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[REVIEW]

The Mammalian Pars Intermedia —Structure and Function—

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1. INTRODUCTION

More than eighty years have now elapsed since Smith (1916) and Allen (1916) discovered striking changes in the pigmentation of the tadpole after hypophysectomy. Since mammals lack a pigmentary effector system in the skin, the structure of the pars intermedia of the pituitary gland and the physiological function of pituitary hormonal peptides derived from the precursor peptide proopiomelanocortin (POMC), including melanocyte-stimulating hormone (MSH), have been mainly investigated in amphibians, fish and reptiles. Therefore, the role of this gland in pigmentary control in these species is well established and has been adequately reviewed (Tonon *et al*., 1988; Lamacz *et al*., 1991). In comparison, the pars intermedia of mammals has regretfully received less attention.

For eighty years, advances in biotechnology and molecular biology have been occurring rapidly, and the study of the pituitary gland has also greatly advanced owing to these new technologies. Furthermore, advancements in endocrinology and immunology have now eliminated many of the traditional barriers between the scientific fields of endocrinology, immunology and neurobiology, and have opened up a new field of research that is regarded as neuro-immuno-endocrinology. Today, many lines of evidence have shown that cellular and molecular neuro-immuno-endocrine interactions take place under normal physiological conditions of mammalian life. Moreover, many interesting findings concerning the pars intermedia of the mammalian pituitary gland of mammals have been made and new clues to its possible role in nature have emerged. Therefore, the physiological functions of the mammalian pars intermedia should be studied and discussed from this new point of view. Nevertheless, physiological roles for the mammalian pars intermedia of the pituitary are still controversial, and no comprehensive review of the structure and physiological function of this part of the mammalian pituitary appears to have been published to date. Therefore, this review will attempt to evaluate the many interesting findings concerning the pars intermedia of mammals from the point of view of neuro-immuno-endocrinology, and suggest lines for future investigations on the pars intermedia. Hence, this review should be interpreted as a landmark for the study of the mammalian pars intermedia.

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2. HISTOCYTOLOGY OF THE PARS INTERMEDIA (FIG. 1)

It has been suggested that some mammalian species that have lost most or all of their body hair such as man, whales, dolphins and elephants lack the pars intermedia of the pituitary (Turner and Bagnara, 1976). Nevertheless, reports reveal that the human pars intermedia is well developed (Murakami *et al*., 1968) and α-MSH is also found in the pituitary of the fetus and the neonate (Silman *et al*., 1978; Mauri *et al*., 1993). Whales and dolphins lack the pars intermedia, however, they do not lack POMC-related peptides that are produced by melanotrophic cells present in the pars distalis (Geiling *et al*., 1940). These results suggest that the pars intermedia of the pituitary gland is an essential organ in mammals and that POMC-related peptides produced by melanotrophic cells may have other important physiological functions in mammals besides melanogenesis.

The pars intermedia of the mouse and the rat pituitary consists of 10–15 layers of densely arranged cells separated into lobules by strands of connective tissue. The principal type of cell in the pars intermedia is the melanotrophic cell, a large polyhedral cell with a smooth ovoid nucleus which secretes α-MSH, β-endorphin, and several other peptide derivatives of a common precursor, POMC. The detailed structure of the pars intermedia of the pituitary gland has been studied in several species of rodents by light and electron microscopy. However, since an appreciation of the structure of the pars intermedia is essential for a proper appraisal of such functional aspects as the cellular origin of POMC and the controlling mechanisms operating on the gland, its histocytology will now be outlined.

(1) *Melanotrophic cells*

The pars intermedia is composed of an almost homogenous population of endocrine cells, the melanotrophic cells that represent the great majority of glandular cells in the tissue. These cells contain abundant secretory granules characterized by a variable electron density that is related to the maturation of their hormone content. Since melanotrophic cells

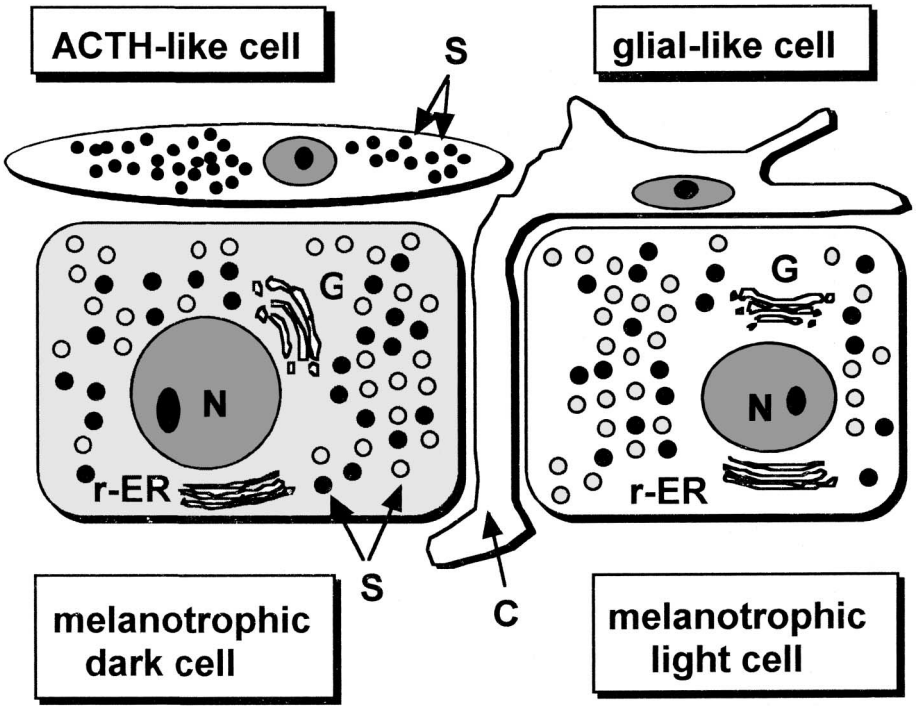

Fig. 1. Schematic representation of the histocytology of the pars intermedia. N : nucleus, S : secretory granules (characterized by variable electron density), G : Golgi apparatus, r-ER : rough surfaced endoplasmic reticulum, C : cytoplasmic processes of glial-like cell.

