of multidrug resistance (MDR). The phenomenon of MDR in

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

cancer cells is mainly associated with overexpression of P-glycoprotein (P-gp) leading to increased efflux of anticancer drugs and down-regulation of apoptosis. The p38 and p44/42 signaling pathways were shown to play pro-apoptotic roles in DOX-induced apoptosis. The cross-talk between MAPK and P-gp pathways in cancer cells is still poorly understood. We aimed to investigate whether the MAPK pathways are involved in P-gp activation and whether their modification can regulate P-gp and DOX-induced apoptosis in Hep3B cells. The p38 and p44/42 cascades in cells exposed or not to DOX (5 mM) were selectively inhibited by SB202190 (SB, 0.1-30 μM) and PD98059 (PD, 10 and 30 μM) respectively. Viability, cell cycle distribution, P-gp activity and content were studied. Both SB and PD strongly stimulated P-gp function as was demonstrated by the Rhodamine-123 efflux assay. Western blot revealed the increase of P-gp expression in cells treated either p38 or p44/42 inhibitors. Combining SB or PD with DOX inhibited apoptosis and increased cell viability. In conclusion, the selective inhibitors SB202190 and PD98059 activate P-gp in Hep3B cells. MAPK pathways p38 and p44/42 can regulate P-gp transporter in cancer cells and modify the cellular response to chemotherapy.
This work was supported by the Dan David Foundation and the Kamea Fund.

6B_08_P

EXPRESSION OF HUMAN HTRA1, HTRA2 AND HTRA3 GENES IN OVARIAN AND ENDOMETRIAL CANCERS

Joanna Narkiewicz[1]*, Dagmara Klasa-Mazurkiewcz[2], Sylwia Lapinska-Szumczyk[2], Janusz Emerich[2], Barbara Lipinska[1]

1 – University of Gdansk, Gdansk, Poland
2 – Medical University of Gdansk, Gdansk, Poland
**jonark@biotech.univ.gda.pl*

The HtrA family of serine proteases takes part in cellular stress response including heat shock, inflammation and cancer. Down-regulation of human HtrA1 and HtrA3 genes has been reported in some cancers, suggesting tumor-suppressor role for both genes. An evidence exists showing that both HtrA1 and HtrA3 regulate biological processes by modulating TGFβ signaling. HtrA2 is a unique HtrA family member playing proapoptotic functions. In the present study expression of human HtrA1, HtrA2, HtrA3 and TGF-β1 genes in ovarian and endometrial cancers was examined by semi-quantitative RT-PCR and Western blotting methods. Analyses were carried out on 98 ovarian and 123 endometrial tissue specimens, including tumors and healthy controls. Our results showed statistically significant decrease of HtrA1 and HtrA3 expression in ovarian and endometrial

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

tumors comparing to normal tissues. For the ovarian tumors, we have found decrease of expression of the HtrA1 and HtrA3 genes in all tested tumor groups including the benign, borderline, malignant and Krukenberg tumors. In the case of HtrA3, the decrease was dramatic both at the mRNA and protein levels; we did not find detectable HtrA3 protein in about 38% of ovarian and 20% of endometrial tumors. Our results showed a slight decrease in HtrA2 expression in the examined tumor tissues. Moreover, our results showed significant negative correlation between HtrA1 and HtrA3, and TGF-β1 relative protein levels in endometrial tissues, suggesting inhibition of TGF-β1 signaling by HtrAs in endometrial cancer. Our results are in agreement with previous reports showing downregulation of HtrA1 and HtrA3 genes' expression in cancers and show additional data on correlation between tumor type and HtrA expression. This is the first report showing correlation between TGF-β1 and HtrA proteins' levels in endometrial cancer.

6B_09_P

HSF-1 IN BREAST CANCER GROWTH, MIGRATION AND METASTASIS

M. Kouspou[1,2], K. Waldeck[1,2], J. Vieusseux[1], J. Harris[1], J. Ojaimi[1], J. Price[1]

[1] Cancer Biology and Metastasis Lab, Monash University, Clayton and [2]Melbourne University, Melbourne Victoria, Australia.
John.Price@med.monash.edu.au

Breast cancer metastasis is the major cause of morbidity and mortality amongst patients. To identify novel mediators of breast cancer metastasis we have identified clones of the human breast cancer cell line, MDA-MB-231, that have differential migratory and metastatic propensities. Gene array analysis identified an increased expression of several HSF-1 target genes in clones possessing a high migratory and metastatic ability. Gene expression data mining of cohorts of breast cancer patient specimens identified HSF-1 to be positively correlated to increased metastatic burden and decreased survival. Therefore, to investigate the role of HSF-1 in breast cancer we stably transfected MDA-MB-231 cells with wild-type HSF-1 ($HSF\text{-}1^{WT}$) and a dominant negative mutant form ($HSF\text{-}1^{DN}$). Characterization of these cells revealed that inhibition of HSF-1 function rather than its expression per se substantially inhibited anchorage-independent growth. In addition to this growth effect, HSF-1 was also identified to be fundamental in the migratory response of the cells towards a number of growth factors, including EGF and IGF-I. Moreover, use of known HSF-1 inhibitors, KNK437 and quercetin, resulted in a potent inhibition of migration. Examination of cell signalling pathways previously described as vital to the migratory behaviour of these cells identified

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

PLCγ1 as being downregulated by HSF-1 inhibition, suggesting an important interplay between these molecules. Therefore, these findings describe for the first time a direct role of HSF-1 in tumour cell migration and identify a novel role for HSF-1 in the metastatic cascade. As such, HSF-1 may represent a potential therapeutic target in breast cancer metastasis.

6B_10_P

UPR ACTIVATION IN THE HUMAN GASTRIC ADENOCARCINOMA CELL LINE AGS ENHANCES RESISTANCE TO THE COMMONLY USED ANTI CANCER AGENTS CISPLATIN, DOXORUBICIN AND 5-FLUROURACIL.

Scriven P, Ritchie J, Wyld L

Academic Surgical Oncology, University of Sheffield, Royal Hallamshire Hospital, Sheffield, United Kingdom. p.scriven@sheffield.ac.uk

Physiological stress such as glucose deprivation adversely impacts on protein folding within the ER and via recruitment of chaperone proteins such as GRP78 activates a signal cascade known as the Unfolded Protein Response (UPR). This mechanism is cytoprotective if the stressor is mild or of short duration, if stress is severe or prolonged then the UPR can initiate apoptosis. The UPR is activated in many solid tumours and has been shown to affect chemosensitivity, specifically increasing resistance to topoisomerase II inhibitors such as doxorubicin whilst possibly enhancing susceptibility to DNA cross linking agents such as cisplatin. By depriving the AGS cell line of glucose our group was able to induce UPR activation as demonstrated by a rise in GRP78 levels on Western Blotting after 24hours of culture in media containing 0.5mmol and 0.2mmol glucose (compared to 11mmol in standard culture). The sensitivity of UPR activated AGS was compared against a standard control for predetermined IC50 doses of the commonly utilised anti cancer agents Cisplatin, Doxorubicin and 5-Flurouracil. Toxicity was determined by MTT assay relative to an untreated control group for each glucose concentration. Relative to standard 11mmol glucose conditions AGS cells cultured in 0.5 and 0.2mmol showed a statistically significant increase in survival ($p<0.05$) when exposed to IC50 doses of doxorubicin, cisplatin and 5-Flurouracil. These findings suggest a link between the glucose deprived tumour microenvironment and resistance to current clinical chemotherapy regimes. UPR activation may become an important marker for targeting chemotherapy regimes appropriately.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

6B_11_P

REGULATION OF TUMOR GROWTH BY HSPBP1, AN HSP70 COCHAPERONE.

Vince Guerriero, Roger Miesfeld, Randy Burd, Shamarie Black and Deborah A. Raynes

Depts. of Animal Sciences, Molecular and Cellular Biology, Biochemistry and Molecular Biophysics and Nutritional Sciences, University of Arizona, Tucson, AZ 85721 Guerrier@email.arizona.edu

The Hsp70 family of heat stress proteins can be regulated by a group of proteins collectively known as cochaperones. HspBP1 is a cochaperone with nucleotide exchange activity that binds to and regulates Hsp70. Hsp70 is elevated in numerous types of cancer cells and has anti-apoptotic activity. Data from this laboratory has shown that HspBP1 levels are also elevated in a variety of cancer types and therefore the HspBP1 to Hsp70 ratio remained constant. HspBP1 and HspBP1 mutants were over expressed in a cancer cell line to determine if increasing the HspBP1 to Hsp70 ratio would alter cell growth and tumor formation. Increased expression of HspBP1 (approximately 3-4 fold) did not alter cell proliferation, cell growth at low densities, cell cycle parameters, apoptosis, or cell migration. However, injection of these cells into SCID mice resulted in a lag period before tumor growth and smaller tumors compared to controls. These results suggest that HspBP1 can alter the tumor host interaction. One possible alteration is a suppression of vascular endothelial growth factor (VEGF) secretion by the tumor cells. Measurement of VEGF secretion revealed that over expression of HspBP1 did not alter secretion rates. Therefore, HspBP1 can alter tumor growth in mice but the mechanism is unknown. Understanding how HspBP1 alters tumor cell growth will provide new information that can be used to develop novel therapies for tumor treatment.

6B_12_P

PROTEIN KINASE Cα ACTIVATION BY LEAD ACETATE LINKS TO THE EGFR/SRC/RAS/RAF/ERK SIGNALING AND PREVENTS CYTOTOXICITY AND MUTAGENICITY IN HUMAN LUNG CANCER CELLS

Chun-Yu Wang, Yun-Wei Lin, and Jia-Ling Yang[1]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Molecular Carcinogenesis Laboratory, Institute of Biotechnology & Department of Life Sciences, National Tsing Hua University, Hsinchu 30013, Taiwan
[1]To whom correspondence should be addressed
E-mail: jlyang@life.nthu.edu.tw

Lead acetate [Pb(II)] exhibits weak genotoxicity in mammalian cells due in part to the enhanced nucleotide excision repair mediated through ERK activation. However, the ERK upstream signaling linking to Pb(II) weak genotoxicity remains unknown. Here we report the essential signaling transducers for ERK activation that prevent Pb(II) cytotoxicity and mutagenicity in human non-small cell lung adenocarcinoma CL3 cells. Pb(II) stimulated the membrane localization associated PKCα activation, the dissociation of Raf kinase inhibitory protein from Raf-1, and the phosphorylation of Raf-1 on S338. The Pb(II)-induced phospho-Raf-1(S338) were markedly decreased by forced expression of a dominant-negative Ras. Pre-treatment with Gö6976 (a conventional PKC inhibitor) or introducing PKCα small interfering RNA (siPKCα) blocked the Raf/ERK activation and significantly increased the cytotoxicity and the *hprt* mutation frequency in Pb(II)-exposed cells. PD153035 (an EGFR inhibitor) or SU6656 (a Src family inhibitor) but not AG1296 (a PDGFR inhibitor) suppressed the Pb(II)-induced activation of tyrosine kinases and PKCα, and phospho-Raf-1(S338) levels. Pb(II) sequentially induced the autophosphorylation of EGFR on Y1173 and Src on Y419. Thus, the PKCα participates in the EGFR/Src/Ras/Raf/ERK signaling cascade and avoids cell death and genomic alteration during Pb(II) exposure.

6B_13_P

DHA INDUCES DISTURBANCE IN CHOLESTEROL HOMEOSTASIS, ER STRESS AND CELL CYCLE ARREST IN COLON CANCER CELLS

GL Størvold, CH Jakobsen, H Bremseth, SA Schønberg

Dept. of Laboratory Medicine, Children's and Women's Health, NTNU Erling Skjalgssons gt 1, 7006 Trondheim, Norway. E-mail, gro.l.storvold@ntnu.no

Epidemiological, animal and cell culture studies indicate an inverse association between long chain omega-3 fatty acids and growth of cancers such as breast, prostate and colon. We have previously shown that docosahexaenoic acid (DHA) induces growth arrest in different colon cancer cell lines, but little information regarding the molecular mechanisms exists. Microarray analysis has revealed that DHA induces cellular stress, and that activation of the endoplasmic reticulum (ER)

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

stress pathway may be one mechanism leading to growth arrest. The main functions of ER are regulation of calcium homeostasis, protein folding and processing, and lipid synthesis. Disturbances in any of these functions may lead to ER stress. We have observed an accumulation of cholesteryl esters in our cell lines after DHA treatment, but only slight increases in total cholesterol levels. In addition we observe increased protein levels of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis, and nSREBP2, the main regulator of genes involved in cholesterol synthesis and uptake. This indicates a cellular demand for cholesterol. Previously we have observed that treatment with DHA leads to an accumulation of cells in G2/M phase of the cell cycle. Progression through G2/M phase is cholesterol-dependent and cholesterol depletion will lead to inactivation of CDK1, downregulation of cyclin B1, and G2/M arrest. In addition activation of ER stress may lead to cell cycle arrest by depletion of cyclin D1. The aim of this study is to investigate the mechanisms by which DHA affects cholesterol homeostasis, induces cell cycle arrest and ER stress, and the possible link between these events.

6C_01_P

HSP90 AND ERBB2 IN THE CARDIAC RESPONSE TO DOXORUBICIN INJURY

Kathleen Gabrielson, Djahida Bedja, Scott Pin, Allison Tsao, Lucio Gama, Bibo Yuan, Nicole Muratore, Yi Xu and Nazareno Paolocci

Department of Comparative Medicine, Johns Hopkins University, Baltimore, MD 21205

A major drawback to chemotherapeutic agent doxorubicin is cardiac toxicity. To understand the signal transduction pathways activated in doxorubicin cardiac toxicity, and the potent synergic effect seen when doxorubicin is combined with anti-ErbB2 (trastuzumab), we developed an in vivo rat model that exhibits progressive dose-dependent cardiac damage and loss of cardiac function after doxorubicin treatment. The hearts of these animals respond to doxorubicin damage by increasing levels of ErbB2 and the ErbB family ligand, NRG1β, and by activating the downstream Akt signaling pathway. These increases in ErbB2 protein levels are not due to increased ErbB2 mRNA, however, suggesting post-transcriptional mechanisms for regulating this protein in the heart. Accordingly, levels of HSP90, a known ErbB2 protein stabilizer and chaperone, are increased by doxorubicin treatment and co-immunoprecipitation reveals binding of HSP90 to ErbB2. Isolated cardiomyocytes are more susceptible to doxorubicin after treatment with HSP90 inhibitor, 17AAG, suggesting that the HSP90 is protective during

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

doxorubicin treatment. Thus, our results provide one plausible mechanism for the susceptibility of the heart to anti-ErbB2 therapy post-doxorubicin therapy in subclinical and clinical conditions. Additionally, these results have lead us to further investigate the biology of HSP90 inhibition in the heart under various conditions. These isolated heart studies will also be included in this presentation.

6D_01_P

BRIEF ACADEMIC STRESS AFFECTS ANTI HSP70 ANTIBODIES, CORTISOL AND PSYCHOLOGICAL FACTORS

Orly Sarid[1], Ofra Anson[2], Arieh Yaari[2]

[1]Faculty of Humanities & Social Sciences, [2]Faculty of Health Sciences; Ben-Gurion University of the Negev, Beer-Sheva, Israel

The objectives of the study were to explore the effect of brief academic stress on anti Hsp70 and cortisol in serum and saliva and on psychological indicators; and to examine the associations between psycho-biological indicators. A brief academic stress of venopuncture and blood withdrawal was chosen as a model for measuring transient mood, endocrine and cell chaperone changes. The study population consisted of 28 healthy students, practicing venopuncture as part of their curricular activity. Sera and saliva were collected and analyzed for the existence of anti Hsp70 and cortisol using an anti-human Hsp70 (IgG/A/M) and cortisol ELISA Kits and following the manufacture's instructions. Pencil and paper questionnaires were administered before (t1) and after (t2) venopuncture and saliva donation. Cortisol and anti Hsp70 antibodies were detected in serum and saliva among all participants. Additionally, a change in some psychological indicators between t1 and t2 took place. Anti Hsp70 in serum was positively correlated with anxiety (t2) ($r=0.47$, $p\leq0.05$) and with anger at t2 (($r=0.54$, $p\leq0.05$). Cortisol in serum was negatively correlated with vigor (t1: $r=-0.44$, t2: $r=-0.48$, $p\leq0.05$).
In conclusion, a brief stress activates endocrine and cell chaperone system in serum and saliva, which is rarely studied in Hsps context. However associations with psychological variables were detected only in the serum.

6D_02_P

THE ROLE OF BACTERIAL DNAJ PROTEIN AND ITS HUMAN HOMOLOG HDJ-2 IN PATHOGENESIS OF RHEUMATOID ARTHRITIS

Stefan Tukaj[1], Agnieszka Kotlarz[1], Jolanta Tyborowska[2], Barbara Lipinska[1]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1. *Department of Biochemistry, University of Gdansk, Kladki 24, Gdansk, Poland.*
2. *Department of Molecular Virology, University of Gdansk, Kladki 24, Poland tukaj@biotech.univ.gda.pl*

Escherichia coli DnaJ (Hsp40) heat shock protein is suspected to participate in pathogenesis of rheumatoid arthritis (RA) in humans by an autoimmune process**.** We have found previously, using ELISA assay, that reaction of the RA patients sera with DnaJ protein and with its human homolog HDJ-2 (produced in bacteria) was at least 2-fold and 5-fold stronger, respectively, than the reaction of the control group, and there was a strong positive correlation between the immune response to DnaJ protein and HDJ-2 in RA. Therefore it is possible that in RA the immune response directed against the bacterial protein could cross-react with the human homologous protein. In this study, similar results were obtained when the HDJ-2 protein produced in insect cells (and therefore modified by farnezylation at the C-terminal end) was used as an antigen. These results confirmed previous findings and indicated also that antigenic properties of HDJ-2 did not change significantly upon farnezylation. We have assayed antibody levels against the N-terminal fragment of HDJ-2 encompassing the "J" domain, and against its C-terminal domain. The humoral response against the C-terminal domain was significantly increased in the RA patients compared to controls, and this response correlated very well with the anti-DnaJ response. The anti-DnaJ monoclonal antibodies recognizing the C-terminal domain of DnaJ reacted very efficiently with the human HDJ-2, indicating that the C-terminal part of HDJ-2 is immunologically similar to its counterpart of DnaJ. These results support a hypothesis that human HDJ-2 may function as an autoantigen in RA and suggest that the C-terminal part of HDJ-2 plays an important role in this process.

6D_03_P

NASAL ADMINISTRATION OF MYCOBACTERIAL HSP70 OR DERIVED PEPTIDES SUPPRESS PROTEOGLYCAN INDUCED ARTHRITIS

Lotte Wieten, Ruurd van der Zee, Elles Klein Koerkamp, Jose Wagenaar-Hilbers, Willem van Eden and Femke Hauet-Broere

Deptartments of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands, r.vanderzee@vet.uu.nl

T cells specific for bacterial Hsp's and cross-reactive with self are involved in regulation of chronic inflammatory diseases. In the present study we

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

investigated the effect of nasal administration of mycobacterial Hsp70 and cross-reactive peptides on proteoglycan induced arthritis, a mouse model for rheumatoid arthritis. Arthritis was induced in Balb/c mice by i.p. immunisation with human proteoglycan (hPG), on day 0 and 21. Nasal treatment with mycob. Hsp70 or peptides was done on day -7, -5 and -3. Mice were sacrificed 3.5 weeks after development of arthritis and splenocytes were stimulated with Hsp70 and PG, and proliferation, cytokine production and mRNA expression were analysed. Intranasal administration of mycobacterial HspP70 led to a delayed onset of arthritis after which the mice developed less severe arthritis than control mice receiving PBS intranasally. The maximum mean arthritis score was significantly ($p=0.026$) lower in Hsp70 treated mice (3.8±3.3) compared to control mice (9.4±2.6). RT- PCR analysis showed that intranasal administration of Hsp70 led to reduced pro-inflammatory cytokine mRNA expression and augmented IL-10 mRNA expression. CD4 mRNA expression was decreased in the joints of Hsp70 treated mice, indicating a reduced (pathogenic) $CD4^+$ T-cell influx in the joint. Similar treatments with mycob. Hsp70 peptides comprising cross-reactive T cell inducing epitopes also ameliorated disease development. Thus, intranasal administration of mycob. Hsp70 induces a protective immune response in the model of PGIA. In addition, we identified Hsp70 peptides capable of inducing regulatory T cells that can inhibit experimentally induced arthritis.

6D_04_P

WHAT MAKES ARTERIAL ENDOTHELIAL CELLS A TARGET FOR THE AUTOIMMUNE ATTACK IN THE EARLIEST STAGES OF ATHEROSCLEROSIS?

Georg Wick

Division of Experimental Pathophysiology and Immunology, Biocenter, Innsbruck Medical University Fritz-Pregl-Str. 3/IV, 6020 Innsbruck, Austria

The autoimmune hypothesis of atherogenesis postulates that preexisting cellular and humoral immunity to either microbial heat shock protein 60 (HSP60) or *bona fide* autoimmunity to biochemically altered autologous HSP60 leads to an attack on stressed arterial endothelial cells (ECs). We have shown in various animal models with spontaneously occurring autoimmune diseases that two essential sets of genes have to be present for an autoimmune disease to develop, i.e. genes that code for autoimmune reactivity of the immune system and genes that are responsible for target organ susceptibility. In the case of atherosclerosis, the arterial ECs express HSP60 that is also transported to the cell surface after being subjected to classical atherosclerosis risk-factors. We have

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

shown the HSP60-inducing effect of most of these risk-factors, including mechanical stress (hypertension), oxygen radicals, oxidized low-density lipoproteins (oxLDL), proinflammatory cytokines (TNFα), and cigarette smoke constituents. Exposure to these classical atherosclerosis risk-factors entail the simultaneous expression of HSP60 and various adhesion molecules (ICAM-1, VCAM-1, P-selectin). Due to their lifelong mechanical pre-stress by the arterial blood pressure, arterial ECs have a lower threshold for the HSP60 inducing effect of atherosclerosis risk-factors as compared to venous ECs. However, when veins are subjected to arterial blood flow conditions, e.g. after arterial-venous bypass operations, HSP60 expression and intimal infiltration with mononuclear cells with subsequent restenosis occurs similar to the pathogenetic events in classical atherosclerosis.

This work was funded by the European Union MOLSTROKE (Molecular basis of vascular events leading to thrombotic stroke) project (LSHM-CT-2004-005206) and the European Vascular Genomics Network (EVGN) (LSHM-CT-2003-503254)

6E_01_P

NITRATED PLASMA ALBUMIN: A POTENTIAL MARKER OF NITRATIVE STRESS LINKED TO ASPHYXIA AND HYPOGLYCEMIA IN NEWBORNS

Jean-Louis Wayenberg[1], Catia Cavedon[1], Valérie Ransy[1], Danièle Vermeylen[2], Eliane Damis[3] and Serge P. Bottari[4]

(1) Department of Paediatrics, Hôpital Français, Université Libre de Bruxelles, Brussels, Belgium, (2) Neonatal Intensive and Non-Intensive Care Unit, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium, (3) Neonatal Intensive and Non-Intensive Care Unit, CHIREC, Brussels, Belgium and (4) Centre Hospitalier Universitaire & INSERM U884, Université Joseph Fourier, Grenoble, France

Several stresses are known to induce an increased generation of peroxynitrite, which is able to nitrate specific cellular and plasma proteins on tyrosines among which albumin. A recent study by Groenendaal et al. showed a strong increase of nitrotyrosine immunostaining in the brains of neonates deceased from perinatal asphyxia, indicating the occurrence of an important nitrative stress. Similarly, neonatal hypoglycemia has been shown to induce nitrotyrosine generation in the brain of animal models. In order to investigate the occurrence of nitrative stress in clinical practice, we have developed a novel sandwich ELISA allowing the quantitative determination of nitrated albumin (nitralbumin) in human plasma as a biological marker of peroxynitrite generation.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

In a currently ongoing study on nitrative stress in human newborns (n = 194), we investigated the plasma nitralbumin concentrations in neonates who suffered perpartal asphyxia or neonatal hypoglycemia. Plasma was prepared from arterial blood obtained during the first hour of life and venous blood at day 1 and day 4 of life.
We found a significant increase ($p < 0.05$) in the nitralbumin concentration at day 1 in children who developed a post-asphyxial moderate or severe neonatal encephalopathy. Newborns who had a normal evolution or who developed a mild neonatal encephalopathy had nitralbumin levels similar to matched controls. Nitralbumin levels were normalized at day 4.
There also was a significant ($p = 0.05$) inverse correlation between glycemia and nitralbumin levels in preterm neonates (n = 51). After exclusion of neonates suffering from concurrent pathologies or confounding factors (IUGR, RDS, gestational diabetes) significance increased to $p < 0.005$. This correlation was found both in arterial blood obtained during the first hour of life and in venous blood obtained at day 1, indicating that nitrative stress is an early but sustained response to hypoglycemia. Nitralbumin levels were normalized at day 4. Subdivision of this cohort into subgroups according to glycemia, i.e. < 1.4, 1.4 - 2.4 and ≥ 2.5 mmol/L, further confirmed the statistical significance of the inverse correlation with nitralbumin levels.
Our data indicate the occurrence of nitrative stress in newborns exposed to perpartal asphyxia and preterm neonates suffering from moderate to severe hypoglycemia. These observations are in agreement with the increased tyrosine nitration observed in brains in post-mortem studies of asphyxiated human and hypoglycaemic animal neonates. These latter studies indicate that nitrative stress carries a serious risk of potential end-organ damage due to protein and lipid nitration. Our findings indicate that the determination of plasma nitralbumine levels with the ELISA we developed is a reliable way of assessing nitrative stress in clinical practice and might therefore have a prognostic value for the medium- and long term outcome of asphyxiated and preterm hypoglycaemic newborns.

6E_02_P

EPIDEMIOLOGICAL STUDY OF POLYMORPHISMS OF *HSP72* PROMOTER GENE AND ITS TRANSLATION PRODUCT

Guisasola MC, Dulin E*, Desco MM, Sanchez MJ, García-Barreno P

*Experimental Med & Surgical Unit.*Clinical Biochemistry Dep. Hosp Gen Univ Gregorio Maranon. Dr Esquerdo 46. 28007.Madrid. Spain. E-mail:mguisasola@mce.hggm.es*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Atheroesclerosis is a chronic inflammatory disease: cells involved are monocytes, macrophages, and T lymphocytes. Neutrophil polymorphonuclear leukocytes (PMNs) are also pathogenic releasing myeloperoxidase tightly linked to atherosclerosis and cardiovascular disease. Candidate molecules/autoantigens proposed include members of the Heat Shock Proteins (HSPs). Polymorphisms of the *hsp72* gene regulator regions, could account for different sensitivities for the development of cardiovascular disease. Epidemiological study of these polymorphisms and its possible correlation with morbi-mortality of population, may lead to identify efficient tools to be used as biomarkers. 92 ♀ (age 49,01 ± 0.68) and 106 ♂ (48,05 ± 0,66) were included randomly. Molecular study of promoter and flanking sequence of *hsp72* gene (**NT_007592**) in PMNs, PMNs[HSP72] and circulating HSP72 were performed. Statistical analysis: Mann-Whitney's U-test, and ANOVA one-way. **Results**: Subjects were divided into 3 groups: G0 without vascular risk factors [n=113 (54 men, 59 women)]; G1 with moderate risk factors (10%), without illness [n=55 (32 men, 23 women)]; and G2 with evident AT disease [n= 30 (19 men, 11 women)].Three SNPs were detected: -325A→C,-95T→C and -27G→C. SNP -325: 71 subjects (35,9%) were *wild type* (AA), 99 heterozygotes (46,5%) (AC), and 35 (17,7%) homozygotes (CC). Homozygotes had the highest [HSP72]i in G2 (p= 0,038 Anova). SNP -27 was co-expressed with -325 in 98,49% of total cases (73 WT, 93 HT, 32 HM).In the same way with SNP –325, CC phenotypes presents significant highest [HSP72]i in G2. SNP -95 was only detected in heterozygosis in 20 subjects (10.1%) and it was related to lowest [HSP72]i in AT (p=0,052, Anova), and to maximal levels in patients with vascular risk factors. With respect to circulating HSP72, the lowest levels belonged to G2 (p= 0,94), independently of the phenotype for any of the SNPs. **Conclusions:** in our study, SNPs -325 and –27 were related in their homozygote form, with highest $[HSP7]_i$, which has been proved previously to be antinmflamatory, and in consequence, protective of AT development and progression. Heterozygosis of –95 may be cardiovascular protective, but when AT is yet established, results in an additional vascular risk factor of AT disease. Grants from FIS 03/1308 and FMM.

6E_03_P

MODULATION OF ANTIOXIDANT STATUS IN A MACROPHAGE CELL LINE DURING CHRONIC EXPOSURE TO GLYCATED SERUM

Anna Bassi

Department of Experimental Medicine, University of Genoa, Italy
ambassi@medicina.unige.it

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Advanced glycation end-products (AGEs) are involved in the pathogenesis of aging and correlated degenerative pathologies, such as diabetic and uremic complications, atherosclerosis and amyloidosis. The aim of the present study was to reproduce those physiopathological conditions that precede the clinical aspects of diabetes or other aging-related diseases and resulted only in mild symptoms such as less appreciable levels of AGE products (i.e. pentosidine). Oxidative stress-related parameters were nalysed in J774A.1 murine macrophage cells during chronic exposure (7 days) to subtoxic concentration of AGE (5% ribose-glycated serum, GS) and subsequently for 48 hours to higher dose (10%GS). To assess the effects of exposure under the different experimental conditions, we analyzed both the cytotoxic and oxidative/glycoxidative effects of cellular damage.
No effects on cell viability were evidenced in either experimental condition. During chronic treatment, glycative markers (free and bound pentosidine) increased significantly in intra- and extra-cellular environments, but the production and release of thiobarbituric acid reactive substances (TBARs), as index of lipid peroxidation, underwent a time-dependent decrease. Exposure to 10% GS, evidenced that glycative markers rose further while TBARs elicited a cellular defence against oxidative stress. Non-adapted cultures showed an accumulation of AGEs, a marked oxidative stress and a loss of viability. During 10% GS exposure, GSH levels in adapted cultures remained constant, as did GSSG/GSH ratio, while non-adapted cells showed a markedly increased redox ratio. A constant increase of HSP70 mRNA was observed in all experimental conditions. On the contrary HSP70 protein expression became undetectable for longer exposure time; this could be due to the direct involvement of HSP70 in the refolding of damaged proteins. The increased levels of Cu/Zn SOD protein and mRNA expressions observed during chronic exposure, was followed by a marked decrease after subsequent 48 hours 10%GS exposure. MnSOD protein and mRNA expressions resulted not affected in all experimental conditions. Our findings suggest an adaptive response of macrophages to subtoxic doses of AGE, which could constitute an important factor in the spread of damage to other cellular types during aging.

6E_04_P

METABOLIC AND CARDIOVASCULAR ALTERATIONS INDUCED BY CHRONIC SOCIAL STRESS IN NORMOTENSIVE AND HYPERTENSIVE RATS

I. Bernatova[1], A. Puzserova[1], M. Barancik[2], M. Ivanova[2] , Z. Csizmadiova[1,3], M. Zeman[3]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[1]*Inst Norm Pathol Physiol,* [2]*Inst Heart Res, Slovak Acad Sci;* [3]*Dept of Animal Physiol and Ethology, Faculty of Nat Sci, Comenius Univ, Bratislava, Slovak Republic,* Iveta.Bernatova@savba.sk

This study investigated the effect of chronic crowding stress on metabolism and cardiovascular functions in normotensive WKY and spontaneously hypertensive (SHR) rats. Adult males were exposed to 8-week crowding (5 rats/cage, 200 cm^2/rat). Controls were kept 4 rats/cage (480 cm^2/rat). Blood pressure (BP) was determined by tail-cuff method. Basal BP of WKY and SHR was 110+/-2 and 180+/-5 mm Hg, respectively and crowding significantly elevated BP only in SHR. No phenotype-related alterations in plasma corticosterone (pCort) were observed in controls. Crowding significantly elevated pCort and reduced blood glucose levels, similarly in both phenotypes. Crowding increased activation (Thr202/Tyr204 phosphorylation) of extracellular-signal regulated protein kinases in the heart of both WKY and SHR, suggesting involvement and similar role of this pathway in responses to crowding stress in both phenotypes. However, phenotype-related differences were observed in cardiovascular responses to stress. Crowding reduced NO production in the heart and aorta of SHR and reduced acetylcholine-induced vasorelaxation of the femoral artery. On the other hand, crowding elevated NO production in the aorta of WKY, without changes in the heart, and improved vasorelaxation of the femoral artery. In conclusion, chronic crowding stress evoked similar metabolic changes in both phenotypes of rats while NO production and endothelium-dependent vasorelaxation were affected differently in WKY and SHR. This suggest impaired cardiovascular adaptation to stress in SHR. Supported by APVV-51-018004 and VEGA SR Nos. 2/7064/27.

6E_05_P

HEART RATE VARIABILITY AND GLYCATED HEMOGLOBIN

Nanna Hurwitz Eller

Department of Occupational Medicine, Hilleroed Hospital, 3400 Hilleroed, Denmark.

Introduction: By the means of spectral analysis of heart rate, the activity in the autonomous nervous system can be expressed as Heart Rate Variability, HRV. Low *Total Power, TP (total variance in HRV, ms^2)* and low *High Frequency power, HF (0,18-0,4Hz)*, the latter known to mirror vagal tone, is associated with higher cardiovascular morbidity.
Aim: To analyse associations between TP, HF and HbA1c.
Methods: The study included seventy-four healthy non-diabetic participants (50 women, 24 men, mean age 49 years). Levels of HbA1c

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

were obtained in 1998 and 2002. In 2002 ECG during the 45 minutes clinical examination were analysed and spectral analysis carried out. LogTP, logHF and logHF /TP were used as dependent variables. The analyses were carried out by means of general linear models, repeated measurements (9 levels of 5 minutes), separately for each gender using HbA1c in 1998, 2002 and expressed as change between 1998 and 2002 as the independent variable, one at a time. Results were adjusted for age and time of examination.
Results: HbA1c in 1998 was only weakly associated with HRV in 2002. HbA1c in 2002 and expressed as change (2002-1998) were significantly associated with HRV, i.e. high HbA1c and high change were associated with low HRV, most pronounced in men.
Conclusions: Increase in HbA1c and actual level of HbA1c was strongly associated with HRV, in non-diabetic women and men. This indicates that the increased cardiovascular risk accompanying insulin resistance may be caused by low HRV.

6E_06_P

EFFECTS OF CHRONIC EXERCISE AND ACUTE STRESS ON CARDIOVASCULAR PERFORMANCE IN RATS

Chauying J. Jen*, Yuan-Chang Hsu, and Hsiun-ing Chen

*Department of Physiology and Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan 70101, Taiwan. *E-mail: jen@mail.ncku.edu.tw*

It is well accepted that the cardiovascular performance is improved by chronic exercise and hampered by stress. However, the detailed mechanisms remain unclear. Wistar rats were divided into sedentary control and chronic exercise groups; the latter were trained on a treadmill for 8 weeks. Rats from either group were exposed to restraint stress and their heart rate (HR), mean blood pressure (mBP) and pulse pressure (PP) were monitored by telemetric measurements under conscious conditions. Moreover, we quantified the acetylcholine-evoked endothelial calcium signaling and the heat shock protein 72 (HSP72) expression in isolated aortic segments. In general, acute stress induced rapid elevations in HR, mBP and PP, which were partially recovered during 1 hr of stress period. However, these parameters did not fully return to resting levels even after 1 hr of post-stress resting. In addition, stress also suppressed endothelial calcium signaling and caused elevated HSP72 expression in aortas. Comparing with sedentary controls, exercise-trained rats showed i) enhanced PP along with reduced HR and mBP at rest and during stress, ii) faster recovery of these parameters during post-stress period, iii) faster adaptation due to repeated stress, iv) minimal stress-suppressed

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

endothelial calcium signaling, and v) augmented stress-induced HSP72 expression. Taken together, exercise training made the cardiovascular system more resistant to stress partially by reducing the stress-induced adverse effects and enhancing the stress-induced protective effects in vessels.

6E_07_P

EFFECTS OF ALFA1-ACID GLYCOPROTEIN ON FATTY ACIDS OVERLOAD IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM DAIRY COWS

N. Lacetera[1], F. Ceciliani[2], G. Kuzminsky[1], C. Lecchi[2], U. Bernabucci[1], A. Nardone[1], P. Sartorelli[2]

1. *Dipartimento di Produzioni Animali, Università della Tuscia, Viterbo, Italy,* ***2.*** *Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Università di Milano, Milano, Italy*

Fatty acids (FA) overload can alter leukocyte functions in dairy ruminants. Alfa1-acid glycoprotein (AGP) may function as a binding protein for hydrophobic molecules. Periparturient cows often experience a concomitant increase of plasma FA and AGP. This study was aimed to assess proliferative response of bovine peripheral blood mononuclear cells (PBMC) cultured in the presence of various concentrations of FA and AGP. Nine pregnant Holstein heifers were utilized as blood donors. PBMC were incubated with a mixture of FA reflecting composition of bovine plasma FA, and at concentrations mimicking those of cows undergoing various degree of lipomobilization (500, 250, 125 e 62.5 μmol/l). AGP was purified from bovine plasma and added to PBMC culture at concentrations, which reflected those found in physiological conditions or during the systemic reaction to inflammation (0.3 and 1.5 mg/ml, respectively). Concanavalin A and pokeweed mitogen (PWM) were utilized as mitogens. PBMC proliferation was assessed by measuring the 5-bromo-2'-deoxyuridine incorporated during DNA synthesis. The highest concentrations of FA significantly impaired proliferative response of PBMC to both mitogens. In PWM-stimulated PBMC only, the addition of 1.5 mg/ml AGP attenuated to a significant extent the negative effects of FA overload on PBMC proliferation. Present results suggest that under conditions of intense lipomobilization, levels of AGP mimicking those observed during the systemic reaction to inflammation may at least partially attenuate the negative effects of FA overload on leukocyte functions.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

6E_08_P

TREATMENT WITH SODIUM PENTOSAN POLIYSULFATE AMELIORATES MORPHOLOGICAL RENAL ALTERATION AND ALBUMINURIA IN DIABETIC RATS

[1]Mathison Y, [2]Finol H.J, [1]Quero Z, [2]Mayora S.

[1]Schools of Medicine J.M. Vargas and [2]Center of Electron Microscopy, School of Science, Central University of Venezuela.
email:yaira57@yahoo.com

Decrease levels of glycosaminoglycans (GAGs) have been observed in kidney and other organs, in human and animal models of diabetes. Long term administration of heparins and other glycosamonoglycans have demonstrated a beneficial effect on morphological and functional renal abnormalities in diabetic rats. We assessed the effect of sodium pentosan polysulfate (SPP), a semi-synthetic orally active glycosaminoglycan with low anticoagulant activity, on renal involvement in streptozotocin diabetic rats. Diabetes was induced in male Sprague-Dawley rats by i.v. administration of streptozotocin (STZ). Animals were randomly allocated in three groups: C=control, STZ and STZ+SPP= pretreated with SPP (15mg/kg, s.c.). After three months of follow-up, blood and 24h-urine samples were obtained and than the animals were sacrificed and the kidney microdissected for morphometric analysis. Urinary albumin excretion was markedly increased in untreated diabetic rats (C= 0,26± 0,03 vs. STZ= 7,75±1,8 mg/24h) and SPP treatment partially prevented the albumin rise (3,7±0,7 mg/24h), without affecting the metabolic control HbA1c (C=3,6±1,7; STZ=8,82±0,47; STZ+PPSNa= 8,63 ± 0,54). Electron microscope observation revealed typical renal lesions described in experimental diabetes (STZ group). SPP administration prevent the glomerular and tubular basement membrane thickening and the lost of cytoarchitecture induced by experimental diabetes. Our data demonstrated that long-term administration of SPP have a favorable effect on morphological and functional abnormalities in kidney of diabetic rats and suggests a potential therapeutic use of this compound. (Supported by CDCH P09-11-5102-2003).

6E_09_P

ANGIOTENSIN II INHIBITS INSULIN-DEPENDENT GLUT4 TRANSLOCATION AND AKT ACTIVATION THROUGH NITRATION IN SKELETAL MUSCLE

Serge Bottari

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Hypoxo-ischemia is known to induce an increased generation of peroxynitrite, which is able to nitrate specific cellular and plasma proteins on tyrosines among which albumin. A recent study by Groenendaal et al. showed a strong increase of nitrotyrosine immunostaining in the brains of neonates deceased from perinatal asphyxia, indicating the occurence of an important nitrative stress. In order to investigate the occurrence of nitrative stress in clinical practice, we have developed a novel double-sandwich ELISA allowing the quantitative determination of nitrated plasma albumin (nitralbumin) in human plasma as a biological marker of peroxynitrite generation.

In a currently ongoing study on nitrative stress in human newborns (n = 194), we investigated the plasma nitralbumin concentrations in neonates who suffered perpartal asphyxia. Plasma was prepared from arterial blood obtained during the first hour of life and venous blood at day 1 and day 4 of life.

We found a significant increase ($p < 0.05$) in the nitralbumin concentration at day 1 in children who developed a moderate or severe neonatal encephalopathy. Newborns who had a normal evolution or who developed a mild neonatal encephalopathy had nitralbumin levels similar to matched controls. Nitralbumin levels were normalized at day 4.

Our data indicate the occurrence of nitrative stress in newborn exposed to perpartal asphyxia. Whereas we did not observe any measurable nitrative stress during the first hour of life it appears to become important during the next 24 hours and to disappear by day 4. These observations are in agreement with the tyrosine nitration observed in neonate brains in a post-mortem study and therefore indicate that nitrative stress carries a serious risk of potential end-organ damage due to protein and lipid nitration. They also indicate that the determination of plasma nitralbumine levels with the ELISA we developed is a reliable way of assessing nitrative stress in clinical practice.

6E_10_P

EFFECTS OF FAT LOSS STRESS IN FAT CELLS

K.T. Tan and Y.H. Lee

Academia Sinica, Institute of Molecular Biology, Taipei 115, Taiwan

Recent years, obesity and the ensuing metabolic disorders such as hypertension, type 2 diabetes mellitus have become the severe healthcare problem in industrial nations. To cope with this problem, efforts such as behavior therapy, surgical intervention and pharmacologic treatment have been applied. Although weigh-reducing drugs, due to their fast effect, tend to be the most popular choice for people wishing to shed a few pounds of their body weight, it was found that there is a high frequency of

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

weight regain
and/or the loss of their effects on reducing weight. In fact, weight recurrence occurs frequently with all kinds of weight-reduction programs, especially with those that produce quick effects. Our evidences show that this phenomenon may be due to the cellular and physiological rescuing-responses triggered by stress from the sudden loss of body weight/fat. We have used both cell and animal systems to study the slim-fast stress and its effects, and our findings indicate that the stressed fat cells are more active than those unstressed in gene expression, glucose uptake, protein synthesis and energy oxidation, etc. In simple words, the stressed cells have become more viable. Accordingly, this increased viability might form a driving force for cells to recover, even to exceed, rapidly their status before stress. The molecular mechanism underlying this rapid recovery was investigated, and the results will be discussed.

6F_01_P

VASCULAR HYPOREACTIVITY IS NOT ASSOCIATED WITH MORTALITY IN RATS WITH ENDOTOXIN STRESS

S.J. Chen[1], P.L. Chiang[2], H.L. Liu[2], R.L. Chen[2], M.H. Liao[3], C.C. Wu[2]

[1]Department of Nursing, Kang-Ning Junior College of Medical Care and Management, Taipei; [2]Institute of Pharmacology and [3]Graduate Institute of Medical Sciences, National Defense Medical Center, Taipei; Taiwan. sjchen@knjc.edu.tw

Sepsis is the systemic inflammatory stress induced by infection. In previous studies, the administration of lipopolysaccharide (LPS) to animals produces a sepsis-like syndrome stress, characterized by low blood pressure and vascular hyporeactivity to vasoconstrictor agents (norepinephrine, NE). The vascular hyporeactivity to vasoconstrictor agents, an important characterization of circulatory failure in septic shock, occurred at the late phase of sepsis. The present study was to compare vascular reactivity between survival and non-survival rats with endotoxic shock. Endotoxemic stress was induced by intravenous administration of LPS in male Wister rats. We measured resting membrane potential (RMP) and NE-induced tension in isolated thoracic aortas by electrophysiology and tension recording experiments. Our results revealed that hypotension, bradycardia, hypoglycemia and hypothermia were found in non-survival septic rats. However, the RMP and vascular reactivity to NE in aorta were not significant different between non-survival and survival septic rats. Thus, our results suggest that the mortality in LPS-induced stress was not associated with vascular hyporeactivity. Indeed, other mechanisms contributed to the mortality induced by LPS should be elucidated in future studies.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

6F_02_P

SESAMOL PROTECTS AGAINST FERRIC NITRILOTRIACETATE-INDUCED OXIDATIVE STRESS IN ACUTE RENAL INJURY IN MICE

Bu-Miin Huang[1], Dur-Zong Hsu[2], Chang-Hsin Wan[2], and Ming-Yie Liu[2]

Departments of Cell Biology and Anatomy[1] and Environmental and Occupational Health[2], National Cheng Kung University Medical College, Tainan, Taiwan

Ferric-nitrilotriacetate (Fe-NTA) has been reported to cause acute nephrotoxicity in animals and in humans. The aim of this study was to examine the protective effects of sesamol on acute renal damage induced by Fe-NTA in mice. Acute renal injury was induced by giving Fe-NTA to mice for 24 h. Renal function was assessed by blood biochemistry, creatinine clearance, and histological examination. Free radicals were determined using a high-performance chemiluminescence analyzer. Fe-NTA (4 mg/kg) increased renal lipid peroxidation and induced acute renal injury. Sesamol (30 mg/kg) attenuated Fe-NTA-induced acute renal injury, decreased renal lipid peroxidation, reduced the generation of renal hydroxyl radical and superoxide anion, and inhibited the activity of xanthine oxidase in mice. Thus, sesamol might ameliorate oxidative-stress-associated-acute renal injury by inhibiting xanthine-oxidase-initiated superoxide anion generation, thereby reducing hydroxyl radical production, at least partially, in acutely iron-intoxicated mice.

