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Authors: Köhidai, László, Lovas, Béla, and Csaba, György

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Effect of Adrenocorticotropic Hormone (ACTH) and Insulin on the Phagocytic Capacity of *Tetrahymena*

LÁSZLÓ KÖHIDAI, BÉLA LOVAS and GYÖRGY CSABA

Department of Biology Semmelweis University of Medicine, H-1445 Budapest, Hungary

ABSTRACT—Adrenocorticotropic hormone (ACTH) and insulin negatively influenced the phagocytic activity of *Tetrahymena*. The two hormones had diverse effects after 4 hr of treatments on no-test-particle containing, "0-cells". At this time the number of "0 cells" was significantly lower in the ACTH-treated groups, while in the insulin-treated groups there was an increase of "0-cells" compared to the control and to the results of the starting experiment. Considering previous results, when small molecular weight hormones, if did at all, positively influenced phagocytosis in *Tetrahymena*, the experiments call the attention to the differences caused by the size of the signal molecules. In the light of the literary data on hormone effects to phagocytosis in mammals and men, the similarity of the effects in species being very far from each other in evolution, could be concluded.

INTRODUCTION

Phagocytosis is one of the most ancestral physiological activities of the cell. According to some theories [23] the phagocytosis associated processes promoted the selection of the first receptor families during early periods of evolution. However the physiological role of phagocytosis is also essential in vertebrate and invertebrate systems at present time. In unicellular eukaryotic cells e.g. *Amoeba, Tetrahymena* [14, 26] the intake of prokaryotic cells as food particles is covered by phagocytosis but this latter is a general way of cellular nutrition in the higher levels up to the primates [20]. Phagocytosis serves not only for nutrition, its receptor mediated character covers wide potencies for immunological responses. In spreading hemocytes of molluscs [28], blood cells of amphibia [30] and in polymorphonuclear cells of mammals [32] phagocytic activity has a close association to the immunological responses evoked. This immune related phagocytic activity of cells itself embodies a well regulated receptor-cooperation. In recognition and targeting of microbes there are many of the subsequent intracellular events during the phagocytosis e.g. Fc gamma receptors direct the particles to phagolysosomes (killing) while e.g. the complement mediated pathway of phagocytosis provides survival to the microbe phagocyted [25].

Receptors, like recognition specific elements of the cell are used not only by food particles and immune-related substances but several hormones can also interact with receptors and some of them are modulators of phagocytic activity, too [19]. The elicited effects are diverse on the different levels of the phylogeny. In the well described unicellular model organism *Tetrahymena* [3] insulin has slight negative or no effect [8] while in the case of human neutrophils insulin and insulin like growth factors are potent stimulators of the phagocytosis [19]. Adrenocorticotropic hormone (ACTH) has also divergent effects as an increased activity was detected in molluscan hemocytes [29] however on the higher level of evolution (in human beings, too) ACTH decreased the phagocytic activity of neutrophils and macrophages [27].

The above mentioned diversities of targeting and hormonal effects elicited draw a special attention to the relations of the phagocytic process and some intracellular pathways induced by hormones. In the case of our eukaryotic protozoan model-*Tetrahymena pyriformis GL* -the effect of hormonal treatment and retreatment was investigated in several experiments. The results pointed to different hormone receptor families responsible for phagocytosis in the *Tetrahymena*. The involvement of a primitive, mu-like opioid receptor was described [2]. In the case of histamine there was a stimulated phagocytosis [11] however it was not H1 or H2 receptor specific action [7]. It was demonstrated that specific receptor-ligand binding was responsible for the phagocytic response of *Tetrahymena* [12]. Other experiments presented histidine [6], arginine vasopressin [18], serotonin [14] or insulin [22] provoked particle intakes and determined that the physiological and the non-physiological mechanisms of intake are dissimilar [22]. Some interactions of hormones (insulin and histamine) [8] and the pretreatment (imprinting) of hormones [15] pointed to receptor mediated mechanisms, too.