recycle their cellular membrane, the electron-lucent granules or vacuoles observed in cytoplasmic areas of melanotrophic cells are thought to be endosomes (Back *et al*., 1993). Ultrastructural changes of activated melanotrophic cells have been well documented by Kobayashi *et al*. (1984) and Takeuchi (1995). An interesting heterogeneity between the tinctorial properties of individual melanotrophic cells (dark cells and light cells) is observed in pituitary tissue sections (Chronwall *et al*., 1987). This melanotrophic cell heterogeneity correlates with various levels of POMC-mRNA expression. Dispersed melanotrophic cells maintain diverse levels of POMC-mRNA, and the content of organelles reflects biosynthetic activity and α-MSH immunoreactivity. Treatment with the dopamine D2 receptor agonist, bromocriptine, highlights the heterogeneity in POMC expression of melanotrophic cells (Beatty *et al*., 1998). Iturriza (1989) reported that dopamine-sensitive and dopamine-insensitive cells might exist in the normal pars intermedia of the pituitary. In vivo, several factors such as the degree of dopaminergic innervation, and gradients of stimulatory and inhibitory stimuli in the extracellular spaces could serve to maintain the heterogeneity of melanotrophic cells.

(2) *ACTH-like cells*

A second type of cell, morphologically quite different from melanotrophic cells is present in the pars intermedia but this type of cell is much less abundant. These cells are mainly found in the rostral zone of the pars intermedia and in the contact zone between the pars nervosa and the pars intermedia. Furthermore, these cells contain highly electron-dense secretory granules, and can be considered as authentic corticotrophic cells (Stoeckel *et al*., 1973). It is of interest that lobular corticotrophic cells are found in the canine pars intermedia of the pituitary (Halmi *et al*., 1981). As the physiological functions of the peptides produced by ACTH-like cells in the mammalian pars intermedia remain unknown, the control mechanisms of hormone secretion and synthesis, and physiological functions of these cells are the major subjects of future research.

(3) *Glial-like cells*

A third type of pars intermedia cell is called glial-like cell, and these are observed lying between the melanotrophic cells and the periphery of the lobules. Glial-like cells express glial fibrillary acid protein (GFAP) and are scattered throughout the pars intermedia. These cells extend their cytoplasmic processes between melanotrophic cells. It is well known that dopaminergic neurons from the CNS innervate the pars intermedia and control hormone synthesis and release by melanotrophic cells. Alterations in expression of GFAP in glial-like cells following lactation, salt-loading, adrenalectomy (Gary and Chronwall, 1995) and D2 receptor agonist treatment (Sands and Chronwall, 1996) suggest that glial-like cells in the pars intermedia also respond to variation in the dopaminergic tone from the hypothalamus. As receptors for neuropeptide Y (NPY) are located on the glial-like cells, axonal NPY may exert an effect on hormone secretion by melanotrophic cells indirectly, via these glial-like cells (See Section 5). Recently, several inflammatory cytokines (IL-1, IL-6, TNF, etc) have been identified in the pars intermedia (See Section 3). It is well known that microglia are macrophage-like cells derived

from mononuclear myeloid progenitors, and activated microglia mediate inflammatory processes by producing inflammatory cytokines (IL-1, IL-6, TNF, etc) and other molecules. These results suggest that hormone synthesis and release by melanotrophic cells are regulated by several cytokines produced by glial-like cells in the pars intermedia. In recent years, it has been reported that IL-18, identified as a new member of the IL-1 family, is also produced by microglia and its expression is enhanced by lipopolysaccharide (LPS) (Conti *et al*., 1999; Prinz and Hanisch, 1999). Therefore, it is possible that many immunological mediators are produced in the pars intermedia.

3. HORMONE CONTENT OF THE PARS INTERMEDIA (FIG. 2)

In the mammalian pars intermedia of the pituitary gland, the first major cleavage of POMC occurs between ACTH and β-lipotropin (β-LPH), yielding the N-terminal POMC fragment ACTH-biosynthetic intermediate (ABI) and β-LPH. The ABI is further cleaved to ACTH and N-POMC (big-γ-MSH/joining peptide). In the pars intermedia, β-LPH is almost completely processed into γ-LPH and β-endorphin, and ACTH is cleaved to α-MSH and corticotropin-like intermediate lobe peptide (CLIP). β-Endorphin is further converted into a variety of endorphin-related products that are not detectable in the anterior pituitary (Loh, 1992). Therefore, the amino acid sequences of both α-MSH and CLIP are found within the ACTH molecule, while the met-enkepharin, β-endorphin, β-MSH and γ-LPH sequences are located in β-LPH molecules (Loh, 1992; Nakanishi *et al*., 1979). Acetylation of α-MSH and β-endorphin is an important process in the regulation of biological activities of α-MSH and β-endorphin. Tissue-specific expression of proopiomelanocortin acetyltransferase in the pars intermedia is consistent with the presence of acetylated forms of β-endorphin, in the pars intermedia only, and not in the pars distalis. An acetyltransferase activity that can acetylate both α-MSH and β-endorphin has been found in the secretory granules of melanotrophic cells.

α-MSH, in its N-acetylated form, has more potent melanotrophic activity than the des-acetyl form. Conversely, N-acetylation of β-endorphin eliminates the opiate activity of this peptide. The physiological functions of N-acetylated forms of β-endorphin produced by melanotrophic cells are poorly understood. Furthermore, the biological functions of other peptide derivatives of POMC produced by melanotrophic cells are still unknown. Nevertheless, it would be expected that these POMC-derived peptides produced by melanotrophic cells are not biologically inactive. It is interesting to note that the pituitary of the human fetus as well as that of the rhesus monkey fetus contains a high concentration of α-MSH, and that the MSH bioactivity decreases dramatically soon after birth (Silman *et al*., 1978). In the human, this may be due to the fact that the fetus, unlike the adult, has a well developed pars intermedia (Murakami *et al*., 1968). Furthermore, morphological studies also suggest that marked hypersecretion of various hormones occurs during the neonatal period (Kobayashi and Takeuchi, 1985). The fact that the fetal and neonatal mammalian pituitaries contain well developed pars intermedia and still have the same developmental changes in α-MSH levels suggests that there may be a physiological role in the POMC system during fetal and neonatal life (See Section 6).