KEYWORDS—Ferric-nitrilotriacetate; acute renal injury, lipid peroxidation, oxygen free radicals, xanthine oxidase, sesamol

---

6F_03_P

PROTECTIVE ROLE OF N-PROCALCITONIN IN ENDOTOXIC SHOCK

Eva Tavares, Rosario Maldonado, Inmaculada Capilla, Cesar Sevillano and Francisco J. Miñano

Pharmacology Research Unit, Valme University Hospital, University of Seville, 41014 Seville, Spain
Corresponding author: jminano@us.es

The overzealous production of proinflammatory cytokines in sepsis can result in shock, multiorgan dysfunction, and even death. Procalcitonin

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

(PCT), a 14-kDa propeptide of calcitonin, is a circulating biomarker of bacterial infection but PCT itself has no known activity. Circulating levels of PCT and its free aminopetide N-procalcitonin (N-PCT), have been found dramatically increased in septic patients, and these rises are correlated with severity and mortality. Importantly, in sepsis, the levels of N-PCT may be even higher than the PCT values. *In vitro* studies suggest that N-PCT may function as a factor suppressing the propagation of inflammation through the inhibition of several processes involved during a response to a bacterial stimulus.These findings together with N-PCT's sequence conservation during evolution, suggest that N-PCT has a critical, and as yet undefined, pathophysiological function. In this study, we assessed the role of N-PCT as a mediator of sepsis in endotoxin-challenged rats. Intraperitoneal administration of an lethal dose (LD_{100}) of *E. coli* endotoxin to normal rats induced a substantial increase in PCT, IL-1β and TNF-α in plasma. The administration of recombinant human N-PCT intraperitoneally significantly protected rats from endotoxin-induced mortality from 100% to 15%, and resulted in a decrease in PCT, IL-1β and TNF-α levels. By contrast, N-PCT did not modify IL-10 levels. These results confirm a critical part for PCT in the pathogenesis of endotoxic shock and indicate that N-PCT is a protective peptide expressed in murine endotoxemia, and does so by down-regulating the systemic production of proinflammatory cytokines in endotoxin-challenged animals.

This work was supported by grants from the Andalusian Government (Consejeria de Salud, 053/05 and 0364/06), Spanish Ministry of Health (FIS 06/1394) and the Valme Foundation.

6F_04_P

UNDETOXIFIED ENDOTOXINS CAUSING PERSISTENT SEPSIS WITH PROGRESSIVE PARKINSONISM (A CASE REPORT)

I. Niehaus

Bad Segeberg, Germany, Ines_Niehaus@gmx.de

A 22-year old female was contaminated with 10 microgram Salmonella minnesota smooth lipopolysaccharides (LPS or endotoxin) by an accident in lab in 1995. 21 days later parkinsonism started after acute sepsis-like symptoms (high fever, chills, myalgia, flu-like symptoms). The LPS-induced neuronal inflammation (6.6 ng LPS/ml cerebrospinal fluid measured in 2001) causes polyneuropathy, myocloni, and reduced metabolism of dopamine and glucose shown by positron emission tomographies of the brain. The chronic inflammatory LPS-induced stress also leads to a multiple chemical sensitivity because of the reduced capacity of the liver, which had up taken a part of the LPS, as a filter

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

organ. The liver was unable to eliminate the still circulating LPS in the blood causing a chronically sub acute sepsis with e. g. tachycardia, orthostatic hypotension. Treatment with endotoxin-binding agents like the antibiotic drug ofloxacin is improving all symptoms including parkinsonism but is unable to cure. After stop taking it the inflammatory reactions are increasing again in the whole body because of the re-binding of LPS to the cells. Acute feverish phases of inflammation provoked by all kinds of stress (e. g. physical or mental exhausting, secondary viral or bacterial infections) are marked by a rapid deterioration of parkinsonism with akinetic crises (muteness, inability to walk), disorientation, difficulties in hearing and seeing. Glucocorticoids, which are not effective in idiopathic Parkinson ’s disease, are able to stop these crises, which is comparable with some patients suffering from post encephalitic parkinsonism. In rats one single i. p. injection of LPS is causing a progressive parkinsonism similar to this case report.

6F_05_P

NON-TOXIC LIPOPOLYSACCHARIDE DERIVATIVES AND EXOGENOUS HSP70 PROTECT AGAINST LETHAL SEPTIC SHOCK AND IMMUNO-METHABOLIC INJURIES INDUCED BY BACTERIAL ENDOTOXIN AND XENOBIOTICS

Sergey Onikienko[1], Alexander Zemlyanoi[3], Irina Guzhova[2], Boris Margulis[2], Maxim Vinokurov[4]

[1] *Military Medical Academy, St Petersburg, Russia, onik@nwgsm.ru*
[2] *Institute of Cytology RAS, St Petersburg, Russia*
[3] *Institute of Military Medicine, St Petersburg, Russia*
[4] *Institute of Cell Biophysics RAS, Pushino, Russia*

Serratia marcescens endotoxin (LPS) modification by electronic beam and CO_2-laser radiation resulted to formation of its non-toxic derivatives. The parent LPS were modified to 1,5-5,0 kDa oligomers with reduced toxicity and enhanced immunogenicity. Treatment of the mice with non-toxic LPS derivatives significantly attenuated the lethality, deleterious immune and metabolic injuries induced by parent LPS (1-7LD_{50},i.p). The protective effect strongly correlated with the degree of LPS molecular structure modification and was significantly enhanced by the combined impact of detoxified LPS and extracellular Hsp70. Detoxified LPS activated antiendotoxin immune response and reduced LPS-induced inflammatory cytokine production. Also it was effective in the treatment of endotoxin-related injuries, inhibited the deleterious effects of ecological and occupational toxicants. Significant exacerbation of whole blood Hsp70 content after its incubation with modified LPS (in vitro) or in response of the patient to high elevation (5000 m) hypoxia and total body

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

hyperthermia reveals the necessity for nontoxic LPS application. Nonresponders need the application of exogenous chaperons. The activation product of yeasts culture Saccharomyces cerevisiae may be a promising source of these cytokines. The yeasts were activated by laser radiation, electronic beam and cavitation. In summary the data prove that application of modified LPS and exogenous chaperones can be promising in treatment of toxin-related injuries.

6F_06_P

REPROGRAMMING OF MACROPHAGES BY ENDOTOXIN TOLERANCE

Schade, F. Ulrich, Butterbach, Katja, Plitzko, Daniela

Surgical Research - Trauma Surgery, University Hospital Essen, D-45122 Essen, Germany
ulrich.schade@uni-due.de

The term "Endotoxin Tolerance" (ET) has been introduced for a state of low responsiveness of experimental animals or humans treated with a low dose of lipopolysaccharide (LPS, endotoxin). ET has attracted a lot of attention since it was shown that cross tolerance exists to other bacterial products such as lipoteichoic acid or lipopeptides. Furthermore, it turned out that ET protected against bacterial infections - Gram-negative, as well as Gram-positive - and ischemia-reperfusion injury. It, therefore, is of potential clinical interest to get insight into the cellular and molecular basis of ET. To this end, we studied different cellular components of the peritoneal cell populations and Kupffer cells of endotoxin tolerant and normal mice with regard to gene expression, intracellular signal transduction and regulation of cytokine production. Corresponding results will be presented. HSP 70 is among the genes that are significantly upregulated in peritoneal cells of ET mice compared to normal mice.

6F_07_P

IMMUNO-NEURO-ENDOCRINE ADAPTATION IN CRITICAL ILLNESS

I.Vermes, A.Beishuizen and A.B. J.Groeneveld

Medical Spectrum Twente, Enschede & Free University Medical Centre Amsterdam,
The Netherlands (i.vermes@home.nl)

Critical illness is a severe stress stimulus that disturbs the milieu interior

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

and induces homeostatic responses specific to the stimulus and generalized responses when the disturbances are prolonged and severe. The immune-neuroendocrine response during critical illness is a dynamic process, differing between the acute and chronic phases. This acute phase lasts typically from about a few hours to several days, depending on the severity of the illness and is characterized by an appropriate hormonal reaction: the mobilization of fuel stores of the organism, together with apparent restrains on their utilization. During the acute phase of critical illness the secretory activity of the pituitary gland is stimulated whereas anabolic target organ hormones are inactivated. Due to the development of intensive care medicine patients who would previously have died during the acute phase nowadays survive and enter the chronic or prolonged phase of the critical illness. This prolonged phase lasts for days and is characterized by an inappropriate hormonal response, resulting in a chronic increase in metabolic rate and a breakdown of body tissue. The secretory activity of the pituitary gland is uniformly inhibited in relation to reduced levels of target organ hormones. These hormonal changes suggest an imbalance between immunosuppressive and immunostimulatory hormones which might be the cause of the increased susceptibility to infectious complications during the chronic phase of severe illness. The suppressed pituitary activity allows the respective target organ hormones to decrease, resulting in a restored balance between the catabolic and anabolic hormonal responses. This recovery phase is characterized by restored sensitivity of the pituitary gland to reduced feedback control. The distinction of acute and prolonged critical illness as different entities with regard to the immuno-neuroendocrine adaptation may be helpful in further understanding of the pathogenesis of critical illness and the targeting of therapeutic intervention.

6F_08_P

IN VIVO IMAGING OF THE EFFECT OF BACTERIAL ENDOTOXINS ON ARTERIAL ENDOTHELIAL CELLS: MOLECULAR IMAGING OF HEAT SHOCK PROTEIN 60 EXPRESSION

Marius C. Wick[1], Christina Mayerl[2], Georg Dobrozemsky[3], Roland Haubner[3], Hermann Dietrich[4], Werner Jaschke[1], and Georg Wick[2]

[1]Department of Radiology, [2]Division for Experimental Pathophysiology and Immunology, Biocenter, [3]Department of Nuclear Medicine, [4]Central Animal Laboratory Facilities,Innsbruck Medical University,Austria

Background. Bacterial endotoxins (LPS) are known to act as stress factors for endothelial cells.
Aims. As a proof of principle, the present project aimed at developing a novel radiotracer-based non-invasive molecular imaging method for *in*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

vivo visualization of early aortal HSP60-expression in a normocholesterolemic rabbit model after induction of endothelial stress.

Material and Methods.

In 14 rabbits, endothelial stress was induced by i.v. injection of bacterial endotoxin (lipopolysaccharide, LPS at 10μg/kg) while 8 animals were untreated controls. Non-invasive *in vivo* molecular imaging was performed 24 hours after intravenous injection of I-124 radiolabelled monoclonal anti-HSP60 antibodies using computertomography (CT) and positron-emission-tomography (PET). *In vitro* correlation of *in vivo* imaging was done by *en face* immunohistochemistry and autoradiography of the aorta.

Results.

Compared to control animals, quantitative analyses of *in vivo* non-invasive molecular PET-CT images of rabbit aortae revealed a significantly increased endothelial binding of I-124 anti-HSP60 antibodies upon application of LPS as an endothelial stressor, especially at sites of aortal branching. This was confirmed by *in vitro* anti-HSP60 immunohistochemistry and autoradiography data.

Conclusion.

Our first results showed, as a proof of principle, that HSP60-expression in normocholesterolemic rabbits is significantly increased after the induction of endothelial stress and non-invasive *in vivo* molecular imaging of early aortal HSP60-expression using radiotracer labelled anti-HSP60 antibodies is possible.

Acknowledgements: This work was supported by the Propter Homines Foundation, Vaduz, FL.

6F_09_P

COMPARISON OF VASCULAR REACTIVITY BETWEEN SURVIVAL AND NON-SURVIVAL RATS WITH SEPTIC STRESS INDUCED BY PERITONITIS

C.C. Wu[1], S.J. Chen[2], P.L. Chiang[1], Z.F. Chen[1], C.T. Chiu[1], W.J. Liaw[3]

Departments of [1]Pharmacology and [3]Anesthiology, National Defense Medical Center, Taipei; Department of Nursing, Kang-Ning Junior College of Medical Care and Management, Taipei; Taiwan. ccwu@ndmctsgh.edu.tw

The sepsis-induced metabolism has two stress phases: hyper-metabolism followed by hypo-metabolism. The vascular hyporeactivity to vasoconstrictor agents, an important characterization of circulatory failure in septic stress, occurred at the late phase of sepsis. This study was to compare vascular reactivity between survival and non-survival rats with peritonitis-induced septic stress and to evaluate whether this is associated with physiological metabolism. Sepsis stress was induced by a surgery of

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

cecal ligation and puncture (CLP) in male Wister rats. We measured resting membrane potential (RMP) and norepinephrine (NE)-induced tension in isolated thoracic aortas by electrophysiology and tension recording experiments. Our results demonstrated that hypotension, hypoglycemia and multiple organ dysfunction syndrome (MODS) occurred in non-survival rats. In addition, not only the RMP was more hyperpolarized in aorta from non-survival rats, but also the vascular reactivity to NE was reduced in non-survival rats than that in survival rats. However, the RMP and vascular reactivity to NE were not significant different in non-survival groups whether they died at early or late phase. Our results suggest that non-survival rats after CLP were associated with vascular hyporeactivity which may lead to MODS.

6F_10_P

SESAME OIL PROTECTS AGAINST HYDROXYL-RADICAL-ASSOCIATED LIPID PEROXIDATION IN LIVER AFTER CECAL LIGATION AND PUNCTURE IN RATS

Ming-Yie Liu[a]; Ya-Hui Li[a]; Se-Ping Chien[b]; Dur-Zong Hsu[a]

Department of Environmental and Occupational Health, National Cheng Kung University, College of Medicin,.Address: 138 Sheng-Li Road, Tainan, Taiwan 704.
Email: myliu@mail.ncku.edu.tw

Sesame oil protects against oxidative-stress-associated hepatic injury in experimental sepsis. The aim of this study was to explore the effects underlying the inhibitory effect of sesame oil on hepatic lipid peroxidation in septic rats. After one-week daily supplement of sesame oil (4 mL/kg/day, orally) to rats, hepatic injury was induced by cecal ligation and puncture. Hepatic oxidative stress was assessed by determining the levels of hepatic lipid peroxidation, hydroxyl radical, superoxide anion, and nitric oxide 12 h after cecal ligation and puncture in rats. In addition, the activity of xanthine oxidase and the expression of inducible nitric oxide synthase were also determined. Hepatic lipid peroxidation, hydroxyl radical, superoxide anion, and nitrite levels were significantly lower in sesame-oil-treated septic rats. Furthermore, sesame oil decreased the activity of xanthine oxidase and the expression of inducible nitric oxide synthase in septic rats. Therefore, sesame oil might reduce hydroxyl-radical-associated hepatic lipid peroxidation by inhibiting superoxide anion and nitric oxide, at least partially, in septic rats.

KEYWORDS—Liver, lipid peroxidation, reactive oxygen substances, nitric oxide, sepsis, sesame oil

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

6G_01_P

THE HSP60-(P.VAL98ILE) VARIANT PROTEIN ASSOCIATED WITH AUTOSOMAL DOMINANT SPASTIC PARAPLEGIA SPG13 DISPLAYS SUBTLE EFFECTS WHEN CO-EXPRESSED WITH WILD TYPE HSP60

Peter Bross[1], Marit N. Nielsen[1], Jane H. Christensen[1], Jakob Hansen[1],Niels Gregersen[1], Debbie Ang[2], Costa Georgopoulos[2]

[1]Research Unit for Molecular Medicine, Aarhus University Hospital and Faculty of Health Sciences, Århus, Denmark, [2]Department of Microbiology and Molecular Medicine, Centre Médical Universitaire, Geneva, Switzerland.
Peter.Bross@ki.au.dk

We have earlier reported that a mutation (c.292G>A/p.Val98Ile) in the human *HSPD1* gene that encodes the mitochondrial Hsp60 chaperonin is associated with dominantly inherited hereditary spastic paraplegia SPG13 [1]. To mimic heterozygosity for the Hsp60-(p.Val98Ile) mutation and to assess a potential dominant negative effect of co-expression of a wild type and the mutant variant of Hsp60 on the function of the 7-meric chaperonin complex, we used a flexible bacterial model system. These cells lack the endogenous chaperonin genes and are maintained alive by expression of a plasmid-encoded Hsp60/Hsp10 operon. The cells harbour a second plasmid with a Hsp60/Hsp10 operon encoding a mutant Hsp60. The two operons can be turned on and off separately. We compared the behaviour of bacterial cells co-expressing either wild type Hsp60 and Hsp60-(p.Val98Ile) or wild type Hsp60 and an artificially constructed Hsp60 ATPase mutant. Induction of co-expression of the Hsp60 ATPase mutant severely inhibited bacterial growth, whereas co-expression of the Hsp60-(p.Val98Ile) mutant variant had only subtle yet specific effects. Additional experiments indicate that both mutant Hsp60 variants form heterologous complexes with wild type subunits. By varying temperature and relative amounts of the wild type and Hsp60-(p.Val98Ile) mutant variant, we have found conditions under which the mutant exerts a clear effect. We propose that the major *in vivo* consequences of heterozygosity for the Hsp60-(p.Val98Ile) variation are due to subtle qualitative and quantitative effects remaining to be discovered.

[1] J. J. Hansen, A. Dürr, I. Cournu-Rebeix, C. Georgopoulos, D. Ang, M. N. Nielsen, C. S. Davoine, A. Brice, B. Fontaine, N. Gregersen and P. Bross, *Hereditary Spastic Paraplegia SPG13 Is Associated with a Mutation in the Gene Encoding the Mitochondrial Chaperonin Hsp60*. Am J Hum Genet *70,* 1328-1332 (2002).

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

6G_02_P

INACTIVATION OF THE HEREDITARY SPASTIC PARAPLEGIA-ASSOCIATED *HSPD1* GENE RESULTS IN A RECESSIVE EMBRYONIC LETHAL PHENOTYPE IN MICE

Jane H. Christensen[1], Marit N. Nielsen[1], Ernst-Martin Füchtbauer[2], Thomas J. Corydon[3], Jakob Hansen[1], Niels Gregersen[1], and Peter G. Bross[1]

1) Research Unit for Molecular Medicine, Aarhus University Hospital, Skejby; 2) Department of Molecular Biology, University of Aarhus; 3) Department of Human Genetics, University of Aarhus, Denmark.
jane.hvarregaard@ki.au.dk

To establish an animal model for the neurodegenerative disease, hereditary spastic paraplegia, we have generated a mouse which is heterozygous for an inactivating insertion in the *hspd1* gene. *Hspd1* encodes the mitochondrial chaperonin Hsp60. Missense mutations in one allele of the gene have been associated with hereditary spastic paraplegia in humans. Heterozygous mice were born at the expected frequency compared to wild type and displayed no obvious severe phenotype. By quantitative RT-PCR we found decreased levels of *hspd1* transcript in all tissues examined, demonstrating that the inactivation of the *hspd1* gene is efficient. By Western blotting analysis, we found that the amount of Hsp60 protein compared to either cytosolic tubulin or mitochondrial VDAC/porin was decreased as well. Breeding of heterozygous animals resulted in reduced litter sizes and offspring homozygous for the inactivated allele were missing. Timed mating revealed a significant proportion of degenerated or growth retarded embryos at 8.5 and 10.5 dpc and normal developed embryos were either wild type or heterozygous. In conclusion, the inactivation of the *hspd1* gene is efficient at the molecular level and it is associated with a recessive peri-implantational embryonic lethal phenotype in mice. We expect that heterozygous mice will develop hereditary spastic paraplegia due to shortage of Hsp60 chaperonin activity, protein quality control failure, and mitochondrial dysfunction in motor neurons with very long axons.

6G_03_P

SMALL HEAT SHOCK PROTEIN HSPB8 AND PROTEIN DEGRADATION IN MOTONEURON DISEASES

Crippa V[1], Simonini F[1], Carra S[2], Bolzoni E[1], Onesto E[1], Rusmini P[1], Sau

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

D[1], Poletti A[1]

1 Institute of Endocrinology, CEND, University of Milan. Via Balzaretti 9, 20133 Milan. ITALY angelo.poletti@unimi.it
2 Centre de recherche en cancerologie de l'Universite Laval. CANADA

In mammals, the small Heat Shock Proteins (HSP) family comprises 10 members called HSPB proteins (HSPB1-10), with chaperone activity. They are upregulated in neurodegenerative disorders exerting a neuroprotective role. Mutations in HSPB1 and HSPB8 have been linked to peripheral neuropathies. We investigated the role of HSPB8 in two motoneuronal diseases: spinal and bulbar muscular atrophy (SBMA) and familial amyotrophic lateral sclerosis (fALS). SBMA is caused by polyglutamine tract expansion (polyQ) in the Androgen Receptor (AR), while fALS is often associated to mutations in Superoxide Dismutase 1 (SOD1). Mutant AR and SOD1 do not share structural or functional domains, but are unstable and tend to aggregate. Immortalized motoneuron, NSC34, expressing mutant form of SOD1 (SOD1G93A) or ARpolyQ (ARQ46) contain intracellular aggregates of the mutant proteins and have a reduced proteasome activity, measured with accumulation of the proteasome reporter YFPu. HSPB8 overexpression decreased the levels of both mutant proteins and reduced YFPu levels, suggesting a desaturation of the proteasome system. Thus, HSPB8 exerts chaperone activity towards both mutated AR and SOD1. By immunoprecipitation, we showed that HSPB8 does not need a protein-protein interaction to reduce mutant AR and SOD1 levels. Moreover, proteasome inhibition with MG132 did not block HSPB8 chaperone activity, suggesting that HSPB8 could activate other degradative pathways, such as the autophagy. Grants Telethon - Italy (#GGP06063), MIUR-FIRB (#RBAU01NXFP), MIUR-Cofin (2005057598_002), University of Milan-FIRST, FONDAZIONE CARIPLO.

6G_04_P

ACTIVATION OF STRESS RESPONSE PATHWAYS IN MOTOR NEURON DISEASES: IMPLICATIONS FOR PATHOGENESIS AND THERAPY

Heather D. Durham, Benoit Gentil, Miranda L. Tradewell, David M. Taylor, Walter E. Mushynski, Laura Senese and Sandra Minotti

Montreal Neurological Institute, McGill University, Montreal QC, Canada.
heather.durham@mcgill.ca

Neural cells differ in their ability to induce heat shock genes in response to thermal or disease-related stresses. Motor neurons have a high threshold for up-regulation of stress-inducible Hsp70 [Manzerra and Brown, 1992;

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Batulan et al, 2003]. However, increasing levels of multiple chaperones is protective in culture and mouse models of motor neuron diseases including amyotrophic lateral sclerosis [Bruening et al, 1999; Kieran et al, 2004; Batulan et al, 2006], spinal bulbar muscular atrophy [Katsuno et al, 2005], and Charcot-Marie-Tooth (CMT) disease type 1A due to missense mutation in *PMP22* [Fortun et al, 2007]. We have demonstrated prevention of the disease-related phenotype in a primary culture model of CMT2 caused by mutations in *nefl* encoding the neurofilament light protein (NFL), as well as of ALS due to *sod1* mutation. Increasing levels of HSPs, including Hsp70 and Hsp40, by over-expression of constitutively active Hsf1 prevented bundling and fragmenting of the neurofilament network and aggregation of CMT-related mutant NFL in motor neurons of dissociated spinal cord cultures. Upregulation of chaperones, including Hsp27, facilitated degradation of NFL mutants in SW13^{vim-} cells. Given the potential for chaperone-based therapies for neurological disorders, our group is studying the mechanisms of *hsp* regulation in motor neurons and is using primary culture models of motor neuron diseases to screen compounds for their ability to increase neuronal HSP levels and for neuroprotection. Differences identified in mechanisms of activating *hsp* expression in neurons included a CaMKIV-dependent pathway for upregulating Hsp70 in motor neurons, but not in fibroblasts.

6G_05_P

DECREASED EXPRESSION OF THE MITOCHONDRIAL MATRIX PROTEASES CLPP AND LON IN CELLS FROM PATIENT WITH HEREDITARY SPASTIC PARAPLEGIA (SPG13).

Jakob Hansen[1], Thomas J. Corydon[2], Alexandra Dürr[3,4], Marit N. Nielsen[1], Jane H. Christensen[1], Niels Gregersen[1], Bertrand Fontaine[5] and Peter Bross[1].

[1]Research Unit for Molecular Medicine, Aarhus University Hospital, Skejby, Denmark.
[2]Institute of Human Genetics, University of Aarhus, Aarhus, Denmark.
[3]INSERM U679, [4]Departement de genetique, [5]INSERM U546, Hôpital de la Salpêtrière, Paris, France.
Jakob.hansen@ki.au.dk

The mitochondrial Hsp60 chaperone promotes folding of proteins in the mitochondrial matrix space, and plays a crucial role in protein quality control. A mutation in the *HSPD1* (SPG13) gene encoding the mutant Hsp60-(p.Val98Ile) protein has been associated with a dominantly inherited form of spastic paraplegia. The Hsp60-(p.Val98Ile) protein is functionally impaired and displays a reduced efficiency in mediating folding of the malate dehydrogenase substrate protein possibly related to

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

a reduced ATP-ase activity of the chaperonin complex, but the molecular defect involved in axonal degeneration in spastic paraplegia is unknown. We have investigated mitochondrial function and gene expression levels of key mitochondrial chaperones and proteases in cultured lymphoblastoid and fibroblast cells from SPG13 patient cells. We found that impaired Hsp60-(p.Val98Ile) function is not related to a severe mitochondrial dysfunction phenotype as indicated by assessment of mitochondrial membrane potential, cell vitality, and sensitivity towards oxidative stress insults. However, a decreased expression of protein quality control proteases Lon and ClpP in SPG13 patient cells was demonstrated. We propose that decreased protease levels may represent an adaptive change of protein quality control giving more time to the folding of proteins whose folding is impaired due to a reduced activity of Hsp60-(p.Val98Ile).

6G_06_P

A NOVEL NUCLEAR DNAJ PROTEIN, DNAJC8, CAN SUPPRESS THE ATAXIN-3-POLYQ AGGREGATION AND CELL DEATH IN A J-DOMAIN INDEPENDENT MANNER

Norie Ito[1, 3], Kenjiro Kamiguchi[1], Toshihiko Torigoe[1*], Katsuya Nakanishi[1], Alisa Sokolovskya[1], Susumu Chiba[3], Shun Shimohama[2], and Noriyuki Sato[1]

Department of Pathology[1] and Neurology[2], Sapporo Medical University School of Medicine, South-1, West-17, Chuo-ku, Sapporo 060-8556, Japan, Sapporo Yamano-ue Hospital[3], torigoe@sapmed.ac.jp[*]

Machado-Joseph disease (MJD), also termed spinocerebellar ataxia type3, is a neurodegenerative disorder characterized by abnormal movement coordination. The disease is fatal and is inherited in a dominant manner. MJD is caused by a pathogenic ataxin-3 protein with an expanded polyglutamine (polyQ) stretch. The neurotoxicity of the expanded polyQ-containing ataxin-3 is closely associated with its aggregate formation. We report here that a novel J-protein, DNAJC8 (JC8) suppresses the ataxin-3-polyQ aggregation in a cellular model of MJD. JC8 was identified by the human EST database search for nuclear J-proteins, since ataxin-3 protein with expanded polyQ stretch forms aggregation in the nucleus of neuronal cells. Overexpression of JC8 in SH-SY5Y neuroblastoma cells significantly suppressed the polyQ aggregation and cell death. JC8 was co-localized with the polyQ-containing protein in the nucleus. To identify a responsible region of JC8 affecting the polyQ aggregation, a series of JC8 deletion mutants were examined for their ability to suppress the aggregation. Interestingly, C-terminal domain of JC8 was essential for the suppression of polyQ aggregation, whereas J-domain was dispensable. These results indicate that JC8 might suppress the polyQ aggregation by a distinct

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

mechanism independent of Hsp70-based chaperone machinery and have a unique protective role against the aggregation of expanded polyQ-containing proteins such as a pathogenic ataxin-3 protein.

6G_07_P

A REVERSE GENETIC OVEREXPRESSION SCREEN REVEALS NON-CANONICAL CHAPERONES AS POTENT SUPPRESSORS OF POLYGLUTAMINE PROTEINS

Harm H. Kampinga, J. Hageman, M. Rujano, M. Vos

[1]*Department of Radiation and Stress Cell Biology, University Medical Center Groningen, Univ. of Groningen, The Netherlands*
Email: h.h.kampinga@med.umcg.nl

As they are able to bind misfolded proteins, Heat Shock Proteins (HSP) may prevent accumulation of toxic poly-Q protein aggregates and as such inhibit disease-associated pathogenesis. Several *in vitro* reports have pointed to the classical Hsp70 chaperone machines and their cofactors as suppressors of polyglutamine-related protein aggregation. Yet, the (limited) *in vivo* work with mouse models has yielded disappointing results from minor delays in disease progression to no effect at all. It has become clear recently that the many different Hsp subfamily members may have different and substrate specific chaperone-like functions. To seek for chaperones that may be particularly effective in dealing with polyglutamine proteins, we conducted a reverse genetic overexpression screen of the human chaperonome. We identified a number of non-canonical Hsp40 family members as well as a new member of the small Hsp family as superior inhibitors of polyglutamine aggregation. Unlike the canonical DnaJB1 (that has mild suppressive activity in vitro), the suppression by the non-canonical Hsp40's was not annihilated by mutating the HPD motif in their J-domain, normally required for interaction with Hsp70 family members. At suboptimal concentrations of the non-canonical Hsp40's, overexpression of none of the Hsp70 family members was able to further reduce the polyglutamine aggregation. All these data suggest that they act independently of the Hsp70 machine. The action of the small Hsp was independent on its N-termimal domain and as such seems to be executed by the crystallin domain.

6G_08_P

OVEREXPRESSION OF HSP27 HAS NO EFFECT ON SURVIVAL IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Krishnan J, Vannuvel K, Kiraly D, Robberecht W and Van Den Bosch L

Laboratory of Neurobiology, K. U. Leuven, B-3000 Leuven, Belgium.
Jyothsna.Krishnan@med.kuleuven.be

Amyotrophic Lateral Sclerosis (ALS) is a chronic neurodegenerative disorder characterized by the selective loss of upper and lower motor neurons. This disease results in gradual atrophy of muscles leading to paralysis and death about 3-5 years after diagnosis. Familial ALS (fALS) is responsible for 10% of cases and mutations in the superoxide dismutase 1 (SOD1) gene cause 2% of fALS. The exact pathogenic mechanism of mutant SOD1 induced motor neuron death is still elusive and is thought to involve oxidative stress and protein aggregation. These two phenomena are known to induce heat shock proteins (Hsp's) which protect cells through their chaperoning and anti-apoptotic activity. Loss of Hsp27 from motor neurons preceding disease has been suggested to contribute to their degeneration in ALS mice. Moreover, several Hsp's, notably Hsp27, have been shown to be protective in a number of in vitro ALS models. However, we recently showed a lack of protective effect of Hsp27 against mutant SOD1-dependent cell death in N2a cells. In this study, we show corroborative results using the ALS mice. Mice that overexpress the human G93A SOD1 mutant developing ALS-like symptoms were crossed with ubiquitous Hsp27 overexpressors and the resulting double transgenic mice (Dtg) were tested. The Dtg mice did not live longer, nor had significant delayed onset of disease compared to their G93A SOD1 littermates. No evidence that motor neurons were protected in these mice was found histologically. Also no difference in the activation, nor in the levels of members of the apoptotic machinery was found in these mice. In conclusion, Hsp27 alone does not seem to be sufficient to protect against the devastating effects of mutant SOD1.

6G_09_P

EXTREMOLYTES: STRESS PROTECTIVE LOW-MOLECULAR-WEIGHT COMPOUNDS AS INHIBITORS OF AMYLOID FORMATION

Jungki Ryu[1], Chan Beum Park[1], Georg Lentzen[2]

[1] Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Republic of Korea
[2] bitop AG, Stockumer Str. 28, 58453 Witten, Germany

Extremolytes are low-molecular weight osmolytes accumulated by extremophilic microorganisms as a response to osmotic and temperature stress and are known to act as chemical chaperones by stabilizing cellular

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

structures (1). Extremolytes have been shown to prevent the misfolding of proteins and to maintain their stability and can reach high intracellular concentrations without interfering with metabolism.
β-Amyloid peptide (Aβ) is the major constituent of senile plaques, the key pathological feature of Alzheimer´s disease. Aβ is physiologically produced as a soluble form, but the aggregation of Aβ monomers causes neurotoxicity. Ectoine, an extremolyte widespread in extremophilic (halophilic) bacteria has been shown to interfere with the formation of amyloid aggregates *in vitro* and amyloid-induced cytotoxicity (2,3).
Using atomic force microscopy and an assay based on thioflavin T fluorescence, we have now tested synthetic ectoine analogs and other osmolytes for their ability to interfere with Alzheimer peptide Aß amyloid formation in vitro. We show that a synthetic analog of ectoine with widened ring size interferes effectively with amyloid fibril formation. The results indicate the possibility of designing synthetic compounds with chemical chaperone properties as potential inhibitors of amyloid formation associated with neurodegenerative diseases.

(1) Lentzen G, Schwarz T (2006) Extremolytes: natural compounds from extremophiles for versatile applications. Appl Microbiol Biotechnol 72:623-634
(2) Arora A, Ha C, Park CB (2004) Inhibition of insulin amyloid formation by small stress molecules. FEBS Lett 564:121-125
(3) Kanapathipillai M, Lentzen G, Sierks M, Park CB (2005) Ectoine and hydroxyectoine inhibit aggregation and neurotoxicity of Alzheimer's beta-amyloid. FEBS Lett 579:4775-4780

6G_10_P

HSP90A AS PART OF THE POLYGENIC RESPONSE TO SCRAPIE DEVELOPMENT IN SHEEP

Marcos-Carcavilla A[1], Calvo JH[2], González C[1], Moazami-Goudarzi K[3], Laurent P[3], Bertaud M[3], Hayes H[3], Beattie AE[4], Serrano C[5], Lyahyai J[5], Martín-Burriel I[5] and Serrano M[1]

[1] INIA, 28040, Madrid, Spain, [2] CITA, 50059, Zaragoza, Spain, [3] INRA, F-78350 Jouy-en-Josas, France, [4]AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand, [5]Universidad de Zaragoza, 50013, Zaragoza, Spain
E-mail: amarcos@inia.es

Prion diseases, including ovine scrapie, are fatal neurodegenerative diseases characterized by the conversion of the host encoded PrP^{c} into its aberrant and aggregate-prone counterpart PrP^{sc}. Molecular chaperones

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

provide the first line of defence against misfolded proteins and probably function at the earliest stages of prion pathogenesis. In the present work, the ovine gene encoding the Hsp90α (*HSPCAA1*), including the promoter and other regulatory regions, has been isolated and characterized. Several sequence polymorphisms have also been identified. Genetic and cytogenetic mapping localized the ovine *HSPCAA1* gene on a chromosome region previously described as a QTL interval for scrapie incubation period in sheep (OAR19q24dist). Quantitative PCR results revealed no changes at *HSPCAA1* mRNA level as consequence of the scrapie infection. Nevertheless, association analyses revealed that several polymorphisms in the 5' flanking region and intron 10 of the ovine *HSPCAA1* gene were differentially distributed between sheep with different responses to scrapie infection. Taking into account the implication of Hsp90 family in triggering stress response under several environmental insults, and in protein degradation, results presented here point to *HSPCAA1* as a good positional and functional candidate gene modulating the response to scrapie in sheep. Its possible importance in other amyloidosis modulation shouldn't be ruled out.

6G_11_P

ONE OF THE IMPLICATIONS OF HSP70 CHAPERONE ANTI-AGGREGATE ACTIVITY IN A CELL MODEL OF HUNTINGTON DISEASE

Irina Guzhova, Anastasia Kaznacheeva, Maria Ippolitova, Alexander Kinev, Boris Margulis

Institute of Cytology RAS, Tikhoretsky, 4, StPetersburg, Russia, margulis@mail.cytspb.rssi.ru

Misfolded proteins and their aggregates is the cause of a great variety of pathologies including polyglutamine-linked diseases, like Huntington's disease (HD). Since most of these diseases can be successfully treated by inducing Hsp70 chaperone expression we have used the intrinsic property of the former to penetrate inside living cells in a cellular model of HD. We found that the incubation of SK-N-SH human neuroblastoma cells overexpressing 103-glutamine expansion with pure Hsp70 substantially lowered the amount of apoptotic cells. This protection was attributed to the anti-aggregate effect of the chaperone that was proved by reduced number and size of aggresomes, especially when the chaperone has been applied prior to the aggregate formation. At the initial stage aggregation of polyglutamine repeats is thought to be accompanied with the latter cross-linking to lysines of certain cellular molecules in a process catalyzed by tissue transglutaminse (tTG). One of the donors of such lysines is glyceraldehydephosphate dehydrogenase (GAPDH), and since the enzyme

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

was shown to specifically bind Hsp70, we suggested that the chaperone can prevent aggregation by depriving tTG of the substrate. Using the same cell model of HD we found that Hsp70 and GAPDH are co-localized with polyglutamine aggregates. Furthermore, the chaperone was shown to physically interact with GAPDH in Hsp70-overexpressing cells. It was found for the first time that the up-regulation of Hsp70 content was accompanied with the reduction of GAPDH quantity to be sequestered by SDS-insoluble aggresomes. In conclusion exogenously administered Hsp70 can prevent aggregate formation in cells overexpressing long polyglutamine tracts and therefore the chaperone itself serves as a potent anti-neurodegenerative drug.

6G_12_P

FORMATION OF HIGHLY TOXIC AMYLOID BETA OLIGOMERS IS MEDIATED BY MOLECULAR CHAPERONES

Masafumi Sakono[1,2]*, Tamotsu Zako[1], Hiroshi Ueda[3], Masafumi Yohda[4], Mizuo Maeda[1]

1 RIKEN, 2 JST, 3 Univ. Tokyo, 4 Tokyo Univ. Agric. Tech.
**RIKEN Bioengineering Lab., 2-1, Hirosawa, Wako, Saitama 351-0198, Japan, e-mail: msakono@riken.jp*

Since the amyloid beta (Aβ) aggregates are the cause of Alzheimer's disease, studies on its formation mechanism is important to develop the methods for prevention and treatment of Alzheimer's disease. In this report, we studied the effect of a molecular chaperone, prefoldin (PFD) from Pyrococcus horikoshii OT3 on Aβ aggregation. Lyophilized Aβ peptide (2 mg/ml) was dissolved in HFIP. The solvent was then dried using a spin-vacuum system, and stored in -80 °C. The HFIP-treated peptide was dissolved in distilled water by vortex. After sonication, 1 mM peptide solution was diluted to 50 μM in PBS buffer with or without 50 μM PFD, and incubated at 50 °C. Transmission electron microscope (TEM) observation and Western blotting analysis showed that the soluble oligomeric (3-50mer) Aβ particles (typically about 10 nm in diameter) were produced by incubation with PFD, while fibrillar aggregates were produced in the absence of PFD. The cytotoxicity of Aβ aggregates against PC12 cells (rat pheochromocytoma cells) was measured by MTT method and terminal deoxynucletidyl transferase-mediated biotin-dUTP nick end labeling (TUNEL) method. The Aβ soluble oligomer formed by the incubation with PFD exhibited higher toxicity than the fibrillar aggregates without PFD. This finding is consistent with the recent results that soluble oligomers, the intermediates of amyloid fibrillation, have higher toxicity than amyloid fibril. We also confirmed that the soluble β soluble oligomer induced apoptosis. Our data suggest that PFD recognizes these toxic

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

soluble oligomers and prevents further fibrillation.

6G_13_P

IMPAIRMENT OF THE UBIQUITIN-PROTEASOME SYSTEM ASSOCIATED WITH EXTRACELLULAR TRANSTHYRETIN AGGREGATES IN FAMILIAL AMYLOIDOTIC POLYNEUROPATHY

Sofia Duque Santos

Institute for Molecular and Cell Biology, Porto, Portugal
sasantos@ibmc.up.pt

The mechanisms developed by cells to acquire protection from the deleterious effects of misfolded proteins are currently well characterized. One essential mechanism is the ubiquitin-proteasome system (UPS) which has been associated with neurodegenerative disorders of intracellular protein aggregation.
We have studied the UPS in familial amyloidotic polyneuropathy (FAP), a neurodegenerative disorder caused by extracellular deposition of mutant transthyretin (TTR). The studies were conducted in TTR synthesizing and non-synthesizing tissues from affected individuals, in transgenic mice models for FAP and in neuroblastoma or Schwannoma cell lines cultured with TTR oligomers. We observed that in human FAP tissues, presenting extracellular TTR aggregates ubiquitin protein conjugates were up-regulated, the proteasome levels were decreased and parkin and alpha-synuclein expressions were both decreased. On the other hand, the liver, that normally synthesizes variant TTR V30M, did not show this response. A similar response was detected in mice models for TTR V30M or TTR L55P. Furthermore, transgenic mice immunized to decrease TTR deposition showed a significant decrease in ubiquitin levels and an increase in parkin and alpha-synuclein levels in comparison to control mice. Studies performed in cell lines with aggregates in medium resulted in increased ubiquitin levels.
The overall results are indicative of TTR deposition as an external stimulus to an intracellular UPS response in FAP.

6G_14_P

ENDOPLASMIC RETICULUM STRESS IN ALZHEIMER'S DISEASE

W. Scheper, J.J.M. Hoozemans, S.M. Chafekar, A. van Aken, M. López-Cavanillas, R. Zwart, P. Eikelenboom and F. Baas

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Neurogenetics Laboratory and Department of Neurology, Academic Medical Center, 1100 DE Amsterdam, The Netherlands.
w.scheper@amc.uva.nl

The unfolded protein response (UPR) is a protein quality control mechanism that initially protects the cells against endoplasmic reticulum (ER) stress caused by the toxicity of unfolded proteins. We have previously shown that the UPR is activated in neurons in Alzheimer's disease (AD) brain. In addition we find upregulation of the levels of the trafficking protein Rab6, which may also be involved in protein quality control. The levels of Rab6 show a very strong correlation with levels of the ER chaperone BiP in AD brain. We find ER stress as well as high Rab6 levels in non-tangle bearing neurons early in AD pathogenesis, suggesting that it precedes the late Aβ (plaques) and tau pathology (tangle formation). Because ER stress is a very early event in AD pathogenesis it is a potential therapeutic target.
Accumulation and aggregation of unfolded proteins is a major neuropathological characteristic of AD, however, no aggregates are found in the ER itself. This suggests that other processes, for example disturbed protein trafficking out of the ER are involved. We use cellular models for early Aβ and tau pathology to delineate mechanistic interactions with the ER stress response, in order to prevent neurotoxicity. We find that early intermediates in Aβ aggregation can induce a mild ER stress response. In addition, preliminary data indicate that increased expression of tau interacts with the ER stress response as well. Our ultimate aim is to modulate the ER stress response in AD to prevent or limit neuronal loss.