The hormone induced phagocytosis has a special feature in protozoa. Data gained from the reviewed references showed that substances with positive effect on the phagocytosis were always small molecules, amino acids or short chain peptides [13, 14] but we have no comparative information about the effect of signal molecules with higher molecular mass on *Tetrahymena*. All the data cited above refers to that phagocytosis has basic physiological properties in unicellular level. Since internalization of hormones in receptosomes was also described in *Tetrahymena* the receptor and postreceptor level consequences of phagocytosis should be

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also significant. Majority of experiments investigates this problem from the aspect of the short term effects of test-particle intake and few information is available about the non-responder cells. Though our knowledge about targeting of receptosomes is increasing [16, 17] the phagocytosis linked pathway and release of the test-particle [1] demand more investigation. Study of both points (non-responder cells and dynamics of test particle release) would provide characteristic insights to the long-term mechanism of phagocytosis related. On the basis of above mentioned facts our questions were: 1. Can large molecular weight peptide hormones influence the phagocytic activity of *Tetrahymena*? 2. Are there any differences in the effect of these hormones compared to each other and to the effects in phylogenetically higher species?

In the experiment the above described problems on the unicellular ciliate *Tetrahymena* was examined in a Chinese ink-phagocytosis model system. We analysed two signal molecules (ACTH and insulin) with a higher molecular mass (FW 4500 and 5780, respectively) compared to the effective molecules tested before. The ratio of non-responder cells was also evaluated.

MATERIALS AND METHODS

Cell cultures

Tetrahymena pyriformis GL cells, cultured in 0.1% yeast extract containing 1% Tryptone medium (Difco, Michigan, USA) at 28°C were used in the logarithmic phase of growth.

Hormones and buffers

The two hormones tested were insulin (Semilente MC, Novo, Copenhagen) and adrenocorticotropic hormone (ACTH, Exacthin, Richter, Budapest). The physiological, inorganic salt solution, Losina-Losinsky solution (hereafter LL solution), used for preconditioning the cells prior the phagocytosis assay was an aqueous mixture of 1% NaCl, 0.1% $MgCl_2$, 0.1%$CaCl_2$, 0.1% KCl and 0.2% $NaHCO_3$ [27].

Assay of phagocytosis

Three hours prior to the assay cells were transferred to the LL solution with the aim to have starved model objects with particle free cytoplasm.

The phagocytic activity of cells was assayed in three groups: 1. control (cells treated with the solvent of hormones); 2. treatment with 10^{-6} M insulin; 3. treatment with 10^{-6} M ACTH

In our previous experiments, physiological tests and binding assays, the responses elicited by 10^{-6} M concentrations of hormones were highly significant [3], that explains the application of this concentration in our present experiment, too. Chinese ink was dissolved in LL solution and filtered immediately before the experiment. Volumes of starved cultures, suspension of Chinese ink and agonists were mixed, (v:v:v=1:1:1). Following 5 min exposure the samples were washed twice with LL solution, then they were divided into two (a and b) subgroups.

Subgroup (a) was fixed with 4% formaldehyde containing LL solution. and the test-particle number was determined by light microscope in 600 cells per group.

Since our pilot experiments demonstrated that four hrs are required to get a significant elimination of test-particles and over this time there is no significant change we followed the next schedule. Cells of subgroup (b) were transferred into LL solution immediately after the second step of washing. After 5 min and 4 hr samples were taken and between this two time points invivo analysis were done by video microscope to detect intracellular mechanism of particle targeting and its release. The number of test particles (food vacuoles containing Chinese ink particles) was determined microscopically with a special consideration to the non-responder cells containing no test particle (hereafter "0-cells").

Statistical evaluation of data

The experiment was repeated in three parallels. The significance of inter-group differences was evaluated by Student`s t-test. Values of linear regression were calculated to evaluate changed phagocytic activity of groups treated with the two hormones.