Recently many bioactive products produced by the pars intermedia have been identified. Cytokines are probably the most interesting products found in the pars intermedia (Fig. 3). Interleukin-6 (IL-6) (Spangelo *et al*., 1994) and tumor necrosis factor (TNF) (Arras *et al*., 1996) are found in melano-

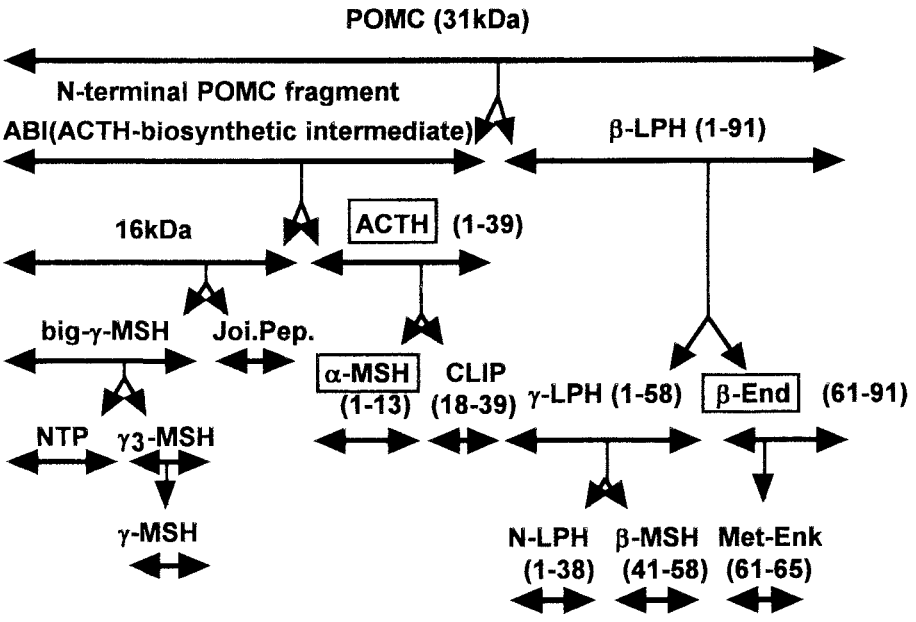

Fig. 2. Proteolytic processing of POMC in the pars intermedia. Joi.Pep.: joining peptide, NTP: N-terminal peptide, CLIP: corticotrophin-like intermediate lobe peptide, End: endorphin, Met-Enk: methionine-enkephalin.

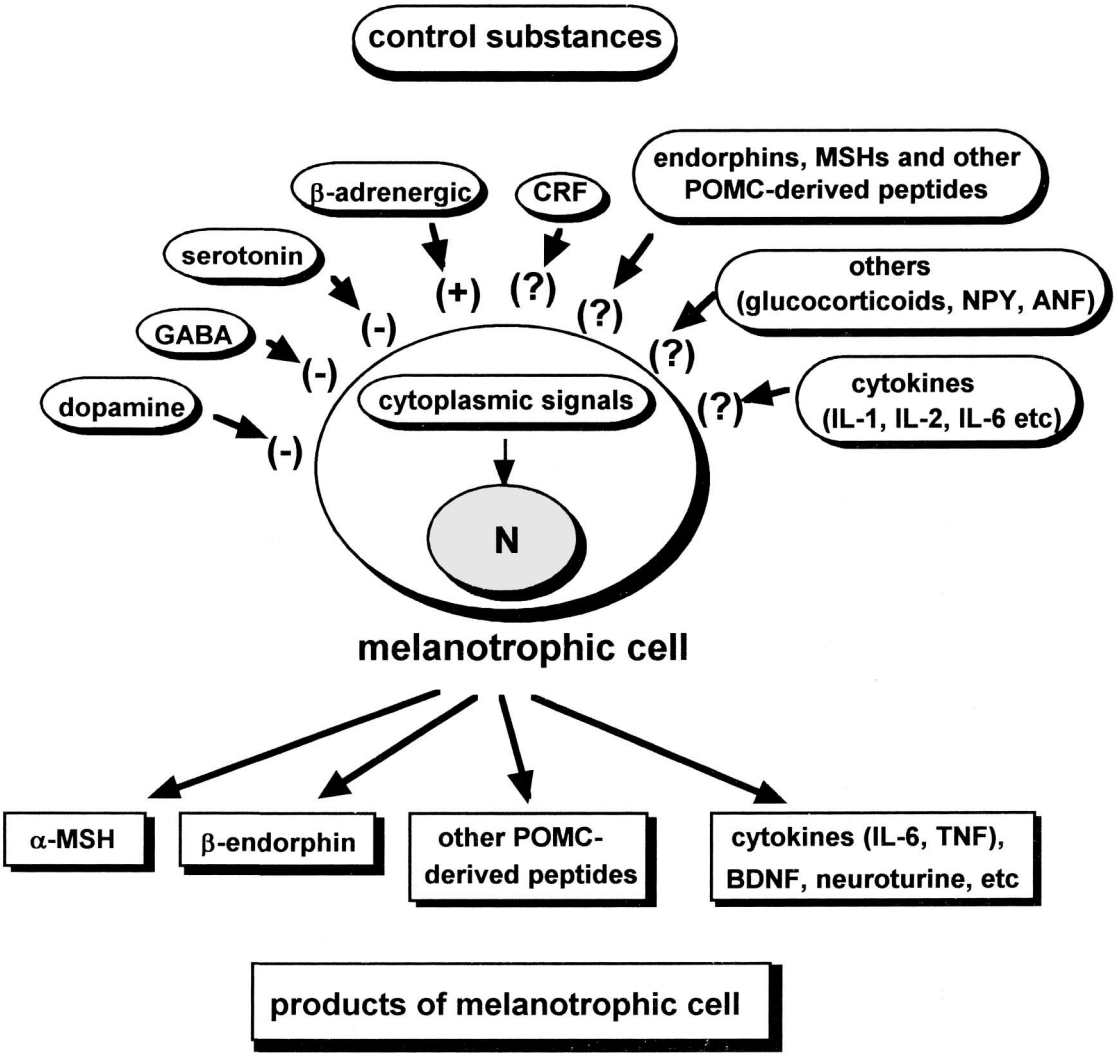

Fig. 3. Schematic representation of control mechanisms acting on melanotrophic cells and bioactive products produced by the melanotrophic cells. (–) : inhibitory control, (+) : stimulatory control, (?) : unknown.