6G_15_P

A DISEASE-CAUSING VARIANT OF SHORT-CHAIN ACYL-COA DEHYDROGENASE PROMOTES OXIDATIVE STRESS

S. P. Schmidt[1], T. J. Corydon[2], N. Gregersen[1];

[1]Research Unit for Molecular Medicine, Skejby Sygehus, Brendstrupgaardsvej 8200 Aarhus N, Denmark, [2]Institute of Human Genetics, University of Aarhus, Bartholin Building, 800 Aarhus C, Denmark.
E-mail of corresponding author: stinne@ki.au.dk

Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is a rare recessively inherited metabolic disorder, affecting the mitochondrial β-oxidation. Patients are usually presenting neuromuscular features such as developmental delay, hypotonia, seizures, as well as a general failure to thrive. To study the pathogenesis of the disease, transduced astrocytic cells stably expressing five different disease-associated SCAD protein

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

variants were established. In the cloning process, the viability of cells expressing each one of two severe SCAD variant proteins was severely reduced, compared with cells expressing the SCAD wild-type (wt) protein. One of these was the rare variation 319C>T (Arg83Cys), which is unable to assemble into catalytically active SCAD tetramers, as well as having aggregational tendencies in vitro. Six out of six SCAD wt cell colonies survived, whereas only four out of seven SCAD Arg83Cys colonies survived colony transfer. To investigate whether this variation inflicts with the ability of the cell to overcome a stress-full situation, cells expressing the wt or the 319C>T variation was subjected to heat stress of 40°C, and the stress-response was followed over a time period of 24 hours, monitored by selected stress response genes (Hsp70, Hsp60 and HO-1 (Hemeoxygenase-1)). The cell line expressing the SCAD Arg83Cys variant protein revealed an elevated production of HO-1 compared with the SCAD wt cells, indicating oxidative stress, elicited by the misfolded mitochondrial SCAD variant protein.

6H_01_P

NUTRITIONAL METABOLIC STRESS AND EPIGENETIC MECHANISM OF SCHIZOPHRENIA

Sahebarao Mahadik

Department of Psychiatry and Health Behavior, Medical College of Georgia, Augusta, GA, USA
SMAHADIK@MCG.EDU

Stress, both environmental (nutrition, toxins, hypoxia) and emotional is ubiquitous and can contribute to epigenetic molecular mechanisms for a wide variety of non-communicable diseases from birth to aging. Maternal as well as fetal nutritional stress (deficiency of micronutrients or oxidative cell damage due to very high caloric intake) has been shown to imprint the risk for adolescent and adult non-communicable disorders such as brain disorders, diabetes and cardiovascular disease. Among these, maternal folic acid and vitamin B12 deficiency has been considered to contribute to the altered gene expression by altered one carbon metabolism and thereby methylation of proteins, DNA and phospholipids, particularly containing omega-3 fatty acids. Role of maternal nutrition has been considered in schizophrenia but evidence is based on epidemiological data. We studied the plasma levels of folic acid, vitamin B12, cortisol and homocysteine, one of the most important factor in oxidative stress-mediated cellular dysfunction, particularly neural cell function during brain development. The drug-naïve patients at the early onset of psychosis (N=28) and matched healthy controls (N=34) were enrolled in a study.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

All the study protocols were properly approved by the Institutional Ethical Committee and each study subject signed the consent to participate in the study. Compared to normal controls, patients had significantly ($p<0.05$) lower levels of folic acid (5.40 ± 3.99 vs 3.46 ± 1.71 ng/ml) as well as vitamin B12 (236.32 ± 132.66 vs 185.52 ± 73.39 pg/ml). These changes were associated with the significantly increased ($p=0.05$) plasma levels of homocysteine in patients (15.99 ± 10.09 vs 11.65 ± 3.88 μmoles/l). It has already been shown that altered methylation of histones, DNA and phospholipids as well as the increased morbidity to diabetes and cardiovascular disease in schizophrenia. Our data have important implications to prevention of these metabolic stress mediated fetal risks for adult disorders including schizophrenia.

6H_02_P

MATERNAL OXIDANT STRESS, NEURULATION, AND THE "BIRTH MONTH" PHENOMENON IN SCHIZOPHRENIA, NEURAL TUBE DEFECTS, AND LEFT-HANDEDNESS

Giovanni Marzullo* and F. Clarke Fraser**

**Per Aspera Research Foundation, New York, NY; ** McGill University, Dept. of Human Genetics, Montreal, QC, Canada*

In the epidemiology of schizophrenia, a very robust but puzzling finding is the "birth month" effect: the tendency of people who develop the disease to be born most often in late winter and least often in late summer. We found the same rhythm among children born with defects of neural tube closure (Marzullo and Fraser, 2005). This shifted our thinking about possible causes from peri-natal to peri-conceptional or early embryonic events in neurogenesis. Using conceptions rather than births, we also observed an interesting correlation with the annual photoperiod; i.e., for both disorders, a conception maximum in May-Jun, a month before the summer solstice (in the northern hemisphere), and a minimum in Nov-Dec, a month before the winter solstice. Such timings, coupled with evidence of functional and anatomical deficits of cerebral asymmetry in schizophrenia, led us to a hypothesis implicating mother's blood-mediated, pro-oxidant effects of sunlight on free-radical-sensitive cellular processes that, during the early-fourth embryonic week, bring about both neural-tube closure and asymmetry development. That is, a sunlight-dependent cycle of maternal oxidant stress would result in a peri-Jun peak of inhibition and a peri-Dec peak of promotion of both processes. This hypothesis predicted a May-Jun conception peak among extreme left-handers, and a Nov-Dec peak among extreme right-handers, in the general population. In the present study, we tested this notion using birth

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

and hand-preference data on over 10,000 American-born professional baseball players. The results were fully consistent with the hypothesis. Evidence relating to UV and visible sunlight effects on human blood antioxidant potential is also discussed.

6H_03_P

THE EFFECT OF SUBCHRONIC KETAMINE TREATMENT AND SOCIAL ISOLATION ON PAIN SENSITIVITY

Tuboly G., Horvath G., Benedek G.

Dept. Physiol., Fac. Med., Univ. Szeged. Dom ter 10. Hungary,
tuboly@phys.szote.u-szeged.hu

Chronic exposure to stressful events precipitates or exacerbates many neuropsychiatric disorders, including schizophrenia, resulting in the stress hypothesis of schizophrenia. Clinical studies have proven that schizophrenia is accompanied by hypoalgesia. Relevant animal models are of decisive importance in the study of psychiatric diseases. It is well-known that subchronic treatment with ketamine or social isolation induces schizophrenia-related alterations, which are ameliorated by clinically used neuroleptics. The purpose of this study was to test nociceptive responses in singly housed and/or subchronic ketamine treatment.
After weaning on postantal day 21, male Wistar rat pups were either group housed (5-6; GR. I and II) or isolated (GR. III and IV) for 20 days. 6 days later (30 days old rats), animals (Gr. II and IV) were injected with 30 mg/kg ketamine daily for 14 days. Control rats (Gr. I. and III) received saline. The tail-flick latency tests were performed at 41. day at 48 and 52 ^{o}C.
At 48 ^{o}C we found a significant effect of housing conditions, whereas ketamine treatment had no effect on reaction time. Both single housed groups of animals had significantly higher reaction times compared with group-housed rats irrespective of ketamin treatment. In contrast, at 52 ^{o}C there were no significant differences between the four groups.
Our study suggests that isolation has effect on acute heat pain sensitivity. Since low temperature activates mainly the C-fibers, while the high temperature the Aδ-fibers, we suppose that this type of stress disturbed primarily the C-fiber linked pain pathways.
This work was supported by a grant of the National Office for Research and Technology (RET-08/04 OMFB-0066/2005) and Hungarian Research Grant (OTKA, K60278).

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

6H_04_P

PSYCHOSIS IN CORRELATION WITH CHRONIC STRESS AND VASOPRESSIN

Dóra Zelena

Institute of Experimental Medicine, Hungarian Academy of Sciences, 1083 Budapest, Szigony 43, Hungary, zelena@koki.hu

The role of stress in the development and maintenance of symptoms in major psychiatric disorders such as schizophrenia is well-established. The biological component of stress is mediated largely through the endocrine system, predominantly by the hypothalamo-pituitary-adrenal (HPA) axis. The two main hypothalamic secretagogues of the axis are CRH and vasopressin with more important role of the latter during chronic stress. Vasopressinergic dysfunction in schizophrenics was also indicated and the naturally vasopressin mutant Brattleboro rats was suggested to be a good model for schizophrenia. Therefore we addressed here the question if the lack of vasopressin in Brattleboro rats will lead to disturbances in chronic stress-induced HPA axis changes parallel with the development of schizophrenia. The classical somatic chronic stress symptoms (body weight reduction, thymus involution, adrenal gland hypertrophy) and also the signs of HPA axis hyperactivity (resting corticosterone elevation and POMC mRNA elevation in the anterior lobe of the pituitary) were present in all three studied situation (repeated restraint, streptozotocin-induced diabetes mellitus, repeated morphine injections) with similar extent in control and vasopressin deficient rats disclosing the involvement of V1b receptors in the process. On the other hand vasopressin as a neurotransmitter may act on other brain regions and there are many compensatory mechanisms in Brattleboro rats (e.g. oxytocin, CRH). Moreover both the adrenomedullary system and the sympathetic nerves are more active in vasopressin deficient rats. So our conclusion is that vasopressin may act on the development of schizophrenia through influencing other neurotransmitters in brain and not the HPA axis. Chronic stress may exacerbate the schizophrenia not through HPA axis changes but e.g. glucocorticoid toxicity in hippocampus.

6I_01_P

STRESS-RELATED GASTRIC ULCERATION: THE GASTROPROTECTIVE ROLE OF GLUCOCORTICOID HORMONES

Ludmila Filaretova[1], Tatiana Bagaeva[1], Koji Takeuchi[2], Gabor Makara[3]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[1]*Pavlov Institute of Physiology, nab. Makarova 6, St.Petersburg, Russia, filaretova@pavlov.infran.ru;* [2]*Kyoto Pharmaceutical University, Japan;* [3]*Institute of Experimental Medicine, Budapest, Hungary*

Various stressors stimulate the production of glucocorticoids, and severe stressors may also induce gastric erosion, "stress ulcer". From the very outset, researchers have focused on the point of view that stress-generated glucocorticoids are causally related with gastric ulcerogenesis. In our studies we have focused on the idea that glucocorticoids released during acute stress have an adaptive effect on the stomach and, therefore, are gastroprotective rather than ulcerogenic. To test this idea, we examined the effect of glucocorticoid deficiency (created by different approaches and followed by corticosterone replacement) or the glucocorticoid receptor antagonist RU-38486 on stress-induced gastric erosion in rats. The data obtained show that the reduction in the stress-induced corticosterone release, or its actions, aggravates stress-caused gastric erosion. It is suggested that an acute increase in corticosterone during stress protects the stomach against injury caused by this stress. Extending this idea, we also hypothesized that glucocorticoids may play a role in the gastroprotective effect of preconditioning stress. It is known that mild, preconditioning, stress may attenuate stress-induced gastric ulceration and this effect is mediated by prostaglandins. We confirmed that mild, preconditioning, stress decrease the gastric ulceration caused by severe stress and demonstrated for the first time that this effect is also provided by glucocorticoids released in response to preconditioning stress. The results support gastroprotective role glucocorticoids released during stress. Supported by FNM RAS-2007, RFBR-07-04-00622.

6J_01_P

ALCOHOL EXPOSURE REGULATES HSF-1 AND HEAT SHOCK PROTEINS 70 AND 90 IN MURINE MACROPHAGES: IMPLICATION IN TNFα PRODUCTION

P. Mandrekar, D. Catalano, V. Jeliazkova and G. Szabo

Dept. of Medicine, Univ. Mass. Med. Ctr, Worcester, MA, USA. pranoti.mandrekar@umassmed.edu

Alcohol use affects innate immune responses, particularly TNFα, and results in alcoholic liver disease. Heat shock proteins, mediators of the stress responses influence TNFα production. Here we hypothesized that alcohol exposure regulates TNFα production and NFκB activation via modulation of the heat shock transcription factor (HSF), hsp70 and hsp90. Murine RAW 264.7 macrophages were exposed to 25mM, 50mm and

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

75mM concentrations of alcohol for 24h, 48h and 72h followed by lipopolysaccharide (LPS) for 15 minutes (for HSF and NFκB binding) or 16h (for hsp70, hsp90) to study the effects of alcohol. At the end of the stimulation, nuclear extracts prepared and subjected to HSF and NFκB binding assays whereas hsp70 and hsp90 in whole cell lysates were determined by Western blotting. Our findings demonstrate that HSF binding was increased after alcohol exposure. Supershift analysis showed the presence of HSF-1 proteins and not HSF-2. Interestingly, hsp70 was increased by short-term alcohol but prolonged alcohol exposure decreased hsp70. Conversely, hsp90 levels were decreased after short-term alcohol treatment and increased by prolonged alcohol treatment in macrophages. Immunoprecipitation experiments of hsp90, a chaperone for IKK, showed decreased hsp90-IKKβ complexes after short-term alcohol whereas prolonged alcohol revealed presence of hsp90-IKKβ and increased IKK kinase activity. Geldanamycin, an hsp90 inhibitor, blocked alcohol-induced increase in TNFα production suggesting an important role for hsp90 in alcohol-induced inflammation. Collectively, our results suggest that alcohol exposure regulates HSF-1 binding, hsp70 and hsp90 and thus could contribute to elevated TNFα and alcohol-induced liver injury.

6J_02_P

HEAT SHOCK MODULATES INFLAMMATORY ACTIVATION OF HUMAN INTESTINAL MICROVASCULAR ENDOTHELIAL CELLS (HIMEC)

Parvaneh Rafiee, Mary F Otterson, David G Binion

Medical College of Wisconsin, Milwaukee, WI, USA

Background and Aims: The heat shock response is an evolutionarily conserved mechanism for the maintenance of cellular homeostasis following sublethal noxious stimuli, where heat shock proteins (HSPs) induced by stress, chaperone intracellular proteins that might otherwise be denatured. HSPs are now appreciated to modulate signaling cascades during periods of repeated stress, including chronic inflammation. Although the microvascular endothelium plays a critical role in chronic intestinal inflammation in IBD, little is known about the role of HSPs in the inflammatory activation of HIMEC, the gut specific microvascular population.

Methods: HIMECs from small and large intestine between passages 8 to12 were used. HIMECs were activated with thermal stress as well as TNF-α and LPS. HSPs and iNOS expression were characterized using RT-PCR and Western blot analysis. HIMEC activation was assessed using whole cell ELISA for detection of cell adhesion molecules, and leukocyte

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

binding under low shear stress flow adhesion.
Results: HIMECs exposed to thermal stress (42C, 1h) with recovery times of 1, 6 and 20h demonstrated induction of HSP family members including HSP32 (HO-1), HSP60, HSP70, HSP90. TNF-α/LPS activation of HIMEC maintained at 37C also demonstrated induced expression of HSP60 and HSP70 at the mRNA and protein levels. To investigate the effect of HSP induction on inflammatory activation, HIMEC preconditioned with thermal stress were assessed with and without TNF-α/LPS activation. Heat shock alone induced significant increases in ICAM 1. TNF-α/LPS activation following heat shock, resulted in the highest levels of ICAM 1 and E selectin expression detected in these cells, which corresponded to significantly increased leukocyte adhesion. Heat shock with and without TNF-α/LPS failed to induce increased iNOS expression, which normally functions to downregulate inflammatory activation in HIMEC.
Conclusions: Heat shock significantly enhanced TNF-α/LPS induced activation of HIMEC. The mechanisms involved increased pro-inflammatory ICAM 1 and E selectin expression, which was not accompanied by an increase in the downregulatory expression of iNOS. Further defining the heat shock response may yield insight into mechanisms which enhance inflammatory activation of HIMEC in the setting of chronic inflammation in IBD.

6J_03_P

A NOVEL ROLE OF HSP70 AS A MODULATORY AGENT FOR DENDRITIC CELLS PHENOTYPE AND FUNCTION

P Stocki, U Holtick, NJ Morris, AM Dickinson

Haematological Sciences, Newcastle University, UK; corresponding author: pawel.stocki@ncl.ac.uk

Heat shock protein 70 (HSP70) is a chaperone proteins which was reported to be implicated in an activation of innate immunity. However, recent data strongly opposes the proposed function of HSP70 as a proinflammatory cytokine highlighting the problem of a bacterial contamination in a purified recombinant human HSP70 (rhHSP70). As it was shown, endotoxin free rhHSP70 is incapable to launch immune activation raising the rhHSP70-endotoxin debate and suggesting that the real role of HSP70 in an immune system, if any, hasn't been reviled yet. To eliminate the problem of endotoxin contamination we purified HSP70 from two different leukemic cell lines which were growing in the endotoxin free environment. In addition, the purification products were tested for LPS content giving negative result. We examined the HSP70 role on monocyte derived dendritic cells (DC) presenting its modulatory effect on

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

a DC activation state. 24h incubation of DC with HSP70 was effective in down regulating surface expression of HLA-DR as well as CD80/86/83. As a control we used an endotoxin-contaminated rhHSP70 (erhHSP70) which in contrast to HSP70 was highly potent DC maturation molecule. We further examined the functional effect of HSP70 treated DC on proliferation of allogeneic T cells, where HSP70 was responsible for its down regulation. The modulatory effect of HSP70 on DC was also investigated on DC cytokine level after CD40L activation, presenting decreased level of IL-4/6/10 and TNFα confirming the observed phenomenon. Although our results which clearly show that HSP70 has an immunological potential as an anti inflammatory agent are contradicting most of the published data they are supported by the most recent publication on the field of HSP70 conscious about possible endotoxin contamination.

6J_04_P

EFFECTS OF ANTI-INFLAMMATORY DRUG SUPPLEMENTATION ON HSP70 EXPRESSION AND RELEASE DURING ANAEROBIC EXERCISE

F. Leoni, T. Coulson, G. Jones, H.E. Ireland, C. Hunter-Lavin, J.H.H. Williams

University of Chester, UK
f.leoni@chester.ac.uk

Hsp70 expression and release increases in response to exercise. To determine whether the inflammatory response is involved in the induction of Hsp70, we analysed the effects of Aspirin and Ibuprofen supplementation and the release of pro and anti-inflammatory cytokines on Hsp70 after an intensive anaerobic exercise (30 second maximal intensity Wingate test). A group of trained males (N=12) were administered Ibuprofen (Ibu, 1200mg) or Aspirin (Asp, 2700mg) and compared to a control group (Con). Blood samples were taken before the exercise, 10 minutes after, and 1 hour after the second blood taking. Hsp70 mRNA, intracellular protein expression, release from leucocytes and target cytokine release (IL-6, TNFα, IL-10) were measured. Flow Cytometry data showed a decrease on general Hsp70+ lymphocyte population after the exercise in Con and Asp but an increase in Ibu group; in particular the B cell expressing Hsp70 shows a decrease in Asp but an increase after 1h after in Ibu. Hsp70+ T helper lymphocytes showed no change in Con, a decrease in Asp and an increase in Ibu 1h after the exercise. Hsp70+ NK population decreased in Asp which was not evident in Con and increased in Ibu group 1h after the exercise. Hsp70+ monocytes decreased straight after the exercise, then returned to normal levels in Con and Asp. An increase in Hsp70+ monocytes was shown after

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

the exercise in Ibu group. No change in Hsp70+ neutrophils was observed. We also showed a slight increase in Hsp70 gene expression straight after the exercise in Con but not in Asp or Ibu.

6K_01_P

C-FOS EXPRESSION IN MEDIODORSAL THALAMIC AREA DURING THE NEONATAL PERIOD: INFLUENCE OF THE MATERNAL DEPRIVATION AND INJECTION-STRESS

Katsumasa Muneoka[1)], Tetsuo Ogawa[1)], Makiko Kuwagata[1, 2)], Seiji Shioda[1)]

[1)] Department of Anatomy I, Showa University School of Medicine, Tokyo, Japan, [2)] Lab.of Pathology, Toxicology division, Hatano Research Institure, FDSC, Kanagawa, Japan, E-mail: kmuneoka@med.showa-u.ac.jp

Mediodorsal thalamic area (MDT) is a brain area innervating the prefrontal cortex (PFC) and implicated as a site of neuropathology in schizophrenia. We investigated the function of MDT in neonatal rats as well as influence of stressful conditions via examining c-Fos expression at postnatal day (PND) 11. We tested three experimental groups that are the home cage- (H), maternal deprivation- (M) and injection-group (I). Rats in H-group were perfused transcranially immediately (within 5 mins) after picking up from their home cages. Rats in M-group were deprived from their mother for 60 mins in a novel cage and thereafter perfused. Rats in I-group were perfused 60 mins after subcutaneous injections of 100μl saline. Removed brains were cut with vibratome and c-Fos- immunoreactivity (ir)-positive cells were visualized with immunohistochemical reactions. Images were digitally taken and the numbers of c-Fos-ir-positive cells in the MDT were counted. Although c-Fos-ir-positive cells were detected in all three groups, the total number was significantly increased in I-group compared with H or M groups. These results suggest that the MDT-PFC neuronal system during the neonatal period is active even under undisturbed environment, and that injection stress but not maternal deprivation stimulate the activity of the MDT. The present findings support the involvement of stress during the development in the pathophysiology of schizophrenia.

6K_02_P

PRENATALLY INCREASED MATERNAL CORTISOL: EFFECTS ON HPA AXIS, BRAIN NEUROTRANSMITTER SYSTEMS, IMMUNE RESPONSES AND BEHAVIOUR IN THE OFFSPRING OF PIGS

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

W. Otten, E. Kanitz, M. Tuchscherer and B. Puppe

Research Unit Behavioural Physiology, Research Institute for the Biology of Farm Animals,
18196- Dummerstorf, Germany, email: otten@fbn-dummerstorf.de

Environmental factors acting prenatally on the developing foetus are important determinants for disorders later in life. Maternal glucocorticoids are considered as major candidates for the mediation of stressful prenatal events to the offspring. We investigated the effects of increased maternal cortisol levels in pregnant sows on central and peripheral alterations of the HPA axis, brain neurotransmitter profiles, immune responses and open-field behaviour of their piglets. Increased endogenous cortisol release was induced in pregnant sows by repeated intramuscular administrations of ACTH every second day during late gestation, whereas control sows received injections of saline. The ACTH treatment of sows significantly increased the birth weight of piglets without affecting gestation length, number of total born piglets or frequency of stillborn piglets. After birth, piglets from ACTH-treated sows showed a suppressed cell-mediated immunity. Decreased corticosteroid-binding globulin levels indicate a higher amount of biologically available free cortisol and elevated noradrenaline levels demonstrate an enhanced activation of the SAM system in the offspring from ACTH sows. Hypothalamic HPA axis feedback seems to be attenuated after birth in ACTH offspring as shown by a slightly reduced glucocorticoid receptor binding. Together with a significantly decreased serotonergic activity in the locus coeruleus region these alterations may account for the increased responses of the HPA and SAM system after an acute restraint stress and the increased emotional reactivity during an open-field test.

6K_03_P

THE EFFECTS OF MATERNAL SEPARATION ON NEUROGENESIS IN THE RAT ROSTRAL MIGRATORY STREAM

[1]Račeková, E., [1]Orendáčová, J., [2]Raček, Ľ.

[1]Institute of Neurobiology, CE, SAS; [2]Institute of Animal Physiology, SAS, Košice, Slovak Republic, racekova@saske.sk

The olfactory bulb is the first relay on the olfactory sensory pathway and the target for neural progenitors generated in the subventricular zone (SVZ) and which migrate along a well-demarcated pathway - the rostral migratory stream (RMS). Although neurogenesis in the SVZ/RMS occurs continuously throughout adulthood, the rate of proliferation and the fate of new cells may be affected by exogenous factors. Maternal separation

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

(MS) is a well-described model of environmental influences on development and subsequent nervous system function. In our study MS has been used as a model of early olfactory deprivation to investigate its effect on neurogenesis in the rat RMS. Pups from postnatal day 1 (P1) to P21 were separated from the dam for 3 hrs daily and compared to pups that remained with the dam. At the end of MS, resp. one week or three months later the rats were injected with BrdU to determine the number of dividing cells in the RMS. Dying cells were visualized by Fluoro Jade-B histochemistry. The RMS on hematoxylin stained sections after all survival times was composed of tightly packed cells as seen in control animals. However, cells proliferation was specifically affected immediately after the finishing of MS resp. after short-term survival. At P21 there was almost complete depletion of dividing cells in the RMS vertical arm. With reduction of dividing cells, the number of dying cells noticeably increased in this part of the RMS. Three months after MS, BrdU-positive cells displayed the similar pattern of density as seen in control animals. These data indicate that adverse experience in life may induce acute site-specific changes in the RMS neurogenesis. This work was supported by the VEGA grants 2/6213/26; 2/5135/25.

6K_04_P

A FLATTENED DIURNAL CORTISOL PROFILE AND DEPRESSION IN ADOLESCENCE ARE RELATED TO ANTENATAL MATERNAL ANXIETY

Bea R.H. Van den Bergh[1], Ben Van Calster[2], Tim Smits[3], Lieven Lagae[4], Sabine Van Huffel[2]

Department of Psychology, K.U.Leuven, Leuven and Flemish Community Brussels, Belgium
bea.vandenbergh@psy.kuleuven.be

In a prospective-longitudinal study maternal anxiety was measured at 12-22, 23-32 and 32-40 weeks of pregnancy (wp) with the State Trait Anxiety Inventory. 68 firstborns of 86 mothers, recruited at the Obstetrical and Gynecological Board consultations of a University Hospital were followed up to age 15. HPAA functioning was measured through establishing a saliva day-time cortisol profile (shortened version); during a week-end day 3 saliva samples were provided, namely immediately after awakening, at noon and in the evening. Severity of depressive symptoms was measured with the Children's Depression Inventory (CDI). We tested the following two hypotheses: (a) that maternal anxiety during pregnancy predicts altered HPAA function (e.g., a flattened cortisol profile) in adolescent offspring; (b) that altered HPAA function mediates the association between prenatal maternal anxiety and depression in these adolescents. Repeated measurements regression analysis and ordinary

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

least-squares regression analyses indicated that maternal anxiety at 12-22 wp was in female and male offspring (all having reached Tanner puberty phase IV), associated with flattening of the diurnal salivary cortisol curve (*P* =.0463). Moreover in female adolescents this flatter cortisol curve was associated with depression (*P* = .0077). Effects remained when controlling for covariates such as postnatal maternal anxiety, smoking during pregnancy, birth weight and obstetrical optimality. Our results indicate that maternal anxiety during pregnancy enhances neurobiological vulnerability to depression - possibly by altering (or programming) foetal physiology - and demonstrate the mediating role of HPAA-dysregulation in linking an adverse foetal environment to depression. These results may lead to a re-orientation of the target of primary prevention and treatment of depression.

6L_01_P

CHRONIC ETHANOL INTAKE IMPAIRS BRAIN RENIN-ANGIOTENSIN SYSTEM FUNCTION. ROLE OF CALCIUM

Martínez-Martos JM, Mayas MD, Cobo MP*, Cortes-Denia P, De la Chica-Rodriguez S, Ramírez-Exposito MJ

*Experimental and Clinical Physiopathology Research Group CVI296, Department of Health Sciences, University of Jaén, Spain. *Servicio de Neurología, Complejo Hospitalario de Jaén, Spain*

Aspartyl aminopeptidase (AspAP) and glutamyl aminopeptidase (GluAP), named together as aminopeptidase A activity (APA) exert angiotensinase activity due to their relation to the metabolism of angiotensins in the regional brain renin-angiotensin-system (RAS). APA converts angiotensin II (AngII) to angiotensin III (AngIII). AngIII possesses most of the properties of AngII and shares the same receptors. Both angiotensins participate not only in the central regulation of blood pressure, but also participate as neurotransmitters/ neuromodulators. Ethanol (EtOH) is a drug of abuse that produces hypertension, and the brain is specially sensitive to hypertension, which causes a variety of vascular and cellular changes. Previous in vitro studies demonstrated that EtOH inhibits APA activity in basal conditions (independently of Ca2+) and also under depolarisation (dependently of Ca2+). In this work, we analyse the effects of chronic EtOH intake on APA activity under basal/resting and K+-stimulated conditions in mouse frontal cortex synaptosomes in a Ca2+-containing or Ca2+-free artificial cerebrospinal fluid (aCSF). The results show that EtOH does not modify APA activity under resting conditions in presence of Ca2+, but is modified in absence of Ca2+. However, EtOH inhibits the K+-stimulated increase in APA activity in presence of Ca2+. In absence of Ca2+, this effect is not observed. Due to brain RAS is related

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

with the pathogenesis of hypertension at different levels, the inhibitory effects of EtOH on angiotensinase activity may explain the changes observed in blood pressure regulation, local blood flow and/or fluid and electrolyte homeostasis, related to alcohol abuse.

Supported by Instituto de Salud Carlos III, Fondo de Investigación Sanitaria (FIS) through grant # PIO 030922.

6L_02_P

CHRONIC ETHANOL INTAKE AND OXIDATIVE STRESS IN MOUSE BRAIN: A CALCIUM-DEPENDENT MECHANISM

Ramírez-Expósito MJ, Mayas MD, Cobo MP*, De la Chica-Rodríguez S, Cortes-Denia P, Martínez-Martos JM

*Experimental and Clinical Physiopathology Research Group CVI296, Department of Health Sciences, University of Jaén, Spain. *Servicio de Neurología, Complejo Hospitalario de Jaén, Spain*

Ethanol (EtOH) is the most widely and frequently used psychoactive drug in modern society. Chronic alcoholism is associated with numerous degenerative and inflammatory disorders of the central nervous system. The purpose of the present work was to study the influence of EtOH in mouse frontal cortex synaptosomes after chronic EtOH intake (15% in drinking water during 30 days), under resting and K^+-stimulated conditions. The neurotoxic effects were analysed by free radicals generation (luminol- or lucigenin- enhanced chemiluminiscence), lipid peroxidation of the membrane lipids (thiobarbituric acid reactive substances -TBARS-) and the oxidation of synaptosomal proteins (carbonyl groups content). In addition, the bioenergetic behaviour of synaptosomes was analysed under the different experimental protocols. Oxidative stress parameters were modified in a different degree after EtOH intake. Thus, under resting conditions, chronic EtOH intake did not modify lucigenin-enhanced chemiluminescence, but increased significantly ($P<0.01$) luminol-enhanced chemiluminescence. Under K^+-stimulated conditions, luminol- and lucigenin-enhanced chemiluminescence were significantly higher ($P<0.01$) after chronic EtOH intake. Changes were observed neither under resting nor K^+-stimulated conditions in TBARS content. Under resting conditions, chronic EtOH intake decreased carbonyl content of proteins ($P<0.01$). Under K^+-stimulated conditions, carbonyl groups content of proteins were significantly lower ($P<0.01$) after chronic EtOH intake. Finally, only K^+-stimulated conditions increased significantly ($P<0.01$) bioenergetic behaviour in control group, whereas decreased significantly ($P<0.01$) after chronic EtOH intake. Although previous *in vitro* studies did not show signs of neurodegeneration after EtOH administration, *in vivo* EtOH intake changes several oxidative stress

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

parameters.
Supported by Instituto de Salud Carlos III, Fondo de Investigación Sanitaria (FIS) through grant # PIO 030922.

6L_03_P

CARDIAC, HEPATIC AND PULMONARY STRESS RESPONSE TO PASSIVE SMOKING PLUS ETHANOL, IN COMBINED RAT TREATMENT. DIFFERENTIAL COINDUCTION OF HSP70.

Mirian Strauss and Zoraida Rodrigues

Sección de Biología Celular, Instituto de Medicina Tropical, Universidad Central de Venezuela. 47019. Caracas 1041-A. Venezuela.

In order to evaluate cardiac, hepatic and pulmonary response to toxic stress, a combined treatment of passive smoking plus ethanol was applied to 60 female Sprague-Dawley rats (80-100g). These were randomized into four groups: control, ethanol (2g/kg), passive smoking (8 cigarettes) and a combined group. 15 days post-treatment, an Hsp70 expression was determined by Western blot analysis. Samples of even protein loading (5.75µg) were separated by SDS-PAGE and blotted onto a nitrocellulose membrane. The samples were then incubated with anti–Hsp70. Bands were detected by an enhanced chemi-luminescence detection kit and exposed to X-ray film for 10 secs. The results were analyzed on a G-800 Densitometer and Quantity One 5.4.2 software. A differential tissular stress response was observed. The greatest recognition of Hsp70 was related to the hepatic tissue of the combined group, followed by the cardiac and pulmonary tissue. The differences observed were statistically significant (Mann-Whitney U test α=0,05). The combined treatment promotes the coinduction of Hsp70 in liver and cardiac tissue, whereas similar coinduction in the pulmonary tissue was not observed. Consistent with this result our previous sub-cellular analysis and its semi-quantification by Image Pro Plus software have shown approximately 60% of alveolar alterations, compared to 8% and 5% of heart and hepatic tissue respectively. The relationship between Hsp70 coinduction and cyto-protection might be important for understanding the role of this protein in organ differential damage, as well as the role of passive smoke components such as nicotine, to possibly enhance thermotolerance by an unknown mechanism.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

6L_04_P

IMPACT OF A CHRONIC ETHANOL TREATMENT IN PRENATALLY STRESSED MALE RATS ON THE ETHANOL APPETENCE AND ON LEVELS OF DELTAFOSB IN THE NUCLEUS ACCUMBENS

Vincent Van Waes[1], Mihaela Enache[1], Stéfania Maccari[1,2] and Muriel Darnaudéry[1]

[1] Unité de Neurosciences & Physiologie Adaptatives EA 4052, Equipe Stress Périnatal, Université de Lille 1,
Villeneuve d'Ascq, France
[2] Department of Experimental Medicine & Pathology, Università di Roma "La Sapienza", Roma, Italy

In rats, prenatal stress affects the activity of the hypothalamic-pituitary-adrenocortical axis and increases vulnerability to several drugs of abuse (amphetamine, 3,4-methylenedioxymethamphetamine and nicotine). Repeated exposure to drugs of abuse persistently induces the transcription factor deltaFosB in the nucleus accumbens, effect hypothesized to contribute to neuroadaptations in dopamine-regulated signalling. The aim of the present work was 1) to determine whether prenatal stress have an impact on ethanol appetence in rats; 2) to assess deltaFosB levels in the nucleus accumbens after a chronic ethanol treatment in prenatally stressed and control rats.
Preference for ethanol was measured in a two-bottle choice paradigm (water versus ethanol 2.5%, 5% or 10%) in adolescent (postnatal days 28-38) naive rats and in adult rats after a chronic oral treatment (ethanol 2.5%, 5% or 10%). The level of motivation for ethanol was determined in adult animals under challenge situations (reduction of ethanol concentration, ethanol withdrawal, concurrent choice with sucrose). After 9 months of ethanol oral treatment, animal were killed, nuclei accumbens dissected and deltaFosB levels assessed by western blot.
Our results indicated that prenatal stress did not modify ethanol preference, neither during adolescence, nor after the forced ethanol drinking period in adulthood. Moreover, animals' motivation for ethanol was weak, in spite of the long-lasting ethanol exposure. In contrast, the chronic oral treatment with ethanol 10% significantly increased deltaFosB levels in the nucleus accumbens. This increase was more important in prenatally stressed than in control animals. The dissociation between the impact of prenatal stress on brain deltaFosB and on ethanol consumption suggests that it would be important to evaluate more finely the motivation for ethanol in our populations of animals, in particular by modulating the palatability of ethanol and/or by considering interindividual differences.

Supported by the 'Conseil Régional du Nord-Pas-de-Calais', the 'IREB' and the 'FEDER'.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

6M_01_P

GENDER-RELATED DIFFERENCES IN RESPONSE OF RAT BRAIN CORTICOSTEROID RECEPTORS AND HEAT SHOCK PROTEINS TO ANTIDEPRESSANT IMIPRAMINE

Gordana Matić, Ivana Elaković & Jelena Brkljačić

Institute for Biological Research, Despot Stefan Blvd. 142, 11000 Belgrade, Serbia; gormatic@ibiss.bg.ac.yu

Sexual dimorphism in the prevalence of major depression[1] has been considered a consequence of sex differences in the HPA axis functioning[2,3]. Antidepressant drugs enhance glucocorticoid signaling and normalize HPA axis reactivity[4], representing useful tools for animal studies on the pathogenesis of this disorder. The aim of the present study was to examine gender-related differences in the response of pituitary and brain corticosteroid receptors (CRs) to a typical tricyclic antidepressant drug, imipramine. To that end, the effects of a long-term imipramine treatment on the protein levels of glucocorticoid receptor (GR), and heat shock proteins Hsp90 and Hsp70, as well as on dexamethasone binding to both GR and mineralocorticoid receptor (MR) in the pituitary, hypothalamus, hippocampus and brain cortex of non-depressed rats were studied. Differences in the GR protein level in the tissues of untreated female vs. male animals were not noticed. However, imipramine led to opposite changes in the cellular level of GR protein in the brain of female and male rats, as well as to gender- and tissue-specific changes in *in vitro* dexamethasone binding to GR and MR in the hippocampus and brain cortex. Gender-related differences in the expression of Hsp90 and Hsp70 were noticed mainly in the hippocampus, only after imipramine treatment. The results suggest that this antidepressant may affect both the GR level and the mechanisms regulating its binding ability in a gender-related manner. [[1]Kuehner C, Acta Psychiatr Scand 2003, 108: 163-174; [2]Young EA, Crit Rev Neurobiol 1995, 9: 371-381; [3]Seale JV et al, J Neuroendocrinol 2004, 16: 516-524; [4]Pariante CM, Miller AH, Biol Psychiatry 2001, 49: 391-404].

6M_02_P

HSP27 IS INVOLVED IN ACTIN BASED CELL MOVEMENT DURING WOUND HEALING

Bindi Doshi, Lawrence Hightower, Juliet Lee

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Department of Molecular and Cell Biology, University of Connecticut, 91 N. Eagleville Rd, U3125, Storrs, CT 06269, USA
Bindi.Doshi@uconn.edu

Heat induction of HSP27 in vertebrate cells causes microfilament stabilization, increased pinocytosis and cell migration. HSP27 plays an important role in cytoprotection, has actin capping activity and inhibits actin polymerization in vitro. The amount of HSP27 expressed is regulated by gene activation and post transcriptionally whereas activity is regulated by phosphorylation in response to stress. Neither phosphorylated nor unphosphorylated multimers alter actin dynamics whereas unphosphorylated monomers inhibit actin polymerization in vitro. Consequently, HSP27 has been proposed as a regulator of cell invasion; however, the direct role of HSP27 in cell motility is unclear. In the present study, we constructed a eukaryotic expression vector using fish DNA to investigate the role of HSP27 in cell motility. Heat shock treatment caused relocation of HSP27GFP from diffuse cytosolic distributions to concentration in perinuclear regions, suggesting a cytoprotective role involving protein aggregation in response to heat. CRL1807, a human colon epithelial cell line, was transfected with HSP27GFP driven by a CMV constitutive promoter to over-express HSP27. In vitro wound assays showed that cells over-expressing HSP27 had a faster wound closure rate compared to control cells. HSP27 levels were knocked down in CRL1807 cells using siRNA. Cells with decreased HSP27 levels showed slower wound closure rates compared to control cells implying a role in regulating motility. Rapidly moving fish epithelial keratocytes were also used as a model cell motility system. Keratocytes over-expressing HSP27GFP had greater cell speed than control cells. Western blotting, immunostaining and co-localization with the actin cytoskeleton will be presented.

6M_03_P

PROHIBITIN IS REQUIRED FOR SURVIVAL OF CARDIOMYOCYTES AGAINST STRESS INDUCED DAMAGE

Lingjia Qian* Xiaohua Liu Zhen Ren Zhihua Yang

Institute of Health and Environmental Medicine, 300050 Tianjin, China
newjia@vip.sina.com

Stress is considered as one of main causes inducing myocardial injury. Mitochondria play a key role in triggering the necrosis and apoptosis pathway of cardiomyocytes under stress. Prohibitin has been shown to stabilize and regulate the respiratory chain enzymes in mitochondria. The present research was designed to investigate the role of prohibitin in

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

mitochondria for cardioprotection. Stress had no influence on prohibitin gene expression in cardiomyocytes, but time-and dose-dependently increased the prohibitin content in mitochondria. The overexpression of prohibitin in cardiomyocytes by transfection of prohibitin gene resulted in an increase of prohibitin in mitochondria. Compared with the non-transfected cardiomyocytes, prohibitin overexpression could protect the mitochondria from stress-induced injury. Consistently, the mitochondria-mediated apoptosis pathway was suppressed in prohibitin overexpressed cardiomyocytes after stress treatment, including the reduced change of mitochondrial membrane permeability transition and inhibited release of CytC from mitochondria to cytoplasma. As a result, stress induced cardiomyocyte apoptosis was inhinbited. Moreover, in stressed cardiomyocytes the interaction between prohibitin and HSP70 was found, and the increase of HSP70 expression promoted the translocation of prohibitin to mitochondria and improved the survival of cardiomyocytes against stress injury. These indicated that prohibitin protected the cardiomyocytes from stress induced damage, increasing prohibitin content in mitochondria via HSP70 may constitute a new therapeutic target for myocardium injury.

The research is supported by national basic Research Priority Program of China (2001CB510206) and National Science Foundation of China (30430590, 30570753)

6M_04_P

ELEVATED HEAT SHOCK PROTEINS 70 AND 27 ARE CORRELATED WITH CNS INJURY IN SURGICAL PATIENTS UNDERGOING DEEP HYPOTHERMIA CIRCULATORY ARREST AND DESCENDING THORACIC AORTIC ANEURYSM RESECTION

James Hecker

Department of Anesthesia, University of Pennsylvania, Philadelphia, PA 19104-4283, USA
Email: heckerj@uphs.upenn.edu

Introduction. The Heat Shock Proteins (HSPs) are members of a highly conserved family of molecular chaperones, some of which are rapidly induced by severe stress. The inducible form of Hsp70 is normally near the lower limit of detection by ELISA in the cerebral spinal fluid (CSF) of humans, and remains so despite exercise to exhaustion Dalsgaard et al (1). The inducible members of the Hsp70 and Hsp27 families are associated with cellular protection and recovery after a near lethal stress. Whole animals, isolated organs and cells subjected to heat shock are protected against a subsequent near lethal ischemic or hypoxic event.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Inducible HSPs, in particular Hsp70 and 27, have also been used as markers for cells and tissues that have been exposed to a near-lethal stress. Although the induction of protective intracellular responses by heat shock is not clinically useful, an understanding of the time course and correlation with injury of HSPs released during brain and/or spinal cord cellular stress (ischemia) is critical in understanding the role of the HSPs in cellular survival, and is potentially very useful as a biomarker of severe cellular stress.

Methods. As a part of the continuing efforts to improve peri-operative management by the Hospital of the University of Pennsylvania Aortic Surgery Research Group, serial human CSF measurements of Hsp70 and 27 during Deep Hypothermic Circulatory Arrest (DHCA) and Descending Thoracic Aorta repair procedures were collected. These patients are at high risk for spinal cord ischemia with surgical resection due to the location of the procedure, and routine invasive monitors therefore includes a lumbar intrathecal ('spinal') drain. In this IRB Approved protocol, CSF samples were collected at the following time points: lumbar drain placement immediately following induction; at time of aortic cross clamp; one, two, and twelve hours after crossclamp; and at regular intervals following, or if signs/symptoms of paraparesis developed post-operatively. Samples were immediately spun and frozen for simultaneous analysis by ELISA. Preliminary results show marked increases in these Heat Shock Proteins.

Results. Of the first 23 DHCA and thoracoabdominal aneurysm patients (not consecutive), nine have large increases in Hsp70 and 27 by 2 hours after crossclamp, nine more have large increases by 48 hours after crossclamp, and of these 18, eight have post-operative paraplegia. All patients with transient or permanent paraparesis had large increases in Hsp70 and Hsp27 at multiple time points in the peri-operative period. One patient had high levels of these HSPs at the initial time point just after induction of anesthesia, and he had had a carotid endarterectomy procedure the preceding day. Reported another way, of the total of 23 patients examined, all 8 patients with post-operative paraplegia had multiple elevated HSPs over the first 48 hours, four patients had multiple elevated HSPs without paraplegia, and seven patients had only a few mildly elevated time points with no post-op paraplegia.

Conclusions. These preliminary results are supportive of Hsp70 and Hsp27 as biomarkers for significant spinal cord and perhaps brain ischemia.

1. Experimental Physiology (2004) 89.3:271-77.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Module 7 – Oral lectures:

7A_01_S

PREDATOR STRESS, MEMORY AND BRAIN PLASTICITY

Diamond, David M.

Departments of Psychology, Molecular Pharmacology and Physiology, University of South Florida, Tampa, Florida 33620 USA
e-mail: ddiamond@mail.usf.edu

It is well-known that the hippocampus is critically involved in the formation of new memories. However, extensive research has provided strong support for the view that hippocampal functioning is impaired in times of strong emotionality. For example, studies have shown that stress suppresses the induction of long-term potentiation (LTP), a well-accepted physiological model of memory, in rats. Moreover, research on people indicates that in times of strong emotionality there is a suppression of hippocampal functioning. Thus, despite the importance of the hippocampus in processes involved in memory formation, in times of stress the hippocampus appears to be inhibited from participating in the formation of emotional memories, including flashbulb and traumatic memories. In this talk I will incorporate rodent and human studies to provide an alternative interpretation of the findings. I will suggest that conditions of strong emotionality actually produce enhanced neuroplasticity in the hippocampus (and amygdala), which underlies the declarative component of emotional memories. The hyperactivation phase of plasticity, which is then followed by an inhibitory phase of hippocampal plasticity, underlies well-described psychological phenomena in emotional memory processing, such as the great durability of flashbulb and traumatic memories, as well as stress-induced amnesia.