RESULTS AND DISCUSSION

On the basis of our data gained insulin and ACTH are effective modulators of phagocytotic activity of the unicellular model cell, *Tetrahymena pyriformis*. The 5 min incubation with the two polypeptide hormones resulted a depressed phagocytic activity (Fig. 1). There were differences in the depression elicited. Insulin had only a slight shift to the left and only in cells containing 6–11 test-particle. ACTH treatment significantly shifted the histogram to the left.

The "0-cell" number at 5 min refers to a special and strong block of phagocytosis. Both hormones increased significantly the number of these cells (Fig. 2a) which suggests a direct negative membrane-level effect of hormones.

At 4 hr the "0-cell" values point to diverse effects of insulin and ACTH on the speed of intracellular targeting and release of test-particle (Fig. 2b). In the insulin-treated group the number of "0-cells" was increased which might be a result of, at least, bidirectional effect of insulin on phagocytosis. The above described weak inhibition on membrane level works at the very time of action. Nevertheless the internalized hormone [16] can stimulate the intracellular pathways of particle movement. This way the release of test-particles will result an increased "0-cell" number after 4 hr. We have to consider also the possibility of the presence of a lasting inhibitory effect as during the 4 hr period the released particles are available to the cells for engulfment, however they are coated with a special layer (Fig. 3). ACTH has opposite effects compared to insulin. Its direct, short-term inhibitory effect was very strong. By the end of the 4 hr period there was a decreased number of "0-cells" compared to the control which might be discussed as an inhibitory effect on the intracellular traffic and release. Here we should not neglect also the theoretical significance of particles released during the 4 hr. Supposing their availability for engulfment this would point also to a stimulative capacity of ACTH on particle uptake.

Considering each parameters studied the effect of ACTH was more expressed than that of insulin on phagocytosis.

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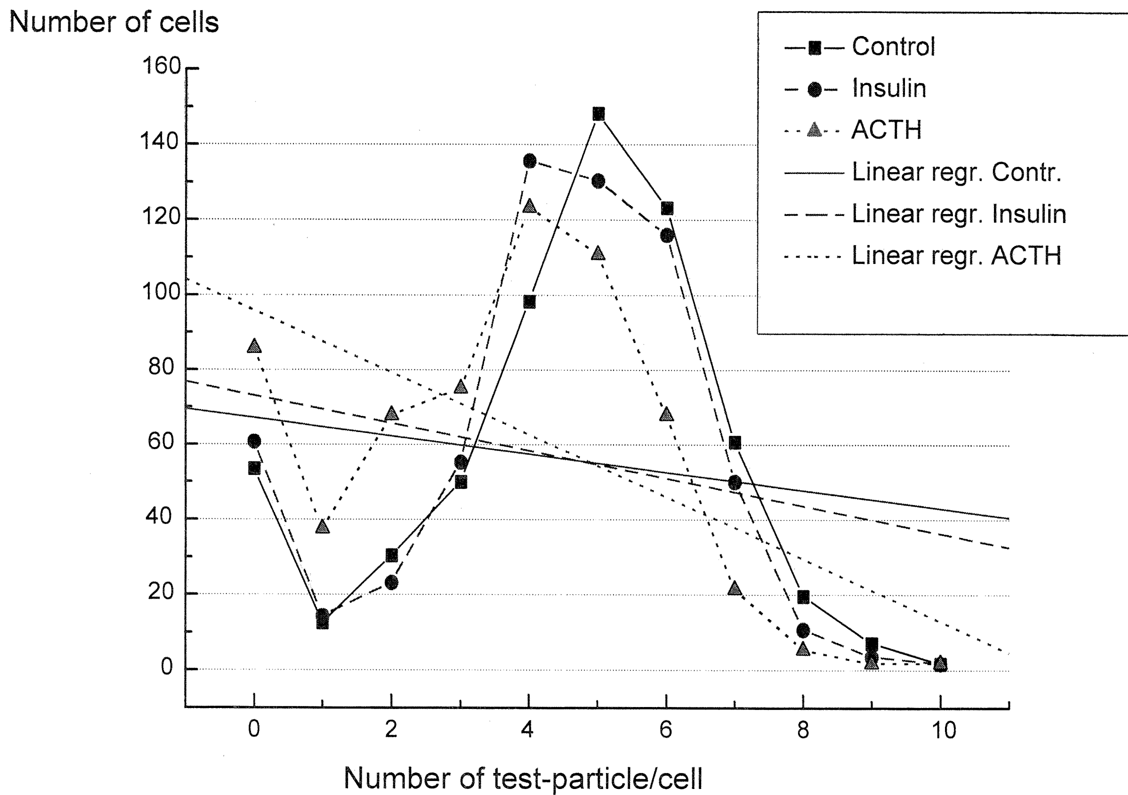