trophic cells and may regulate hormone synthesis and the cellular functions of the pars distalis via the intra-adenohypophyseal portal system (See Section 4). More recent histological studies revealed that the pars intermedia produces several neurotrophic factors such as brain-derived neurotrophic factor (BDNF) (Hopker *et al*., 1997) and neuroturin (Xian *et al*., 1999). Surprisingly, BDNF expression in melanotrophic cells is regulated by dopaminergic stimulation. So far, the biological and physiological functions of BDNF and neuroturin produced by melanotrophic cells are unknown.

It is well known that marked changes in the plasma concentrations of pituitary hormones are observed after peripheral challenge with the bacterial endotoxin lipopolysaccharide (LPS). A key step in these responses may be the induction of inflammatory cytokines, because administration of cytokines such as interleukin-1 (IL-1) can induce similar effects. Furthermore, IL-1β converting enzyme (ICE ; also called caspase-1) transforms the immature precursor form of IL-1β to the mature form. Whiteside *et al*. (1999) reported that systematically administered LPS enhances the production of ICE in the pituitary gland. These results suggest that members of the IL-1 family may be produced in the pituitary gland and regulate the cellular functions of the pars distalis and pars intermedia, although the actual IL-1 producing cells have not yet been identified. Therefore, we must take off our old coat and commence study of the mammalian pars intermedia on the basis of neuro-immuno-endocrinology.

4. VASCULATURE OF THE PARS INTERMEDIA

The vascularity of the pars intermedia is variable. In the pig, capillaries are quite obvious within the gland, and in the cat, goat and rabbit, the pars intermedia is relatively well vascularized. However, in many species including man, the pars intermedia has few blood vessels and there appears to be a paucity of blood vessels (Turner and Bagnara, 1976). The pars intermedia of laboratory animals such as the mouse and rat, and that of the small desert gerbil (Gerbillus pyramidum) also have few blood vessels (Lebaili *et al*., 1999). This sparse

vascularity in the pars intermedia of the pituitary gland is unusual in the endocrine system. Therefore, this feature raises intriguing questions concerning the route of egress of POMC-derived peptides produced by melanotrophic cells. There may be another outflow system between the parenchymal cells such as an irrigation system for nutrition and secretion.

Recently, a scanning electron microscope study of cast samples of the rat pituitary gland suggested that the capillary bed of the rat pars intermedia is a fairly independent unit, and a constant portal drainage system into the pars distalis (Murakami *et al*., 1985). The pars intermedia of the newborn rat pituitary gland is poorly vasculated. In the newborn rat, few vessels or capillaries are present between the vascular beds of the pars distalis and the pars nervosa. In the adult rat, on the other hand, a number of capillaries are present in all areas. These results suggest that the capillary bed of the rat pars intermedia begins to develop after birth, and that complete extension of this capillary bed from the caudal to the rostral end of the pars intermedia is completed after puberty. The capillary network of the pars intermedia receives arterial or afferent vessels from the middle and posterior hypophyseal arteries. These run into the pars intermedia and continue directly into the superficial plexus of the pars intermedia. The vascular network of the pars intermedia continues into the sinusoidal capillaries of the pars distalis and the blood stream is usually directed from the pars intermedia to finally drain into the pars distalis. Therefore, it is possible that hormonal substances such as POMC-related peptides in the pars intermedia act on the pars distalis via the intra-adenohypophyseal portal vessels.

5. CONTROL MECHANISMS OF THE PARS INTERMEDIA (FIG. 3)

(1) *Neuronal control mechanism*

In all vertebrate classes, the pars intermedia of the pituitary gland is poorly vascularized but richly innervated by a dense plexus of fibers that originate from neurons located in the hypothalamus. Immunocytochemical studies have revealed that these axon terminals contain classical neurotransmitters such as dopamine, γ-amino butyric acid (GABA) or serotonin, and moreover, various neuropeptides such as corticotropin-releasing factors (CRF), neuropeptide Y (NPY) and endorphins.

Among these transmitters, dopamine is regarded as an inhibitor of the synthesis and secretion of POMC-related peptides. The secretion of α-MSH and β-endorphin by melanotrophic cells is primarily regulated by dopaminergic terminals that emanate from neurons in the rostral arcuate nucleus of the basomedial hypothalamus and terminate directly on melanotrophic cells (Björklund *et al*., 1973; Holzbauer and Racke, 1985). This innervation of the pars intermedia of the pituitary occurs during the first postnatal week (Kobayashi and Takeuchi, 1985; Takeuchi, 1995). Morphometric electron microscopy revealed cytological signs of hyperfunction in the pars intermedia cells up to 3 days after birth. These included a rise in the percentage volume of the r-ER indicative of increased protein synthesis, an increase in the number of Golgi granules per unit Golgi area showing induction of granule-forming activity, and a decrease in the numerical density of secretory granules reflecting extracellular release of the granules. Cytologically, this hyperfunction was significantly inhibited by day 7 as evidenced by a decrease in the percentage volume of the r-ER, a decrease in the number of Golgi granules and an increase in the numerical density of secretory granules (Figs. 4a, 4b and 4c). Furthermore, the number of nerve fibers observed in the pars intermedia significantly increased from days 5 to 7 (Fig. 4d). The dopamine receptor antagonist haloperidol stimulates the secretion of α-MSH and β-endorphin peptides and accelerates the synthesis of POMC-mRNA. Conversely, treatment with the dopamine receptor agonist bromocriptine decreases the synthesis and secretion of POMC-derived peptides and reduces POMC-mRNA levels in the melanotrophic cells as evidenced by in situ hybridization (Fig. 5). Furthermore, dopaminergic neurons may regulate POMC gene expression and cellular proliferation of melanotrophic cells (Chronwall *et al*., 1987). The mechanism responsible for peptide and/or dopaminergic influence on the mitotic rate of melanotrophic cells, whether it is direct through the POMC-related receptors or via stimulation of an oncogene, has yet to be resolved. Interestingly, the expressions of the IL-1 receptor (Spangelo *et al*., 1994) and of the glucocorticoid receptor (Antankly *et al*., 1985) are inhibited by dopamine. These results suggest that expression of other cytokine or hormone receptors may also be inhibited by dopaminergic neurons. Therefore, in order to understand the biological functions of the mammalian pars intermedia, we must study the control mechanisms of hormone synthesis and secretion by melanotrophic cells that are not under the inhibitory control of dopaminergic neurons in vitro.