7A_02_S

HYPER- AND HYPO-AROUSAL INT HE CONTROL OF AGGRESSIVENESS: THE ROLE OF GLUCOCORTICOIDS

Haller J, Halasz J, Mikics E, Toth M, Barsy B.

Institute of Experimental Medicine, Budapest, Hungary

We will show that - beyond chronic HPA-axis hyperfunction -, *decreased* glucocorticoid production also leads to psychopathologies. This point will be exemplified by studies on aggressive behavior. Psychopathology-associated human aggression types are induced by a variety of conditions,

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

are behaviorally variable, and show a differential pharmacological responsiveness. Thus, there are several types of abnormal human aggression. This diversity was not reflected by conventional laboratory approaches that focused on the quantitative aspects of aggressive behavior. Recently, several laboratory models of abnormal aggression were proposed, which mainly model hyperarousal-driven aggressiveness (characteristic to intermittent explosive disorder, posttraumatic stress disorder, depression, chronic burnout, etc.) and hypoarousal-driven aggressiveness (characteristic mainly to antisocial personality disorder and its childhood antecedent conduct disorder). Findings obtained with these models suggest that hyperarousal-driven aggressiveness has at its roots an excessive acute glucocorticoid stress response (and probably an exaggerated response of other stress-related systems), whereas chronic hypoarousal-associated aggressiveness is due to glucocorticoid deficits that affect brain function on the long term. In hypoarousal-driven aggressiveness, serotonergic neurotransmission appears to lose its impact on aggression (which it has in normal aggression), certain prefrontal neurons are weakly activated, whereas the central amygdala (no, or weakly involved in the control of normal aggression) acquires important roles. We suggest that the specific study of abnormal aspects of aggressive behavior would lead to important developments in understanding the specific mechanisms underlying different forms of aggression, and may ultimately lead to the development of better treatment approaches.

7A_03_S

EARLY ADVERSE EXPERIENCE AND RISK FOR PSYCHOPATHOLOGY

Christine Heim

Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA, USA
cmheim@emory.edu

Childhood trauma is a potent risk factor for developing depression in adulthood, particularly in response to additional stress. We here summarize results from a series of clinical studies suggesting that childhood trauma in humans is associated with sensitization of the neuroendocrine stress response, glucocorticoid resistance, increased central corticotropin-releasing factor (CRF) and decreased oxytocin activity, immune activation, and reduced hippocampal volume, closely paralleling several of the neuroendocrine features of depression. Neuroendocrine changes secondary to early-life stress likely reflect risk to develop depression in response to stress, potentially due to failure of a connected neural circuitry implicated in emotional, neuroendocrine and

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

autonomic control to compensate in response to challenge. However, not all of depression is related to childhood trauma and our results suggest the existence of biologically distinguishable subtypes of depression as a function of childhood trauma that are also responsive to differential treatment. Other risk factors, such as female gender and genetic dispositions, interfere with components of the stress response and further increase vulnerability for depression. Similar associations apply to a spectrum of other psychiatric and medical disorders that frequently coincide with depression and are aggravated by stress. Taken together, this line of evidence demonstrates that psychoneuroendocrine research may ultimately promote optimized clinical care and help prevent the adverse outcomes of childhood trauma.

7A_04_S

A SYSTEMS-LEVEL MODEL OF STRESS EFFECTS ON COGNITION

Jeansok J. Kim and Taejib Yoon

Department of Psychology and Program in Neurobiology & Behavior, University of Washington, Seattle, Washington, U.S.A.

Stress is a biologically significant social-environmental factor that plays a pervasive role in our lives, from impacting our daily behaviors to producing and exacerbating myriad physical and mental illness. An accumulating body of evidence from human and animal studies reveals that while the acute response to stress (i.e., heightened cognition) is an adaptive mechanism, exposures to *uncontrollable* (unpredictable and inescapable) stress can subsequently produce detrimental neurocognitive effects, particularly in the hippocampus. Rodent studies further indicate that stress impairs long-term potentiation (LTP), a leading candidate cellular mechanism of information storage, in the hippocampus. We have recently discovered that amygdalar lesions/inactivation and prefrontal cortex lesions block and exacerbate, respectively, stress-induced impairments in hippocampal LTP and spatial memory. Moreover, single unit recording data indicate that stress alters the firing rate of place cells recorded from dorsal hippocampus, providing an empirical bridge between stress effects on synaptic plasticity and spatial memory. Based on these findings, we will present a conceptual model of the central stress mechanism (a neural-endocrine network comprising of amygdala, prefrontal cortex and glucocorticoids) regulating hippocampal functioning.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7B_01_S

LOW-ENERGY STRESS-RELATED DISORDERS. DIAGNOSTIC UTILITY OF BIO-PSYCHOSOCIAL MARKERS

Bengt B. Arnetz[1,2] , Ulla Maria Anderberg[2], Ingrid Anderzen[2], and Christina Halford[2]

[1]Division of Occupational and Environmental Health, Department of Family Medicine and Public Health Sciences, Wayne State University, Detroit, Michigan 48009, USA. barnetz@med.wayne.edu
[2] Division of Social Medicine, Department of Public Health Sciences, Uppsala University, Uppsala Sciences Park, 751 05 Uppsala, Sweden

It is commonly difficult to differentiate low energy states due to stress-related disorders from depression.[1] The aim of the present study was to evaluate possible differences in symptoms, psychiatric co-diagnosis, and biological stress markers in patients suffering from stress-related disorders versus depression. 150 patients that had been referred by primary care physicians to an academic stress medicine center were evaluated by a physician using structured psychiatric tools, the center's validated stress-assessment visual analogue scales, biological stress markers, blood pressure, and Body Mass Index. Patients with depression, >20 points on MADRS, scored significantly lower on the global energy scale, self rated health, and quality of sleep as compared to patients suffering from stress-related disorders. Depressed patients scored higher on the global stress scale. Depressed patients rated themselves to be less rested following a night's sleep. Depressed patients scored lower with regard to satisfaction with their family and job situations. It is suggested that studies involving either stress-related disorders or depression more carefully screen the participants with regard to biopsychosocial characteristics. A substantial number of patients being referred for stress-related disorders also suffer from psychiatric co-morbidity.

[1] Arnetz, BB, Ekman R. (Eds). Stress in health and disease. Wiley-VCH. Weinheim, Germany. ISBN-13 978-3-527-31221-4.

7B_02_S

STRESS AND THE HEART: THE PHYSIOLOGICAL BASIS FOR THE DEVELOPMENT OF CARDIAC RISK IN DEPRESSION AND ANXIETY DISODERS

Gavin Lambert

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Baker Heart Research Institute, Melbourne, Australia
(gavin.lambert@baker.edu.au)

Until recently it was thought that no more than 50% of clinical coronary heart disease was explicable in terms of classical cardiac risk factors such as dyslipidemia, cigarette smoking, high blood pressure and diabetes. Recent large scale epidemiological studies have increased our understanding of the mechanisms generating cardiac risk and have provided evidence indicating that psychosocial factors, particularly depressive illness (MDD), anxiety states, and acute and chronic mental stress are involved here, "triggering" clinical cardiovascular events, and possibly also contributing to hypertension and atherosclerosis development. Although the underlying mechanisms in play are most likely multi factorial in origin the sympathetic nervous system is undoubtedly paramount in many cases. Using noradrenaline isotope dilution methodology and direct nerve recording coupled with invasive blood sampling techniques we have examined cardiac sympathetic activity in patients with MDD and also panic disorder (PD). By sampling blood also from the internal jugular vein we have been able to gain insight into brain monoamine turnover in these conditions and have directly evaluated the effects of SSRI therapy on brain monoamines in these conditions. The pattern of sympathetic activation is very different in patients with MDD (bimodal) or PD (normal). Brain monomaine turnover is different in each group, with brain noradrenaline turnover being reduced in MDD yet normal in PD. Interestingly, in both MDD and PD brain serotonin turnover, surprisingly, is markedly elevated before treatment and is significantly diminished following SSRI therapy. Clearly, the role of brain monoamines and their relation to generating increased cardiac risk merits further attention.

7B_03_S

STRESS AND COMMON HEALTH COMPLAINTS

Holger Ursin

Unifob Health, University of Bergen, Norway

According to the Cognitive Activation Theory of Stress (CATS – Ursin and Eriksen 2004), a formal system of systematic definitions, the term "stress" is used for stress stimuli, the stress experience, the non-specific, general stress response, and the experience of the stress response. The stress response is normal, healthy, and necessary alarm. If sustained there may be a risk of illness and disease. The level and duration of the alarm depends on the expectancy of the outcome of stimuli and the specific responses available for coping.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

The most common health complaints are subjective health complaints like muscle pain, tiredness and mood changes. These are normal aches of short duration and low intensity for most people. For some the pains and complaints are substantial and long-lasting with serious implications for functioning. There are no sharp or obvious limits in the distribution of health complaints, separating 'normal' and endurable pain and complaints, and intolerable complaints that need professional help. These conditions are most often unspecific, and are the most common reason for encounters with health professionals, and the most frequent reason for sick leave and disability. There is a striking comorbidity for all these conditions. This may be explained by psychobiological sensitization within neural loops, which has been suggested as a mechanism for these conditions (Ursin 1997).

7B_04_S

INTERMITTENT NEUROGENIC STRESS DELAYS ONSET OF DIABETES IN RATS: SELYE'S EUSTRESS?

Holly E. Bates, A Sirek, MA Kiraly, JTY Yue, D Goche Montes, SG Matthews, and M Vranic

University of Toronto, Toronto, Canada. holdoug@yahoo.ca

Acute stress deteriorates glycemic control in diabetes. We hypothesized that chronic intermittent restraint stress (1hr/d, 5d/wk for 13wks) of male ZDF rats, a model of Type 2 Diabetes, would accelerate development of diabetes. Intermittent stress lowered food intake by 15%. Thus, pair fed rats were included to distinguish between the effects of reduced food intake and of stress *per se*. Surprisingly, intermittent stress delayed development of fed and fasting hyperglycemia, effects mediated partly by reduced food intake, but also by intermittent stress *per se*. The stress-induced reduction in food intake improved insulin secretion and β-cell mass, suggesting these as mechanisms for reduced food intake to improve glycemia. However, intermittent stress *per se* also led to HPA axis adaptations. Although basal ACTH levels did not differ, intermittent stress prevented the 30% increase in basal corticosterone (CORT) with food restriction, which could predict 20% of the variation in fed glycemia. In addition, intermittent stress led to habituation of restraint-induced CORT responses to lower levels than those induced by food removal. These CORT adaptations with intermittent stress were consistent with adaptation of mRNA levels for hippocampal MR, paraventricular nucleus AVP, and anterior pituitary POMC. Thus, intermittent restraint stress *per se* delays hyperglycemia, presumably via adaptations in the HPA axis that prevent the hypercorticosteronemia caused by food restriction. Since hypercortisolemia deteriorates glycemic control, adaptation to repetitive,

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

predictable stress may be beneficial for glucose regulation. Thus, as Hans Selye noted when coining eustress, not all stress is deleterious.

7C_01_S

MATERNAL STRESS AND THE PRENATAL PROGRAMMING OF INFANT IMMUNITY

Christopher L. Coe, Gabriele R. Lubach

University of Wisconsin, 22 N. Charter Street, Madison, WI, USA, *ccoe@wisc.edu*

Background. While stress can influence immune responses at any point in the life span, our research has shown that the effects are larger and more lasting in the young and aged host. In particular, during fetal and infant development, the maturational trajectory of immunity can be permanently altered. This presentation will focus on changes seen after manipulation of the *in utero* environment and the role of placental and neuroendocrine processes in mediating the immune alterations. Methods. Pregnancy conditions of rhesus monkeys were manipulated in several ways, including by psychological disturbance of the gravid female, administration of dexamethasone acutely, dietary treatments, or viral infection. The impact on the infants' immune, endocrine, brain, and behavioral development was assessed.
Results. Maternal stress, or antenatal administration of corticosteroids, significantly affected immune responses at birth and continued to have an effect on lymphocyte proliferation and cytokine responses up to 2 years of age. These immune changes were associated with changes in the gut microbiota (including reduced Lactobacilli and Bifidobacter), which increased the risk for infection with enteric pathogens. A novel pathway mediating the effect of maternal stress on the fetus was also identified: a reduction in the placental transfer of iron. Lower iron stores at birth increased the risk for an iron deficiency anemia (IDA) in the growing infants. The anemia emerging at 4-8 months of age provided a second postnatal hit to the immune system. In addition, infant monkeys from stressed pregnancies evinced behavioral and neural changes, including immature neuromotor reflexes at birth, greater emotionality during the first year of life, and a smaller hippocampus as juveniles. Smaller hippocampal size was associated with less neurogenesis, and a more reactive hypothalamic-pituitary-adrenal axis. Conclusions. Stressful and challenging events during fetal life can significantly impact the development of immunity at a vulnerable point in ontogeny and change the regulatory set points for several physiological systems postnatally.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7C_02_S

CYTOKINE ACTIONS IN THE BRAIN MEDIATE THE INCREASED PREVALENCE OF DEPRESSION IN DISEASES WITH AN INFLAMMATORY COMPONENT

Robert Dantzer

Integrative Immunology & Behavior Program, University of Illinois at Urbana-Champaign, USA

Physically ill patients experience a high prevalence of affect disorders that are exacerbated by relatively minor infections. Activation of the peripheral innate immune system induces signs of sickness that culminate in depressive-like behavior in adult mice. This response is exaggerated in aged mice whose innate immune system is chronically activated. Peripheral inflammation is relayed to the brain by both the sensory nerves that innervate the site of inflammation and the overflow of inflammatory mediators that enters the general circulation. In response to these stimuli, brain macrophage-like cells produces cytokines that directly or indirectly affect neuronal function. Activation of indoleamine 2,3 dioxygenase (IDO), a key enzyme in the metabolism of tryptophan, is an important mechanism for the depressive-like effects of immune activation. Enhanced activity of this enzyme in response to tumor necrosis factor-alpha and/or interferon-gamma induces the production of neurotoxic kynurenine metabolites and decreases the bioavailability of tryptophan, which ultimately impacts on serotoninergic neurotransmission. The development of depressive-like behavior in mouse in response to acute or chronic activation of the peripheral innate immune system is temporarily correlated with IDO activation in the brain and at the periphery. Furthermore pretreatment with 1-methyl-tryptophan, a competitive antagonist of IDO, abrogates depressive-like behavior but not sickness behavior in mice during the course of inflammation. These preclinical findings emphasize the role of cytokines and their metabolic effects in the pathophysiology of inflammation-associated depression. Supported by NIMH (R01 MH-71349 and MH-079829).

7C_03_S

LONG PHOTOPERIOD-INDUCED GLUCOCORTICOID RESISTANCE AND DECREASED NEGATIVE SELECTION IN DOUBLE-POSITIVE THYMOCYTES OF FEMALE BUT NOT MALE MICE. MECHANISMS AND POSSIBLE IMPLICATION IN THE GEOGRAPHICAL DISTRIBUTION OF AUTOIMMUNE DISEASES

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Georges Maestroni

Center for Experimental Pathology, Cantonal Institute of Pathology, 6601 Locarno, Switzerland

The mammalian immune response is circadian and is regulated through daily alterations of darkness and recently defined specific pathway of non-visual light. The endogenous pace maker that drives this circadian cycle is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Light reaches the SCN through non-rod, non-cone intrinsically photosensitive retinal ganglion cells. The nervous pathway connecting the non-visual light detection in the SCN of the hypothalamus leads to a release of neurohormones from the pituitary, pineal, adrenal glands, and the gonads that finally reach the lymphoid organs. We found that exposure of adult female but not male mice to long photoperiod (22 hours light: 2 hours dark, LP) for 8 days decreased the glucocorticoid sensitivity of the thymus gland. Dexamethasone –induced apoptosis was decreased in double positive thymocytes and this effect was apparent only in vivo but not in vitro. This effect was not observed in prebubertal female mice and in adult gonadectomized mice as well as in beta-2 adrenergic receptor gene deficient mice. We exposed to the LP DO11.10 mice transgenic for a T cell receptor recognizing an ovalbumin peptide. The peptide was then injected intraperitoneally to induce intrathymic negative selection. The LP-exposed mice showed a significantly reduced negative selection of double positive thymocytes. Intrathymic negative selection is mediated by endogenous glucocorticoids and generates tolerance to self –antigens. As the seasonal variation of the photoperiod increases with the latitude, we might have disclosed a basic mechanism explaining the gender and geographical distribution of autoimmune disorders which are more frequent in women and show a north-south gradient of incidence.

7C_04_S

ALTERATIONS OF THE CATECHOLAMINE-CYTOKINE BALANCE IN DEPRESSION

Szelényi J. and Vizi E.S.

Institute of Experimental Medicine, Laboratory of Neuroimmunology, 43. Szigony str., H-1083 Budapest, Hungary

Cytokines are involved both in various immune reactions and in controlling certain events in the central nervous system. In our earlier studies it was shown that monoamine neurotransmitters, released in stress situations, represent a tonic sympathetic control on cytokine production and on the balance of proinflammatory/antiinflammatory cytokines. Basic and clinical

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

studies have provided evidence that the biophase level of monoamines, determined by the balance of their release and uptake, is involved in the pathophysiology and treatment of depression, while inflammatory mediators might also have role in its etiology. In this work we studied the role of changes in norepinephrine (NE) level on the LPS evoked TNF-α and IL-10 response both in the plasma and in the hippocampus of mice. We demonstrated that the LPS induced TNF-α response is in direct correlation with the biophase level of NE as it is significantly higher when the release of NE of vesicular origin was completely inhibited in an animal model of depression (reserpine treatment) and it is significantly lower in case of increasing biophase level of NE by genetical (NETKO) or chemical (desipramine) disruption of NE reuptake. IL-10 was changed inversely to the TNF-α level only in the desipramine treated animals. Our results showed that depression is related both to changes in peripheral and in hippocampal inflammatory cytokine production and to monoamine neurotransmitter levels. Since several antiinflammatory drugs have also antidepressant effect we hypothesized that inhibitors of the monoamine uptake system might have multiple targets and are also able to modulate the LPS-induced inflammatory responses, which might contribute to their antidepressant effect.

This work was supported by grants OTKA T-046896 and ETT 298/2006

7D_01_S

SOCIOECONOMIC AND PSYCHOSOCIAL DETERMINANTS OF CHRONIC STRESS IN A CHANGING SOCIETY

Maria S. Kopp

Institute of Behavioral Sciences, Semmelweis University, H-1089 Budapest, Nagyvárad t. 4, Hungary kopmar@net.sote.hu

In the last decades in the transforming societies of Central and Eastern Europe (CEE) premature mortality increased dramatically, especially among men. In Hungarostudy 2002 survey 12.640 persons were interviewed in their homes. They represent the Hungarian population according to age, sex and 150 sub-regions above age 18. Socioeconomic, psychosocial, work and family related factors, behavioral and self-reported health measures were recorded. From the latest Hungarostudy 2006 follow-up study 1130 men and 1529 women who in 2002 were between the age of 40-69, were included into the present study. By 2006 99 men (8.8%) and 53 women (3.6%) died in the 40-69 age group. After adjustment for traditional risk factors, work related measures, such as job insecurity and low social support from co-workers predicted significantly the all-cause mortality among men. Low education and low personal

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

income were connected with premature mortality only among men. Living with the spouse was significant protective factor against early death only among men, whereas dissatisfaction with personal relations was a significant risk factor among women. Beck Depression score and self-rated health were much more important predictors of premature death among men than among women. In the transforming society of Hungary middle-aged men are much more vulnerable to the work and socioeconomic deprivation related chronic stress factors than women in the same age groups. Chronic stress is proposed as an integrating model that can be applied to understanding the gender differences in premature mortality during social transformation.

7D_02_S

JOB STRAIN COMPONENTS AND ADRENERGIC ALFA-2 AND B_1-RECEPTOR-POLYMORPHISMS - A PUTATIVE STRESS-GENE INTERACTION AFFECTING OFFICE BLOOD PRESSURE LEVELS IN MEN

Bertil Öhlin, Peter M Nilsson, Olle Melander

Department of Clinical Sciences, Lund University, University Hospital, Malmö, S-205 02 Sweden.

Background: Job strain and the polymorphisms of both the alpha-2 and beta-1-adrenergic receptor genes have both been linked to blood pressure elevation, respectively. We aimed at studying a potential interaction between job strain and its components (job demand and decision latitude), and the beta-1 receptor (Arg389Gly) polymorphism in relation to office blood pressure.
Methods: From the Malmö Diet and Cancer population cohort a total of 6095 subjects were randomly selected to be followed regarding cardiovascular risk factors. From this group, employed individuals with baseline questionnaire data regarding work characteristics were included (1338 men, 1707 women). Determination of the adrenergic β_1-receptor Arg389Gly polymorphism was possible in 1271 men and 1601 women, and these individuals formed the study group. Associations with the alpha-2 receptor have already been reported (Öhlin B, et al. J Hypertens 2007, accepted for publication)
Results: Men with job strain and the Arg389Arg genotype had a non-significant higher SBP (p=0.18) and DBP (p=0.34) than those with a Gly allele with or without job strain. The interaction term genotype x job strain was of borderline significance for SBP (p=0.07) after adjustments for age, country of birth, and job status. The demand score was significantly interacting with genotype in men (p=0.01 for SBP, and p=0.009 for DBP), after adjustments for age, country of birth, job status, antihypertensive

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

treatment, and BMI. Men with a Gly allele had lower blood pressures with increasing demand score, whereas men homozygous for the Arg allele had lower blood pressures with increasing latitude score. In women, those with job strain had borderline significantly higher blood pressures than those without job strain, regardless of genetic variants.
Conclusions: Job strain, and in particular job demands, seems to interact with the Arg389Gly polymorphism in men, resulting in higher blood pressures in men with genotype Arg389Arg. The genotypes interact differently with the components of job strain (demand and decision latitude) in terms of blood pressure levels. These preliminary findings need to be addressed in future studies.

7D_03_S

WORK STRESS AND HEALTH IN THE CULTURAL CONTEXT OF GLOBALISATION

Johannes Siegrist

University of Duesseldorf, Germany

Economic globalisation has far-reaching impact on the socio-cultural life and the health of populations in modern and rapidly developing societies. Technological progress goes along with the spread of a Western lifestyle that increases the risk of non-communicable diseases. Importantly, a globally expanding market of capital, goods and labour results in an increase of stressful working conditions among large parts of employed people, in addition to the afflictions of unemployment.
Based on the discoveries of two leading theoretical models, a wide range of health-adverse effects of stressful work has now been identified. In these models, stressful work is defined as either a combination of high demand and low control in one's job, and as an imbalance between high effort spent and low reward received in turn, where rewards include money, esteem, and promotion prospects including job security. This latter model is of particular interest in the context of globalisation as it points to violations of a fundamental principle of economic and social exchange, reciprocity.
Major research findings from epidemiological and experimental studies of work stress and health are demonstrated, with special emphasis on rapidly transforming societies. In the final part, policy implications of current scientific evidence are discussed, with a focus on worksite health promotion, and promising preliminary findings from intervention studies are reported. Yet, given the scale of challenge, coordinated international strategies towards healthy work will be needed.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7D_04_S

PSYCHOSOCIAL STRESS MANAGEMENT PROGRAMS IN TRANSFORMING SOCIETIES

Redford B. Williams

Duke University Medical Center, Durham, NC, USA

Populations living in societies undergoing major transition can be subjected to mounting levels of stress that have the potential to produce serious health problems in some or all segments of the population. Since the breakup of the Soviet Union in 1989, for example, there has been a marked increase in annual mortality in countries of the former Soviet Block, most marked in Russia where the annual mortality increased from 600/100,000 to 900/100,000 – producing an excess of 400,000 deaths per year. Another example is India, where a continuing dramatic expansion of the economy has created another set of social, political and economic upheavals. A recent WHO report estimates that by 2010, 65% of the world's cardiac cases will be persons of Indian descent, both in India and elsewhere. It is likely that a significant proportion of these increasing mortality and cardiac problems are a result of the stresses encountered in these transforming societies. While all might agree that the ideal solution would be to ameliorate the social, political, and economic conditions that are creating the health-damaging stress, that may be a solution that is easier to contemplate than to implement. In this presentation I will review experiences with one structured stress management program, the Williams LifeSkills Workshop, that has been implemented, with appropriate adaptations to the different cultures, in the Far East and Hungary. In both regions, results indicate that training in coping skills can be implemented with results that clearly document reduced stress levels. To have a favorable impact on the health problems in these regions at the public health level, however, it will be necessary to develop and implement delivery systems that can disseminate this training on a mass scale. I will conclude by considering how mass media and the internet might be used to do this.

7E_01_S

ACHIEVING STRESSLESS PREDICTABLE PERIO / IMPLANT EXCELLENCE

André P. SAADOUN

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

The creation of an esthetic implant restoration with gingival architecture in harmony with the adjacent dentition is a formidable challenge. The process of soft and hard tissue healing must be understood and incorporated into a carefully coordinated sequence of therapy.
The essential prerequisites in order to establish an optimal aesthetic implant restoration should always remain a precise, comprehensive biological and prosthetic diagnosis as well as the choice of the most conservative, appropriate, and least traumatic treatment for the patient to prevent any injury to the periodontal and dental structure and achieve a successful outcome.
The final objective is to achieve an optimal crown restoration surrounded by its natural gingival environment, using delicate ossoeus and/or muco-gingival plastic surgery.
A major evolution in implantology has taken place with tapered and rough surface implants, inserted in a one-step, non-submerged surgical protocol.
It is important to identify complications and clinical mistakes and their implications on the final esthetic outcome.

7E_02_S

THE ROLE OF STRESS IN PERIODONTAL DISEASE

B.Willershausen, A.Kasaj, A.Pistorius

Department of Operative Dentistry, Johannes Gutenberg University, Mainz Germany

In the last two decades several investigations have been carried out on associations between stress factors and periodontitis and a number pathogenesis models have been discussed and examined. As a physiologic measure of stress urin levels of corticosteroids have been used, and increased free cortisol levels in the urine of periodontitis patients as compared with controls have been encountered. It was shown that emotional stress may interfere with normal immune function and may result in increased levels of circulating hormones that can effect the periodontium. Stressful life events appear to lead to a greater prevalence of periodontal disease and individuals with financial worries, distress, depression or inadequate coping mechanisms have a more sever loss of clinical attachment. Furthermore, it could be demonstrated that anxiety and stress may have a negative influence on the response to periodontal therapy and that patients responding less well to periodontal treatment have more psychosocial strain and a more passivedependent personality. Although epidemiologic data on the association of stress and periodontal disease is still limited, it appears that stress may be a putative risk factor for periodontitis.
The purpose of this presentation is to provide an overview, based on own

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

data obtained from controlled clinical studies, on the association of stress, distress and coping behaviours with periodontal disease.

7E_03_S

HYPNOSIS IN DENTISTRY

Krause W.R.

Dept. of Psychiatry and Psychotherapy, Harz-Klinikum Wernigerode-Blankenburg, Germany
psychiatrie@harz-klinikum.de

In daily practice dentists are faced with a wide array of psychological problems including dental fear, gagging, psychogenic denture intolerance, bruxism, atypical facial pain etc. Although several hypnotherapeutic methods can be used rather effectively to solve most of these problems [1,2], only few dentists use such techniques in his/her practice. Therefore the aim of the lecture is to introduce the possibilities of hypnotherapy in dentistry and to inspire the audience's effort to use this method in the dental practice. For this purpose author will summarize the theoretical background and basic clinical knowledge of hypnotherapy, and will describe how hypnosis can be used for a wide spectrum of dental problems. References: (1): Krause, W-R.: Hypnose und Autogenes Training. In Schultz, JH.: Hypnosetechnik. Praktische Anleitung zum Hypnotisieren für Ärzte. 9. Auflage, bearbeitet und ergänzt von G. Iversen und W-R. Krause. Gustav Fischer Verlag, Stuttgart, 1994. (2): Staats, J., Krause, W-R.: Hypnotherapie in der zahnärztlichen Praxis. Hüthig Verlag, Heidelberg, 1995.

7E_04_S

PSYCHOSOMATIC DENTISTRY IN HUNGARY. TRENDS AND PROGRESS IN THE LAST 10 YEARS

Fábián T.K.

Clinic of Prosthetic Dentistry, Semmelweis University, Budapest, Hungary

Following a rather long brake, an increase of interest in psychosomatic dentistry occurred in the middle of nineties in Hungary. From this time up to the present 67 written publications were published from the Faculty of Dentistry of the Semmelweis University Budapest related to this topic.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

During this time all together 32 researchers participated in this multidisciplinary project including 13 dentists, 6 psychologists, 5 biochemists, 1 psychiatrist, 1 teacher, 5 dental student and 1 psychology student. Research topics included epidemiology of dental fear, clinical treatment of dental fear and several oro-facial psychogenic symptoms, and stress related biochemical changes of saliva. The lecture will summarize the most important data and results of this 10 years project and give a broad outline of research possibilities in the next future.

7F_01_S

GENE - ENVIRONMENT INTERACTIONS IN PTSD AND OTHER STRESS-RELATED DISORDERS

Marcus Ising

Max Planck Institute of Psychiatry, Kraepelinstr. 2-10, 80804 Munich, Germany
ising@mpipsykl.mpg.de

Stress related disorders can be defined as illnesses whose causation, onset, or development is substantially influenced by stress and its neurobiological correlates. Among mental disorders, depression, anxiety, and post-traumatic stress disorder (PTSD) are typical examples for stress-related disorders. They are characterized by a moderate heritability suggesting the involvement of genetic vulnerability factors. Additionally, environmental influences including exposure to stressful life events and trauma contribute to the disease risk and can act as triggers for the onset of the disorder. Taking both into account, the investigation of gene – environment interactions is an important approach to elucidate the aetiology of stress-related disorders. We will discuss different approaches to examine gene – environment interactions in depression, anxiety, and PTSD, summarize the findings from the literature and present own results. The investigation of gene – environment interactions could prove as a promising approach to extend our knowledge about the aetiology and to identify new treatment targets in stress-related disorders.

7F_03_S

THE SEARCH TO CURE PTSD: EVIDENCE FOR NOVEL MOLECULAR TARGETS

Christoph Czermak, MD, Alexander Neumeister, MD

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Yale University, Dept of Psychiatry, West Haven, CT, USA,
alexander.neumeister@yale.edu

Indirect support exists for an involvement of the noradrenergic system, the serotonergic system and nicotinic acetylcholine receptors in the pathophysiology of posttraumatic stress disorder (PTSD). To untangle the relative contribution of these transmitter systems to the pathophysiology of PTSD and to identify potential novel targets to treat PTSD, we conducted a series of functional imaging studies using positron emission tomography (PET) and single photon emission computed tomography (SPECT). Using novel radioligands we studied for the first time the expression of serotonin (5-HT) receptors types 1A and 1B, the norepinephrine transporter and nicotinic acetylcholine receptors in the brain of PTSD patients, trauma-exposed and non-traumatized healthy control subjects. We also studied changes in electrodermal skin activity and neurochemistry following infusion with the α_2-antagonist yohimbine in PTSD subjects relative to healthy controls. We found evidence for a role of the 5-HT1B receptor but not the 5-HT1A receptor in PTSD suggesting the 5-HT1B receptor as a potential target for drug development. We further substantiated the important role of noradrenergic mechanisms in PTSD. Altogether, our studies provide evidence for the involvement of monoaminergic mechanisms in the pathophysiology of PTSD and identify novel targets for drug development.

7F_04_S

THE PSYCHOBIOLOGY OF COPING WITH PTSD

Miranda Olff

Center for Psychological Trauma, Academic Medical Center University of Amsterdam, Meibergdreef 5, 1105 AZ Amsterdam, The Netherlands,
m.olff@amc.uva.nl

Although many people are exposed to trauma, only part develop post traumatic stress disorder (PTSD) or other posttrauma psychopathology. It is possible that humans differ in the degree to which trauma induces neurobiological perturbations of their threat response systems, which may result in a differential degree of psychopathology. Posttraumatic stress disorder (PTSD) has been associated with dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis as well as of the hypothalamus-pituitary-thyroid (HPT) axis. Findings have not been consistent and may depend on methodological issues like controlling for relevant variables. Results of a meta-analysis will be shown. In this paper we also present data on six HPA and HPT-axis related hormones in civilian chronic PTSD patients. Comparing chronic PTSD patients to healthy

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

volunteers we found that patients had significantly lower plasma cortisol, prolactin and TSH levels. When studying the effects of psychotherapy in chronic PTSD patients we found that after Brief Eclectic Psychotherapy (BEP) significant changes occurred in levels of cortisol and DHEA. Responders showed an increase of cortisol and DHEA levels, while in non-responders both hormone levels decreased. Differences were only found after controlling for depressive symptoms. In conclusion, learning to cope with trauma through psychotherapy for PTSD may alter dysregulations in the HPA-axis, but comorbid depressive symptoms should be taken into account. Continued study of the psychobiology of trauma and PTSD will enhance our understanding of adaptation to psychosocial stressors and support efforts to treat associated psychological and biological sequelae.

7G_01_S

STRESS-TRIGGERED REPRODUCTIVE SUPRESSION: AN EVOLVED ADAPTATION?

Pablo Nepomnaschy

National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA
nepomnaschyp@niehs.nih.gov

It is estimated that only about 23% of women trying to conceive will begin a successful pregnancy during the first menstrual cycle of their attempt. This low rate of reproductive success has led some health scientists to describe human reproduction as inefficient and, therefore, an evolutionary paradox. In contrast to this pathological vision of women's reproduction, evolutionary theorists propose that reproductive suppression may have originated as an adaptation that, in dire circumstances, helps prevent pregnancy. As practitioners' positions on this issue can affect both diagnoses and treatments, the debate of these opposing paradigms is of critical importance.
This talk contributes to the aforementioned debate through an examination of the physiologic effects of stress on women's reproductive function. Stress is commonly reported to lead to reproductive suppression. Most of the available evidence to support this claim, however, has been derived from animal and clinical or retrospective studies. Here I present data from a population-based, longitudinal study I conducted among Kaqchikel Maya in the southwestern highlands of Guatemala. The relationship between stress axis (hypothalmic-pituitary-adrenal) activation and ovarian function, implantation and early pregnancy fate is discussed. Evolutionary and pathologic aspects of the effects of stress on reproductive function are evaluated.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7G_02_S

THE RELATIONSHIP BETWEEN PSYCHOLOGICAL STRESS AND FEMALE FERTILITY

Sheiner Eyal, M.D

Department of Obstetrics and Gynecology, Faculty of Health Sciences & Soroka University Medical Center, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

Fertility treatments carry emotional burdens on women and their partners. Although the psychosocial aspects of infertility have not been adequately addressed in clinical practice, there is consensus in the literature that attention to the psychological aspects of infertility is strongly advisable. Psychological factors such as depression, state-anxiety, and stress-induced changes in heart rate might be predictive of a decreased probability of achieving a viable pregnancy in various types of infertility and fertility treatments. Previous intervention trials did not include screening for psychological nor physiological stress markers known to predict pregnancy in women experiencing difficulties achieving pregnancy. It is not clear whether the psychological stress is a part of the infertility etiology as a causative factor (psychogenic hypothesis), or rather represents a development of the infertility problem (psychological consequences hypothesis). Nevertheless, it is more likely that there is an interactive causal association between infertility and psychosocial distress.

7G_03_S

THE HYPOTHALAMIC-PITUITARY-ADRENAL AND THE HYPOTHALAMIC-PITUITARY-GONADAL AXES INTERPLAY IN REPRODUCTION

George Mastorakos

Endocrine Unit, Second Department of Obstretics and Gynecology, Aretaieion Hospital, Athens University Medical School, Athens; mastorak@mail.kapatel.gr

Vertebrates respond to stress with activation of the HPA axis, the adrenergic and the autonomic nervous systems. The principal central nervous system regulators of the HPA axis are corticotropin releasing hormone (CRH) and antidiuretic hormone (AVP). Apart central nervous system, CRH has been found in the adrenal medulla, ovaries,

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

myometrium, endometrium, placenta, testis and elsewhere. The activation of the HPA axis during stress affects all body systems. The reproductive axis is inhibited by HPA axis, for the sake of saving energy. The changes of the HPG axis during stress are species-specific, and depend on type and duration of the stimulus. Several conditions may be associated with altered regulation of the HPA axis. Polycystic ovary syndrome, anorexia nervosa and pregnancy in the third trimester are all characterized by HPA axis activation. In contrast, during postpartum period, HPA axis suppression is implicated in the "postpartum blues". The actions of CRH are also essential in fetal development and neonatal survival.

7G_04_S

STRESS DURING PREGNANCY: CONSEQUENCES FOR MOTHER AND CHILD

PC Arck [1], M Ruecke [1], K Nakamura [2], H Fliege [1], M Rose [3], S Blois [1], M Pincus [1], BF Klapp [1]

[1] *Charité, University Medicine Berlin, Germany*
[2] *University of Vancouver, Canada*
[3] *Qualitymetric, USA*

Maternal stress perception has long been suspected as a possible cause of infertility, implantation failure, late pregnancy complications and impaired fetal development, notions that exist since ancient times and across all cultures. In view of the enormous complexity of the regulatory nervous, endocrine and immune mechanisms involved in pregnancy maintenance, it is evident that pregnancy failure is not a single entity condition, but most likely the result of complex dysregulation. This dysregulation can be initiated or aggravated by stress. While there are still more open questions than answers, the neuro-endocrine-immune circuitry of the stress response during pregnancy is becoming increasingly defined, e.g. due to the development of particularly instructive rodent models and prospectively designed human cohort trials. Subsequently, clinicians are becoming far more attentive to the effect of psychological stress on pregnancy complications. As a reseult of emerging basic science research endeavours elucidating hierarchical, temporal and spatial interactions of key parameters during central and peripheral responses to psychological stress, a list of candidate molecular targets for clinically useful therapeutic intervention has become available by and should be tested in interdisciplinary research approaches.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Module 7 – Poster lectures:

7A_01_P

THE STRIP-SEARCH VICTIM AND STRESS: A SOCIAL-PSYCHOLOGICAL ANALYSIS

Thomas Blass

Department of Psychology, University of Maryland Baltimore County, Baltimore, MD 21250, USA
blass@umbc.edu

Spanning a 9-year period from 1995-2004 a similar hoax was perpetrated on 70 fast-food restaurants across the United States. A man, claiming to be a policeman (in actually he was not) would telephone a restaurant and tell the manager to strip-search a particular employee (usually female) in search of money she supposedly stole. The managers typically complied with the "policeman" whose instructions sometimes went beyond having the employee remove all her clothes, to searching her body cavities, and even sexual abuse. Visible stress reactions by the victims were common (e.g., crying). Drawing on police reports, court records, and journalistic accounts, I present a systematic analysis of the victims' stress reactions in a sample of such incidents. This analysis will be presented within the framework of a broader, overarching inquiry about the surprising willingness of the managers to carry out such reprehensible orders—an inquiry informed by the groundbreaking experiments on obedience to authority by social psychologist Stanley Milgram conducted at Yale University in 1961-62.

7A_02_P

COGNITIVE DEFICITS CAUSED BY CHRONIC STRESS CAN BE PREVENTED BY THE *GINKGO BILOBA* AND *HYPERICUM PERFORATUM* ADMINISTRATION

Jan J. Braszko, Anna Walesiuk, Emil Trofimiuk

Department of Clinical Pharmacology, Medical University of Bialystok, Waszyngtona 15A, 15-274 Białystok, Poland

Exposure to chronic restraint stress in rats as well as the psychosocial stress in humans has been shown to alter cognitive functions such as learning and memory and has been linked to the pathophysiology of mood and anxiety disorders. Anxiolytic/sedative/antidepressant agents used in the management of the stress-related disorders have several side effects

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

and are not cost-effective.
Therefore, in this study we investigated efficacy of the orally given two natural medicines: extract of *Ginkgo biloba* (EGB 761, 100 mg/kg) and dried, standardized confection of crude *Hypericum perforatum* (350 mg/kg), in the prevention of the post-stress memory dysfunctions.
Male Wistar rats (150-160 g) were stressed for 21 days by placing them for 2 h daily in a tight restraint tubes. There were separate sets of experiments testing effects of EGB 761 and *H. perforatum*. To define specific role of the rat stress hormone (cortisol in humans) we ran also groups injected daily with corticosterone (5 mg/kg subcutaneously).
Each experimental set consisted of 6 groups of animals: CONTROL, STRESS, corticosterone (CORT), EXTRACT (EGB 761 or *H. perforatum),* EXTRACT + STRESS, EXTRACT + CORT.
Both, STRESS and CORT groups displayed considerable and statistically significant deficits in memory: spatial [measured in the Morris Water Maze (MWM) ($p<0.05$) and the Barnes Maze (BM) ($p<0.05$)], visuo-spatial [measured in the Object Recognition (OR) ($p<0.01$) Test] and non-spatial associative [measured in an inhibitory avoidance (IA) ($p<0.01$) Test].
Both, EGB 761 and *H. perforatum* administered daily during the stress procedure or chronic corticosterone treatment totally abolished most of these deficits. Interestingly, in naïve rats EGB 761 improved memory of the positively (curiosity - OR) reinforced behaviour more than that of the negatively (water immersion – MWM, exposure – BM, electric current – IA) reinforced ones. The latter, however, were better remembered by the animals receiving *H. perforatum*. There were only minor differences between the beneficial actions of EGB 761 and *H. perforatum* on the deficits caused by stress- and corticosterone.
In conclusion, *G. biloba* and *H. perforatum* appear to contain agents able to effectively counteract increasingly important stress-induced cognitive deficits.

7A_03_P

ARE EXTREMES IN ANXIETY LINKED TO COGNITIVE ABILITIES?

[1]Bunck M., [2]Sartori SB, [1]Zurmühlen R., [1]Wotjak CT., [2]Singewald N. and [1]Landgraf R.

[1]Max Planck Institute of Psychiatry, Munich, Germany, [2]Department of Pharmacology and Toxicology, University of Innsbruck, Austria; Bunck@mpipsykl.mpg.de

Anxiety and learning/memory are hypothesized to be linked phenomena, as patients suffering from anxiety disorders and depression display changes in cognitive abilities. To mimic the clinical situation, wild type

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

CD1 mice (NAB) were selected and inbred for extremes in anxiety-related behaviour in the elevated plus maze test to generate (HAB) versus low anxiety-related behaviour (LAB) mice. To study the interplay between emotionality and cognition, HAB, NAB and LAB mice were subjected to various social and non-social learning tasks. The general ability to recognise a familiar stimulus animal was assessed by the social recognition test. As a more sophisticated measure of social memory, the social discrimination task (SD) was performed. Furthermore, we used a contextual and cued fear-conditioning (FC) paradigm to investigate extinction abilities. The results clearly indicate that all three lines show social recognition abilities with a similar decline in olfactory investigation of the same stimulus. Remarkably, in the SD only HAB mice succeeded in identifying a familiar animal even after 120 min interexposure interval, indicating that the SD can be used to reveal differences in cognitive abilities between the mouse lines. Interestingly, in both FC paradigms HAB mice displayed delayed extinction compared to both, NAB and LAB animals. The data suggest that hyper-anxiety typical of HABs is linked to enhanced social memory abilities and resistance to extinction. Further analyses of the interaction between emotionality and cognition will focus on anxiety- and cognition-related neuropeptide systems, the effects of antidepressants and extinction-facilitating drugs.

7A_04_P

UPREGULATION OF SYNAPTOTAGMIN AND TRKB IS INVOLVED IN CHRONIC EXERCISE-ENHANCED LEARNING AND MEMORY BEHAVIOR IN MICE

Hsiun-ing Chen[1]*, Yu-Fan Liu[2], and Chauying J. Jen[1]

*Department of Physiology[1] and Institute of Basic Medical Sciences[2], College of Medicine, National Cheng Kung University, Tainan 70101, Taiwan. *E-mail: hichen@mail.ncku.edu.tw*

Acute severe exercise elevates stress hormone levels and impairs cognitive function in rodents. In contrast, chronic exercise improves cognitive function without affecting resting stress hormone levels. It is known that synaptotagmin, a Ca^{2+}-dependent synaptic vesicle protein, brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin-related kinase B (TrkB) play important roles in hippocampus-dependent learning and memory behavior. However, whether chronic exercise can improve learning and memory by upregulating these molecules remains unraveled. To answer this question, male BALB/c mice were used as the animal model. After 4 weeks of treadmill exercise training, the ability of learning and memory was evaluated by one-trial passive avoidance test (PA), an aversive learning task. Hippocampal synaptotagmin and TrkB

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

protein expressions were determined by Western blotting, and BDNF was measured by ELISA. Our results showed that after chronic treadmill exercise, 1) the retention latency of PA was increased; 2) protein levels of hippocampal TrkB and synaptotagmin were elevated; 3) TrkB or synaptotagmin protein expression was positively correlated with PA performance. These data suggest that the upregulation of synaptotagmin and TrkB may contribute to the chronic exercise-facilitated hippocampus-dependent cognitive function.