FIG. 1. Distribution of test-paticle containing *Tetrahymena* cells following a 5 min incubation with control medium, 10^{-6} M insulin or 10^{-6} M ACTH. Values of linear regression are: $R_{cont}=0.163$; $R_{ins}=0.239$; $R_{ACTH}=0.631$, where the confidence limit is 0.95.

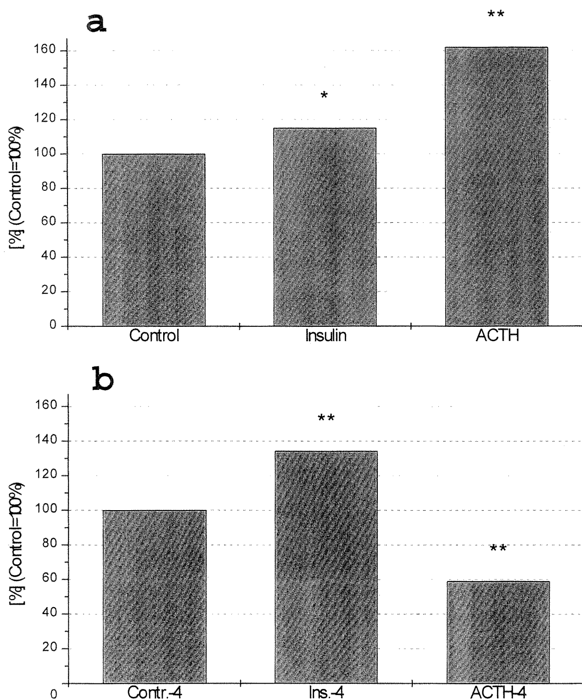

FIG. 2. (a) Ratio of no test-particle containing "0-cells" immediately after the 5 min treatment with 10^{-6} M insulin and 10^{-6} M ACTH (control group=100%). *: $p<0.05$; **: $p<0.01$. (b) Ratio of no test-particle containing "0-cells" after 4 hr the treatment with 10^{-6} M insulin and 10^{-6} M ACTH (control group=100%). **: $p<0.01$

Insulin has a more or less sepecific receptor in *Tetrahymena* which becomes more specific after the first encounter with the hormone [21]. ACTH receptors had not been studied in *Tetrahymena*. However the presence of opioid receptors [33] was justified and endogeneous opioids contain the same amino acid sequences as ACTH. So it is possible, that ACTH could act to the phagocytic activity by using these receptors. As it is known from the literature, opioids also can inhibit phagocytosis in *Tetrahymena* [31].

It seems to be clear from the experiments that large molecular weight hormones have opposite effects on phagocytosis, than small molecular ones, studied in previous experiments. The differences in the direction of influence would be dependent on the molecular weight indeed, however the quality of hormones also must be considered. The *Tetrahymena* can select between the polypeptide molecules used, as the effect of ACTH was more expressed than those of the insulin. It is important, that not only the uptake of particles was influenced by the hormones, but the release, too, what means that the whole process of endo- and exocytosis is touched by them.