Immnunohistochemical studies with antibodies against the GABA molecule itself have demonstrated that a dense network of GABA-like immunoreactive fine varicose nerve fibers are present in the pars intermedia of the rat pituitary gland, surrounding melanotrophic cells (Sakaue *et al*., 1988). GABA has also been shown to act as a direct inhibitor on the pars intermedia and to reduce hormone secretion (Hadley *et al*., 1977; Oertel *et al*., 1982; Tomiko *et al*., 1983). Furthermore, dopamine and GABA are colocalized in axons of the pars intermedia (Vuillez *et al*., 1987). These observations raise the question, why are two inhibitors packaged in the same nerve terminal when one inhibitory substance should be enough to regulate the melanotrophic cells. It is possible that these neurotransmitters may interact either at the pre-synaptic or post-synaptic level.

Serotonin receptor expression has been demonstrated on the pars intermedia, suggesting a physiological role for serotonin in regulating secretory activity of the pars intermedia (Westlund and Childs, 1982; De Souza, 1986; Friedman *et al*., 1987). There is, however, disagreement among reports studying the physiological role of serotonin. One study supports a stimulatory effect of serotonin (Randle *et al*., 1983), while another study indicates no effect (Jackson and Lowry,

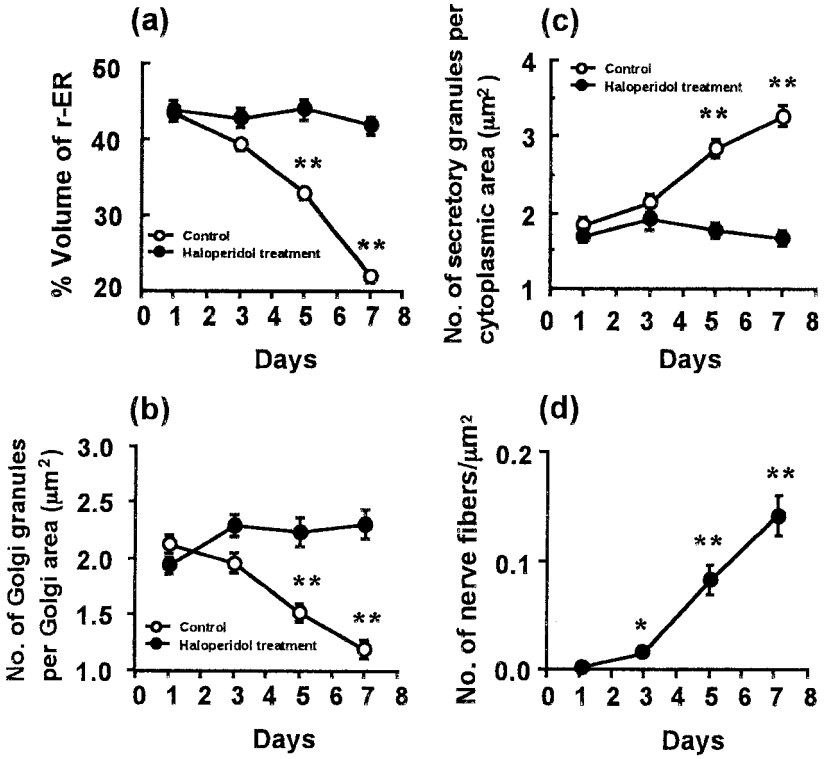

Fig. 4. The kinetics of ultrastructural, morphometrical parameters in the pars intermedia cells of neonatal mice and effect of haloperidol on the pars intermedia cells of neonatal mice. (a) The kinetics and effects of haloperidol on the percentage of the cytoplasm occupied by the rough endoplasmic reticulum. (b) The kinetics and effects of haloperidol on the numerical density of immature Golgi granules per Golgi area (μm^2). (c) The kinetics and effects of haloperidol on the numerical density of secretory granules per cytoplasmic area (μm^2). (d) The number of nerve fibers in the pars intermedia of neonatal mice. The nerve fibers in the pars intermedia of neonatal mice were observed by electron microscope. The number of nerve fibers observed in the pars intermedia significantly increased from day 5 to day 7. Data represent mean±SE. *, significantly different from control ($p<0.05$). **, significantly different from control ($p<0.01$).

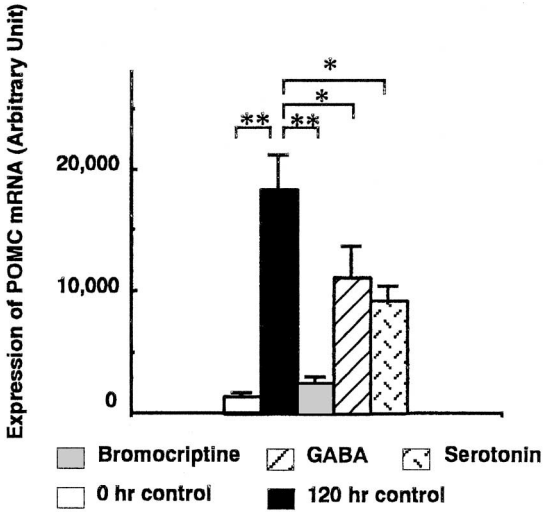

Fig. 5. Expression of POMC-mRNA in the dispersed pars intermedia cells of 0 hr controls ( ), 120 hr controls ( ), and bromocriptine-treated ( ), GABA-treated ( ) and serotonin-treated ( ) cells. Data represent mean±SE. *, significantly different from control ($p<0.05$). **, significantly different from control ($p<0.01$).