7A_05_P

TOWARDS THE GENETIC DISSECTION OF ANXIETY

Czibere L, Bunck M, Frank E, Keßler MS, Kohli M, Bettecken T and Landgraf R

Max Planck Institute of Psychiatry, Kraepelinstraße 2, 80804 Munich, Germany
Landgraf@MPIPSYKL.MPG.DE

To investigate the genetic background of anxiety-related and depression-like behaviour in mice, two lines, selectively inbred for high ("HAB") and low anxiety-related behaviour ("LAB"), starting out from outbred CD1 mice. As mutations in the genome are assumed to make up for the majority of genetic differences, we plan to pinpoint them with a linkage analysis approach. For this, the 'Mouse Medium Density Linkage Panel' by Illumina, was used to genotype 1449 single nucleotide polymorphisms (SNPs) in HAB and LAB mice, as well as in F1 progeny (HABxLAB).
The animals tested were derived from generation 20 or higher, providing a solid basis for genetically homogenous lines.
In a first analysis, out of the 1449 loci tested, for 225 autosomal loci HAB and LAB mice both displayed homozygosity, but for different alleles. Cross-mated animals (F1, HABxLAB) were all heterozygous for these loci.
Compared to an inter-strain analysis approach using two standard inbred mouse strains (e.g. BALB/c and C57BL6), where animals do not only differ in their anxiety-related behaviour, our intra-strain analysis considerably reduces the number of informative SNPs. Therefore, in our mouse lines, the number of informative false positive SNPs, relevant to the behavioural phenotype, will be much lower.
Interestingly, about 1/3 of the opposite homozygous SNPs are concentrated on only 5 chromosomes, whereas two chromosomes contained just 3 and 6 of these SNPs.
Altogether, the Illumina Mouse Medium Density Linkage Panel seems to provide a sufficient number of informative SNPs as to allow the identification of a linkage region, causative for the development of the anxiety trait. We are planning to genotype a larger number of freely

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

segregating F2 generation animals (F1xF1). From this we expect being able to identify true candidate regions for anxiety-related behaviour.

7A_06_P

A REWARD-SEEKING MODEL FOR DISTINGUISHING ADAPTATION AND STRESS

Seamus Decker

Department of Anthropology, University of Massachusetts, 215 Machmer Hall, Amherst, MA 01003 USA
sdecker@anthro.umass.edu

Lack of a unifying concept for distinguishing adaptation from stress with research on psychoneuroendocrine (PNE) processes such as hypothalamic-pituitary-adrenal axis (HPA) response to psychosocial conditions has led to at least four distinct types of errors: (i) failure to distinguish adaptive PNE activation, i.e., "arousal," from maladaptive PNE activation, i.e., "stress;" (ii) failure to distinguish response from conditions that provoked the response, i.e., either stressors or a vulnerability of some sort; (iii) assumption that subjective distress and psychobiological activation are equivalent and interchangeable; and (iv) failure to distinguish differences in the causes and consequences of response which is episodic, ephemeral and moderated compared to response which is repetitious, prolonged, or exaggerated. Distinguishing adaptive from maladaptive response depends on a clear understanding of the adaptive purposes of PNE processes such as HPA response to psychosocial conditions, and attention to the context and consequences of any specific pattern of response. This paper presents a novel and unifying model in which episodic cortisol response to psychosocial factors is conceived as an adaptation to perceived loss of reward. Reward is conceived broadly as being the myriad of social or behavioral factors that may lead to specific psychobiological states of "reward" (e.g., increased activity in specific dopaminergic regions of the pre-frontal cortex). By testing this conceptual model, researchers stand to make substantial contributions to understanding of how the HPA works in everyday life, and effects on human health and well-being.

7A_07_P

RESPONSIVENESS OF THE OK-LIST: A NEW RATING SCALE FOR CLINICAL DIAGNOSIS AND TREATMENT EVALUATION OF PSYCHOLOGICAL SYMPTOMS OF CHRONIC STRESS

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

E Dorant MD PhD, AJM Schmidt PhD, GM van der Molen PhD

Maastricht University, Health Organization, Policy and Economics, PO Box 616, 6200 MD Maastricht, Netherlands; E.Dorant@beoz.unimaas.nl

Objective. A new rating scale for clinical diagnosis and treatment evaluation of complaints of chronic stress was developed in The Netherlands. This OK-list consists of 22 universally applicable complaint-like items, scorable on a 5-point Likert scale. Psychometric evaluation showed high validity, reliability and unidimensionality. In this study we evaluate the responsiveness of the OK-list in patients suffering from chronic stress syndrome, equivalent to the clinical end state of severe burnout.
Methods. A single group repeated measurements design was used. Intake and end-of-treatment information on level of chronic stress complaints was collected with the OK-list. Responsiveness was assessed using paired T-test, SRM and a graphical illustration presenting means for each chronic stress complaint. For parallel comparison SCL-90R data were used. Results were calculated for two subgroups defined by concurrent depression using data collected with the Beck Depression Inventory.
Results. Comparison with SCL-90R revealed parallel lowering of OK-scores between intake and end-of-treatment in both subgroups. All paired T-tests were statistically significant (p-values <0.001) and SRMs were close to 1.5, well above the cut-off score for high responsiveness. Differences between intake and end-of-treatment mean item scores were nearly all greater than 1 point on the Likert scale.
Conclusion. The OK-list proved to be highly responsive to change in patients treated for chronic stress syndrome and thus provides an excellent alternative to questionnaires on chronic stress complaints or burnout when evaluation of natural course or treatment effect is important.

7A_08_P

WORK STRESS AND COPING RESOURCES: THE STUDY OF THE MEDIATING ROLES OF CAUSAL ATTRIBUTIONS

Sayyed M Haybatollahi

University of Helsinki, Unioninkatu 37, PL 54, 00014, Helsinki, Finland.

The lecture of psychological stress contains a large number of studies, investigating the mediating and moderating effects of dispositional factors such as type A behavior pattern, Negative affectivity, and Locus of Control over the stress-strain, stress-coping and stress-live events relationships (Cooper et al, 2001). Attribution theory, in this respect, has widely been

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

utilized to study the mediating roles of such dispositional variables as there was a strong need to identify the individuals' pattern of reasoning for different aspects of live events. Hence, In order to investigate the mediating roles of causal attributions over the association between coping resources and work stressors as well as verify a number of path models of the relationships between the concerned variables, 564 fulltime stuffs, working at the Universities' administrative office in the Helsinki capital area, participated the study. In this study work stressors were comprised of five role conflicts i.e., Overload, Insufficiency, Ambiguity, Boundary, and responsibility. Whereas, coping resources consisted of Recreation, Self-Care, Social Support, and Cognitive Coping. Mediating variables were basically concerned with four dimensions of causal attributions (i.e., Locus of control, Stability, Globality, and Controllability) for negative and positive events. To measure occupational stress and personal resources, using Lazarus' transactional model of stress (Lazarus & Folkman; 1984), 72 items were derived from Occupational Stress Inventory Revised Edition (Osipow, 1998). The employees' attributional style was also measured by Occupational Attributional Style Questionnaire (Furnham, 2004). The primary analysis of the hypotheses indicated the existence of such mediation by causal attribution for personal resources and different levels of occupational stressors; as such the general path model was supported. However, the result of further analysis using hierarchical regression analysis and structural equation modeling identified more details of testing the hypotheses. For instance, Responsibility did not appear to be a significant predictor for the variances of personal resources. Hence, the results of this study were mostly in line with the results of the same studies in the field of causal attribution (Weiner, 1986).
NB. The analysis of the data hasn't been finished yet and the further results of testing the hypothesis will be ready for the oral presentation in the conference.

7A_09_P

THE EFFECTS OF SEX, AGE AND POPULATION ON BASELINE GLUCOCORTICOID LEVELS IN SPINY MICE (*ACOMYS CAHIRINUS*)

Nováková M.[1], Kutalová H.[1], Palme R.[2] Frynta D.[1] & Janský L.[3]

[1]Dept.of Zoology, Faculty of Science, Charles Univ., Prague, Czech Republic
[2]Institute of Biochemistry, Dept. of Natural Sciences, Univ. of Veterinary Medicine, Vienna, Austria
[3]Dept. of Animal Physiology, Faculty of Biological Sciences, Univ. of South Bohemia, České Budějovice, Czech Republic
Corresponding author: frynta@centrum.cz

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Baseline glucocortiocoid (GC) levels may be related to the social status in small animal species. Here we examined GC levels in a moderately social rodent the Egyptian spiny mouse (*Acomys cahirinus*). Studied animals originated from 2 populations, differing considerably in their ecology: the population from Abu Simbel (S Egypt) is a desert-dweller, while that from Cairo is commensal. To assess baseline GC levels we collected faecal samples from 68 individuals belonging to 10 social groups. Each social group consisted of animals of both sexes and two age grades (directly corresponding to dominance ranks). Levels of GC metabolites were assessed by a 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassays (EIA). The results showed no effect of rank and only a small effect of sex (higher levels in females) on GC metabolites levels. Thus, the hypothesis that dominant males are more stressed than other functional groups may be rejected. Surprisingly, there was a considerable difference among groups. This may be interpreted as a substantial effect of social relationships within each particular group. The commensal population exhibited much higher levels of GC metabolites than the desert one. This is consistent with behavioural differences of studied populations - commensals are more active, but simultaneously also more anxious. Evolutionary adaptation to living in buildings is suggested as the ultimate cause of these physiological and behavioural differences.

7A_10_P

EFFECTS OF STRESS APPLIED AT THE LATE STAGE OF PREGNANCY ON THE FEEDING BEHAVIOR IN THE RAT

Mari Kimoto[1*], Rie Amano[1], Reiko Ishikawa[1],Yuki Ooe[1], Mie Shimura[1],Tami Niinuma[1], Hiromi Hirakawa[1], Yasuhiro Kumei[2], Kazuo Toda[1,3]

Physiological Lab., Japan Women's University, Mejirodai, Bunkyo-ku, Tokyo 112-8681[1], Biochemistry, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo113-8549, Japan[2], Integrative Sensory Physiology, Nagasaki University, Nagasaki 852-8588, Japan[3].
**kimoto@fc.jwu.ac.jp*

The purpose of the present study is to examine how stress that is imposed at the fetal stage could influence the same Wistar rat at the adult stage. The pregnant rats were exposed to either gravitational stress (1.5 and 2.0G) or wire-net restraint during the 15th through 21st day of pregnancy for 10 min daily. The male offspring of the wire-net restraint mother rat showed significant decrease of slope in growth curve at the 15th through 66th day. The 1.5G gravitational stress on mother rats did not influence the body weight of the male offspring, while 2.0G gravitational stress on mother rats increased the slope of growth curve of 6 to 10 day-old male offspring rats. By contrast, 1.5G gravitational stress and wire-net restraint

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

did not influence the feeding behavior in the adult stage of the female offspring rats. However, 2.0G stress increased the slope of the growth curve. The somatostatin level in the blood was decreased in gravitational stress group in the male, but not in the female offspring rats. Data showed that the stress imposed at the fetal stage modified the growth pattern even at the adult stage and that the gender difference was observed in the anti-stress response. In addition, type of stress may be an important factor for producing emotional changes, such as, feeding behavior.

Supported by JSPS grants (No.18500630 and No.18613011, 2006-2007).

7A_11_P

THE ROLE OF CORTICOSTERONE IN EXERCISE-INDUCED HIPPOCAMPAL NEUROGENESIS

Yu-Min Kuo, Ya-Ting Chang, Yi-Chieh Chen, Chih-Wei Wu

Department of Cell Biology and Anatomy, Medical College, National Cheng Kung University, 1 Ta-Hsueh Road, Tainan, Taiwan.
kuoym@mail.ncku.edu.tw

Neurogenesis has been suggested to correlate with certain aspects of brain cognitive function. Several extrinsic stimuli, including physical exercise is known to have advantageous effects on neurogenesis and brain function. However, why exercise promotes neurogenesis remains unknown. Previously, stress-induced high level of corticosterone was shown to inhibit neurogenesis, while adrenalectomy enhance neurogenesis. As exercise modulates the level of serum corticosterone, we hypothesize that corticosterone signaling pathway is involved in the exercise-induced neurogenesis. In this study, hippocampal neurogenesis is estimated by double-labeling of mitotic marker (BrdU) and neuronal progenitor marker (DCX) in the subgranule zone. Our results showed that treadmill running exercise (TRE) significantly enhanced neurogenesis. The levels of serum corticosterone were elevated immediately and decreased 4 h after TRE. Five-week TRE down-regulated the protein levels of mineralocoticoid receptor (MR), while glucocorticoid receptor (GR) remained unaltered. To mimic the exercise-elicited corticosterone response, mice were injected with low dose of corticosterone (4mg/kg/day) for three weeks. Our results showed that mice received corticosterone injection had slightly more BrdU/DCX positive cells than those of vehicle controls with no changes in the MR and GR levels. Alternatively, mice received MR antagonist, spironolactone, treatment 90 min before TRE exhibited more neurogenesis than that of TRE. Spironolactone alone also expressed significant effect on neurogenesis. Taken together, our results suggest that reduced corticosterone signaling

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

pathway is involved in the TRE-induced hippocampal neurogenesis.

7A_12_P

ADOLESCENTS COPING WITH PERSONAL SECURITY STRESS: STRATEGIES AND ADJUSTMENT

Dr. Hanna Laufer

The Academic College of Emek Yezreel, Dept. of Human Services
Address correspondence to: Hanna Laufer, P.O. box 277, Timrat, 23840, ISRAEL (E-mail: hlaufer@research.haifa.ac.il)

Living in areas of intensive combat and conflict zones, may be accompanied by psychological stress among adolescents.
The present study makes an attempt to explore the influence of the combat zone geographical proximity on the stress assessment, and to detect and define the specific resulting responses of adolescents.
A comparison was done between adolescents living in communities along the seam-zone, and adolescents living in communities remote from Israel's borders, in the purpose to investigate whether a difference exists between the adaptation levels of adolescents exposed to military stress - to those who are not.
The test sample included 279 males and females, ages 14 to 18.
It was found that adolescents living in the seam-zone vicinity reported a primary stress evaluation level higher than that of adolescents from the comparison group. In spite of this finding - no significant differences on the secondary stress evaluation (control estimation level) was observed between the two groups. It was found that adolescents, who revealed the lowest adaptation level, were those who reported the highest level of uncontrollability.
Another finding indicates that stress situations, evaluated as uncontrollable, lead to an intense use of the secondary control engagement strategies (e.g. distraction, positive thinking, cognitive restructuring and acceptance) and the disengagement coping strategies (e.g. avoidance, denial, wishful thinking). It was also found that for both groups - coping strategies of the primary control types (problem solving; emotional regulation and emotional expression) predict a higher level of adaptation.
Based on these findings it was concluded that the stress type (controlled or uncontrolled) has an important effect on the prediction of selected coping strategies and adaptive results. Gender differences were observed: the males, living in the vicinity of the seam-zone, revealed the highest, while the females - the lowest level of adaptation.
It will be possible to utilize the present study findings in their adaptation to the treatment and the education policy for the more vulnerable part of

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

the population of adolescents: the females.

Keywords: psychological stress; adolescents; coping; adaptation

---

7A_13_P

EFFECTS OF A PSYCHOSOCIAL STRESSOR ON SELF-INJURIOUS BEHAVIOR AND HYPOTHALAMIC-PITUITARY-ADRENOCORTICAL FUNCTION IN RHESUS MONKEYS

J.S. Meyer, M.D. Davenport, C.K. Lutz, S. Tiefenbacher, and M.A. Novak

Univ. of Massachusetts, Amherst, MA 01003 USA; New England Primate Research Center, Southborough, MA 01772 USA; jmeyer@psych.umass.edu

Self-injurious behavior (SIB) is a disorder that occurs in both clinical and non-clinical populations. Although a role for stress in the etiology and/or maintenance of SIB has been proposed, there is little empirical evidence for this hypothesis. Our laboratory has been studying the pathophysiology of spontaneously occurring SIB (manifested as self-biting and occasional self-wounding) in singly housed male rhesus monkeys at the New England Primate Research Center. In the present study, we examined the short- and long-term effects of an administratively mandated relocation of our monkeys (both SIB and controls) on the animals' behavior and hypothalamic-pituitary-adrenocortical (HPA) function. The relocation involved movement of the subjects to different cages in a new colony room with unfamiliar animals. Daytime and nighttime behaviors were recorded, as well as time-dependent changes in salivary, serum, and hair cortisol concentrations, and serum corticosteroid binding globulin (CBG). Relocation stress induced long-lasting behavioral abnormalities in the SIB group, including an increase in self-biting and a disruption in sleep. Both groups exhibited rapid increases in salivary and serum cortisol following the move. Hair cortisol, which we have validated as an index of long-term HPA system activity (Davenport et al., 2006), was elevated over baseline at 4 months post-move but not at 1 year. In contrast, serum cortisol remained increased over baseline at 1 year, a point at which we also observed a compensatory rise in serum CBG. These results may have relevance for humans exposed to major life stressors. Supported by RR11122 and RR00168.

7A_14_P

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

ASSOCIATION OF STAGE OF STRESS MANAGEMENT BEHAVIOR WITH PERCEIVED STRESS AND COPING IN JAPANESE COLLEGE STUDENTS

MURAYAMA, Hiroyoshi[1)] , TANAKA , Yoshiyuki[2)] , ITO Sakurako[1)], YAJIMA, Junpei[3)] TSUADA, Akira[1)], TSUDA, Shigeko[4)]

[1)] *Kurume University, Kurume, Japan*
[2)] *Tokyo University of Social Welfare, Isezaki, Japan*
[3)] *Beppu University, Beppu, Japan*
[4)] *Fukuoka Jogakuin University, Fukuoka, Japan*
hiro-murayama@umin.net

Background Transtheoretical model (TTM) is a theory of behavior change that can be applied to single, multiple, and complex behavior targets (Prochaska & DiClemente, 1986). The TTM is best known for its applications to smoking, high-fat diet, and drinking. TTM assumes five stages of change, which are named as Pre-contemplation (PC), Contemplation (C), Preparation (P), Action (A), and Maintenance (M) stage. There are thus far few attempts that apply TTM to change in effective stress management behavior in Japan. To understand change in stress management behavior by TTM, this study is to examine association of stage of stress management behavior with perceived stress and coping in Japanese college students. **Method** Participants were 699 (243 male and 456 female) college students in Fukuoka. The mean ages were 19.7 and 19.3 years, respectively. The measures were 1) Stage of change algorithm, 2) a Japanese version of Rode Island Stress and Coping Inventory, and 3) a Japanese version of Stress Management Behaviors Inventory consisting of four subscales such as setting a limit, planning, reframing, and unhealthy behaviors. **Result and Discussion** Portions of students were 35 % in PC, 15 % in C, 16 % in P, 11 % in A, and 23 % in M stage, respectively. Degree of perceived stress declined as stage proceeds, whereas perceived frequency of effective coping increased across proceeding of stages. Subjects in A and M stage showed lower frequency of unhealthy behaviors, and higher planning and reframing behaviors. These results replicate previous findings using American sample that effective stress management behaviors are associated with lower levels of stress and higher levels of appropriate coping, and provided support for usefulness of applying TTM to stress management behavior in Japan.

7A_15_P

RELIABILITY AND VALIDITY OF LAYERED VOICE ANALYSIS TECHNOLOGY IN THE DETECTION OF MENTAL STRESS

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Nemoto K[1], Tachikawa H[1], Takao T[1], Sato H[1], Ashizawa Y[1], Endo G[1], Tanaka K[1], Ishii R[1], Ishii N[1], Hashimoto K[1], Iguchi T[1], Hada S[2], Hori M[3] and Asada T[3]

[1]Psycholosoft, 1-1-1 Tennodai, Tsukuba, Japan; [2]Alegria Co., Ltd., Tokyo, Japan; [3]Dept. of Psychiatry, Univ. of Tsukuba, Tsukuba, Japan. kiyotaka@nemotos.net

It is known that speech signal contains features which provide information about a human speaker. Although several technologies to detect stress using human voice are available, reports on the reliability and validity of these technologies are controversial. In this study, we investigated the reliability and validity of the Layered Voice Analysis (LVA) technology. Methods: One-hundred and six healthy subjects participated this study. First, stress was assessed by using Speilberger State-Trait Anxiety Inventory (STAI). Blood pressure (BP) was also measured. Then, subjects were randomly assigned to the anagram task group and control group. Before task begins, all of the subjects were asked to answer 10 questions vocally, and they were all recorded. After answering questions, task group underwent anagram task whereas control group just read aloud series of words. After the task, STAI-S and BP were measured again. Answers to each question were analyzed using LVA and 22 parameters were computed. The internal consistency was assessed for each parameter using answers before task. Two-sample t-test was performed to see if parameters change significantly due to anagram task. Results: Of 22 parameters, Cronbach's alpha of 18 parameters was more than 0.6. Two-sample t-test showed that 10 of 18 parameters along with STAI-S and systolic BP changed significantly during the anagram task. Conclusion: Most of the parameters LVA computed are reliable and the value of these parameters changed significantly under stressful conditions. LVA might be useful in the detection of mental stress.

7A_16_P

WIDOWHOOD INCREASES RISK FOR SUBSEQUENT DEMENTIA, ESPECIALLY FOR WOMEN: THE CACHE COUNTY STUDY

Norton MC [1], Tschanz JT [1], Østbye T [2], Corcoran C [1], Zandi PP [3], Breitner JCS [4], Welsh-Bohmer KA [2]

[1] Utah State University, [2] Duke University, [3] The Johns Hopkins University, [4] University of Washington

A host of life stressors increase risk for depression in late life, most notably widowhood. Although depression, in turn, increases risk for dementia, few studies have examined the direct association between late-

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

life stressors and subsequent dementia onset. Even more rare are large, population-based longitudinal studies addressing this research question, with their ability to minimize the selection bias inherent in clinic based samples. In Cache County, Utah (USA) elderly (aged 65+) residents were assessed for dementia (90% of entire county participated) with a multistage case ascertainment protocol in 1995-6 (baseline) and those without dementia at baseline were again assessed in 1998-9 and 2002-3. The longitudinal sample with at least two evaluations included 3,117 persons and of these, 2,231 were married, 789 were widowed, and 97 were separated or divorced at baseline. Cox proportional hazards regression was employed to model effect of marital status, on dementia-free survival (years) before and after adjustment for gender, age, and presence of APOE e4 allele. Before adjustment for covariates, widowhood (at baseline) was associated with significantly higher incident dementia compared to being married (HR=2.37, 95%CI: 1.96-2.87). A trend for the marital status*gender interaction was observed (p=.140) after covariate adjustments. Compared to being married, widowhood was associated with increased hazard for dementia in women only (HR=1.77, 95%CI: 1.00-3.12). Future studies will examine factors that may mitigate increased risk, and the effects of more recent widowhood and multiple marital transitions.

7A_17_P

STRESS AND MEMORY INTERACTION : DECLARATIVE MEMORY IMPAIRMENT FOLLOWING A 5-DAY MILITARY COMMANDO OPERATION.

C. Piérard[1], M. Pérès[1], J.C. Jouanin[1], P. Liscia[1], C.Y. Guézennec and D. Béracochéa[2]

1 : IMASSA - BP 73 - F-91223 Brétigny-sur-Orge Cedex
2 : LNC - Université Bordeaux 1 - F-33405 Talence Cedex

A commando operation is composed of multiple routines and skills that require alertness, attention to external environment, memory performance and reactivity to events. As the declarative memory system is very sensitive to environmental interactions and stress-generating situations, we decided to specifically study the effects of a 5-day commando operation on this system.

Cognitive and memory performance of 21 male cadets was examined 3 weeks before and at the end of the operation, using an original computerized cognitive test battery allowing field investigation. In a first step, the battery evaluated psychomotor and cognitive performance with : a subjective vigilance and mood testing, a visuomotor coordination test, a selective attention test, and finally a planning test. In a second step, more

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

specific memory tests were administered to investigate short-term components on the one hand, and long-term components on the other hand. For each item, the battery measured the reaction time and calculated the percentages of good and wrong responses, as well as the percentage of omissions.
Our results evidenced that a 5-day commando operation significantly increased reaction times. In accordance with the relative but significant hypovigilance of the subjects, selective attention and short-term memory were affected (memory span, visual memory and audiovisual association). More surprisingly, long-term memory (semantic memory) was also impaired. On the contrary, spatial working memory and planning tasks were spared.
Finally, this field study demonstrates that the stress induced by a prolonged commando operation is able to selectively impair several components of declarative memory.

7A_18_P

LEARN TO SURVIVE CHRONIC STRESS! LONG-TERM BENEFITS OF A STRUCTURED BEHAVIOURAL INTERVENTION PROGRAM

Adrienne Stauder*, Piroska Balog*, Tamás Martos*, Mónika Kovács*, Virginia Williams**, Redford Williams***

** Semmelweis University, Institute of Behavioural Sciences, 1089 Budapest, Nagyvárad tér 4, staadr@net.sote.hu; **Williams LifeSkills Inc, Durham, NC, USA, ***Psychiatry and Behavioural Sciences, Duke University Medical Center, Durham, NC, USA*

We studied the effectiveness of a 16-hour structured stress management training, the Hungarian version of the Williams LifeSkills program. Since November 2004 more than 400 persons of diverse background were trained. We monitored short-term and long-term outcomes in a sample of 24 people with high distress (mean age 34,4 ys, sd=10,35). Data were collected before (t1), right after (t2) and 6 month after (t3) the intervention. We used standardized questionnaires: Cohen Perceived Stress scale (PSS), Spielberger Trait Anxiety Inventory (STAIT), shortened Beck Depression Inventory (BDI), Patients Health Questionnaires (PHQ15), 5-item WHO Well-being Index (WWB), Rahe Life Meaning (MEAN) scale, and 1-item on "satisfaction with life" (SAT). Statistics: paired sample T test, levels of significance are * $p<0.05$ ** $p<0.01$. After the intervention perceived stress decreased and remained at normal level at 6 month follow-up (PSS1=31.8, PSS2=22.7**, PSS3=24.3**).* Stress related symptoms also decreased (BDI1=19.1, BDI2=9.6**, BDI3=11.1**; STAIT1=54.8, STAIT2=42.6**, STAIT3=46.2**; PHQ1=8.5, PHQ2=7.1, PHQ3=5.6**). Indices of well-being improved

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

(WWB1=6.0, WWB2=8.6**, WWB3=7.9**; MEAN1=9.6 MEAN2=12.1**, MEAN3=11.3**; SAT1=5.4, SAT2=7.3**, SAT3=7.0*). Our results confirm that stress management skills can be learned via this short behavioural intervention, high initial perceived stress and related psychological and somatic symptoms decreased, well-being increased, and these favourable changes were maintained at 6 month follow-up.

7A_19_P

STRESS COPING STRATEGIES IN A 3D ESCAPE MAZE REVEAL TWO DISTINCT TYPES OF SOCIAL FEAR LEARNING

Cliff H Summers, Russ E Carpenter

Biology, Neuroscience Group, University of South Dakota, Vermillion, SD 57069 USA cliff@usd.edu

Rainbow trout interact aggressively, and form distinct social hierarchies. We hypothesized that social interaction against a larger opponent provides the impetus and information necessary to stimulate goal-oriented learning in subordinate individuals. A small (~100 g) juvenile rainbow trout in one tank compartment was adjacent to a large fish (~300 g; US) in another. A small (5 cm) hole leading to an empty compartment, large enough only for the smaller fish to pass was only available when the larger fish was present. Once a day the water inflow was turned off (CS); 15s later dividers were removed and fish interacted for 15 min. Larger fish were aggressive, and the learning curve for subordinate fish was dramatic (600%) over seven days. Escape time improved daily until fish escaped in approximately one minute. Plasma was taken 3 days before and 1 day after social interaction and learning trials. For samples taken after the trials, fish were presented with inflow water off but no large fish challenge. Fish learning to escape showed no change in plasma cortisol. However, fish that did not learn to escape exhibited a four-fold increase in cortisol, although no large fish was presented as a social challenge. Turning off inflow water acts as a conditioned stimulus to provoke increased cortisol, and elevated dopamine and serotonin in amygdala, hypothalamus and raphé. Increased anxiety induced by CRF stimulates increased attacks, but also increased retreat and escape behavior. Coping strategies appear to be influenced by CRF_1 receptor activity. Fear conditioning in non-escaping fish is manifest by increased neural and hormonal stress responsiveness, whereas those that learn to escape utilize spatial learning to cope with social stress.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7A_20_P

CIRCULAR CAUSATION BETWEEN MAJOR LIFE EVENTS, DEPRESSION, AND WELLBEING

Gabor Szabo, Zsuzsa Szanto, Maria Kopp

Institute of Behavioural Sciences, Semmelweis University, H-1089 Budapest Nagyvarad ter 4, Hungary, szabgab@net.sote.hu

Object: Study of the circular causation between Rahe-Holmes Major Life Events (MLE), depression and wellbeing using data from a Hungarian follow-up survey Hungarostudy Epidemiological Panel (HEP2006). Sample and methods: From participants of a nationwide questionnaire survey in 2002, 4528 adult persons participated in the second wave of the survey in 2005. Data were collected about the MLE experienced during the periods preceding the first and the second waves, and the shortened WHO Wellbeing Score (WS) and Beck Depression Inventory (BDI). The follow-up enabled us to examine the circular cause-and-effect relationships between MLE and psychological states. Results: The relationships between MLE stress scores and the number of stressful MLE were uncertain because they did hardly or not correlated with the BDI and the WS. However, including only the number of the negative MLE we found strong relationships. (1) In cross-sectional terms, in both waves we found strong relationships between the number of negative MLE, BDI (0,23***; 0,17***) and WS (−0,15***,−0,14***). (2) The number of negative MLE, BDI and WS showed stability between waves (0,15***; 0,27***; 0,26***). (3) The cross-correlations showed that previous negative MLE were strongly related to later BDI and WS (0,17***, −0,12***) while earlier BDI and WS showed a weaker but significant relationship with later negative MLE (0,09***, −0,07***). Discussion: Negative MLE result in permanent stress while the effects of positive MLE are uncertain. Although the psychological status has some effect on MLE much more robust effects can be found in the opposite direction: negative MLE result in the increase of BDI and decrease of WS.

7A_21_P

SUBJECTIVE WELL-BEING AFFECTION TOWARD DEVELOPMENT OF STRESS-MANAGEMENT BEHAVIORS

Tanaka Yoshiyuki[1], Tsuda Akira[2], Egami Yuko[3], Jingu Sumie[3]

[1]*Tokyo University of Social Welfare, Isesaki, Japan*
[2]*Kurume University, Kurume, Japan*
[3]*Fukuoka Health Promotion Foundation, Fukuoka, Japan*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

ytanaka0519@yahoo.co.jp

The present study describes how development of stress-management behaviors (SMBs) was different depend on the level of individual subjective well-being. The subjects were 282 participants (56 male and 226 female) in the 3 months health promotion program. Before and after the program, subjects were asked whether they had each of 8 SMBs. Subjective well-being was assessed at the pre-program by the revised version of Psychological Lively Scale (PLS-R) which consists of 4 subscales: life satisfaction, negative mood, vitality to challenge, and emotional stability. In the result, the development depended on the level of PLS-R scores. Most of the subjects with high pre-life satisfaction could have passed off their weariness easily, got something to change their bad mood or daily stress, and had some pleasure after the program; even though, low pre-life satisfaction subjects could not. Same relation was found between the level of vitality to challenge and their weariness, emotional stability and their weariness, emotional stability and their good sleep. The low pre-negative mood, conversely, related to the change of their good sleep, some pleasure, and nice way to pass of weariness. The augmentation of SMB number was also related to the PLS-R score. This result was also related to the pre-number of SMBs. All subjects having few pre-SMBs multiplied the number, though subjects with high pre-PLS-R changed much more (4.3 to 6.2). While the number of SMBs with low pre-PLS-R in many pre-SMBs was not significantly changed, high pre-PLS-R subjects developed their SMBs even their pre-SMBs were many. These results revealed that high subjective well-being accelerate the development of SMBs.

7A_22_P

IS AN INCREASE IN STRESS TOLERANCE CAPACITY CONDUCIVE TO LESSENING PSYCHOSOCIAL STRESS IN AN APPARENTLY HEALTHY POPULATION?

Kazuhiko Yamamoto[1], Yoko Sakamoto[1], Masahiro Irie[1], Susumu Ohmori[2], Mototaka Yoshinari[2]

1. Institute of Health Science, Kyushu University, Fukuoka, 815-8540 Japan, 2. Kyushu Central Hospital, Fukuoka, 815-8588 Japan

The aim of this study was to examine the associations between stress tolerance capacity measured using IMST (Inventory to measure stress tolerance capacity with 20 items) and a stress score measured using IMPS (Inventory to measure psychosocial stress with 40 items) among apparently healthy adults.[1)] A total of 684 male (47.1 ± 7.9 years) and 517 female public school workers (47.5 ± 7.0 years) responded to

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

questionnaires which assessed the degree of stress response (i.e., the stress score) and the degree of stress tolerance capacity (i.e., the stress intolerance score). [2)] The higher the stress intolerance score was, the higher the stress score was for both men and women. Stress scores of men who answered yes to 17 items on the IMST and of women who answered yes to 14 items were lower than those of men and women who answered no to the same items on this test. The higher the stress intolerance scores of men and women were, the higher were the proportions of men who answered yes to 38 items and women who answered yes to 39 items on the IMPS. A stepwise regression model yielded 8 items on the IMST which influenced the stress score for men and 4 items which influenced it for women. An inverse association between the stress score and physical exercise was found to be significant in men, while this association was not found in women. The results suggest that efforts to increase the stress tolerance capacity, such as having appropriate social support, a healthy lifestyle, and a positive attitude toward life, may be conducive to lessening psychosocial stress among an otherwise healthy population. They also imply that a stress management program needs to treat men and women differentially. References: 1) Yamamoto K (2005) Development of the inventories to measure psychosocial stress and stress tolerance. Jpn J Physiol Anthropol 10: 67-77 [*in Japanese*], 2) Yamamoto, K. el al. (2007) The Relationship between IMPS-measured stress score and biomedical parameters regarding health status among public school workers. J Physiol Anthropol (in press).

7A_23_P

DETERMINANTS OF ALCOHOL USE AMONG UNIVERSITY STUDENTS: THE ROLE OF STRESS, COPING AND EXPECTANCIES

Cecilia Chau

Katholieke Universiteit Leuven, Belgium
Promotor: Prof. Dr. Stephan Van den Broucke

The purpose of this investigation were two: (1) to obtain a description of alcohol consumption patterns among Peruvian university students in general, and of subgroups defined by gender and socioeconomic status (SES); and (2) to investigate the determinants of non-problematic and problematic alcohol use among Peruvian university students, taking into account both characteristics of the social context and the psychosocial variables which mediate their influence.
We consider as predictor variables: Socioeconomic status, gender, parents' use of alcohol and best male and female friends. The mediating variables were: perceived stress, coping styles and alcohol expectancies. These two sets of variables were related to use of alcohol during the last

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

six months. Questionnaires were administered to the participants from three universities (*N* = 1081): one public university and two private universities.
The main findings indicate that most university students in Peru consume alcoholic beverages. The prevalence of alcohol use appears to be lower for students who attend a public university, whereas almost all students from the private universities drink alcohol. Students present a low frequency of drinking during weekdays, but consumption increases during the weekends, beer being the preferred beverage. Consumption of alcohol is also related to tobacco and marihuana. Significant differences in alcohol consumption patterns were found for gender and SES. Parental drinking was found to have a significant influence on their children's alcohol use in general. More best male friends than best female friends drink alcohol.
To discriminate between drinkers and non-drinkers, the most significant variables were: alcohol consumption of best female or male friends, positive expectancies in personal/social aspects of drinking, SES, negative expectancies in personal/social aspects of drinking, and gender.
The results of a discriminant analysis between risky and non-risky drinkers indicate that positive alcohol expectancies in personal/ social aspects play a major role, along with gender, female and best female friends' alcohol use, and avoidant coping styles.

7B_01_P

DEPRESSION IN RELATION TO DIFFERENT SOURCES OF STRESS.

Piroska Balog, Szilvia Adam, Tamas Martos, Anna Susanszky, Adrienn Stauder, Krisztina Neculai, Maria S. Kopp

Semmelweis University, Institute of Behavioral Sciences, 1089, Budapest, Nagyvarad ter 4., balopir@net.sote.hu

The aim of this study was to investigate the role of different sources of stress (work-, marital stress, and the role of life goals for both sexes, and gender-role stress for men, and work-family conflict for women) in connection with depressive symptoms. The study is based on Hungarostudy Epidemiological Panel 2006 (N 4528). We analyzed a subsample of N= 1679, aged 18-65, actively working and living with partner (men 47.4% women 52.6%. Among them 17% men and 20% women reported elevated depression score (Shortened Beck Depression Scale). To measure sources of stress we used shortened versions of: Marital Stress Scale, Effort – Reward Imbalance Questionnaire, Aspiration Index, Masculine-Gender Role Stress Scale, and one question for work-family conflict. Hierarchical logistic regression analyses were performed to study the effect of different sources of stress on depression, and we calculated odds ratios (OR) with 95% confidence intervals. Age and

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

education were also included in the model. For women age (OR 1, 04 (1,02-1,06), education (OR 0,86 (0,75-0,98), marital stress (OR 1,97 (1,24-3,12), work stress (OR 2,40 (1,66-3,45), and work-family conflict (OR 1,27 (1,05-1,55) were all related to elevated depression scores at step 2. At step 3 when extrinsic life goals were included in the model, age, marital stress, work stress and stress from work-family conflict were still significantly related to depression, but not the education. We found extrinsic life goals as possible mediator between education and depression (OR 2,14 (1,44-3,18). In men extrinsic life goals have no effect on depression. Neither age nor education was related to depression. We found in men only marital stress (OR 1,91 (1,05-3,48) and work stress (OR 2,45 (1,60-3,73) significantly related to depression.

7B_02_P

LOW-ENERGY STRESS-RELATED DISORDERS. DIAGNOSTIC UTILITY OF BIO-PSYCHOSOCIAL MARKERS.

Bengt B. Arnetz[1,2] , Ulla Maria Anderberg[2], Ingrid Anderzen[2], and Christina Halford[2]

[1]Division of Occupational and Environmental Health, Department of Family Medicine and Public Health Sciences, Wayne State University, Detroit, Michigan 48009, USA. barnetz@med.wayne.edu
[2] Division of Social Medicine, Department of Public Health Sciences, Uppsala University, Uppsala Sciences Park, 751 05 Uppsala, Sweden

It is commonly difficult to differentiate low energy states due to stress-related disorders from depression.[1] The aim of the present study was to evaluate possible differences in symptoms, psychiatric co-diagnosis, and biological stress markers in patients suffering from stress-related disorders versus depression. 150 patients that had been referred by primary care physicians to an academic stress medicine center were evaluated by a physician using structured psychiatric tools, the center's validated stress-assessment visual analogue scales, biological stress markers, blood pressure, and Body Mass Index. Patients with depression, >20 points on MADRS, scored significantly lower on the global energy scale, self rated health, and quality of sleep as compared to patients suffering from stress-related disorders. Depressed patients scored higher on the global stress scale. Depressed patients rated themselves to be less rested following a night's sleep. Depressed patients scored lower with regard to satisfaction with their family and job situations. It is suggested that studies involving either stress-related disorders or depression more carefully screen the participants with regard to biopsychosocial characteristics. A substantial number of patients being referred for stress-related disorders also suffer from psychiatric co-morbidity.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[1] Arnetz, BB, Ekman R. (Eds). Stress in health and disease. Wiley-VCH. Weinheim, Germany. ISBN-13 978-3-527-31221-4.

7B_03_P

DAILY WORRY PREDICTS HOSPITALIZATION AFTER CORONARY ARTERY AND VALVE SURGERY

Piroska Balog, Zsuzsa Cserep, Aniko Malik, Eszter Losoncz, Andrea Szekely

Semmelweis University, Institute of Behavioral Sciences and Gottsegen Gyorgy Hungarian Institute of Cardiology, balopir@net.oste.hu

The relationships between psychosocial factors and future cardiac events have been investigated mainly in population-based studies. Few data are available about the long-term effects of non-clinical variables as worry on the outcome after cardiac surgery. 180 patients who underwent cardiac surgery using cardiopulmonary bypass were prospectively studied and followed up for 4 years. Age, education, living status, worry (4 items from Spielberger State-Trait Anxiety Inventory), depression (Beck Depression Inventory), perioperative characteristics and clinical risk factors (EUROscore, postoperative congestive heart failure, duration at ICU and hospital stay) were also assessed. Psychological self-report questionnaires were completed preoperatively and 6, 12, 24, 36, 48 month after discharge. Clinical end-points were cardiac events requiring hospitalization during follow-up. Hierarchical logistic regression analyses were performed to study the effect of daily worry on hospitalization, and we calculated odds ratios (OR) with 95% confidence intervals. In the last step were all analyzed variables were included in the model, postoperative congestive heart failure (OR 4,77 (1,27-17,93)), EUROscore (OR 1,84 (0,71-1,004)) and the mean of daily worries measured at different time points (OR 1,40 (1,03-3,18)) were significantly related to hospitalization during follow-up. Our results support the notion that daily stressors as worry would predict hospitalization after coronary artery and valve surgery.

7B_04_P

GENETIC VARIATION IN THE HUMAN NORADRENALINE TRANSPORTER GENE

Richard Bayles, Elisabeth Lambert, Josefin Forstrom, Katherine Shields*, Amanda Eddy*, Jeremy Jowett*, John Blangero**, Tye Dawood and Gavin

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Lambert

*Baker Heart Research Institute & *International Diabetes Institute, Melbourne, Australia. **Southwest Foundation for Biomedical Research, San Antonio, TX, USA*
Corresponding author: richard.bayles@baker.edu.au

The functional integrity of the noradrenaline transporter (NET) is essential for the inactivation of neuronally released noradrenaline. A number of clinical conditions have been identified in which there is phenotypic evidence of a cardiac defect in NET function, as determined by the extraction of tritiated noradrenaline across the heart. An abnormality in neuronal noradrenaline reuptake could sensitise the heart to sympathetic activation. Patients with panic disorder, major depressive disorder, postural orthostatic tachycardia syndrome and essential hypertension were identified as having phenotypic evidence of a defect in function of NET. The mechanism of reduced NET activity in these patients remains unknown but it might have a genetic origin. The aim of this project is to investigate the possible genetic mechanisms responsible for impaired NET function. In the present study, 97 publicly available single nucleotide polymorphisms (SNPs) were selected from NCBI's dbSNP database within the NET gene. SNPs were selected based on their probability of having a functional role. These were genotyped in 210 unrelated individuals and variation assessed for their influence on several phenotypic measures related to noradrenergic metabolism. Of the 97 SNPs selected, 37 were found to be polymorphic in our sample group. Based on preliminary robust Bayesian quantitative trait nucleotide analysis, several SNPs show consistent evidence for association with clinical diagnosis and catecholamine levels.

7B_05_P

PSYCHOSOCIAL STRESS IN AN OUTPATIENT REHABILITATION CLINIC

Benkö, E., Kovacs, C., Novy, B., Kargl, D., Beier,G., Kowatsch, S., Kurz, RW

Zentrum für Ambulante Rehabilitation der Pensionsversicherungsanstalt
Ärztlicher Leiter: Prim. Univ.-Doz. Dr. Robert Kurz
Wehlistr. 127, 1027 Vienna
e-mail: edith.benkoe@pva.sozvers.at

Psychosocial stress plays an important role in the development of physical ailments. Significant gender effects have been shown to exist before the manifestation of these ailments. The following study seeks to explore the

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

type and extent of acute and chronic stress among male and female patients with chronic illnesses in outpatient rehabilitation clinics. It also provides normative data for the measurement of acute stress using the analog scale "stress barometer " and the Trierer Inventory for the Assessment of Chronic Stress.
Method: Cross-sectional study using a self-administered questionnaire
Instruments: Trier Inventory for the Assessment of Chronic Stress (TICS), an analog scale "stress barometer" as a measure of acute stress
Statistics: Multivariate analysis of variance
Participants: 353 patients of an outpatient rehabilitation clinic (ZAR: Zentrum für Ambulante Rehabilitation) in Vienna, Austria with cardiovascular diseases and orthopedic illnesses. 58% of participants were male, 31% were currently employed. The mean age was 59.4 years.
Results: 654 questionnaires were distributed, 509 returned, 353 complete data sets (response rate 77 %). After correcting for the influence of age, multivariate analyses showed significant differences between men and women ($p < .001$), as well as between employed and retired patients ($p < .001$). In terms of chronic stress, female patients reported being more overworked ($p < .01$), experiencing more social stress ($p < .05$), feeling a stronger sense of insufficient support ($p < .05$) and having more chronic anxiety ($p < .001$). They also showed higher overall chronic stress screening scores than male patients ($p < .01$). Employed patients reported higher levels of acute stress than retired patients ($p < .001$). In terms of chronic stress, they also had higher overall screening scores ($p < .001$). They reported being more overworked ($p < .001$) and experiencing more social stress ($p < .001$), more intense pressure to succeed ($p < .001$) as well as a stronger sense of insufficient support ($p < .05$). . While working men reported the highest levels of stress from pressure for success, working women were most burdened by social stress. For both sexes, chronic anxiety was the second most important source of stress.
Discussion: Psychosocial stress can be found not only before the manifestation of illness, but also during rehabilitation. There are significant differences depending on gender and employment. Psychological interventions aimed at helping patients cope with stress should take these differences into account.