As it was mentioned in the Introduction, in mammals and human beings the phagocytosis inhibiting effect of ACTH is more expressed, related to the effect of insulin. There is the same situation in *Tetrahymena*. Similar parellelism was observed in the case of other hormones earlier [5, 9, 10, 21]. The present observation provides new data to our evolution-

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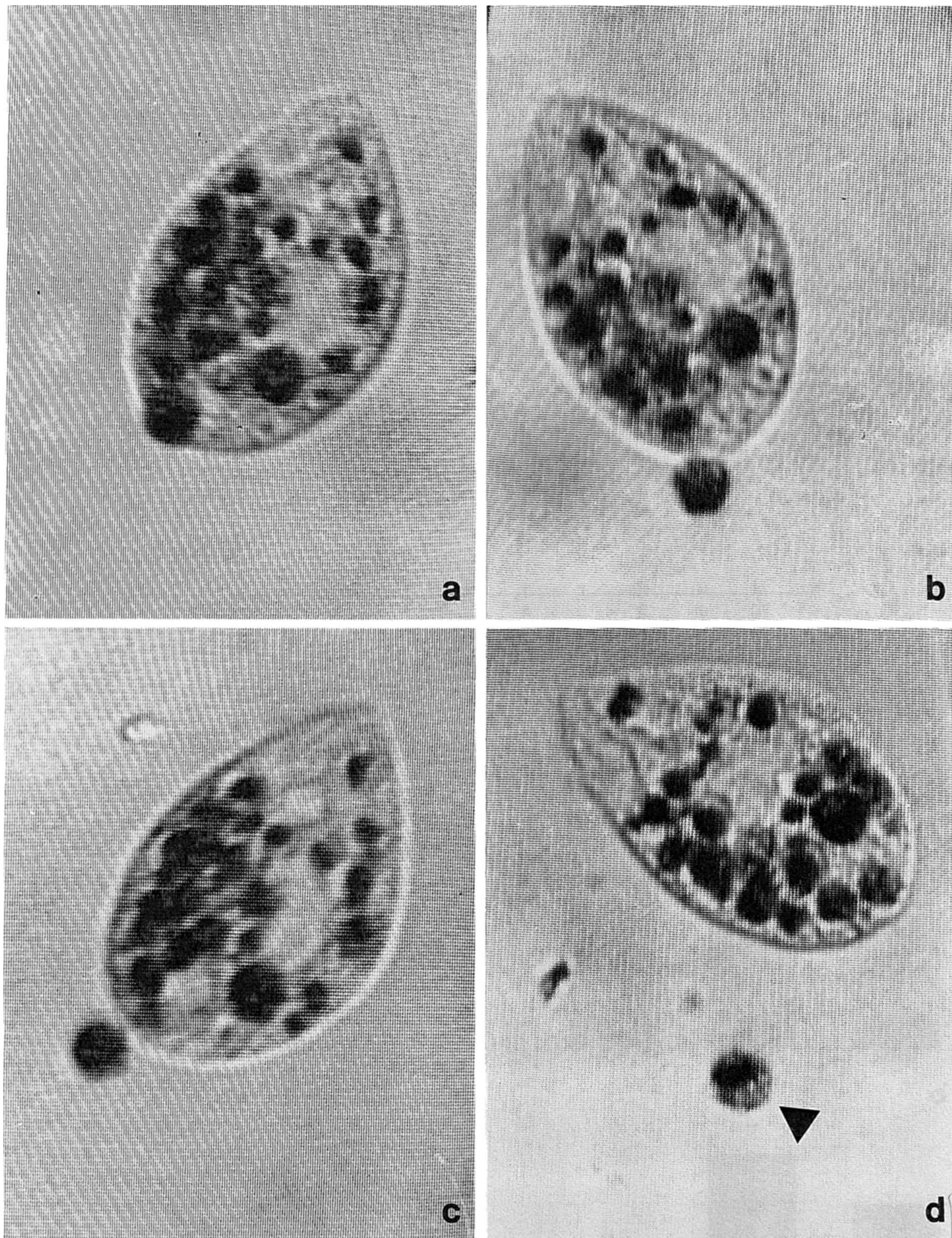

Fig. 3. Steps of test-particle elimination via cytoproct of *Tetrahymena* analysed by video microscope. Note the coverage of the leaving particle.

ary conception on the presence, similar binding capacity and response provocation of receptors for mammalian hormones in the lowest levels of evolution.

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