1983). In vivo studies have shown that administration of serotonin agonist stimulated α-MSH release and pretreatment with a dopamine agonist blocked serotonin-induced release of α-MSH from the rat pars intermedia (Carr *et al.*, 1991). Nevertheless, an in vitro study reported that short-term treatment with serotonin failed to alter peptide secretion by the perfused pars intermedia (Jackson and Lowry, 1983). These results suggest that the complicated effect of serotonin on the pars intermedia is masked by a dominant strong inhibition by dopamine. Recently Takeuchi(1995) observed that serotonin directly inhibits hormone synthesis and release by cultured melanotrophic cells free from inhibitory control by dopamine in vitro (Fig. 5).

The physiological functions of NPY also appear to be complicated. Though it has been reported that the pars intermedia does not contain NPY receptors (Torda and Saavedra, 1990), NPY inhibits the release of POMC from melanotrophic cells (Blasquez *et al.*, 1995). Thus, many questions about mechanisms of neural control on the pars intermedia remain unanswered. Furthermore, nonsynaptic and receptor-mediated regulations of melanotrophic cells are illustrated by the presence of β-adrenergic receptors and corticotropin-releasing factor (CRF) in the pars intermedia. There is evidence that sympathetic noradrenergic axons are sparse in the pars intermedia of the pituitary and are only present in the vicinity of blood vessels (Björklund, 1968), and that the

pars intermedia is controlled by stimulatory β-adrenergic input in addition to inhibitory controls (Munemura *et al*., 1980; Cote *et al*., 1982). While the receptor for CRF is also observed in the pars intermedia (Grigoriadis and De Souza, 1989), CRF is localized in the axons of the pars nervosa, but not those of the pars intermedia (Burlet *et al*., 1983).

Atrial natriuretic factor (ANF), histamine, somatostatin, bombesin, glucocorticoids and nerve growth factor have been demonstrated to have inhibitory or stimulatory roles in the pars intermedia. Multiregulatory mechanisms of various neurotransmitters may act on hormone synthesis and release from the pars intermedia. Recently it has been reported that β-endorphin levels and processing in the pars intermedia are also regulated by a T-cell specific factor (IL-2), secreted in animals undergoing transplantation immunity (Zakarian *et al*., 1989). In addition, cytokines also regulate hormone synthesis and release from the pars intermedia (See Section 6).

Synaptic and asynaptic receptor-mediated controls of melanotrophic cells are important regulatory mechanisms for hormone synthesis and release not only by the stimulatory β-adrenergic receptors, but also by the inhibitory dopamine and GABA receptors. An interesting heterogeneity in tinctorial properties among individual melanotrophic cells exists in rat and mouse sections, which correlates with the different levels of proopiomelanocortin observed (See Section 2). Molecular heterogeneity of POMC may be controlled by a combination of synaptic and asynaptic receptor-mediated neurotransmission, cytokines and other humoral factors acting upon individual melanotrophic cells. As yet, it is unclear why inhibitory and stimulatory transmitters are simultaneously present in the pars intermedia of mammals. Thus, subpopulations of melanotrophic cells expressing different biochemical molecules are present, suggesting differential regulatory influences at the level of individual cells. I think that there are several avenues for the control of melanotrophic cells namely, synaptic transmission, classic endocrine regulation and asynaptically released regulatory transmitters acting by mechanisms similar to parasynaptic transmission (Schmitt, 1984).

(2) *Paracrine and autocrine control mechanisms*

Chronic administration of morphine reduces the secretion and biosynthesis of POMC-related peptides in melanotrophic cells. In contrast, acute morphine treatment stimulates secretion and synthesis of POMC-related peptides by the cells. Stimulation of POMC-related peptide secretion by opioid peptides occurs, at least in part, as a result of direct interaction with opioid receptors in the pars intermedia of the pituitary. In fact, activation of μ-opioid receptors stimulates α-MSH and β-endorphin secretion by the pars intermedia (Carr and Lovering, 2000). This result suggests that secretion of POMC-related peptides by melanotrophic cells appears to be under tonic μ-receptor control in regions where inhibitory dopaminergic innervation to the pars intermedia is poor. Five subtypes of melanocortin receptors (MC-1 receptor ~ MC-5 receptor), which are G-protein linked receptors, are observed in the different organs and tissues of mammals (Cone *et al*., 1996). Recently, the MC-3 melanocortin receptor has been identified on melanotrophic cells (Lorsignol *et al*., 1999). More detailed studies are necessary to determine expression of melanocortin receptors on melanotrophic cells and their role in the physiological control mechanisms which act on melanotrophic cells. These results suggest that melanotrophic cells in the pars intermedia may be regulated by POMC-related peptides (at least by endorphins and MSHs) in autocrine and paracrine manners.

GFAP-positive glial-like cells with long cytoplasmic processes are present in the pars intermedia interspersed among the melanotrophic cells. Surprisingly, alterations in the glial-like cell GFAP expression following lactation, salt-loading, adrenalectomy and D2 receptor agonist treatment (Gary and Chronwall, 1995; Sands and Chronwall, 1996) suggest that glial-like cells in the pars intermedia respond to variations in dopaminergic tone. On the other hand, glial-like cells produce a number of cytokines, and these cytokines are found in the pars intermedia (See Section 3). Therefore, cytokines produced by the glial-like cells in the pars intermedia may also regulate POMC synthesis and release by melanotrophic cells in a paracrine manner.