7B_06_P

FUNCTIONAL DECLINE: PHYSIOLOGICAL AND PSYCHOSOCIAL STRESS IN OLDER HOSPITALIZED PATIENTS

Cheryl Chia-Hui Chen, Guan-Hua Huang, & Charlotte Wang

National Taiwan University School of Nursing; National Chiao Tung University Institute of Statistics; National Taiwan University, Institute of Epidemiology, Taipei, Taiwan. cheryl.chen@ha.mc.ntu.edu.tw

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Hospitalization remains a stress event for older people. Functional decline is a leading complication of hospitalization for older patients. Such decline is very stressful both in economic and human terms. The purpose of this study was to describe and identify patterns of functional decline during and 6-month post hospitalization and to ascertain discrete indicators that signal different patterns of functional trajectories, using latent class analysis.
A perspective cohort study was conducted on 296 older hospitalized patients aged 65 years and older who consecutively admitted to five surgical and medical units at a tertiary medical center in northern Taiwan. Participants were assessed during their hospitalization (48 hours within admission and before discharge) and 3 and 6 months post discharge. Demographics, medication taken, co-morbidities, cognitive status, oral health, nutritional status, presence of social support and depressive symptoms, admission diagnosis, and length of stay were assessed in order to test their relationship with patterns of functional trajectories.
Functional decline was common with the worse point occurred at discharge. Six months post discharge, more than half of subjects (n=149, 50.3%) never returned to their admission functional status. Three patterns of functional trajectories were identified and age, gender, number of co-morbidities, cognitive status, nutritional status, and oral health status at admission were statistically associated with patterns of decline.
Visualizing three different patterns of functional trajectories and studying indicators that signal such differences will help care providers understand how function changed and possible ways to mitigate physiological and psychosocial stress in older hospitalized patients.

7B_07_P

ACTIVATION OF THE HPA AXIS FOLLOWING SSRI ADMINISTRATION IN PATIENTS WITH MDD

T Dawood, D Barton, E Lambert, A Tilbrook*, J Loose*, M Esler, D Haikerwal, D Kaye, G Lambert

*Baker Heart Research Institute and *Monash University, Melbourne, Australia* tye.dawood@baker.edu.au

Major depressive disorder (MDD) has been linked with hypothalamic-pituitary-adrenal (HPA) axis activation. However, the interactions between the sympathoadrenal system and HPA axis in MDD are equivocal. Further, the actions of selective serotonin reuptake inhibitors (SSRIs) on these systems are yet to be identified. Nineteen patients with MDD, but otherwise healthy, and 18 healthy controls were recruited from the community for plasma cortisol, ACTH and adrenaline measurements.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Following 12 weeks of SSRI treatment, levels of these hormones were again measured in MDD patients. Compared with control subjects, patients with MDD had considerably higher state (32±2 vs 58±2, p=0.001) and trait (33±2 vs 63±1, p=0.001) anxiety scores. Indicative of HPA axis activation, plasma cortisol concentrations were significantly increased in untreated MDD [53(37-68) in controls and 69(64-92) in MDD, p=0.01, median (25-75 percentile)]. Following treatment, state and trait anxiety levels were reduced in MDD patients (p=0.001). Consistent with the decrease in anxiety scores, arterial adrenaline concentrations decreased in MDD (49±6pg/mL to 36±5pg/mL, p=0.05). Surprisingly, and contrary to adrenaline concentrations, cortisol concentrations increased following therapy (to 103±60ng/mL, p=0.075). Plasma ACTH levels also increased slightly after treatment. No correlations between HPA axis activity, symapthoadrenal activity and degree of depression or anxiety were observed, except for a significant relationship between plasma cortisol and ACTH following SSRI administration (r=0.7, p<0.01). These results indicate that the HPA axis and sympathoadrenal system are dissociated in untreated and SSRI-treated MDD.

7B_08_P

ASSESSMENT OF PERCEIVED STRESS: VALIDITY AND PROGNOSTIC PERFORMANCE OF THE ORIGINAL AND A SHORTER VERSION OF THE PERCEIVED STRESS QUESTIONNAIRE (PSQ)

Herbert Fliege, Ricarda Joachim, Eva Peters, Petra Arck, Susan Levenstein*, & Burghard F. Klapp

Dpt. of Psychosomatic Medicine, Charité Universitätsmedizin Berlin, Luisenstr. 13 A, D-10117 Berlin, Germany, herbert.fliege@charite.de
** Aventino Medical Group, Via della Fonte di Fauno, 22, 00153 Rome, Italy*

The Perceived Stress Questionnaire (PSQ) by Levenstein et al. (1993) assesses subjectively experienced stress independently of a specific occasion. It has been translated from English into many other languages, with validated adaptations in Italian, Spanish, Portuguese, and Swedish, and translations in Thai and Korean. In our own studies we translated, adapted and validated it in German.
The paper presents data from several studies that give evidence of the psychometric qualities in different groups. A special emphasis is on how well the slightly shortened 20-item version (PSQ-20) performs with respect to stress and somatic outcomes as compared to the overall 30-item version (PSQ-30).
Samples comprise patients with asthma, atopic dermatitis, tinnitus, inflammatory bowel diseases, somatoform, affective or eating disorders, women during pregnancy, after spontaneous abortion or after regular

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

delivery, healthy adults, and students, with over 3000 participants. Comparisons between the 20-item and the original 30-item versions include a re-analysis of the original ulcerative colitis sample's prospective data.
A structure of 4 factors ("worries", "tension" and "joy" (reversed) as indicating stress reaction, and "demands" indicating perceived stressors) proved fairly stable across different groups. Psychometric properties were good. Associations between stress, disease symptoms, physiological functioning (e.g. lung function in asthmatics) and immunological parameters are reported. A comparison between the 30-item and the 20-item versions shows that the prognostic performance concerning an exacerbation of ulcerative colitis over a period of up to 68 months is maintained despite item reduction.
We propose the PSQ as a valid and economic tool for research on perceived stress. The overall score of the revised PSQ-20 permits comparison with results from studies with the PSQ-30.

7B_09_P

DIFFERENTIAL EFFECTS OF FLUOXETINE ON ENERGY BALANCE AND CORTICOSTERONE RESPONSES TO REPEATED RESTRAINT STRESS IN 40D- AND 60D-OLD MALE RATS

Sheila Pinto[2] and Francisca Gómez[1,2]

[1]Pharmacology Department, Faculty of Pharmacy and [2]Brain Mapping Unit, Pluridiciplinary Institute, Complutense University of Madrid, Paseo Juan XXIII, 1, 28040 Madrid, Spain
Corresponding author: Francisca Gómez
e-mail: gomezof@farm.ucm.es

Fluoxetine (FLX) is widely used to treat depression in adolescent and adult depressive patients. FLX affects activity of the hypothalamic-pituitary-adrenal (HPA) axis in rats but little is known about whether age-dependent differences may exist regarding the effects of FLX on HPA axis activity, as well as the possible interactions with the stress response. We investigated the pubertal (40d) and adult (60d) rodent energy balance and corticosterone (B) responses to repeated restraint stress [(3h/day) x 3days], after daily injection of either vehicle (VEH) or FLX (10mg/kg; 1ml/kg, ip). We show that independently of age, the effect of FLX on food intake and body weight of unrestrained rats was comparable to the effect of restraint in VEH-treated rats. Combination of FLX and restraint resulted in additive-like effects. However, 40d rats maintained positive ponderal growth whereas 60d rats lost weight. Acutely, FLX blocked restraint-induced acute weight loss during the 3h of daily restraint only in 60d-old rats suggesting that FLX reduced energy expenditure. It is likely that this

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

effect may alter compensatory mechanisms involved in energy balance regulation. FLX increased plasma corticosterone (B) levels, measured 1h after injection, similarly in 40d- and 60d-old rats. However, FLX prolonged the acute and repeated restraint-induced increases in plasma B levels only in 60d-old rats. Nevertheless, the B response adapted with repetition. We conclude that acute and repeated FLX treatment in unrestrained rats has stress-like effects on energy balance and HPA axis activity. Moreover, acute and repeated FLX treatment alters energy balance and HPA axis responses to acute and repeated restraint in an age-dependent manner. Thus, 40d-old male rats are able to maintain positive energy balance and normal B response to restraint whereas 60d-old male rats lose weight and show prolonged B responses to repeated restraint.

7B_10_P

DIFFERENTIAL ELICITATION OF THE SALIVA LEVEL OF 3-METHOXY-4-HYDROXYPHENYLGLYCOL (MHPG) , A METABOLITE OF NORADRENALINE, INDUCED BY MENTAL STRESS TESTING

Satoshi Horiuchi, Akira Tsuda, Hisayoshi Okamura, Jumpei Yajima

Graduate School of Psychology, Kurume University
Address: 1635, Mii-machi, Kurume-shi, Fukuoka-ken, 839-8502, Japan
E-mail: satosato.007@nifty.com

3-methoxy-4-hydroxyphenylglycol (MHPG) is a principal metabolite of noradrenaline derived from the brain. Therefore, to assess the saliva levels of MHPG is considered as a non-invasive tool for measuring central noradrenergic activity. From previous findings, although it is suspected that changes in the saliva level of MHPG varies among modes of mental stress testing, very few studies have tested this possibility directly. Therefore, the purpose of this study was to test different elicitation of the saliva level of MHPG induced Stroop Color Word Conflict Test (SCWCT) and Uchida-Kraepelin Test (UKT) as modes of mental stress testing in ten male healthy volunteers. The participants performed these two tasks at about the same time on two different days according to their schedule available. The order of the tasks was counterbalanced. MHPG responses varied among tasks; only loading SCWCT resulted in the significant increase in MHPG level in the saliva even while both SCWCT and UKT induced similar degree of subjective stress state. These results provide evidence that mental work load induces increase in levels of MHPG specifically, suggesting that there are specific situational dimension(s) which activates the central noradrenergic nervous system. Combined with previous studies focusing on individual difference, future attempts to clarify the dimension (s) would improve our understanding of brain noradrenergic activity induced by acute stress.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7B_11_P

NEONATAL ENVIRONMENTS DIFFERENTIALLY AFFECT ANXIETY BEHAVIOUR AND MAMMARY GLAND DEVELOPMENT IN BALB/C MICE: A NEW LOOK AT THE STRESS AND BREAST CANCER RELATIONSHIP

Leslie R. Kerr[1], Ayehsa Salleh[1], Tricia Ralph[1] & Chi Yip Ho[2]

[1] *Trent University, Peterborough, Ontario, Canada, K9J 7B8*
[2] *Microarray Laboratory, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada*

Early life stressors may increase the risk of breast cancer. Although, several studies have produced inconsistent support for the role of stressors in breast cancer risk. Because the mammary gland is one of a few organ systems that completes its development postnatally, it may be uniquely susceptible to genetic and epigenetic modifications caused by neonatal experiences. In rodents, as in humans, mother and infant interactions influence the development of the HPA axis, impacting on hormone levels, immune responses, reactivity to stressors, and possibly mammary gland development. Here, we examine how early life experiences differentially influence both anxiety-related behaviour and mammary gland development. Similar to other studies, we demonstrated that immobility in the forced swim test was higher in maternally separated (MS; 4hrs/d, PND 2-22) than in handled (H; 15min/d; PND2-22) and unmanipulated, typically reared (TR) females, indicating increased anxiety and HPA axis reactivity in MS mice. Gross morphological analyses of mammary glands demonstrated that differentiation rates (LAU /TEB) in mammary glands of adult H mice were higher than in both TR and MS mice ($p's<0.01$). Unexpectedly, protein levels of ERα were higher and p53 were lower in both neonatally manipulated conditions than in the TR condition ($p's<0.01$), whereas ERα and p53 gene expression profiles among the three conditions were similar. Studies are on-going to better elucidate the complex interaction among mother-offspring interactions, stressor reactivity, mammary gland development, and breast cancer risk.

7B_12_P

RELATION BETWEEN ATTACHMENT QUALITY AND AUTONOMIC REACTIONS TO ACUTE PSYCHOSOCIAL STRESS IN ADOLESCENCE

La Marca[1], R., Nguyen[1], T. T., Ehlert[1], U. & Zulauf-Logoz[2], M.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[1]*University of Zurich, Department of Clinical Psychology and Psychotherapy, Zurich, Switzerland*
[2]*University of Zurich, Department of Child and Adolescent Psychiatry, Zurich, Switzerland*
Email to corresponding author: r.lamarca@psychologie.unizh.ch

Stress is known to influence the HPA axis and the autonomic nerve system. Furthermore it is related to several disorders. Little is known about the effect of attachment quality on acute stress reaction. The purpose of this study was to investigate the relation between attachment quality and biopsychological stress reactivity. Thirty healthy children (18 female) participated at the age of 15 on a attachment interview (CAI, Child Attachment Interview) and a psychosocial stress test consisting of a public speaking and a mental arithmetic task in front of an audience (TSST-C). Salivary cortisol and amylase were repeatedly collected before and after the stress test. Heart rate and heart rate variability were measured continuously. The data are based on a dissertation project which was performed by Zulauf-Logoz (1997). The TSST-C resulted in a significant increase in cortisol ($p<.001$), amylase ($p<.01$), heart rate ($p<.01$), LF/HF-ratio of heart rate variability ($p<.01$) and a significant decrease of HF ($p<.01$). Secure attached adolescents showed lower cortisol-AUC_I during stress ($p<.05$) and lower heart rate and higher HF during baseline conditions (both $p<.05$), when considering the attachment to the mother but not to the father. In the present study the TSST-C induced an increase in the activity of the HPA axis and the sympathetic nervous system, while reducing the activity of the parasympathetic nervous system. Attachment quality was shown to influence baseline and stress reaction of different biological stress systems. These data show the importance of attachment quality in stress research. Further research is needed to investigate the role of attachment quality on negative effects of stress. Zulauf-Logoz, M. (1997). *Die desorganisierte Mutterbindung bei einjährigen Kindern. Die motivationspsychologische Bedeutung der D-Klassifikation im ‚Fremde-Situations-Test'.* Bern: Peter Lang.

7B_13_P

SHAKER STRESS – A PSYCHOGENIC MODEL OF STRESS WITH USE IN TOXICOLOGY

James B. Lucot[1], Mojmir Mach[2], Michal Dubovický[2]

[1]*Dept. Pharmacology, Wright State University, Dayton, OH, USA, james.lucot@wright.edu*
[2] *Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava 84104, Slovakia*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Animal models of stress can provide information about the course and etiology of stress related disorders, such as depression and anxiety. The incidence of these disorders constantly increases, with serious medical and socio-economic consequences. Shaker stress with an unpredictable onset can be delivered over long periods of time and is a model of chronic psychogenic disturbances. It is thus useful in studies for which classic stress models are not able to be used. We refined this model and increased its duration to a week for our studies of the Gulf War Syndrome, where non-predictability of stress was necessary to mimic battlefield uncertainties. Our results showed that exposure to chemical warfare agents (i.e. sarin) is more dangerous in combination with chronic intermittent shaker stress and that some toxicities have a delayed onset, as was observed in those with the Syndrome. This model also was used for behavioral phenotyping of stress responses in oxytocin knockout (OTKO) mice. Acute shaker stress in OTKO male mice induced a larger stress response compared to wild type mice. Neurochemical analysis revealed neural substrates for both psychopathology and interactions with toxins. These data demonstrate that this stress model is reproducible and well controlled. It is a relevant and flexible stress model which can be used in various studies in toxicology and behavioral phenotyping.

7B_14_P

A QUANTITATIVE STUDY ON STRESS OF DISABLED AND RETIRED PEOPLE IN EYES OF POPULATION OF THE CZECH REPUBLIC

Michal Matyska

Masarykian University, Joštova 10, 602 00 Brno, CZE. balu@mail.muni.cz

Sociological research is focused on relations of public opinion and real stress status of disabled and retired people. Research was done by answering questions by targeted persons. First part was completed on healthful population (age +18, economically productive, physically unhandicapped, total number of informants: 150 for disabled and 132 for retired). Those people were put the question: „Do you think, that disabled/retired people are suffering from stress?" Five-points scale was every time used. Answer results: disabled people: 61.3% yes (92), 16.7% rather so (25), 2% do not know (3), 16% rather not (24) and 4% no (4). Retired people: 64.4% yes (85), 14.4% rather so (19), 1.5% do not know (2), 13.6% rather not (18) and 6% no (8). As a whole, disabled people are supposed to be suffering from stress by 78% of population and retired people by 78.8% of population. Second part was done with disabled (total 48) and retired (total 75) people. Firstly, they were inquired, if they suffer from stress. Method is the same as above. As a whole, 31.2% (11+4)

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

disabled people subjectively think, that they suffer from stress at least sometimes and 68.6% of disabled people state the minimal incidence of stress (25+8). 49.3% of retired people subjectively think, that they suffer from stress (6+31) and 50.6% of retired people do not confirm this statement (26+12). Finally, psychological stress analysis was done (method in Cungi, Ch.. 1998. Savoir gérer son stress. Paris: RETZ). Objectively 27.1% (13) of disabled and 56% (42) of retired people suffer from stress. Result is, that the most of informants in unhandicapped population do not correctly apprehend the real stress situation of disabled people, but the opinion was right about retired.

7B_15_P

PSYCHOSOCIAL STRESS AND CHRONIC HEPATITIS C VIRUS (HCV)

Micali Elvira, Squadrito Giovanni, Amaddeo Giuliana, Callipari Serena, Abbate Carmelo, Barbaro Mario and Raimondo Giovanni.

University of Messina. Medicine and Surgery. Messina (Italy)

Chronic hepatitis C produces extra-hepatic disorders and troubles. Symptomatic patients, that haven't got absolute and/or relative contro-indications, could be underwent to pharmacological treatment with interferone. The study means to give prominence to the forms with which the HCV chronic subjects (with genotype 2 and 3) react to the appearances of their physics functionality and the neuro-psychic symptomatology that comes out after the therapy with the interferone. It was administred by a clinical psychologist to 20 patients naïve (M=12 F=8) mean age 46,15 years, mean of schooling 12 years, all sick with chronic HCV, the Illness Behaviour Questionnaire (IBQ) mental reactive standardized and validated ;62 items, that form 7 scales, carry on the reactions and the feeling of the patients respect the disease and the perception of psychosocial situation. The evalutation was carried out at the beginning of the treatment with interferone and after 6 months of therapy. IBQ, at the beginning of the pharmacological treatment, with positive value of HCV-RNA, registered that 9 subjects of 20 had significant score for the scales "*conviction of disease"* and "*affective inhibition"*.
After six months of assumption of interferone the haematologic exams gave prominence to a persisten reduction of HCV-RNA, while the IBQ scale: *"hypochondria", "psycho-somatic perception of disease", "dysphoria", "negation", " irritability"*, gave prominence to a significant clinically score for all of the sample. Our results, obtained with IBQ, they make assume that aspecific physiological answer of the human physical and the complicated reactions that follow to a pathological condition and the specific pharmacological treatment produce psycho-emotional modifications and psyco-somatic stress.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Keywords: chronic hepatitis C, dysphoria, psychosocial stress, irritability, IBQ

7B_16_P

THE LONG-TERM IMPACT OF PERCEIVED STRESS ON SLEEP QUALITY

Leif W. Rydstedt
University West, Trollhättan, Sweden

Jason J. Devereux, Mark Cropley, Georgia Michalianou
University of Surrey, Guildford, UK

The purpose of the present study was to investigate the long-term impact of perceived stress on sleep quality. The sample consisted of 70 British white-collar workers (age m=42, 31% females) who participated in a survey study and also volunteered for a field study about 4 years later. Hierarchic regression analysis was used with age and gender entered in the first step and an indicator of baseline sleep quality in the second step of the equation, while perceived stress at baseline (measure by the 10-item version of PSS, aiming at stress the last month) was entered in the third step. The result showed that perceived stress level at baseline significantly predicted (R^2 =.049, $p<.05$) sleep quality (measured by PSQI) 4 years later. This finding suggest that even more momentarily perceived stress may reflect a more chronic strain influence.

7B_17_P

THE STRESS-ASTHMA RELATIONSHIP IN CHILDREN: SOME PROGRESS TOWARDS SOLVING THE PUZZLE

Seija Sandber

Department of Mental Health Sciences, University College London

While there is still an open verdict for the role of stress in the pathogenesis of childhood asthma, a growing body of research suggests that psychosocial stress is a contributory factor development of asthma especially during early childhood. However, the area of study where the evidence is fairly robust concerns stressful experiences exacerbating the symptoms of asthma in children.
Despite this robust pattern of findings, little is known about the underlying

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

mechanisms responsible. Experimental studies have produced inconsistent findings. One reason for this may lie in the difficulty of experimentally creating true stress conditions – instead, the effects of emotional arousal have mostly been measured. Only animal studies have so far managed to examine mediators of stress-induced pathways in some detail.
The presentation will first briefly review the results of a prospective study by the author and colleagues (Sandberg et al, 2000, 2004) in conjunction with those of a subsequent case - control study by Miller & Chen (2006). The former showed that a severely negative life event significantly increases the risk of an acute exacerbation of asthma immediately afterwards, and again after a period of a few weeks. Chronic background stress both magnified the risk related to stressful life events and affected the timing of maximum risk. The latter added a possibly important validation of these results. It showed that children with asthma who experienced a severe life event in the context of chronic stress had diminished expression of genes encoding glucocorticoid and β2-adrenergic receptors relative to children without comparable stress exposure.
An attempt will then be made to integrate the above findings in the context of stress affecting the immune processes implicated in asthma, and within the framework of emotional/ physiological dysregulation, possibly reflecting a common underlying genetic vulnerability to atopy and asthma.

7B_18_P

MPM, A SYNTHETIC CCK ANTAGONIST, ANTAGONIZED THE EFFECTS OF STRESS ON HIPPOCAMPAL DENDRITIC REMODELING IN RATS

Sattayasai Jintana[1], Boonprakob Yodchai[2], Lattmann Eric[3]

[1]Department of Pharmacology, Faculty of Medicine, [2] Department of Physical therapy, Faculty of Associated Medicine,Khon Kaen University, Khon Kaen, Thailand, 40002
[3] The School of Pharmacy, Aston University, Aston Triangle, Birmingham B4 7ET, UK.

Stress may be defined as any type of threat, either real or perceived, that requires compensatory responses for the maintenance of homeostasis and the responses may be adaptive or maladaptive. The maladaptive responses to a stressor could become a stress and a pathophysiological cascade may ensue, for example, depression, increased anxiety or post-traumatic stress disorder. The hippocampal formation is one of a plastic and vulnerable brain structure and highly susceptible to stress and glucocorticoids. Stress and chronic glucocorticoid administration have

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

been shown to induce hippocampal damage, such as neuron death, gliosis and dendritic atrophy found in many animal models. In this study, MPM [N-(5-methyl-3-oxo-1,2-diphenyl-1H-pyrazol-4-yl)-N'-3-methoxyphenylurea], a synthetic CCK (cholecystokinin) antagonist, was tested for the antagonistic effect against stress. Male Spraque-Dawley rats were subjected to chronic restraint stress for 6 h/day, for 28 days. Prior to the restraint sessions, rats were orally fed with either 5% DMSO or MPM (0.5 mg/kg). On day 1, 7, 14, 21 and 28, the animals were tested in elevated plus maze and forced swim model. At the end of the treatment, rat's brains were removed and processed for Golgi-Cox method and the dendritic trees of CA3 hippocampal pyramidal neurons were observed under light microscope. The results showed that MPM could antagonize all the effects of chronic restraint stress, including mood disorders and hippocampal dendritic atrophy. According to the receptor binding property, MPM might antagonize stress effects through both types of receptors at hippocampus, pituitary and adrenal glands and break the LHPA axis in response to stress.

7B_19_P

LIVING WITH HIV- IS IT STRESSFUL AS IT SOUNDS?

Dr. Batia Sheffy

Caesarea, Israel

HIV can produce psychosocial stress. HIV/AIDS has characters that are associated with stress, like secrets and stigma along with other variables of coping with chronic-terminal disease.
Living with disease, can produce psychological stress, and since the relations between mind and body are known, awareness of coping mechanisms, and using certain coping styles can reduce stress and improve life satisfaction and psychological well being.
The study presented collected data from 100 people living with HIV/AIDS in Israel. The study examined correlations between ways of coping (emotional focused or problem solving) and quality of life and well being.
The main assumption was that using problem solving coping style will lead to higher life satisfaction, which are both rational variables and on the other hand, emotional focused coping style will lead to well being which is an emotional variable. Also were examined stress related variables and their association with the depended variables.
The assumption was partly confirmed and the correlations between the affective variables were not confirmed. Other variables, related to psychological stress were also correlated with the depended variables.
Key words: psychological stress, HIV, coping, life satisfaction, psychological well being.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7B_20_P

STRESS, QUALITY OF LIFE AND MULTIPLE CHRONIC DISEASES: PATTERNS EMERGING FROM A LARGE NATIONAL SAMPLE, AUSTRALIA

Agnes Walker

Australian Centre for Economic Research on Health, Australian National University CANBERRRA 0200 AUSTRALA, Agnes.Walker@anu.edu.au

OBJECTIVES: to study the associations between multiple chronic diseases and quality of life and psychological distress scores.
METHODS: analysis of unit record cross sectional data from Australian national surveys for the population aged 20 years or more. Identification of an appropriate indicator of multiple chronic diseases (ie comorbidities). Use of logistic regression techniques to study associations between (a) comorbidities and demographic, socioeconomic and risk factor variables and (b) quality of life (general and psychological distress) and demographic, socioeconomic and health status indicators.
RESULTS: older people, obese persons, women, persons with low socioeconomic status and those living alone had significantly greater probability of having three or more chronic illnesses than did other 20+ year olds ($p<0.0001$). Also people with comorbidities and/or with poor self-rated general health; those living alone; people with low educational qualifications; and persons with low socioeconomic status were more likely to feel dissatisfied, unhappy or terrible about their lives and of having moderate, high or very high psychological distress scores than did the rest of the 20+ year old population ($p<0.0001$).
CONCLUSIONS: age and obesity were found to be major risk factors for comorbidities. In turn, comorbidities and self-rated health were negatively associated with quality of life - whether indicated by how people felt about their lives generally, or by the extent of their psychological distress.

7B_21_P

PSYCHOLOGICAL RISK FACTORS IN RHEUMATOID ARTHRITIS. PRELIMINARY STUDY

Agnieszka Wlazło[1], Jacek Kleszczyński[2], Grażyna Dolińska[1], Jerzy Leszek[2]

[1]*Uniwersytet Wrocławski*
[2]*Akademia Medyczna we Wrocławiu*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

e-mail: aga.ag@vp.pl

Introduction:
Psychological factors – by intact on the one hand on assessment of difficult situations and on the other on stress coping - can promote pathophysiological reactions, which are associated with rheumatoid arthritis. Up to now, features of personality and temperament, typical for rheumatoid arthritis patients, were not assessed.
Aim of the study:
Appreciation psychological risk factors characteristics for rheumatoid arthritis patients.
Methods:
Thirty patients (16 F, 14 M) in age 16-19 (mean age 46) with diagnosis of rheumatoid arthritis (according to ARA) were included to the study. Control group were health people with family load with autoimmunological disorders. The all patients in preliminary period of the disease treated with pharmacotherapy and rehabilitation and people belong to control group used following questionnaires:

- Life Orientation Test (M. F. Scheier, C. S. Carver, M. W. Bridges)
- The Sense of Coherence Questionnaire (A. Antonovsky)
- Formal Characteristic Behavior: Temperament Questionnaire (B. Zawadzki, J. Strelau)
- Eysenck Personality Questionnaire – Revised (S. B. G. Eysenck, H. J. Eysenck, P. Barett)
- Syndrome of Aggression: Psychological Questionnaire (Z. B. Gaś)

Results:
Firstly, we have found that level of optimism, sensivity, activity and endurance were statistical significantly lower in group of rheumatoid arthritis patients; secondly level of emotional reactivity and neurotism were statistical significantly higher.
Conclusion:
These results seem to support hypothesis that psychological factors may play significant role in induction and progression of rheumatoid arthritis. However, taking into consideration multifactor etiopathogenesis of this disease, our results should be confirmed in future investigations.

7B_22_P

POSTTRANSLATIONAL MODIFICATIONS AND SUBCELLULAR REDISTRIBUTION OF GLUCOCORTICOID RECEPTOR IN RESPONSE TO ACUTE STRESS IN WISTAR RAT BRAIN

Miroslav Adzic[1], Jelena Djordjevic[1], Ana Niciforovic[1], Marija Radojcic[1] and Marija Krstic-Demonacos[2]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

[1]*Dept. Mol. Biol. Endocrinol., VINCA Inst. Nucl. Sci., Belgrade, Serbia;*
[2]*Faculty of Life Sciences, University of Manchester, Manchester, UK;*
marija@vin.bg.ac.yu

Prefrontal cortex (PFC) and hippocampus (HIP) are main limbic structures involved in inhibitory feedback to HPA axis during stress. Inhibitory feedback is provided by glucocorticoid receptor (GR), which acts as suppressor of excessive stress response. The level of blood plasma corticosterone is elevated in response to stress, leading to GR activation. Furthermore, the GR functions are controlled by inhibitory phosporylation at ser246 (S246) by JNK and stimulatory phosphorylations at ser224 and ser232 (S224, S232) by CDK. Molecular mechanism of GR posttranslational modifications, as well as, subcellular location of its phosphorylated isoforms in response to acute stress *in vivo* have not been precisely defined yet. We studied these mechanisms in PFC and HIP of male Wistar rats exposed to acute stress by immobilization. The decreased level of total GR in the cytoplasm and increased level in the nucleus was accompanied by marked decrease of its phosphorylated isoform S246pGR in both compartments. The levels of active, phosphorylated JNK isoforms were elevated in the cytoplasm and significantly decreased in the nucleus. The GR activation by acute stress was indicated by the elevated phosphorylation of GR at S232 in the nucleus of HIP. Overall, GR cytoplasmic-nucleus shuttling in PFC and HIP is accompanied by changed ratios of inhibitory and stimulatory phosphorylation of GR, pointing out to their significance for GR subcellular location and response to acute stress.

7C_01_P

STRESS TRIGGERED NEURONAL PLASTICITY IN SPLEEN IN INTERACTION WITH IMMUNOCYTES

C. Liezmann[1], M. Daniltchenko[1], S. Pavlovic Masnikosa[1], B.F. Klapp[1], and E.M.J. Peters[1]

[1] *University-Medicine Charité, Charité Center 12 for Internal Medicine and Dermatology, Psychoneuroimmunology, Campus Virchow, Berlin, Germany; eva.peters@charite.de*

Stress is considered as a factor which induces or aggravates inflammatory skin diseases such as atopic dermatitis. An influence of stress on the interaction of peripheral nerve fibers with cells of the cutaneous immune system (mast cells, dendritic cells) with following modulation of an inflammation reaction could be proven recently. In this context, Substance P (SP) - a sensory neuropeptide - was revealed as an important stress mediator with its own stress axis in the skin. Here we postulate stress-dependent communication between nerve fibers and immune-competent

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

cells with effect on the course of inflammatory skin diseases in the spleen. To address this question, we employed a combined mouse model of experimental allergic dermatitis (AD) and stress. AD was induced in C57BL/6 mice by double sensitization (i.p) and an intradermal challenge using chicken egg ovalbumin. Animals were additionally exposed to sonic stress for 24h prior to challenge. In this model stress leads to a relative hyperinnervation of the immune-competent areas of the spleen. At the same time, an increased number of antigen-presenting cells (APC) can be observed in these arreas and contacts between nerve fibers and APC were found. Further analysis of quality and function of neuro-immune interaction will reveal the role of the observed stress-induced alterations in the spleen in atopic disease .

7C_02_P

STRESS POTENTIATES MIGRATION AND MATURATION OF SKIN DENDRITIC CELLS IN ALLERGIC DERMATITIS

S. Pavlovic Masnikosa[1], M. Daniltchenko[1], S. Blois[1], B.F. Klapp[1], and E.M.J. Peters[1]

[1] *University-Medicine Charité, Charité Center 12 for Internal Medicine and Dermatology, Psychoneuroimmunology, Campus Virchow, Berlin, Germany; eva.peters@charite.de*

Altered TH1/TH2 immune responses are held responsible for atopic disease. Stress appears to be a potent immunmodulator as well as aggravator of atopic disease. To examine stress effects on immunity in atopy, we employed a combined mouse model of experimental allergic dermatitis (AD) and sound stress. In a previous study we had shown that stress applied prior to challenge induced neuronal plasticity, increased neurogenic inflammation. We also observed an altered cytokine balance indicating altered antigen processing. Here we show, that stress prior to sensitization alters dendritic cell (DC) function. In stressed animals the number of $MHCII^+$ cells in epidermis and dermis is reduced 48h after challenge. DCs, mainly Langerhans cells, migrate more frequently from epidermal sheets cultured from treated-skin biopsies obtained immediately after challenge when AD animals were exposed to stress. The like was observed after treatment of AD epidermal sheets with the stress mediator substance P (SP), but not with calcitonin gene related protein. Moreover, the effect of stress was abolished when animals were treated with NK1 antagonist prior and after stress application. Using flow cytometry, we found that these cells migrate to the draining lymph nodes, where DCs (CD11c+) from stressed AD animals show significant up-regulation of co-stimulatory molecules CD80 and CD86. This correlated with an enhanced expression of LFA-1 and VLA-4, adhesion molecules

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

implicated in facilitation of TH1 and TH2 responses, respectively. Taken together, we show that stress activates DC migration and maturation in atopic dermatitis-like allergic dermatitis in a SP dependent fashion. Stress may therefore be involved in the sensitisation of atopic individuals to an allergen and thereby determine the course of the disease.

7C_03_P

STRESS AND ASTHMA - FROM MICE TO MEN

Ricarda A. Joachim, David Quarcoo*, Oliver Noga, Eva M. Peters, Petra C. Arck, Viktoriya Sagach, Q.Thai Dinh and Burghard F. Klapp

*Department of Internal Medicine and Psychosomatics and *Department of Pediatric Pneumology and Immunology, Charité Universitaetsmedizin Berlin, Germany*
ricarda.joachim@charite.de

Despite the well documented clinical association of stress and bronchial asthma morbidity, experimental data on mechanisms is still limited. To explore pathways linking stress and asthma we employ an animal model that combines an established protocol of allergic airway inflammation with sound stress exposure. Our findings demonstrate that stress dramatically enhances airway reactivity and airway inflammation via the neurokinin-1 receptor, the main receptor for the tachykinin substance P (SP). After stress increased SP expression is found in airway innervating neurons. Mice with allergic airway inflammation show higher levels of PPT-I mRNA (encoding for SP)in lung tissue under stress conditions. In lymph nodes the percentage of TNF-alpha^{+} T-cells is higher in stressed mice or after application of SP. Furthermore, we identified the chemoattractant eotaxin to be involved in stress increased eosinophilic airway inflammation.
To prove the relevance of our findings we investigated the correlation of stress perception and the cytokine profile of circulating lymphocytes in humans. In asthmatic patients stress perception correlated with percentages of TNF-alpha^{+} CD3^{+} T-cells in peripheral blood. Stress perception further correlated with serum BDNF levels in asthmatics but not in controls. Interestingly, higher stress perception was associated with decreased lung function (FEV1) in asthmatics.
The results of our studies support the hypothesis that stress deteriorates bronchial asthma by inducing a pro-inflammatory cytokine profile - probably via neuro-immunological pathways - in asthmatic individuals.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7C_04_P

MOLECULAR BASIS FOR PSYCHOSOCIAL DISTRESS MEDIATED IMMUNE DYSREGULATION.

Herbert L. Mathews and Linda W. Janusek

Loyola University Chicago, Maywood, IL USA hmathew@lumc.edu

Women diagnosed with breast cancer experience psychosocial distress that is associated with immune dysregulation. For this investigation, epigenetic modification of peripheral blood subsequent to breast cancer diagnosis was evaluated. A pre-post, two-group design was used with initial data collected 3–4 weeks after diagnosis. This time period, P1, was post surgery and was before other therapies. This time period provided for an analysis of the effects of psychosocial distress without confounds. Women were also sampled 2 months post cancer treatment, designated time period P2. Results were compared P1 to P2 and were also analyzed with respect to a comparison group of matched women without cancer. At P1, women diagnosed with cancer exhibited increased psychosocial distress and immune dysregulation, characterized by reduced natural killer cell activity and interferon gamma production. Histone analysis of the peripheral blood of women diagnosed with cancer at P1 showed reduced acetylation of histone H4-K8, K-12 and K-16 when analyzed with respect to histones derived from peripheral blood of the comparison group of women. At P2, women diagnosed with cancer exhibited decreased psychosocial distress and normalized immune function. Furthermore at P2, acetylation of histone H4-K8, K-12 and K-16 was greater than at P1 for women diagnosed with cancer, and normalized in that the degree of acetylation was similar to that of the women of the comparison group. This was in contrast to the acetylation pattern of histone H4-K5, which was unchanged P1 to P2. These results, coupled with our previous observations, demonstrate peripheral blood epigenetic modification of histones to be associated with the immune dysregulation characteristic of breast cancer patients who experience psychosocial distress.

7C_05_P

PSYCHO-IMMUNE RESPONSE OF WOMEN WITH BREAST CANCER TO A MINDFULNESS BASED STRESS REDUCTION PROGRAM

Linda Witek Janusek, Kevin Albuqureque, and Herbert L. Mathews

Loyola University of Chicago, Maywood, IL USA ljanuse@luc.edu

Women diagnosed with breast cancer have psychological distress that is

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

accompanied by dysregulated immune function. In this study a mind-body intervention was evaluated for its capacity to decrease psychological distress and to improve immune function. Women treated with breast conserving surgery, radiotherapy, and adjuvant endocrine therapy were enrolled into an 8-week mindfulness based stress reduction (MBSR) program or into a control group (Non-MBSR) who received usual care. Immune and psychological outcomes were assessed pre-, mid-, at completion, and post-MBSR. Both groups of women, MBSR and Non-MBSR exhibited increased mood disturbance, anxiety, and perceived stress when compared to age-matched cancer free women. Peripheral blood mononuclear cells (PBMC) from both groups of women produced more IL-4, IL-6 and IL-10 and less interferon gamma as well as reduced natural killer cell activity when compared to the PBMC of age-matched cancer free women. When the two groups (MBSR and Non-MBSR) of women were compared, the MBSR women showed a more sustained decrease in anxiety and an earlier decrease in perceived stress than did the Non-MBSR women. Immunologically, MBSR women showed a quicker and more profound recovery of PBMC NKCA as well as interferon gamma production. NKCA at the post-MBSR time point was equivalent to that of cancer free women while the Non-MBSR women still exhibited reduced NKCA. These results suggest that MBSR may permit an earlier return to psychological and immunological normalcy in women undergoing diagnosis and treatment for cancer.

7C_06_P

SALIVARY CORTISOL AND LONG TERMED STRESS: TWO YEARS FOLLOW UP ON LONG TERMED STRESSED EMPLOYEES

Bo Netterstrøm & Aase Marie Hansen

Clinic of Occupational Medicine, Hillerød Hospital, Denmark
Danish National Center for Work Environment Reasearch

Aim: To explore in the development of awakening cortisol reactivity during recovery from long termed stress.
Methods: Seventy employed persons aged 25 to 61 years were referred to a stress management programme at the Clinic of Occupational Medicine in 2003 and 2004.
Questionnaires regarding stress symptoms, SF36 and depression (MDI-10, WHO scale) were filled out at baseline, after 4 months and one and two years. Salivary cortisol was collected at awakening and ½ hour later. TSH, HbA1C, fibrinogen and serum lipids were measured at baseline and 4 months later.
Results: Behavioural and somatic symptoms as well as perception of general health (SF36) correlated to awakening cortisol reactivity (ACR).

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

ACR decreased during the follow up period and correlated to decrease in symptom score. ACR did not correlate to TSH, HbA1C, fibrinogen or serum lipids.
Conclusion: An almost complete recovery was obtained in one year. The HPA axis function seems to be depressed during long-term stress. A change in metabolism in an anabolic direction was followed by recovery.

7C_07_P

RELATIONSHIP BETWEEN PSYCHOPATHOLOGICAL SYMPTOMS AND PSYCHONEUROENEDOCRINIMMUNOLOGICAL INDICATORS IN PATIENTS WITH PANIC DISORDER.

Hisayoshi Okamura[1], Akira Tsuda[2], Jumpei Yajima[4], Hiroo Fukuyama[3]

[1]Cognitive and Molecular Research Institute of Brain Diseases, Kurume, Japan, oka1013@yahoo.co.jp [2]Department of Psychology, [3]Department of Social Welfare, Kurume University,4Department of Human Studies, Beppu University

Many studies have reported that the acceleration of the noradrenergic neurons in the brain have been recognized on the patients with panic disorder. There is substantial evidence that the anxiety state in human is associated with a high level of 3-methoxy-4-hydroxyphenylglychol(MHPG) in the plasma. These finding suggested that saliva level of free-MHPG seemed to be useful indicator for assessing the pschopathological symptoms of panic disorder.
The purpose of this study is to investigate the relationships between the psychopathological symptoms with SSRI treatment and the saliva of Psychoneuroendocrinimmunological (PNEI) indicators. Participants were 10 first-visit outpatients with panic disorder (8 males, 2 females, 18-46 of age) and 20 age-matched healthy volunteers. In order to assess the level of free-MHPG, cortisol and s-IgA, the saliva was collected. In addition to this, the participants' anxiety and mood levels were evaluated with HAS, POMS, and GHQ-28 by the psychiatrics in charge at their first visit, first week, third week and fifth week after beginning of SSRI drug treatment.
The saliva levels of free-MHPG and cortisol at subjects' first visit to the hospital were significantly higher and saliva level of s-IgA was lower than those of control subjects. Following the 5 weeks of SSRI treatment, the saliva levels of free-MHPG and cortisol were decreased, while the saliva level of s-IgA was increased and the scores of HAS, POMS and GHQ-28 subscale of anxiety were improved. In addition to this, the levels of free-MHPG and cortisol were associated with the reduced anxiety and depression.
These results indicated that free-MHPG, cortisol and s-IgA level can be

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

useful indicators for assessing the pschopathological symptoms of panic disorder, and also the response to drug treatment in these patients.

7C_08_P

ALLERGIC DERMATITIS REDUCES STRESS-COPING SKILLS: INDICATIONS FROM A MOUSE MODEL

M. Daniltchenko[1], S. Pavlovic Masnikosa[1], A. Örsal[1], , B.F. Klapp[1], and E.M.J. Peters[1]

[1] University-Medicine Charité, Charité Center 12 for Internal Medicine and Dermatology, Psychoneuroimmunology, Campus Virchow, Berlin, Germany; eva.peters@charite.de

Stress is said to cause, trigger and aggravate allergic diseases such as atopic dermatitis. Atopic patients show an altered HPA-axis reactivity and increased depression and anxiety which have been related to disease severity. However, if stress affects dermatitis or dermatitis causes stress remains unclear. To address this question, we employed a combined mouse model of experimental allergic dermatitis (AD) and stress. AD was induced in C57BL/6 mice by double sensitization (i.p) and an intradermal challenge using chicken egg ovalbumin. Animals were additionally exposed to sonic stress for 24h prior to challenge. We monitored the alterations in anxiety- and depression- like behaviour, locomotor activity, exploration, and "approach/avoid conflict" behaviour using elevated plus maze (EPM) and tail suspension test (TST). AD induction caused slight increase in anxiety-like behaviour with tendency to avoid conflict situation. Interestingly, stress itself promoted locomotion and exploration. However, this effect of stress was reduced when AD mice had been exposed to stress. No significant differences in depression-like behaviour were observed among the examined groups as measured in TST. At the same time we observed a significant decrease in c-Fos+ activated neurons in the hypothalamus in AD mice exposed to stress. Taken together, the presence of a cutaneous inflammation in the AD mice affected both behaviour and HPA reactivity suggest that altered reaction to stress stimuli and coping abilities is a result of AD. Enhancement of stress-coping skills therefore appears a useful measure to balance AD-induced behavioural changes.