6. PHYSIOLOGICAL FUNCTIONS OF POMC-DERIVED PEPTIDES (FIG. 6)

(1) *Host defense during fetal and neonatal life*

Significant alterations in the synthesis of the numerous hormonal peptides derived from POMC may occur during fetal and neonatal life. For example, pituitary α-MSH and CLIP predominate during the fetal life of humans and monkeys but are absent in the adult while the levels of β-endorphin may also be more prominent during fetal life (Swaab *et al*., 1976; Silman *et al*., 1978; Mauri *et al*., 1993). Morphological studies also suggest that immaturity of the hypothalamic inhibitory control mechanisms on the pars intermedia cause marked hypersecretion of various hormones (Kobayashi and Takeuchi, 1985). The presence of these POMC-derived peptides has been suggested to reflect a physiological function in the fetus and in neonatal life. In this regard, it is important to substantiate current hypotheses about the pituitary-adrenal axis in development. The mammalian adrenal cortex possesses a so-called fetal zone that is of a great size during gestation (Rudman *et al*., 1980). This steroidogenic zone undergoes rapid involution at parturition, whereas the outer cortical zone hypertrophies to produce the definitive cortical zone. There is evidence that in some animals the fetal zone is responsive to α-MSH rather than to ACTH, and that the definitive cortical zone is responsive to ACTH, but not to α-MSH. Near the time of parturition, there is a sharp increase in ACTH production compared with neuropeptides of lower molecular weight, such as α-MSH and CLIP. It is postulated, therefore, that the increase in ACTH synthesis rather than parturition is responsible for the increase in the production of cortisol by the definitive adrenal cortex. Thus the key mechanism in the chain of events controlling parturition may be the switch in pituitary peptide synthesis from α-MSH production in the pars

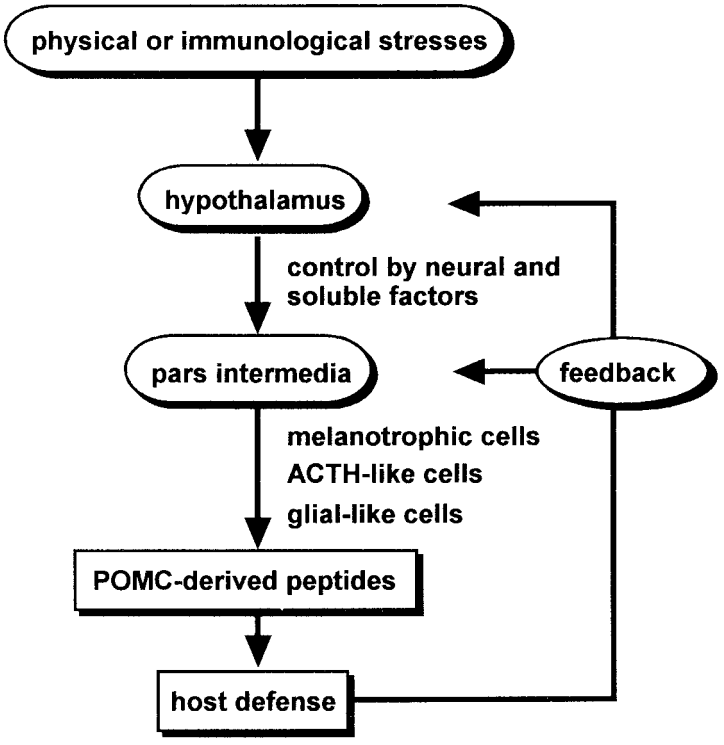

Fig. 6. Schematization of the hypothetical pathways underlying the host defense effects of POMC-derived peptides.

intermedia to ACTH synthesis in the pars distalis. Furthermore, the neonatal period is a critical time for survival in all mammals, and neonatal animals require an ability to cope with acute exogenous infections, dehydration and hypoglycemia (Cornblath and Schwartz, 1993). Recently, it has been reported that repeated hypoglycemic stress enhances the secretory activity of the pars intermedia in mice (Takeuchi and Takahashi, 1995). These results suggest that POMC-related peptides are necessary for the host to survive during the postnatal period of mammals.

(2) *Hydro-mineral regulation*

Previous electron microscopic and morphometric studies have demonstrated ultrastructural changes showing marked hypersecretion by the cells of the pars intermedia of the mouse pituitary gland in response to dietary sodium restriction (Kobayashi, 1974; Kobayashi and Takema, 1976), suggesting a new role for POMC-related peptides as a major pituitary factor in the regulation of aldosterone secretion by the adrenals (Silman *et al*., 1978). Furthermore, experimental copious drinking has the effect of causing marked hypersecretion by melanotrophs of the mouse pars intermedia (Kobayashi *et al*., 1984). Morphological changes in melanotrophic cells of new-born mice indicate hypersecretion when, after 5 hr of separation, pups are returned to their mother and allowed to breast feed for 1 hr (Kobayashi and Takeuchi, 1985). Leenders *et al*. (1990) demonstrated that this experimental copious drinking causes a rapid increase in POMC synthesis in melanotrophs, whereas dehydration has the opposite effect. Studies have demonstrated that aldosterone secretion is stimulated by POMC-derived peptides such as α-MSH (Vinson *et al*., 1984), β-MSH (Yamakado *et al*., 1980) and γ-MSH (Griffing *et al*., 1985). These results suggest that the pars intermedia may be involved in regulating plasma aldosterone levels under severe conditions of low plasma sodium conditions, and hydro-mineral metabolism in mammals. In fact, it is well known that camels and llamas, which have a well developed pars intermedia that completely surrounds the pars nervosa, drink water copiously after dehydration due to their long travels in desert country. Similarly, a well-developed pars intermedia is also found in other mammals adapted to arid environments. Lebaili *et al*. (1999) reported that in hydrated Gerbils, marked hypersecretion by melanotrophic cells was morphologically evident. Nevertheless, Kobayashi suggested that excess drinking of supplemental NaCl at a concentration of up to 0.9% in a 5% glucose drinking solution, still evokes significant melanotrophic cell activity (unpublished data). Thus, it is probably correct to assume that NaCl intake is not solely an inhibitory stimulus on the function of melanotrophic cells, but changes in hydro-mineral conditions of the animal after copious drinking may be a major factor for controlling melanotrophs.