7C_09_P

CNS-IMMUNE INTERACTIONS DURING STRESS: POSSIBLE ROLE FOR FREE RADICALS

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Arunabha Ray and Kavita Gulati

Department of Pharmacology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-110 007, India
(E-mail : arunabha14@yahoo.co.in)

Free radicals are highly reactive moieties and both ROS and RNS have been implicated in a variety of pathophysiological states. CNS-Immune interactions are important regulators of stress responses and the present study was designed to investigate the possible role of free radicals and their interactions during stress-induced behavioral and immunological responses in rats. Exposure to restraint stress (RS) persistently suppressed behavioral activity in the EPM test, as evidenced by the reduced open arm entries and time spent in the open arms as compared to the control (no RS). Pretreatment of rats with the antioxidants, ascorbic acid, melatonin and NO mimetics, L-arginine and isosorbide dinitrate, all differentially reversed RS-induced behavioral suppression in the EPM test. The NO synthase inhibitors, L-NAME and 7-nitroindazole, on the other hand, either had no influence or induced aggravations in the neurobehavioral suppression in the EPM test. Assay of brain homogenates showed that RS-induced behavioral changes were closely paralleled by enhanced brain MDA levels, and lowered brain NOx and glutathione levels. These changes were significantly modified by the antioxidant and NO mimetic pretreatments. In rats immunized with sheep RBC, RS suppressed both humoral and cell-mediated immune responses, and differentially modulated cytokine (TNF-α and IL-4) levels, which were attenuated by the anti-oxidants and NO precursors/releasers and aggravated by NO synthase inhibitors, These immunological changes were accompanied by corresponding changes in plasma MDA, glutathione, and NOx. These behavioral, immunological and biochemical data in response to RS and its alterations by antioxidants and NO-ergic agents are strongly suggestive of the involvement of ROS and RNS, in the CNS-Immune interactions during stress.

7C_10_P

WALKING THE LABYRINTH: EFFECTS ON MIND, BODY, AND SPIRIT

M. Kay Sandor, Ph.D., RN

The University of Texas Medical Branch, School of Nursing
301 University Blvd.
Galveston, Texas 77555-1029
ksandor@utmb.edu

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Background: This project examines the physiologic and psychospiritual responses to a walking meditation—the labyrinth. Meditation is a complementary health practice that integrates the mind, the body, and the spirit. Labyrinths have been reported to reduce stress and induce relaxation, but no studies testing the effects of a labyrinth walk appear in the literature. The purpose of this pilot study is to examine the physiologic and psychospiritual responses to a program of walking the labyrinth compared to a program of walking on a track at a slow pace. The **specific aims** of this study are as follows:

(1) Evaluate the effects of a walking meditation (labyrinth) program on physiological markers of stress (blood pressure, pulse, respirations, Cortisol, IL-6 and CRP) in women 55-70 years of age.

(2) Determine the effects of a walking meditation (labyrinth) program on psychospiritual outcomes (affect, anxiety, aggression, depression, and spiritual well-being) in women 55-70 years of age.

Method: An experimental, pretest-posttest, repeated-measures design was chosen for this pilot study. The phenomena of interest are the physiologic and psychospiritual responses of women (55-70 years old) to a facilitated group labyrinth walking program [Intervention] and a facilitated group track walking program [Comparison]. Data was collected prior to walking (Time 1), at the end of a month-long facilitated group walking program (Time 2) and at the end of the a month of self-managed walks (Time 3).

Results: Data analysis is in progress and findings will be reported.

Discussion and Conclusions: Holistic and innovative community-based interventions addressing disease prevention and health promotion in women 55-70 years of age, are an important complementary adjunct to traditional care.

7C_11_P

CHARACTERISTICS OF PSYCHOBIOLOGICAL STRESS RESPONSIVENESS ON MENTAL STRESS TESTING IN DEPRESSIVE SUBJECTS

Yajima, Jumpei, Okamura, Hisayoshi, Horiuchi, Satoshi, Tsuda, Akira

Department of Human Studies, Beppu University, yajima@mc.beppu-u.ac.jp

Our previous study indicated that the acute stress caused increases in the saliva level of free-3-methoxy-4-hydroxyphenylglycol (MHPG) and secretory-immunoglobulin-A (s-IgA), cortisol, and the score of questionnaire (DSSQ: Dundee stress state questionnaire). We have clarified that the health state and the daily stress influence the Psychoneuroendocirnoimmunological stress response according to the

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

model of Experimental-field studies. This study investigated relationship between the psychobiological stress response induced by mental stress testing and the depression which was evaluated by Beck Depression Inventory (BDI).Subjects were healthy 226 volunteers (20.3±3.2 years old). At first, the subjects completed BDI. They took 10 minutes rest in an armchair prior to the stress session, and were exposed to 15 minutes mental stress testing. Before and after the stress session, saliva was collected and the subject was assessed a written inquiry subjective scales. Mental stress testing increased the saliva free-MHPG level, s-IgA level, cortisol level and then gradually reduced to the normal range after the stress session. Subjects also showed stress responses such as the tense arousal, the self consciousness, and task-irrelevant thought. The saliva s-IgA level of the depression group (BDI>7) was lower than that of the normal group (BDI<4) during the session. And, the negative mood and the task-relevant thought of the depression group were higher than that of the normal group. In addition, the performance of the task of the depression group was lower than the normal group. These results suggest that the depression group strengthens cognitive confusion by task, recognizes subjective stress strongly along with it, and the immunity activity is inhibited.

Key words: PNEI index, mental stress testing, depression

7D_01_P

INTERNET MAIL COUNSELING FOR SOCIAL WITHDRAWAL IN JAPANESE YOUTH

Kuramoto Hidehiko, M.D.

Kita-no-Maru Clinic, Tokyo

Social withdrawal (SW) of the youth, defined by 1) remaining at home for a duration of six months or longer with no social participation and by 2) not being diagnosed as schizophrenia or other mental disorders, has become a serious problem in Japan. One of the major therapeutic difficulties resides in the SW case's reluctance to meet the others. The internet mail counseling helps overcome those difficulties. In order to support SW cases, "*the net counseling room campaign*" was carried out intermittently by NHK (*Japan Broadcasting Corporation*) from October 2002 to March 2005. The characteristics of the cases consulted from April 2004 to March 2005 were analyzed. Out of the total 767 individual cases, there were 334 males (43.5%) and 433 females (56.5%). The average ages were 26.9 years old for males and 24.8 years old for females (p<.001). The average durations of SW were 49.6 months for males and 31.1 months for females (p<.001). Other items for analysis were "*reasons for consultation*", "*contents of consultation*", "*experience of school*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

refusal", and "*how to spend a day*". Some new findings on the actual conditions of the SW in Japanese youth were obtained.

7D_02_P

UKRAINE, UNEMPLOYMENT, STRESS

Natalia Logvinova

University of René Descartes, Paris 5, France
International University of Solomon, Ukraine
Fellow, American Academy of Experts in Traumatic Stress Studies

Unemployment in the former Soviet Union was a direct result of the fundamental social and economic changes, which took place in 1990's, and has become a major social phenomenon. Prsently we can but confirm the existence of unemployment, which affects a significant number of Ukrainians (43% in 2003).
These studies answered mainly two questions: Who is unemployed and what measures do they undertake to find work. On the other hand there have been no psychological or psyhopathological studies to answer the question, what is the impact of unemployment on the population of the SIC? Hoping to at least partially fill the void of research in this area, we conducted first study the former Soviet Union that focused on the potential psychological consequences, which the existing situation might have on the Ukrainian unemployed.
We have examined 108 unemployed persons and 50 employees: the relation between the unemployment situation and the level stress, and the social intelligence, workability, attitude to work. We have assed the variables according to the length of the unemployment period, the degree of stress and the presence of illegal work.
We have chosen the fallowing methods: The social intelligence test of Guilford and O'Sullivan (1977), the test of workability diagnosis of Landolt (1997), the social readjustment rating scale of Holmes and Rahe (1967).
Our study has revealed important fact that can be considered to be particular to Ukrainian unemployment - about 50% of the group showed low level of stress. This was an unexpected result because the western culture considers the unemployment as one of the worst misfortunes, after death and disease. We can also state the cultural differences concerning the life events.
The values of social intelligence and of workability (the overall estimate and the qualitative characteristics: reliability and accuracy) are significantly lower in the group of unemployed compared to the works. The unemployed persons' attitude to work is very specific. The length of the unemployment and the level of stress affect both the workability of individuals and their attitude to work.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7D_03_P

PSYCHOSOCIAL STRESS AT WORK AND OUTSIDE WORK AND PROBLEM DRINKING: THE HAPIEE STUDY

Pikhart H[1], Bobak M[1], Pajak A[2], Malyutina S[3], Kubinova R[4], Peasey A[1], Marmot M[1].

[1]Department of Epidemiology and Public Health, UCL, UK; [2]Department of Epidemiology and Population Studies, Jagiellonian University, Krakow, Poland; [3]Institute of Internal Medicine, RAMS, Novosibirsk, Russia; [4]NIPH, Prague, Czech Republic

Background: It has been shown in the past that psychosocial factors (at work and outside work) are associated with various health outcomes. It is thought that they influence health partly through health behaviours. Aims: To examine the association between the effort-reward imbalance (ERI), overcommitment and control over general life, and several alcohol related measures in three populations in Central and Eastern Europe in the HAPIEE Study. Methods: The sample of population aged 45–69 years old in Novosibirsk (Russia), Krakow (Poland), and 6 Czech towns completed a questionnaire in 2002-2005 that included ERI, overcommitment (OC), control over life, and a number of sociodemographic variables. Annual alcohol intake, annual number of drinking sessions, the mean dose of alcohol per drinking session, and binge drinking were calculated from graduated frequencies in the questionnaire. Data were also available on problem drinking (>2 positive answers on CAGE questionnaire). Only men who were full-time working at the time of interview were used for the analysis (N=6700). Results: After controlling for age and country, the measures of problem drinking were associated with ERI, OC and control over life. Adjustment for socioeconomic position did not substantially change the results. When all 3 psychosocial constructs were used at the same model, the estimated effects were reduced but still associated with the alcohol related outcomes. Conclusions: Stress at work and outside work expressed by ERI, OC and control over life is associated with problem drinking in these populations.

7D_04_P

STUDY THE INFLUENCE OF INDIAN AND WESTERN MUSIC ON EMOTIONAL MATURITY TO REDUCE THE STRESS OF INDIVIDUALS

Urvashi Shrivastava

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Department of Guidance and Counseling, Urvashi Kala Srishti, Pune, India

The psychology of music has inspirational, therapeutic and spiritual values which are useful for stress management. Music not only pleases the mind but also gives pleasure and ecstasy to the soul. The present study intends to explore the influence of Indian and western music on Emotional Maturity to reduce 'STRESS" of individuals. The sample was comprised of 60 adolescents (13-20 years), which had 30 Indian-music and 30 Western-music listeners. Following hypotheses was made:

(1) There would be difference in the levels of Emotional maturity of Indian and Western music listeners.
(2) There would be difference in the levels of Emotional maturity of male and female Indian and Western music listeners.

"Musical Interest Test" was used to differentiate the music listeners. To measure the levels of Emotional Maturity "EMS" (Emotional Maturity Scale – Mahesh Bhargava) was used. The important attributes of "Emotional Maturity (EM)" are;

(1) Firm sense of reality
(2) Flexibility
(3) Adoptability

As per the EMS scale higher the score lesser is the maturity level. The results indicated that by taking the consolidated data of mean values of "EMS", the highest emotional maturity is indicated by male listeners of Indian music with the value of 71.2 and the lowest Emotional maturity is indicated by male listeners of western music with the value of 86.4. Just by considering the "Mean Values" of listeners, Indian music listeners are highly emotionally matured than western music listeners. "t – test" was applied on the data for the levels of significance. Results also indicated that the maximum significant t-values in the grand total of "Emotional Instability, Emotional Regression, Maladjustment, Personality Disintegration and lack of Independence" is by the Male listeners of Indian and Western music with value of 7.73. The lowest significant value is observed between the "Female" listeners of Indian and Western music with t-value of only 2.4.

7D_05_P

MODELLING HOLISTIC HEALTH AND PREVENTION ETHICS IN MENTAL CARE

Hannele Ylilehto [1], M.D. Ph.D., Arja Rautio [2], M.D. Ph.D.

[1] Department of Public Health Science and General Practice, Box 5000, 90014 University of Oulu, Finland,

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

email: hannele.ylilehto@oulu.fi tel. +358 (0)8 537 5655.
[2] Centre for Arctic Medicine, Thule Institute, University of Oulu

Understanding of the aetiology of postpartum mood disorders requires the integration of both psychosocial and biological risk factors. The purpose of the previous study was to examine the time after a child is born from the salutogenic perspective. The study evaluated the mood of the mothers using the Edinburgh Postnatal Depression Scale (EPDS) during pregnancy and after childbirth. The relationships of the couples were studied using parts of Spaniers's Dyadic Adjustment Scale and part of Bienvenu's Marital Communication Inventory. If the relationship was considered bad, the risk of developing depression during pregnancy was 4.7 times higher (RR = 4.7, 95%, CI 2.8–8), and after childbirth 5.5 times higher (RR = 5.5, 95%, CI 3.1–9.6). The qualitative section of the study identifies the resources for recovery used by the subjects. A focused interview was carried out with 29 mothers 3–10 months after childbirth. Many of those who had exceeded the cut-off point felt they had suffered from passing melancholy or they had problems in their marital relationship. The objective is to study stress and recovery in a patient date consisting of subjects who feel they live in a difficult relationship. Themes of research: Recovery from depression, connection between breast infection and postpartum depression, connection between postpartum depression and violence, relations between recovery and molecular phenomena. Prevention ethics section will be applied in the background during the entire research project. This application supports a wider project in modelling holistic health.

7E_01_P

AUTOGENIC TRAINING IN THE TREATMENT OF ATYPICAL FACIAL PAIN.

[1]Krause M., [2]Müller O., [3]Nguyen M.T., [3]Fábián T.K., [3]Fejérdy P., [1]Krause W.R..

[1]Dept. of Psychiatry and Psychotherapy, Harz-Klinikum Wernigerode-Blankenburg, Germany
[2]Clinic of Pediatric Dentistry and Orthodontics, Semmelweis University Budapest, Hungary
[3]Clinic of Prosthetic Dentistry, Semmelweis University, Faculty of Dentistry, Budapest, Hungary

In this study 10 female patients (age between 29-58 yr.) with atypical oro-facial pain (ICD-10 category: F40-48) were treated with autogenic training in a small group, once a week for 10 weeks. The method was administered as an initial therapy in the early phase of treatment. The

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

first two basic exercises (feeling of heaviness and warmth) were practiced only, in sitting position [1]. Suggestions of the therapist [1,2] were given via microphone with the use of loudspeakers. Relaxing background music was also provided. Symptom palliation was measured with numerical analogue scale. In 3 cases (30%) symptoms disappeared totally. At least partial symptom palliation occurred in another 4 cases (40%). In 3 cases (30%) the treatment was unsuccessful. Belief of the patients in psychotherapy and patient-therapist relationship strong enough for entering to the exploratory part of psychotherapy were developed in all cases (100%). References: (1): Krause, W-R.: Hypnose und Autogenes Training. In: Schultz, JH.: Hypnosetechnik. Praktische Anleitung zum Hypnotisieren für Ärzte. 9. Auflage, bearbeitet und ergänzt von G. Iversen und W-R. Krause. Gustav Fischer Verlag, Stuttgart, 1994 Pp. 71-80. (2): Staats, J., Krause, W-R.: Hypnotherapie in der zahnärztlichen Praxis. Hüthig Verlag, Heidelberg, 1995. Pp 120-123.

7E_02_P

INFLUENCE OF EXPERIMENTAL DEVIATED MANDIBULAR POSITIONING ON PREFRONTAL CORTEX -ANALYSIS BY FUNCTIONAL NEAR-INFRARED SPECTROSCOPY

Mami Shibusawa[1], Keiichi Ishigami[1], Kazuhiko Takayama[1], Tomotaka Takeda[1], Kazunori Nakajima[1], Kaoru Sakatani[2]

Department of Sports Dentistry, Tokyo Dental College[1], Chiba, Japan.
sibusawa@tdc.ac.jp
Department of Neurological Surgery Nihon University School of Medicine[2], Tokyo, Japan

Exposure to stress increases dopaminergic activity in several regions of the brain. This phenomenon is most profound in the mesocortical dopamine system, where stress has been shown to increase selectively the release and turnover rate of dopamine. Recent *in vitro* studies employing micro dialysis have indicated that amino acids can increase the release of dopamine from prefrontal cortex (PFC) in rat or small animals. Disturbance of mastication following occlusal dysfunction has been reported to induce mental stress. However the relationship between a dysfunction of the stomatognathic system and stress remains to be clarified in human. Functional near infrared spectroscopy (fNIRS) is a powerful tool for noninvasive imaging. We used fNIRS to examine the PFC in healthy volunteers (N =5) under experimental deviated mandibular positioning (EDMP) achieved with a resin bite block. The block was positioned where the non-masticating-side canine cusp came into contact. The trial, 300s in total, started with a 60s pre-rest block, and was followed by a 180s EDMP task block and a 60s post-rest block. We found that

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

EDMP resulted in an increase in Oxy Hb in the bilateral PFC, indicating neural activation in these areas. However, the area activated and degree of increase in Oxy Hb varied among subjects. Many studies have found that stress resulted in activation of the PFC. Taken together with our results, this suggests that the EDMP cause stress, leading to activation of the PFC.

7F_01_P

ABSENCE OF ASSOCIATION BETWEEN GENETIC POLYMORPHISMS IN CRFR1 AND PTSD

Dinko Pavlinić[1], Goran Ćurić[1], Marijana Braš[2], Marta Đurković[3], Rudolf Gregurek[2], Pavo Filaković[3] and Gordan Lauc[1]

[1]University of Osijek School of Medicine, Department of Chemistry and Biochemistry, Osijek, Croatia. glauc@pharma.hr
[2]University of Osijek School of Medicine, Department of Psychiatry, Osijek, Croatia
[3]University of Zagreb School of Medicine, Clinic for Psychological Medicine, Zagreb, Croatia

One of the main mechanisms in the activation of the hypothalamo–pituitary–adrenocortical (HPA) axis is the release of corticotropin releasing hormone hormone (CRH) from the hypothalamus and it's binding to the corticotropin releasing hormone receptor (CRHR1) in the pituitary gland. Recently it was reported that two haplotype tagging SNPs (htSNP) in CRHR1 are associated with patterns of human alcohol drinking and could potentially contribute to the development of alcohol dependence. Aiming to see whether same genetic variations are associated with potential predisposition for the development of post-traumatic stress disorder (PTSD) we have investigated association of these two haplotypes of CRHR1 and PTSD. DNA was isolated from blood of 200 patients with diagnosed PTSD and 200 matching control individuals. TaqMan Pre-designed SNP genotyping assays were used to genotype two htSNPs (rs242939 corresponding to T to C exchange at position 44371356 and rs1876830 corresponding to C to T exchange at position 44386772 of Chromosome 17). Contrary to the situation observed in alcoholism, we were not able to find any association of CRFR1 genotype with PTSD since frequencies of all alleles were nearly the same in both studied populations. Interestingly, the observed frequency of the major allele at rs242939 corresponded to the frequency reported to correlate with low alcohol consumption, while the observed frequency of the major allele at rs1876830 was between the reported frequencies for low and high alcohol consumers.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7F_02_P

RISK FACTORS FOR POSTTRAUMATIC STRESS DISORDER

Virginia Olga B. Emery, Ph.D and Paul E. Emery, M.D.

Dartmouth Medical School, Department of Psychiatry HB 7750 U.S.A.

Research on risk factors in the development of Posttraumatic Stress Disorder (PTSD) can be conceptualized using three major paradigms: (1) risk due to preexisting psychopathology; (2) risk due to preexisting traits or characteristics, which fall along a normal-subclinical continuum; (3) risk due to preexisting childhood stress factors in family of origin. The research to be reported can be categorized under this third paradigm. The study to be reported was designed to compare antecedent stressors in homes of origin in a group of 20 Vietnam veterans diagnosed as having PTSD and 20 Vietnam veterans not meeting criteria for PTSD.
Samples were balanced on demographic variables, draft status, branch of service, rank, and type of discharge. Also, exposure to combat was controlled across samples. Findings suggest that veterans who developed PTSD had greater childhood stress related to parental alcoholism and parental unemployment than did non-PTSD counterparts. Additional findings are discussed as are methodological refinements needed to better hone in on relationship between family system stressors during primary socialization and risk for PTSD.

7F_03_P

RTM - NON VERBAL APPROACH IN HELPING CHILDREN VICTIMIZED BY THE TSUNAMI

Moshe Farchi

Tel Hai Academic College, Israel

Rauma means a circle in the local Singale language in Sri Lanka.
During January 2005 through march 2005 three Israeli delegation experts in post trauma where sent to assist the children victimized by the tsunami to cope with the trauma and the post trauma symptoms. The delegations where all sponsored by IsraAID – an NGO Israeli voluntary organization . Rauma-Trauma Model (R.T.M) was the main intervention model that was developed on sight and adjusted to the local community
Theoretical Background:
Literature describes several trauma intervention models. The described

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

intervention was based on two that integrates both the needs in one hand and the coping recourses on the other hand:

The Conservation of Resources (COR) Model (Hobfoll, 1989) is an integrated model of stress that encompasses several stress theories. According to the model, individuals seek to acquire and maintain resources, including objects (e.g., homes, clothes, food), personal characteristics (e.g., self-esteem), conditions (e.g., being married or living with someone provides social support, more financial security), and energies (e.g., time, money, and knowledge). Stress occurs when there is a loss of resources, or a threat of loss. For example, the model proposes that the tsunami victims – both children and adults lost all their belongings. Since belongings include the fishing boats livelihood recourses are gone. This leads to the loss of family roles, anxiety, passiveness and depression.

The conservation of resource theory posits that resources operate within an ecological context where feedback, sharing, and exchange operate between the individual, social context, and environment. Given these principles, it is reasoned that community interventions must acknowledge the solid base of most problems and accept that interventions must target resources and be intensive enough to change the ecology in which resources operate.

Lahad (1993) suggests a multi-modal model to understand mental resilience in stressful situations. The model was developed through ongoing work with a population living in the shadow of constant threat on their lives. Lahad states that through observing and interviewing people under stress it can be seen clearly that every individual has their own special combination of coping activities and resources.

The model relates to the six major characteristics or dimensions that he believes to be at the core of the individual's coping style, as summarized in the major theories: Belief and values, Affect and emotion, Social, Imagination, Cognition and thought, and Physiology and activities. Lahad called this model BASIC PH and stated that this integrative multi-modal model relates to the individual's coping style as a combination of all six dimensions.

Foa (1985) adds and suggests the prolonged exposure as a PTSD intervention and prevention program containing 3 major steps:

1. Psycho education interventions concerning common reactions to trauma and the cause of chronic post trauma difficulties.
2. Imaginable exposure repeated recounting of the traumatic memory (emotional reliving)
3. In-vivo exposure—gradually approaching trauma reminders (e.g., situations, objects) that, despite posing no harm, are feared and avoided.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7F_04_P

POSTTRAUMATIC STRESS DISORDER AND TERRORISM: WHAT DO WE KNOW?

Jonathan Laugharne

School of Psychiatry and Clinical Neurosciences, University of Western Australia, 16 The Terrace, Fremantle, WA 6160, Australia
jonathan.laugharne@uwa.edu.au

There is an increasing body of data relating to the psychological effects of terrorism, in particular relating to Posttraumatic Stress Disorder (PTSD). Evidence includes studies in the wake of the 9/11 attacks in the US in 2001, the Oklahoma bombing, as well as terrorist activity in Israel, and Kenya. Some caution must be exercised in drawing general conclusions from the data as numerous variables are operating including different socio-political contexts within which the attacks occur. Elevated rates of PTSD in the general population follow terrorist attacks but soon normalize whereas directly exposed populations have higher rates (20-38%) and more persistent symptoms. An increased risk of PTSD is associated with direct exposure, geographical proximity, female sex, low income, poor education, poor social supports, prior psychotropic drug use and high-level media reporting of events (for vulnerable individuals). Studies to date indicate a high degree of psychological resilience in all populations affected by terrorism.

7F_05_P

RISK AND PROTECTIVE FACTORS IN RELATION TO PTSD SYMPTOMS

Beth Spenciner Rosenthal and W. Cody Wilson

York College-City Univ of NY, 94-20 Guy R. Brewer Blvd., Jamaica, NY 11451, rosenthal@york.cuny.edu

The issues of risk and protective factors in relationship to manifesting PTSD symptoms have begun to be raised in the literature. A cross-sectional correlational study of 848 adolescents recently graduated from high school in New York City tested three hypotheses: 1) *witnessing* the physical assault of another (independently of being a victim) is traumatic and is associated with PTSD symptoms; 2) *having a sympathetic adult confidant available* is a protective factor with regard to PTSD symptoms for older adolescents; and 3) *having a sense of personal self efficacy* is a protective factor with regard to PTSD symptoms. Theoretical analysis

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

indicates that a "protective factor" may operate at three levels: 1) by reducing exposure to the "trauma event"; 2) by reducing levels of PTSD symptoms; and 3) by buffering or moderating the impact of the traumatic event on PTSD symptoms. The results of the empirical study indicate: first, that witnessing the physical assault of others produces PTSD symptoms (medium effect size); and second, that both "availability of an adult confidant" and "feeling of self efficacy" reduce the likelihood of an adolescent being exposed to physical assault (small effect size), reduce the level of PTSD symptoms (medium effect size), but do not significantly moderate the impact of exposure to physical assault on PTSD. Thus, both "having available an adult confidant" and "feeling of self efficacy" act as protective factors for older adolescents with respect to manifesting PTSD symptoms. The principal protective mechanism of both "availability of an adult confidant" and "feeling of self efficacy" appears to be through a general reduction of the level of PTSD symptoms.

7F_06_P

RELATIONSHIP BETWEEN TRAUMATIC BIOGRAPHIC EVENTS AND CURRENT PERSONALITY ORGANIZATION IN A SAMPLE OF REFUGEES AND ASYLUM SEEKERS

Lony Schiltz

Centre de Recherche Publique- Santé, Luxembourg

To estimate the effect of traumatic events, from the beginning of life up to recent stressors linked to natural catastrophes, war, political persecution and migration, we have undertaken an integrated clinical and experimental study with a sample of 73 refugees and asylum seekers. Every person had been seen individually for at least 8 hours.
We used a mixed methodology, combining a semi-structured biographical interview, a projective test, i.e. the Sentences Completion Test (Rotter & Willerman, 1949), for which we have developed a new manner of interpretation, and psychometric scales, i.e. the HADS (Zigmond & Snaith, 1983) and the Index of Wellbeing (Campbell & al, 1976). Furthermore, we analysed the artistic production (pictures, stories written under musical induction), realized by the people during the arts therapeutic sessions we offered to them after the test session, with the objective of helping them overcoming the break-up of their life project.
As our data belonged partly to the nominal and ordinal level of measurement, we largely used non parametric multidimensional statistics, like Non Linear Principal Components Analysis and multidimensional scaling. We could ex tract two profiles of personality functioning, linked either to repeated breaks, negligence and maltreatment from the beginning of life, or either to a recent external catastrophe, interrupting a

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

continuous life course. Through the evaluation of the arts therapeutic sessions, we could note the first signs of a resumption of the blocked process of subjectivation.
Our theoretical discussion, focused on the mode of action of arts psychotherapy, is based on an integration of recent research results in psychopathology and neurobiology. The results of the study allowed us proposing differential psychotherapeutic measures for the two profiles of traumatized people.

7F_07_P

THE INFLUENCE OF PRECLINICAL ANALGESIA AND SEDATION ON THE ONSET, SEVERITY AND COURSE OF POSTTRAUMATIC STRESS SYMPTOMS IN ACCIDENT VICTIMS

Michael Schönenberg, Ursula Reichwald, Kathrin Wilk, Heike Jacob, Gregor Domes, Andreas Badke & Martin Hautzinger

Department of Clinical and Developmental Psychology, University of Tübingen, Germany
Corresponding author: michael.schoenenberg@uni-tuebingen.de

We have previously demonstrated a strong association between the application of a single or fractionated dose of the N-methyl-D-aspartate antagonist ketamine and symptoms of acute (Schönenberg et al., 2007) and posttraumatic stress disorder (Schönenberg et al., 2005) in moderately injured accident victims. Ketamine has analgesic and sedative properties and for that reason it is widely administered in emergency care. Based on our previous data there is evidence to suggest that ketamine might profoundly augment early stress symptoms, probably via the enhancement of glutamatergic neurotransmission, resulting in worsened long-term posttraumatic sequelae. The objective of the study to be portrayed at the conference is to extend prior findings by prospectively examining ketamines' effects on psychological, endocrinological and neuro-cognitive variables. Moderately injured accident victims were consecutively recruited in two urban trauma centres. Based on their preclinical analgosedation, patients were divided into groups (ketamine vs. opioids vs. no medication). Initial assessments of posttraumatic psychopathology were carried out within 48 hrs post-event during hospital-stay and were repeated two months, six months and one year post-event. Further, neuropsychological assessments and a diurnal profile of the biological stress markers cortisol and dehydroepiandrosterone (DHEA) were determined at each time point. First results of the ongoing study will be presented at the conference.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7F_08_P

CORTISOL METABOLIC CLEARANCE IN POSTTRAUMATIC STRESS DISORDER

G.H. Trevor Wheler, David Brandon, Aaron Clemons, Crystal Riley, John Kendall, D. Lynn Loriaux, J. David Kinzie

Oregon Health and Sciences University, Portland, Oregon 97239 USA

Studies of glucocorticoid status in Posttraumatic Stress Disorder (PTSD) have hypothesized that these patients are maladapted to stress due to distorted feed back regulation of the HPA axis and/or altered sensitivity to glucocorticoid. However, cortisol levels in various biological fluids from PTSD subjects have been reported to be low, high or unchanged compared to controls. To address this issue, we have studied a group of patients with active PTSD symptoms, not currently on medication, in a controlled clinical study to determine the metabolic clearance rate of cortisol. PTSD symptomatology was confirmed by DSM IV criteria, and by the Clinician Administered PTSD Scale (CAPS). The production rate of cortisol (PR) was measured after constant infusion of tri-deuterated (d3) cortisol in normal saline administered at 20 µg per hour. Isotope dilution ratios of cortisol (d0) and d3-cortisol were determined by stable isotope dilution gas chromatography mass spectrometry using pooled serum samples as previously described (JCEM 91(9): 3486, 2006). Blood samples (3ml) were taken every 30 minutes for 24 hours. Serum cortisol was measured by radioimmunoassay. Daily metabolic clearance rate (MCR24h) was calculated from the daily PR using the following formula: MCR24h=PR/[cortisol]24h. The MCR24h for PTSD and controls was 122 ± 46.7 L/d·m2 (n=10) and 94.6 ± 33.4 L/d·m2 (n=10) respectively in agreement with reported values. There was no statistically significant difference between the MCR24h in PTSD compared with controls. We conclude that in the chronic and unprovoked state there is no demonstrable difference in production rate or metabolic clearance rate of cortisol, measured on a daily basis, in this group of PTSD subjects. This method provides a way to measure cortisol dynamics more specifically, and should be valuable in detailed assessments of psychological conditions such as PTSD.

7F_09_P

WORKLOAD, STRESS AND HEALTH AMONG MEDICAL STUDENTS

Eda Merisalu, Vuokko Vahtera, Martin Täll

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Deparment of Public Health, University of Tartu
Str Ravila 19, Tartu 50411, Estonia

The aim of the study was to analyse relationships between work load, stress and health complaints among medical students in Estonia
Method. The student study was carried out in late autumn 2005. The total study group consisted of 650 participants from the second to sixth course, whereas 470 medicine, 110 dentistry and 70 pharmacy students were included. Because of short learning experience the first course was not included into the study. The adapted anonymous questionnaire *Influence of studying on medical student's health* was used. The instrument was based on the questionnaire, used in official IFEMSA project since 1998. There were 66 questions subdivided into 8 subgroups: demographic data, general health evaluation, health, life style, work load, working conditions, communication and future vision. *Yes/no* answers, 10-point scale for stress and mainly 5-point scales were used. Also, opened questions were included.
Results. The response rate was 65,7%. Study group consisted of 427 respondents - 69% medicine, 19% dentistry and 12% farmacy students. Most part of them (82%) were females. Medicine students of younger courses had significantly higher work load than their older colleagues. A half of them spent on studies 8-10 and more hours. The students with long working days described significantly higher stress than the others ($p<0,001$). Very high stress (>9) was measured among 7%, high (6-8) - 45% and moderate (3-5) - 35,5% of respondents (average 5,4±2,3 points). The most part of students assessed their health good (61%) and average (22%). Tiredness (85%), sore eyes (57%) and headache (54%) were the main health complaints among medical students. Than more health complaints, the higher stress and lack of physical activities and hobbies ($p<0,001$).
Conclusion. Implementation of stress managing programs for medical students is very important.

7G_01_P

REPRODUCTION OF SOUTHERN MURIQUIS IN CAPTIVITY (*BRACHYTELES ARACHNOIDES*): ENDOCRINOLOGICAL ASSESSMENT OF REPRODUCTIVE FUNCTION BY MEASUREMENT OF FECAL STEROID METABOLITES

LIMA, A.B.F.[1]; VIAU, P. [1]; PISSINATI, A.[2]; CHELINI, M.O.M. [1]; OLIVEIRA, C.A. [1*]

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1. Laboratory of Hormone Measurements of the Department of Animal Reproduction of the Faculty of Veterinary Medicine of the University of Sao Paulo, Brazil; 2. Primate Center of Rio de Janeiro, Brazil
cadolive@usp.br

The southern muriqui (*Brachyteles. arachnoides*), the largest Neotropical primate species, is on the verge of extinction with critically endangered status (IUCN 2000). We validated noninvasive endocrine monitoring techniques for this species by quantifying fecal estrogens and progestins in 4 adult females as well as fecal metabolites of testosterone and glucocorticoids in 4 adult males and 1 sub-adult male. The study was conducted over a period of 11 months under two different environmental conditions: a) Two adult males, two adult females and a young male living on an island of 600 $m^{2,}$ with natural vegetation, at Curitiba Zoo (PPC) and b) two adult couples housed in a large cage of 15.40X5.85X4.70m at Rio de Janeiro Primate Center (CPRJ). Fecal samples were collected at least every other day over four continuous periods of 20 to 24 days with 45-day intervals between to subsequent sampling periods. Reproductive behavior was observed on the same schedule. Fecal steroids were extracted by dilution in ethanol. We used radioimmunoassay (RIA) in solid phase to quantify fecal estradiol, progesterone, testosterone, and glucocorticoid metabolites. Estrogen and progestin concentrations in female feces varied individually over a wide range (0.03 to 145.56µg/g of dried feces for progestins and 0.002 to 71.57µg/g of dried feces for estrogens). Three females did not show any ovarian activity over specific periods of the study, while ovarian cyclicity was observed over all sample periods in one female from CPRJ. Despite of their low levels of fecal reproductive steroids, all females displayed proceptive behavior and copulations. Surprisingly, despite of their semi-free ranging condition, the PPC males showed significantly higher concentrations of fecal glucocorticoids and lower concentration of fecal testosterone than the caged CPRJ males ($p<0.05$). As expected, the lowest concentration of testosterone metabolites ($p<0.05$) was found in the feces of the sub-adult male. For all males, copulations occurred when testosterone levels were the highest and peaks of glucocorticoid concentration were related to stressful situations such as copulation or fight. Therefore, this study shown that the quantification of fecal steroid metabolites was effective for reproductive monitoring as well as for assessing the physiological response to stressful events.

Key words: Endocrinology. Steroids. Metabolites. Primates. Stress.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7G_02_P

THE IMPACT OF SECONDARY SEX CHARACTERISTICS (SSC) AND SOCIAL BEHAVIOR TO CORTISOL AND SEXUAL HORMONES IN FEMALE PRIMATES

B. Wallner[1], J. Dittami[2] and I. H. Machatschke[2]

[1]Dept. of Anthropology, [2] Dept. of Behavioural Biology, University of Vienna, Austria

Primates living in multi male–female associations can develop SSC during sexual active periods. Size of SSC is dependent on fluctuating sex-steroids. Males intensify socio-sexual contact with females showing exaggerated SSC.
We investigated the socio-endocrine impact of SSC in three different species: Facial and anogenital redness in Japanese macaques (JM) during the breeding and non-breeding season, perineal swellings in Barbary macaques (BM) treated with the contraceptive levonorgestrel during non-sexual periods, and perineal swellings in chimpanzees (Ch) during intact cycle periods. Data from JM and BM were collected under semi-natural, from Ch under caged conditions. Fecal samples were used for analyses of sex steroid and cortisol (CORT) metabolites.
In BM, multiple regressions showed a negative relationship between SSC size and CORT and a positive one between swelling size and intersexual socio-positive contact. Females with enlarged SSC had lower progesterone levels and increased estradiol-progesterone ratios.
Ch females housed with males showed decreased CORT during late and decreasing tumescence of the SSC. In single housed individuals, increased CORT was related to decreased plasma FSH. Paired females had shorter cycle length.
In JM, a light and a dark group could be discerned with regard to redness. In the dark group, intensity of redness increased during the breeding season and correlated with the amount of socio-sexual behavior. These females had significantly elevated CORT and sex steroid titers.
In conclusion, reduced HPA activity in females is associated with exaggerated SSC and intensified male contact when male mating competition for females is diminished (e.g., BM, Ch).

7H_01_P

COMPLEMENTARY AND ALTERNATIVE MEDICINE (CAM) USE AFFECTS STRESS AS REPORTED BY RETINITIS PIGMENTOSA (RP) PATIENTS

Ava Kiser; Liancheng Yang; Gislin Dagnelie

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Johns Hopkins Wilmer Eye Institute; 550 N. Broadway, 6th floor; Baltimore, MD 21205; abittne1@jhmi.edu

Benefits of CAM-related interventions have been demonstrated for patients with chronic diseases in which stress and disability are prevalent. RP patients experience nightblindness and a slowly progressive loss of vision, which eventually leads to bare or no light perception in advanced stages. They commonly indicate that they have "good" and "bad" vision days, stating that stress causes a decrease in vision, and that vision improves when the stress is alleviated. We assessed CAM use by RP patients and its perceived effectiveness. A survey posted on an anonymous internet forum was completed by 96 RP patients internationally. 95% of respondents tried at least one of 9 CAM areas, and 85% of these respondents felt that CAM affected their stress/anxiety levels. 31%, 47%, 17% and 74% of respondents used yoga, meditation, mind-body therapy, and spirituality/religion, respectively. Stress/anxiety levels were subjectively affected in the following proportions of those who used these therapies: 93% Yoga, 92% Meditation, 87% Mind-Body Therapies, 84% Spirituality/Religion, 80% Massage Therapy, 78% Energy Therapies, 78% Herbal Therapies/Aromatherapy, 64% Movement Therapies, and 56% Acupuncture. A slim majority (~50%) felt that stress was affected: a little with acupuncture/mind-body/aromatherapies/herbal therapies; moderately with meditation/yoga/spirituality/religion/massage/energy therapies, and by a large amount with movement therapies. RP patients are using CAM modalities, and a large majority attributes some impact upon stress/anxiety; therefore clinicians and researchers should be aware of their use. Clinical trials with CAM interventions for RP patients are necessary to attempt to validate these findings.

7H_02_P

THE INFLUENCE OF PRACTICING TAI CHI CHUAN AND AEROBIC EXERCISE ON JOB-RELATED STRESS AND BURNOUT AMONG HIGH-TECH WORKERS IN ISRAEL

Eitan Raveh, M.OccH, Joseph Ribak, PhD

Occupational Health Department, Faculty of Medicine
Tel Aviv University, Israel
Eitan76@hotmail.com

Background: Working in high-tech companies is characterized with long working hours and high demands, factors that strongly effect the levels of occupational stress and burnout. The use of aerobic exercise or relaxation

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

techniques, in order to reduce Job-related Stress and burnout was described in medical literature. Tai Chi Chuan, an ancient Chinese martial art, combines slow movements with controlled breathing, and requires concentration and serenity. Studies have shown that the practice of Tai chi Chuan has similar effects on the cardiovascular system as in moderate aerobic exercise, but it also generates additional benefits of stress reduction and peacefulness.
Objectives: To evaluate the influence of practicing Tai Chi Chuan compared to other aerobic exercise on occupational stress and burnout among high-tech workers in Israel.
Methods: 40 Israeli high-tech workers, practicing Tai Chi for 1-15 years, have received self-report questionnaires in order to evaluate levels of occupational stress and burnout. The questionnaires included a demographic questionnaire regarding daily work and leisure-time physical activity habits, the Karasek job-related stress questionnaire (JCQ) and the Shirom-Melamed Burnout Measure (SMBM). 87 other Israeli high-tech workers, who don't practice Tai Chi, received identical questionnaires. According to their answers, the control group was divided to two groups: the people on the first group are doing physical aerobic exercise, and those on the second group don't practice any exercise.
Results: Statistical analysis showed significant differences between the study group and the control groups in cognitive burnout outcomes. In addition, the control group not doing any physical exercise, was found to have larger percentage of workers with burnout values higher than normal values. A positive correlation was found between job-related stress and burnout in all three groups. Among Tai Chi practitioners, relationships were found between age, years and level of practice and burnout outcomes. No significant difference was found between the three groups in the stress outcomes.

7I_01_P

THE IMPACT OF A SURVEY BASED WORKPLACE INTERVENTION PROGRAM ON EMPLOYEE HEALTH, BIOLOGICAL STRESS MARKERS, AND PRODUCTIVITY

Ingrid L. Anderzén, PhD, and Bengt B. Arnetz[1], MD, MPH, MScEPI, PhD

Uppsala University, Uppsala Sweden and [1]Uppsala University and Wayne State University, Detroit

Increasing employee health and participation have been identified as crucial to achieve productive organizations. However, there are few studies assessing methods to improve psychosocial working conditions, employee health and well-being as well as productivity. This study assessed the impact on health, biological stress markers, absenteeism and

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

productivity from a one year structured intervention program based on group-specific psychosocial results from 22 work units in five different offices. White collar employees representing 22 different work units were assessed before, and one year after, a one-year intervention program based on group-specific psychosocial work-related results. Self-rated questionnaire data (QWC), biological blood measurements and hard-core registry data on sickness absenteeism and productivity were analyzed at baseline and at the one-year follow-up. Employee ratings of the psychosocial work environment showed a significant statistical improvement in performance feedback, participatory management, employeeship, skills development, efficiency, leadership, employee well-being, and work-related exhaustion, ($p < 0.05$). Restorative hormones, such as serum testosterone levels, increased during the intervention ($p < 0.01$) and changed levels of serum testosterone were significantly correlated to overall changes in organizational well-being during the year ($r = .51$, $p<.01$). Absenteeism decreased, and productivity improved. The study presents practical means for occupational health practitioner to participate in the management process of creating healthy work organizations. Fact-based psychosocial workplace interventions are suggested to be an important process for enhancing employee well-being as well as organizational performance.

7I_02_P

BOOSTING PROGRAM FOLLOWING STRESS INOCULATION: EFFECTIVE MEANS TO REDUCE MEDICAL STUDENTS STRESS

Stephanie Bughi, Hoang-Anh Phan, Devi Navamani, Stephanie Rothman, Zaw Aung, & Stefan Bughi

Rancho Los Amigos National Rehabilitation Center, Downey, California, USA

Introduction: Stress is known to be associated with the medical profession and starts during the medical training. Stress inoculation was reported to significantly decrease the students reported stress level. However, "resistance" to stress inoculation is common, if students do not practice stress coping techniques. To help students manage stress a boosting program: students to educate patients on stress management (SPESM) was implemented. Present study reports the results of this program. **Methods**: The study subjects (n=28) are 3rd and 4th year medical students (MS), who had one-month rotation in a medical-surgical unit, at a university affiliated program in Southern California. MS' stress level was measured at the beginning and at the end of the rotation by using the General Well-Being Scale (GWBS). The total GWBS stress score

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

(SS) ranges from 0 to 110. A score <72 = stress, and < 60= distress. Following the stress inoculation the MS were advised to implement SPESM. **Results**: 16/28 (57%) of students implemented SPESM once/week (Group 1), and 12/28 (43%) more than twice/week (Group 2). The SS significantly increased in group 2 (78+/-13.2 vs 84.8+/-11.2, p<0.005), but not in group 1. The prevalence of stress/distress did not change in group 1, but significantly decreased in group 2, from 41.7% to16.7%, p<0.05. **Conclusion:** "Resistance" to stress inoculation can be successfully overcome by using a boosting program (SPESM). Adequate management of stress during the medical training will serve as blueprints for managing stress later on during medical profession with beneficial effects on mental and physical health and subsequent improvement in health care delivery.

7I_03_P

JOB STRESS AND PREHOSPITAL DELAY IN WOMEN AND MEN WITH ACUTE CORONARY SYNDROME

Yoshimi Fukuoka,[1] PhD, Kathleen Dracup[1] DNSc, Linda Groah[1] MSN, Erick Kyriakidis[2] MD[3]

[1]University of California San Francisco CA, [2] Saint Thomas Hospital, Nashville TN, USA
Yoshimi.Fukuoka@nursing.ucsf.edu

Approximately 50% of all heart attack deaths occur before the patient reaches the hospital. Shortening prehospital delay time from symptom onset to hospital arrival and calling an ambulance are important to reduce mortality and morbidity during cardiac events. The purpose of the study is to examine the association between job stress/working hours and prehospital delay (time interval from symptom onset to hospital arrival) and ambulance use in patients with acute coronary syndrome (ACS) in employed women and men. **Methods:** In this cross-sectional study, a total of 152 consecutive patients (40 women and 112 men) who were admitted with ACS and worked more than 20 hours/week were recruited. A structured interview was conducted to assess their job stress (job demand, job control, social support at work), working hours and symptom onset time while patients were still in the hospital. The average age for women and men were 50.6 (SD8.5) and 51.0 SD (6.7) respectively. **Results:** Women had significantly lower job control and shorter working hours/week compared to men (p < .05). The median prehospital delay from symptom onset time to hospital arrival was 6.4 hours in employed women and 4.0 hours in employed men. While only 10.0% of employed women used an ambulance, 20.5% of men used an ambulance during cardiac events. Female gender and daytime shift work were significant

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

predictors of not calling an ambulance during ACS, after controlling for age, health insurance, severity of illness, working hours, and job control ($p < .05$).
Conclusions: These reported gender differences in job stress and other job factors support the need to educate women as to how to respond to acute cardiac events.

7I_04_P

WORK-RELATED STRESS AMONG FEMALE STAFF IN CROATIAN HOSPITALS

Rajna Golubic[1], Bojana Knezevic[2], Milan Milosevic[1], Ana Radonic[2], Marin Belak[3], Predrag Knezevic[4], Jadranka Mustajbegovic[1]

[1] *Department for Environmental and Occupational Health, "Andrija Stampar" School of Public Health, Rockefellerova 4, Zagreb, CROATIA*
[2] *Clinical Hospital Centre Zagreb*
[3] *Clinical Hospital Sisters of Mercy, Zagreb*
[4] *Clinical Hospital Dubrava, Zagreb*
rajna.golubic@gmail.com

Aim: To determine the difference in stress perception among female staff at various hospital departments. Methods: 984 female employees in 4 Croatian hospitals anonymously and voluntarily completed the Occupational Stress Assessment Questionnaire. According to the factor analysis the stressors were classified into 6 groups: Work management, Public criticism, Shift work, Professional demands, Interpersonal communication and Professional health hazards. The differences were analyzed by Mann Whitney U test and $P<0.05$ was regarded as significant. Results: 200 (20.3%) of the analyzed female staff belonged to the category physicians, while the remaining 784 (79.7%) were technicians and nurses. At all hospital departments physicians made the minority of the entire female staff, with the exceptions of anesthesiology and pathology, where they made 62% and 53.8% of the total number of female employees, respectively. Public criticism ($P<0.001$), shift work ($P=0.03$) and professional demands ($P=0.003$) were considered significantly more stressful by physicians than by nurses and technicians. However, nurses and technicians reported professional health hazards as significantly more stressful than physicians ($P=0.002$). The perception of stress related to work management and interpersonal communication didn't significantly differ among professional categories. Conclusion: Physicians, on one hand and nurses and technicians on the other hand find their job stressful, but the perception of the same stressors among female staff is different.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7I_05_P

THE EFFECT OF ORGANIZATIONAL CHANGES ON HEALTH AND SICKNESS ABSENCE AMONG HEALTH CARE EMPLOYEES - A LONGITUDINAL STUDY MEASURING BIOLOGIC STRESS HORMONES, INDIVIDUAL AND WORK SITE FACTORS

Ann-Sophie Hansson[1] , Eva Vingård[2] , Bengt B Arnetz[3], Ingrid Anderzén[1]

[1] Department of Public Health and Caring Sciences, Uppsala University, Sweden;
[2] Department of Medical Sciences, Uppsala University, Sweden; [3] Division of Occupational and Environmental Medicine, Wayne State University, Detroit, USA.

A series of organizational changes during the last decades in health care organizations have resulted in an uncertain and turbulent working life with negative effects on health and job satisfaction. The aim of this longitudinal study was to assess affects on health and sickness absence among health care employees after organizational changes. The population consisted of 278 employees divided in a study group, structurally concerned of the changes and a control group. The response rate was 81% and 74% respectively at baseline and follow up measurements. Group differences were analyzed both for an open (n= 226) and closed cohort (n= 156) using one-way ANOVA as well as a two-way ANOVA for repeated measurement in a closed cohort. To explain predictors for changed health a stepwise linear regression analysis was used. The results in the open cohort showed that the study group experienced significantly worse self-rated health and worse work satisfaction after the reorganization compared with the control group. The study group had also increased their level of work related exhaustion. The results from the closed cohort showed that the recovery hormone DHEA-S had significantly decreased and sickness absence increased among employees in the study group compared to those in the control group. Factors that best predicted changed health after the reorganization were *work related exhaustion*, *age* and *coping ability.* Sickness absence had increased significant for the study group at the 1-year follow up (7% and 2% respectively).

7I_06_P

STRESSOR TRAITS&STRESS-REDUCTION IN WORKING AND NON-WORKING MOTHERS

Mohammad Hatami

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

The present study aims to evaluate the effect of stress-reduction meichenbaum technique on working nurses, teachers and non-working housewife mothers according to **mother–child** stressor traits- **Adaptability, Acceptability, Demandingness, Mood, Hyperactivity / Distractibility, Reinforces, Depression, Attachment, Restrictions of role, Sense of competence, Social isolation scale, Relationship with spouse, Parental health.**
First, 228 working and non-working mothers were matched and they were evaluated using "parenting stress index" and "occcupational stress index". Second, 24 mothers with high level stress shared in two matched groups of experimental and control. Experimental group were under treatment of stress-reduction technique for 13 weeks but control group didn,t received any treatment. The level of stress in both group was assessed before and after the treatment. In order to analysis of data,besides of the basic statistical methods. The result of this study showed that
(1) there was significant difference between stress among working mothers and non-working mothers as well.
(2) the nature of stressor in expanding of the level of stress in mothers had different effects, while, **demandingness** and **relationship with spouse** in nurses, **demandingness and restrictions of role** in teachers and **demandingness** and **depression** in non-working mothers had more contribution in these subjects.
(3) the result of stress-reduction technique was effective in reducing of life, occupational and a few stressor traits.

7I_07_P

WORK STRESS OF SOCIAL WORK MANAGERS IN TURKEY *

Vedat Isikhan **

University of Hacettepe, Department of Social Work, Ankara, Turkey

Management process of the institutions which offer service to solve the problems of individuals and families may have some particular difficulties. Foremost among these are problems faced by the managers when they try to bring together the different type of professionals on behalf of individuals who have constant psycho-social and economic problems . Managers are constantly experiencing stress when trying to deal with

* ***Vedat Isikhan**, Associate Professor University of Hacettepe, Department of Social Work, Ankara Turkey*
** *This research was supported by Research Fund of Hacettepe University, Turkey (Project Number: 0.01.705.001).*

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

these problems.
This study aims to determine the factors that influence work stress of social work managers in Turkey. The data have been collected by questionnaries sent to 554 social work managers by mail. For statistical analysis, questionnary forms of 320 managers were considered to be eligible.
The important results of the study are as follows: The great majority of managers have high level of work stress and Type A personality. In work stress, marital status, job satisfaction, overall duration of work, administrative position, lack of autonomy, lack of personal support from the colleagues and obligation to attend many meetings and interviews are found to be influential factors ($p<0,05$).
Further studies are required to increase both the quality of service offered and the quality of life of social workers and social work managers and in the long term to determine the organizational and non-organizational sources of stress.

This research was supported by Research Fund of Hacettepe University, Turkey (Project Number: 0.01.705.001).

7I_08_P

WORK-RELATED STRESS AND GENDER DIFFERENCES AMONG CROATIAN HOSPITAL PHYSICIANS

Bojana Knezevic[1], Milan Milosevic[2], Rajna Golubic[2], Ljiljana Belosevic[3], Lana Matec[4], Jadranka Mustajbegovic[2]

[1]Clinical Hospital Center Zagreb, Salata 2, Croatia, bojana.knezevic@inet.hr
[2]Department for Environmental and Occupational Health, "Andrija Stampar" School of Public Health, University of Zagreb, Croatia
[3]Croatian Ministry of Defense
[4]Medical School University of Zagreb

INTRODUCTION: Psychological distress among healthcare professionals has negative effects on the quality of care provided to patients. Physicians are in general subjects to multiple stressors. AIM: To reveal the differences in reporting work-related stressors between female and male physicians in hospitals. METHODS: 370 physicians (200 females and 170 males anonymously) and voluntarily completed the *Occupational Stress Assessment Questionnaire*. The stressors were related to: Work management, Shift work, Professional demands, Interpersonal relationship, Public criticism and law suits, and Professional hazards. Questions were graded with Likert Scale from 1 to 5. The differences were statistically analyzed by Mann Whitney U test and $P<0.05$ was considered

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

significant. RESULTS: An average age of female physicians is 41 years (26-60) and average age of male physicians is 43 years (26-63). Male physicians are more frequently clustered in surgery and radiology. Both groups of physicians reported: Work organization, Public criticism and law suits and Professional demands as stressful or very stressful. Shift work is significantly more frequently (*P*=0.003) reported by male doctors vs. female doctors. CONCLUSION: Both groups, male and female physicians, in hospitals are experiencing high level of stress. Male physicians considered shift work more stressful than females, because they are clustered in more demanding specialties.

7I_09_P

HEART RATE VARIABILITY IN PHYSICIANS WORKING ON NIGHT CALL

Malmberg, B. MD[1], Persson, R. PhD[2], Ørbaek, P. MD[2]

1 Department of Occupational and Environmental Medicine, Lund University Hospital, Sweden
2 National Research Centre for the Working Environment, Copenhagen, Denmark.
Corresponding author: Birgitta.Malmberg@med.lu.se

Shiftwork and stress have negative effects on performance, well-being and sleep, and are associated with an increased risk for ischaemic heart disease and metabolic disturbances. Physicians often work in shifts, but little is known about possible physiological effects of night call duty. The aim of this study was to investigate the impact of physicians' night call duty on autonomic balance, measured by heart rate variability (HRV), and to determine whether there were differences between physician specialities. For this reason 19 anaesthesiologists were compared with 17 paediatricians and ENT surgeons. Monitoring by digital holter electrocardiogram was made during night call, the recovery period after night call, and on an ordinary workday. HRV analyses were made in 24 hour periods for SDNN (time domain measurement of total variability), and in 10 min strips during 1 h at 21:00-22:00 for HFn (frequency domain variable, reflecting vagal influence on HRV). Statistically significant effects of day in HFnu were seen only among the anaesthesiologists, who had 21% lower levels during night call compared with day work, and 38% lower compared with post night call (P<0.001 for all). Further, the anaesthesiologists had 27% lower levels than the pediatricians/ENT-surgeons during night call (p=0.008). Total variability (SDNN) for 24 hours, did not differ between pshysician groups or day work versus night call. In conclusion, the lower relative influence of parasympathetic activity (HFnu) in the evening during night call for anaesthesiologists may indicate

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

a higher stress activation compared with pediatricians and ENT surgeons.

7I_10_P

DIFFERENCES IN STRESS PERCEPTION BETWEEN PHYSICIANS IN SURGICAL AND NON-SURGICAL SPECIALTIES

Milan Milosevic[1], Bojana Knezevic[2], Rajna Golubic[1], Jadranka Mustajbegovic[1], Lana Matec[3], Maja Debeljak[3]

[1]Department for Environmental and Occupational Health, "Andrija Stampar" School of Public Health, Rockefellerova 4, Zagreb, CROATIA; e-mail: milan.milosevic@mef.hr
[2]Clinical Hospital Centre Zagreb
[3]Medical School University of Zagreb

Aim: To investigate differences in stress perception between physicians in surgical and non-surgical specialties. Subjects and Methods: 212 physicians in surgical and 158 in non-surgical specialties anonymously and voluntarily completed the Occupational Stress Assessment Questionnaire in four Croatian clinics. Questions regarding stress were graded by the Likert Scale from 1 to 5 and due to factor analysis classified in six items: Work management, Public criticism, Shift work, Professional demands, Interpersonal relationship at workplace and Professional health hazards. Items were used as dependent variables in multivariate analysis of variance. Results: Significant difference was found between surgical and non-surgical specialties on the combined dependent variables: F (6, 363)=11.6, $P<0.001$; Wilks Lambda=0.84. When the results for the dependent variables were considered separately, the only differences to reach significance, using a Bonferoni adjusted alpha level of 0.008 were: Professional health hazards: F (1, 368)=41.4, $P<0.001$, Public criticism: F (1, 368)=7.5, $P=0.006$ and Shift work F (1, 368)=33.7, $P<0.001$. Inspection of mean stress scores (M ± SD) confirms that surgical specialties, compared to non-surgical, have higher grades regarding professional health hazards (2.7 ± 0.9 vs. 2.1 ± 0.7), public criticism (3.4 ± 0.9 vs. 3.1 ± 0.9) and shift work (3.1 ± 1.1 vs. 2.4 ± 1.2). Conclusion: Physicians in surgical specialties perceive higher level of stress due to higher exposure to professional health hazards, public criticism and shift work.

7I_11_P

INTENSIFYING INFLUENCE OF NIGHT SHIFT WORK ON CATHECHOLAMINE METABOLISM

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Fumihisa Miyauchi

Ehime Rosai Hospital, 13-27 Minamikomatsubara, Niihama, Japan
miyauchi@ra2.so-net.ne.jp

"Working at night affects not only physical condition but also ovarian function" has been reported previously. To investigate the influence of working at night on adrenergic system, concentrations of catecholamine in plasma and urine were examined during the daytime and at night. Seventy seven nurses, who enrolled voluntarily in this study, involved shift works rotating from 8:15 to 17:00 (daytime shift), from 15:45 to 0:30 (evening shift) and from 0:00 to 8:45 (night shift). Blood was obtained before and after shift work, and urine was collected for 24 hours from the start of shift work. Concentrations of dopamine, noradrenaline and adrenaline were measured by HPLC. Plasma concentrations of dopamine were decreased after both works of daytime shift (16.3 +/- 3.2 to 9.7 +/- 1.1 pg/ml) and night shift (13.3 +/- 3.8 to 9.4 +/- 0.9). Plasma concentrations of noradrenaline were decreased after daytime shift work (316.1 +/- 17.0 to 263.6 +/- 16.5 pg/ml), but not changed after night shift work (264.3 +/- 29.6 to 259.0 +/- 15.2). Plasma concentrations of adrenaline were increased after both works of daytime shift (31.5 +/- 3.0 to 48.3 +/- 7.1 pg/ml) and night shift (34.7 +/- 6.4 to 40.8 +/- 5.0). Total amounts of urinary secretion of dopamine, noradrenaline and adrenaline were significantly lower in nurses of night shift work than those in nurses of daytime shift work, respectively. These results suggest the possibility that working at night would affect central nervous system and adrenal function, and intensifies metabolic clearance rate of catecholamine.

7I_12_P

DAILY LIFE STRESS AND MENTAL HEALTH IN HOSPITAL EMPLOYEES

Ohtani, Yoshio [1], Shimada, Eiko [2], Suzuki, Makihiko [3]

1) Kitasato University Schol of Allied Health Sciences; Sagamihara-Shi, Kanagawa, Japan; y-ohtani@kitasato-u.ac.jp
2) Knanagawa Prefectural Center of Psychiatry, 3) Kitasato University College of Liberal Arts and Sciences

Introduction: Workers in a medical facilities may be greatly affected in their mental and/or somatic conditions, although they labor to help patients with various diseases. In this study we investigated how daily life stresses in the hospital employees influence on their mental health, sleep

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

and several phases of anger.

Methods:

1）) Subjects: One hundred and forty-eight emloyees in number of the Hospital in a big city of Japan (Male 39, Female 80, Unclear 29; 46.2 years old of mean age) consist of 18 in Medical, 94 in Nursing, 17 in Secretary Division and Nuclear 19.

2) Materials for the assessment of mental and/or psychological conditions

(4) Daily Life Stress Scale (Munakata et al., 1985)
(5) Genral Health Questionaire (GHQ-60)
(6) Athens Insomnia Scale
(7) State-Trait Anger Expression Inventory

3) Statistical analysis: Multiple comparison by Bonferoni method using the score of Daily Life Stress Scale in 4 step groups [Weak(W);0-4 points, Moderate(M); 5-9, Rather Strong(RS);10-18. Strong(S);□19] as an independent variable and the total points of GHQ, Insomnia scale and 5 phases of STAXI[State(STX-S),Trait(TX-T□,Anger Expression-Control (AE-C)□AE-Out (AE-O)□AE-In (AE-I)] as dependent variables:

Results and Discussions:

1. In the total score of GHQ, the points of S group was significantly higher than those of W, M and RS, as well as RS group's was higher than W group's□p<0.01□.This means that the higher score in
2. As to insomnia, the score of S group was significantly higher than those of W, M and RS groups, and M's was higher than W'sp<0.01. And RS's was higher than W'sp<0.05. So the data proves the positive corelatio between the degrees of stress and insomnia.
3. Regarding anger, S group showed a significant high score to W, M and RS's in STX-S and STX-T, as well as to W in AE-Ip<0.01. Moreover, RS group tended to show a higher score to M's in AE-Ip<0.05. There were not staistically found any significant corelation either in AE-C or AE-O. These findings indicate that those who have a strong stress are greatly angered at that time, have an irascible personality and tend to hold in their temper without expressing outside.

7I_13_P

JOB STRAIN AND CARDIOVASCULAR RISK FACTORS IN PHYSICIANS

Laura Poanta[1], Mihai Ober[2], Anca Farcas[2]

[1]Department of Internal Medicine II, [2]Department of Internal Medicine I, Clinicilor 2-4 street, Cluj-Napoca, Romania, correspondence e-mail: laurapoanta@yahoo.com

Background: with the development of modern civilization, occupational

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

stress becomes increasingly important. Physicians are an exposed category and they are prone to develop stress-related disease and risk behaviors.

Methods: we conduct a study among 118 physicians, 78 women and 40 men. The Romanian version of Job Content Questionnaire (JCQ), was mailed to all the subjects. We investigate the main scales of JCQ: decision latitude (DL), psychological job demands (PJD) and social support (SoS), along with the prevalence of smoking, sedentary behavior, cholesterol, triglycerides, fibrinogen, CRP and plasma cortisol.

Results: The final response rate was 112 (94.9%), 72 (64.2 %) women and 40 (35.7 %) men aged between 30 and 60. Results are shown in the table.

Smoking (32% of women and 56% of men) and sedentary behavior (over 60 % both in women and men) positively correlated with PJD and negatively with SoS. Total cholesterol positively correlated with PJD in both women (r=0.33) and men (r=0.51), and negatively with coworker support and depression (r=-0.21, r=-0.28 in women, and r=-0.57, r=-0.19 in men, respectively). We didn't find significant correlations of those items with proinflammatory state, but we did find interesting correlations with plasma cortisol (e.g. negative correlation with coworker support both in men and women, $P<0.05$).

Discussions: The issue of professional stress is very important in modern medicine. Age and gender can be determinants of job strain, but also individual job characteristics. Many studies shown positive relations between job strain and some harmful habits such as tobacco smoking and sedentary lifestyle, as well as with other risk factors like dyslipidemia and proinflammatory state; we have to outline again the importance of organizational factors in managing both stress and satisfaction at workplace.

ITEM	*DL*	*PJD*	*SoS*	*Cholesterol (mg%)*	*Triglycerides (mg%)*	*HDLc (mg%)*	*Fibrinogen (mg%)*	*CRP mg/l*	*Cortisol mcg/dl*
mean	71.41	35.25	23.63	180.48	81.40	56.04	333.40	4.84	412.23
SD	8.90	5.53	6.41	31.81	41.83	12.30	135.58	6.80	171.30

Legend: DL = decision latitude, PJD = psychological job demands, SoS = social support, HDLc high density lipoprotein, CRP = C-reactive Protein

---

7I_14_P

TREATING PUBLIC SPEAKING ANXIETY IN BRAZILIAN ORGANIZATIONS: A COMPARATIVE EVALUATION OF TREATMENTS

Ana Maria Rossi

International Stress Management Association - Brazil (ISMA-BR)

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Rua Padre Chagas, 185 cj. 1104
90.570-080 Porto Alegre, RS Brazil
Phone: (55)(51) 3346.2568 / Fax: (55)(51) 3222.8598
E-mail: stress@ismabrasil.com.br

Research on public speaking anxiety suggests that choice of therapeutic treatment should be made according to specific individual needs and personal characteristics. Specific research on treatment efficacy indicates that while some individuals do benefit somewhat from any specific treatment, results can be significantly increased by using the therapeutic treatment that is specifically adapted to the particular needs of each person. This study was designed to evaluate an integrative approach (IA) that aimed to address the different individual needs and allowed the individual to use his/her own creative powers of visualization to overcome public speaking anxiety at work. 150 public speaking anxious employees were randomly assigned to two treatments and a control group. Measurements included self report and physiological measures. Physiological measures (skin temperature, galvanic skin response and heart rate) were taken before and after treatment. Results indicated that the IA approach produced significantly lower self report measures of stress than either the control group or the PMR group. All treatment groups showed a significant lowering of psysiological measures from pre-test to post-test.

Keywords: PSA, integrative approach, physiological measure

7I_15_P

OCCUPATIONAL STRESSORS AND ITS ORGANIZATIONAL AND INDIVIDUAL CORRELATES: A NATIONWIDE STUDY OF NORWEGIAN AMBULANCE PERSONNEL

Tom Sterud[a], Erlend Hem[a], Øivind Ekeberg[a], Bjørn Lau[b]

[a]*Department of Behavioural Sciences in Medicine, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Norway*
[b]*National Institute of Occupational Health, Oslo, Norway*

Corresponding author:
Tom Sterud, Department of Behavioral Sciences in Medicine, PO Box 1111 Blindern, NO-0317 Oslo, Norway
e-mail: tom.sterud@medisin.uio.no

Objectives: We compared the severity and frequency level of organizational and ambulance-specific stressors, and studied their

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

relationship to structural conditions and individual differences in a nationwide sample of operational ambulance personnel (n = 1180). *Methods*: The questionnaire included the Job Stress Survey, the Norwegian Ambulance Stress Survey, the Basic Character Inventory, General Self-Efficacy Scale, and questions addressing structural work conditions. *Results*: Serious operational tasks and physical demands were identified as the two most severe stressors. Lack of support from co-workers was the most severe and frequent organizational stressor. Higher frequency of stressors was most strongly associated with size of service districts (β ranging between .18 and .30, $p < .01$) and working overtime (β ranging from .13 to .27, $p < .05$). Neuroticism (β ranging from .09 to .17, $p < .01$) and low general self-efficacy (β ranging from −.12 to −.16, $p < .001$) were equally strongly related to severity of stressors, as were structural conditions. Conclusions: Ambulance-specific stressors were reported as both more severe and more frequently occurring stressors than were organizational stressors. Structural working conditions were more strongly related to frequency of job stressors, but higher levels of neuroticism, and lower levels of self-efficacy were equally strongly related to stressor severity, as was structural working conditions.

7I_16_P

WORKPLACE STRESS AMONG HEALTH CARE WORKERS

Nóra Szabó[1,2], Katalin Hegedűs [2], Gábor Szabó[2], Krisztina D. Neculai[2], Gyöngyvér Salavecz[2]

[1]Institute for Basic and Continuing Education of Health Workers, Budapest, Hungary
[2]Institute of Behavioral Sciences, Semmelweis Universit,y, Budapest, Hungary
1089, Budapest, Nagyvárad tér 4, Hungary
E-mail: enoraszabo@freemail.hu

Antecedents: Work of nurses is extremely stressful and is combined with low professional and social acknowledgement, but is there any difference in terms of workplace stress between two groups of nurses in similar position? Aim and hypothesis: The aim of the survey is to investigate comparatively hospice nurses and nurses caring for elderly patients. Hypothesis: due to interdisciplinary approach hospice nurses are in a more favourable situation than nurses caring for elderly patients in terms of degree of workplace stress and social support. Methods: A cross-sectional study was performed among hospice nurses (N=25) and nurses caring for elderly patients (N=50) using a test battery comprising the Brief Stress and Coping Inventory (Rahe, Tolles, 2002), the Vital Exhaustion

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Questionnaire (Appels, Mulder, 1988), the Support Dimension Scale (Caldwell, 1987), and the Effort-Reward Imbalance Questionnaire (Siegrist, 1996). Expansion of the sample is being in progress with data of Hungarostudy Epidemiological Panel. Results: In terms of social support hospice nurses are in a more favourable position (P=0.048). Workplace stress is significantly higher in nurses caring for elderly patients than in hospice nurses (P=0,035 for overcommitment scale, P=0.034 for extrinsic effort scale). Conclusions: Interdisciplinary approach of hospice may promote nurses' acceptance and appreciation, whereas higher social support may reduce nurses' vital exhaustion and the degree of workplace stress. This model might be applicable for other groups of health care staff as well.

7I_17_P

STAFF STRESS IN BATHING RESIDENTS IN JAPANESE AGED CARE FACILITIES

Edit Nagy-Tanaka, Mio Ito, Ryutaro Takahashi

Tokyo Metropolitan Institute of Gerontology, 35-2 Sakae-cho, Tokyo 173-0015 Japan,
E-mail: edit@tmig.or.jp

Bathing residents in long-term aged care facilities, especially residents with dementia, is a stressful task. Bathing residents in a bathtub, usually twice a week, which is a common practice in Japan, is particularly demanding. The purpose of this study was to examine the reasons of staff stress in the bathing situation in long-term aged care facilities in Japan. Three large-scale (LS) settings and 3 small-scale (SS) settings were studied. The methods used were observation of the bathing routines of 21 staff (19 LS staff and 2 SS staff) and a questionnaire survey to 80 staff (61 LS staff and 19 SS staff). Data analysis was done by descriptive analysis and content analysis of free answers. The observation results revealed a stressful atmosphere in the LS settings, with noisy environment, hurried pace of work and confusion about task division. The questionnaire ratings showed that the LS staff were significantly more dissatisfied with most aspects of bathing, especially with the number of residents to be bathed a day and bathing time for each resident. According to preliminary results of the free answers, the LS staff felt task-oriented stress, such as large number of residents, time constraints and the responsibility to prevent accidents, while the SS staff mentioned person-oriented stress, like persuading demented residents to bathe and handling reluctant and violent residents. Nursing implications and stress coping methods will be discussed.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7J_01_P

THE ROLE OF PERSONAL RESILIENCE AND COGNITIVE APPRAISAL IN UNDERGRADUATES' COPING WITH DAILY HASSLES

B. M. S. Bruneau

University of Greenwich, London, United Kingdom.
B.S.Bruneau@gre.ac.uk

The personal resources a person brings to a situation determine how an event is appraised (Lazarus & Folkman, 1984; Frydenberg, 2002; Devonport & Lane, 2006). The role of the process in how undergraduates manage stress has yet to be established. The present study hypothesised that when undergraduates cope with daily hassles their cognitive appraisal is mediated by their resilience (optimism, self-esteem, and perceived control) levels. Undergraduates are a relevant study group as there are concerns that their health is more at risk than that of other people, after controlling for age and sex (Stewart-Brown *et al*., 2000). The present longitudinal study involved 511 undergraduates across two UK universities who completed a questionnaire at four periods during the first fourteen months of their degree programme. The questionnaire comprised the Student-Life Stress Inventory (SLSI), Coping Operations Preference Enquiry (COPE), Resilience Indicator (RI) and the General Health Questionnaire (GHQ-12). Undergraduates' mean stress score was repeatedly at around 2.45, on a scale of 1 to 5, where 5 represents extreme stress. High self-esteem and the seeking of instrumental support were the only variables which correlated significantly at each time period ($r(373)=.11$, $p<.05$ time 1) ($r(263)=.16$, $p<.05$, time 2) ($r(168)=.21$, $p<.01$ time 3) ($r(157)=.16$, $p<.05$ time 4). High optimism was correlated with high psychological well-being only at time one ($r(374)=.18$, $p<.01$). This research contributes to health psychology as it demonstrates that paying attention to undergraduates' self-worth may promote their health.

7K_01_P

DIFFERENCES BETWEEN MEN AND WOMEN IN MENTAL AND PHYSICAL HEALTH IN THE RURAL POPULATION

Aparicio García, Marta Evelia; Sánchez-López, Mª Pilar; Cardenal Herráiz, Violeta; y Dresch, Virginia.

Universidad Complutense de Madrid

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

In this research project differences have been analysed in physical and psychological health between men and women in the Spanish rural population, taking into account the work situation (workers, unemployed or housewives), psychological variables (anxiety, self-esteem and satisfaction) and physical variables (substance abuse, number of ailments and self-perceived state of health). 1000 subjects have taken part, representing the Spanish rural population and belonging to the five labour categories studied. The findings show that women have worse health than men, since they score higher on variables such as Anxiety or Ailments). Depending on the labour situation of the participants, unemployed men prove to be the healthiest and unemployed women and housewives the least healthy.

7K_02_P

CONTRIBUTION OF SENSORY NEURONS TO SEX DIFFERENCE IN THE DEVELOPMENT OF STRESS-INDUCED GASTRIC MUCOSAL INJURY IN MICE

Naoaki Harada, Kenji Okajima, Nobuhiko Shimozawa

Departments of Biodefense Medicine, Nagoya City University Graduate School of Medical Sciences, Nagoya, JAPAN

Background and Aims: Sensory neurons play a critical role in reducing stress-induced gastric mucosal injury by releasing calcitonin gene-related peptide (CGRP) through an increase in gastric mucosal levels of prostacyclin (PGI_2). Since estrogen enhances nerve growth factor-mediated CGRP production in sensory neurons, we hypothesized that stress-induced gastric mucosal injury occurs less in females than in males. ***Methods:*** Gastric ulcer index (UI), gastric myeloperoxidase (MPO) activity, gastric tissue levels of CGRP and 6-keto-$PGF_{1\alpha}$, a stable metabolite of PGI_2, were determined in male and female wild-type ($CGRP^{+/+}$) mice and CGRP knockout ($CGRP^{-/-}$) mice subjected to water-immersion restraint stress. ***Results:*** In $CGRP^{+/+}$ mice, UI and MPO activities were lower and gastric tissue levels of CGRP and 6-keto-$PGF_{1\alpha}$ were higher in females than in males, but there were no such sex differences in $CGRP^{-/-}$ mice. Sex differences in $CGRP^{+/+}$ mice were eliminated by pretreatment with SB366791 (500 μg/kg, i.p.), a vanilloid receptor antagonist, and by ovariectomy (OVX). Reversal of sex differences by OVX was not observed in female $CGRP^{+/+}$ mice with estradiol replacement (1 mg/kg/week for 3 weeks). Levels of CGRP mRNA in dorsal root ganglion neurons isolated from female $CGRP^{+/+}$ mice were

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

decreased by OVX and these decreases were reversed by estradiol replacement. ***Conclusions:*** Estrogen-mediated increases in CGRP levels in sensory neurons might contribute to reduce stress-induced gastric mucosal injury by attenuating inflammatory responses. This might at least partly explain the sex difference observed in the development of stress-induced gastric mucosal injury in mice.

7K_03_P

EXTRINSIC LIFE GOAL ORIENTATION IS ASSOCIATED WITH MARITAL STRESS FOR WOMEN BUT NOT FOR MEN - EVIDENCE FROM THE HUNGAROSTUDY EPIDEMIOLOGICAL PANEL 2006

Tamas Martos, Piroska Balog, Maria S. Kopp

Semmelweis University, Institute of Behavioural Sciences, 1089, Budapest, Nagyvarad ter 4. martos.tamas@btk.ppke.hu

Marital stress has been proved to be an important factor in predicting various physical and mental health outcomes, including cardiovascular morbidity, depression etc. Motivational factors, like life goals and aspirations were also associated with health outcomes. Pursuing intrinsic goals (e.g. personal growth and good relationships) was shown to be associated with improved psychological functioning and better health outcomes while extrinsic aspirations (financial success and good appearance) were not. Connections between life goals and marital stress have not been studied before. The study is based on Hungarostudy Epidemiological Panel 2006 (N=4528). We analysed a subsample of married/cohabiting people (male N=1550, female N=1875) assessing marital stress (MS, via Shortened Marital Stress Scale) and life goal orientation (relative importance of extrinsic vs. intrinsic life goals, via Aspiration Index). 167 men (10,8%) and 267 women (14,2%) showed elevated level of MS. Hierarchical logistic regression analyses were performed, and odds ratios for marital stress (with 95% CI) were calculated both for men and women. For men relative importance of extrinsic life goals was not significantly related to MS (OR=1,31, 95% CI 0,94-1,81). For women relative importance of extrinsic life goals was significantly related to MS (OR=1,38, 95% CI 1,06-1,80) even after controlling for sociodemographic variables (age and education) and average importance of life goals. Results indicate gender differences regarding the relationship between marital stress and life goals: perceived marital stress may be connected to extrinsic life goal orientation for women but not for men.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

7K_04_P

RISK FACTORS OF MASCULINE GENDER ROLE STRESS AMONG MIDDLE-AGED MEN IN HUNGARY

Anna Susanszky, Eva Susanszky, Maria S. Kopp

Semmelweis University, Institute of Behavioral Sciences, 1089, Budapest, Nagyvarad ter 4., susana@net.sote.hu

Aim: To investigate relations of masculine gender role stress to work-, marital-, social stress, rival behavior, self rated health and socio-economical status. Methods: Survey among middle-aged (age 36-64) men (N=614) selected from Hungarostudy Epidemiological Panel 2006 (N= 4528) using the shortened Masculine Gender Role Stress Scale (Eisler&Skidmore, 1987), the shortened marital Stress Scale, shortened Effort – Reward Imbalance Questionnaire, and Bergen Social Relationship Scale. Stepwise linear regression analyses (stepwise method) were performed to study the effect of different sources of stress and the rival behavior on the masculine gender role stress. Age, health and socio-economical status were also included in the model. Results: Using stepwise linear regression analyses, work stress, (standardized beta=0.10), educational level (standardized beta=-0.12), and rival behavior (standardized beta=0.08) predicted masculine gender role stress best (F=6.68; p=0.00). Conclusion: These results highlight that rival behavior and work stress may serve as risk factors of gender role stress. High educational level emerged as a protective factor of gender role stress.

7K_05_P

GENDER DIFFERENCES IN ATTITUDES TOWARD DEATH AND ANXIETY

Agnes Zana – Katalin Hegedus – Gabor Szabo

Semmelweis University, Institute of Behavioural Sciences
4. Nagyvarad ter, 1024. Budapest, Hungary. E-mail: zanagi@net.sote.hu

Objective: The aim of our research is to investigate whether there are gender differences in anxiety and death related attitudes. According to our hypothesis the attitudes and anxiety toward death and the pattern of fear

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

of death differs for the two genders; we assume that women have higher fear of death and anxiety compared to men.
Method: For the questionnaire survey (N=246, female:167, male 76 /3 missing/, average age: 41,5) we used the *Neimeyer and Moore's Multidimensional Fear of Death Scale* (MFODS, Neimeyer and Moore 1994, Zana 2006) and the *STAI-T* (State-Trait Anxiety Inventory, Spielberger 1970, Sipos 1978).
Results: The three death fear factors rated highest are *Fear for significant others, Fear of the dying process* and *Fear of the dead* in both groups. However, we found significant gender differences in fear intensity on these factors ($p<0.04$). Overall fear of death ($p=0.005$) and trait anxiety ($p=0.021$) was significantly higher among women. Older participants reported significantly lower fear of death and anxiety compared to the younger ones.
Conclusion: Our results are consistent with previous findings showing that women and younger individuals have higher anxiety and death fear levels. Men's lower anxiety and death fear values may be explained by their gender specific suppression of emotions, which may be stress inducing.

Module 8 – Poster lectures:

8_01_P

GENETICS OF SALINITY TOLERANCE IN WHEAT

H. Dashti[1], M. Naghavi[2], A. Tajabadipur[3]

1,3-Valiasr University, Rafsanjan, Iran
2 -Tehran University, Karaj, Iran

Six generations (P1, P2, F1, F2, BC1 and BC2) of cross Rovshan × Falat was grown in greenhouse condition in order to evaluate gene effects of resistance to salinity in wheat. Five characters including: sodium and potassium concentrations, K/Na ratios, plant height and heading date were recorded and subjected to estimate means and variances pooled over replications. Gene effects were calculated by using the six parameter model, viz., m (average effect), d (additive), h (dominance), i (additive_additive) j (additive_ dominance) and l (dominance_dominance) according to Hayman (1958) after testing adequacy of additive-dominance (three parameter) model by using joint scaling test of Cavalli (1952). The six-parameter model was adequate in most of the cases to explain genetic variation among the generation means. Generation mean analysis revealed that additive components played a major role, but that dominance components also contributed significantly in controlling the variability of the recorded characters. The dominant and dominant × dominant components of the model for most characters showed values in

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

the opposite direction as it indicated the duplicate epistasis, which denoted little role of this epistasis in selection as it decreased heterosis in selection. This study revealed that these characters showed all three types of gene action i.e. additive, dominance and epistasis and should be considered to cumulate the resistance genes to salinity in one genotype. In order to utilize all three types of gene effects simultaneously, reciprocal recurrent selection breeding procedure would be the best option. Nevertheless, the information obtained in this study provides a better understanding of the genetic resistance to salinity and, it is a prerequisite to apply pyramiding of resistance genes. The development of appropriate markers linked to resistance genes would greatly enhance the feasibility of such a strategy.

Key words: salinity resistance, generation mean analysis, wheat

---

8_02_P

INVOLVEMENT TUMOR SUPPRESSOR P53 AND HEAT SHOCK PROTEINS IN NUCLEOTIDE EXCISION REPAIR AND BASE EXCISION REPAIR

Yu-Ching Chang and Yin-Chang Liu

Institute of Molecular Medicine, National Tsing-Hua University, Hsin-Chu, Taiwan 30043, Corresponding author: ycliu@life.nthu.edu.tw

Tumor suppressor p53 and heat shock proteins are DNA damage inducible proteins. We consider p53 like heat shock proteins as stress inducible proteins, and we are interested in their involvement in nucleotide excision repair (NER) and base excision repair (BER). Although p53 known as genomic guardian may facilitate the DNA repair via its transactivation on the expression of repair-related proteins, our recent study and the studies of others have implicated the direct involvement of p53 in the repair pathways of NER and BER. Our approach differs from others; we have used the comet-nuclear extract incubation assay to detect the dependence of excision activity of nuclear extract on p53 in vitro. Furthermore, the immunofluorescence experiment indicates that p53 is necessary for the recruitment of NER repair proteins XPB to the UV-induced damage site. We used the similar approach to study hsp70 and found that hsp70 is necessary to the activity in the nuclear extract to excise DNA damages induced by oxidative stress but not the DNA damages induced by UV irradiation. Both hsp70 and hsp27 have been shown in the previous studies to enhance the cell survivals upon UV or oxidative stress and have been implicated to facilitate NER and BER, however, the molecular mechanisms remain unexplored. Our approach may shed some light in this regard.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

8_03_P

A NOVEL HAND-HELD MONITOR OF SYMPATHETIC NERVOUS SYSTEM USING BIOMARKER

Masaki Yamaguchi, Kazutaka Teramura, Yusuke Tahara, Josaku Sakakima

Graduate School of Science and Engineering, University of Toyama, Japan, yamag@eng.u-toyama.ac.jp

Objective: Salivary amylase activity (sAMY) can be a useful index of plasma norepinephrine concentration under a variety of stressful conditions, since it appears that increased sympathetic nervous activity is a major stimulator of amylase secretion. In order to realize a hand-held monitor of the sympathetic nervous system, we fabricated a completely automated analytical system for sAMY using a dry-chemistry system. Materials and Methods: The monitor consisted of a disposable test-strip and an optical analyser (126 × 130 × 48 mm3; 350 g), which was incorporated within an automatic saliva transfer device. The test-strip consisted of a collecting paper and a reagent paper containing 2-chloro-4-nitrophenyl-4-O-β-D-galactopyranosylmaltoside (Gal-G2-CNP), a substrate for amylase. The collecting paper is directly inserted into an oral cavity, and approximately 30 μl of whole saliva is collected from under the tongue. When Gal-G2-CNP is hydrolyzed by amylase, the hydrolyzed product (CNP) develops a yellow color and the reflectance is measured by the optical analyser. Results and Discussion: When this monitor was used, it took 30 s for saliva sampling, 30 s for saliva transfer and measurement, and a total of one minute was enough to measure the sAMY. The calibration curve of the monitor was measured for sAMY. Within a range of sAMY between 10 and 140 kU/l, the calibration curve showed a coefficient with R^2 = 0.97. With regard to reproducibility of the measured results for the saliva transfer volume of the same samples, the coefficient variation (CV) was 5.5%. Conclusion: It was demonstrated that the manufactured monitor enabled a user to automatically measure the sAMY with a high accuracy.

8_04_P

PREJUNCTIONAL AT_1 RECEPTOR MEDIATES SYMPATHETIC RESPONSE TO COLD PRESSOR STRESS IN HUMANS

Zavala L.E.*, Cierco M.*, Gutiérrez A.**, Garrido MR***, e Israel A.***

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Unidad de Investigaciones Farmacológicas y Toxicológicas, UNEFM ** Postgrado de Anestesiología, Hospital Universitario "Dr. Alfredo Van Grieken", Coro, Estado Falcón * Laboratorio de Neuropéptidos, Facultad de Farmacia UCV, Caracas, Venezuela. email: lidazavala@hotmail.com*

Angiotensin II (ANG) has a role in stress-induced cardiovascular response by supporting sympathetic outflow through the stimulation of prejunctional AT_1 receptors. Thus, blockade of AT_1 receptor should decrease sympathetic response to stress. To assess this hypothesis, we compared the effect of losartan with the effect of eprosartan (EPRO), a selective prejunctional AT_1 receptor antagonist, on the cardiovascular response to cold pressor test (CPT) applied to healthy human volunteers. CPT is a model of acute stress known to increase arterial blood pressure (BP) and heart rate (HR). CPT was performed by placing the subject's left hand up to the wrist in iced water (4°C) for 90 sec. 82 healthy normotensive volunteers were recruited for the study. Informed consent was obtained from all subjects. They were divided in three treatment groups: placebo (n=37), losartan (50 mg) (n=14) and eprosartan (600 mg) (n=18). Cardiovascular parameters (BP and HR) were measured in a double-blind placebo-controlled fashion in the groups before (basal), 175 minutes after oral single treatment (basal-2) and post CPT. CPT was accompanied by a significant increase in HR, systolic, diastolic and mean BP in placebo-treated subjects. Pretreatment with any of the AT_1R antagonists completely suppressed the pressor response to CPT without changes in HR response. Our results demonstrate a role of prejunctional AT_1 receptor in the regulation of sympathetic response to acute stress.

8_05_P

WHAT CHARACTERIZES MEN AND WOMEN WITH HIGH LEVELS OF STRESS?

Line Nielsen, Tine Curtis, Tage S. Kristensen and Naja Rod Nielsen

National Institute of Public Health, Øster Farimagsgade 5A, DK-1399 Copenhagen K, Denmark,
lin@si-folkesundhed.dk

Background: Despite the fact that stress is a growing public health problem little is known about what characterizes individuals with stress. In order to prevent stress it is essential to be aware of factors that are predictive of stress. The objective of the study is to assess individual and neighborhood level factors that are associated with stress in a national representative sample of the Danish population.
Methods: The National Health Interview Survey 2005 consisted of a representative sample of the Danish population. The 9.708 men and

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

women were asked about perceived stress, health related behavior, working conditions, and sociodemographic factors. Data on neighborhood factors like crime rate or material deprivation were derived from national registries. Stress was assessed by the Perceived Stress Scale. Data were analyzed by means of logistic regression models.
Results: Women had a higher mean score of stress than men. Low education, heavy smoking, physical inactivity, lack of social network, and poor working conditions were associated with higher perceived stress among both men and women. Living in a deprived neighborhood was associated with higher stress among women, while living in a neighborhood with a high rate of crime was associated with higher stress among men.
Conclusions: This study contributes to the understanding of what characterizes individuals with high stress and may thereby help in guiding future prevention. To strengthen the knowledge of the relation between individual factors, neighborhood factors and stress prospective studies and intervention studies are needed.

Downloaded From: https://bioone.org/journals/Cell-Stress-&-Chaperones on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use