(3) *Immuno-modulatory activity of POMC*

One of the oldest vertebrate host defense mechanisms for survival is the innate immunity. The skin is constantly subjected to injury and threatened invasion by pathogens. Therefore, the epithelial tissue of the skin is a barrier and a first line of defense against infection. Recently it has been reported that keratinocytes release large amounts of IL-1 (Mizutani *et al*., 1991) and constitutively express IL-18 (Naik *et al*., 1999). These cytokines promote inflammatory reactions in the epidermis and dermis. POMC-derived peptides exhibit immunomodulatory properties. α-MSH decreases fever and all major forms of experimental inflammation. The anti-pyretic potency of α-MSH in reducing fever caused by endogenous pyrogen is surprisingly more than 25,000-fold greater than that of acetaminophen on a molar basis (Murphy *et al*., 1983; Catania and Lipton, 1993). α-MSH inhibits the production of interferon-γ (IFN-γ), tumor necrosis factor (TNF), inducible NO synthase (iNOS) and thus NO production (Chiao *et al*., 1996), and inhibits the inflammatory effects of IL-1 (Cannon *et al*., 1986). Furthermore, non-opioid fragments of β-endorphin enhance lymphocyte natural killer (NK) cell cytotoxicity (Kay *et al*., 1987). These results suggest that in mammals, POMC-derived peptides play an important role in the first line of host defense against infection together with inflammatory cytokines as immuno-modulators. Surprisingly, it has been reported that macrophages produce immunoreactive POMC-derived peptides that inhibit the production of proinflammatory cytokines by macrophages in an autocrine or paracrine manner (Lyons and Blalock, 1995). It is well known that the macrophage has phagocytic ability and is regarded as a primitive type of cell in host defense. α-MSH is considered to be an ancestor peptide, since its amino acid sequence has been highly conserved throughout evolution. These observations suggest that POMC-derived peptides play a role in the innate network of basic adaptive mechanisms that are essential for survival.

Moreover, transplantation immunity stimulates POMC biosynthesis and processing in the pars intermedia, and similar phenomena are induced by IL-2 administration in the rat (Zakarian *et al*., 1989). Immunological stress can also enhance the production of POMC-derived peptides by melanotrophic cells and it is well known that α-MSH inhibits the immunological activities of IL-1. Conversely, administration of IL-1 enhances the release of CRF in the median eminence (Berkenbosh *et al*., 1987). Interferons (IFNs) stimulate the differentiation of melanocytes by increasing the expression of surface α-MSH receptors (Kameyama *et al*., 1989). These data support the notion of the existence of an immunoregulatory feedback circuit between the immune system and the pars intermedia. Previously Chiao and collaborators reported interesting results showing that α-MSH prevents LPS-induced hepatic inflammation by inhibiting the production of TNF-α, IL-8 and MCP-1, which then modulate the infiltration of inflammatory cells (Chiao *et al*., 1996). Interestingly, their experimental protocol stimulates the production of IL-18 in the liver, and the administration of anti-IL-18 antibody also inhibits parenchymal cell damage in the liver (Okamura *et al*., 1995). The intestinal epithelium is also the first line of host defense in the gut. Surprisingly, intestinal epithelial cells also have opioid receptors (Quito *et al*., 1991; Zagon *et al*., 1996) and the production of IL-18 is markedly enhanced by physiological stresses (Takeuchi *et al*., 1997, 1999). These results also suggest that POMC-derived peptides play a role in host defense together with inflammatory cytokines. Nevertheless, the physiological roles of POMC-related peptides produced by melanotrophic cells are still unclear. Further studies are required for us to understand the physiological functions of POMC-related peptides.

7. CONCLUSIONS

In endocrinology, studies on the physiological functions of the mammalian pituitary gland have been mainly through reductionism, and molecular biology and biotechnology have contributed greatly to the study of the pituitary gland. The scientific basis for studying the pars intermedia is developing rapidly and more appropriate approaches may be developed in the near future. Nevertheless, the reason for the existence of the pars intermedia of the mammalian pituitary gland is still unknown. Therefore, for the first time, I have applied the new concept of neuro-immuno-endocrinology to the studies of the pars intermedia of the mammalian pituitary gland in this review. Accordingly, this review should be interpreted as a landmark for the study of the mammalian pars intermedia. Since mammals lack a pigmentary effector system in the skin, it seems very obvious that POMC-derived peptides including α-MSH produced by melanotrophic cells have other unknown functions. So far, α-MSH and endorphins are the subjects of a relatively large number of research reports, because these hormones have been thought to be the main products of the pars intermedia. Moreover, various conditions and factors that influence the activity of the pars intermedia have been also well discussed. Notwithstanding, there are many molecular and biochemical events in the pars intermedia, and physiological functions of POMC-derived peptides produced by mammalian melanotrophic cells that are still unclear. Mammals require an increase in POMC-derived peptides secretion during their life span. The inhibitory control on the pars intermedia can be attenuated resulting in a rise in POMC-derived peptides secretion. Actually, the pars intermedia of mammals show marked hypersecretion of various POMC-derived peptides during the fetal and neonatal periods. Since neonatal animals are susceptible to exogenous infections, POMC-derived peptides secreted during the fetal and postnatal periods may play a role in host defense. The pars intermedia of the desert mouse (Gerbil) shows interesting morphological changes in nature. These results demonstrate that there are many avenues for studying the mammalian pars intermedia. It is interesting that lymphocytes also express authentic POMC-mRNA, and this mRNA is translated and the resulting protein is processed and secreted as POMC-derived peptides. Physiological functions of these peptides produced by lymphocytes are also still unknown. Consequently, POMC-derived peptides are now thought to have multiple physiological functions and ubiqui-

tously contribute to host-defense against exogenous infections and environmental stresses.

Lastly, I would like to emphasize that the pars intermedia of the pituitary gland should be regarded as a neuro-immuno-endocrine gland in mammals. Advances in neuro-immuno-endocrinology are quite rapid. Novel physiological functions of the mammalian pars intermedia will be identified through this novel point of view.

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