Effects of hormones on chemotaxis in *Tetrahymena*: investigations on receptor memory

L. Köhidai, J. Karsa and G. Csaba

Department of Biology, Semmelweis University of Medicine, POB 370, Nagyvárad tér 4, H-1445 Budapest, Hungary

Abstract

The peptide type hormones such as insulin and adrenocorticotropic hormone (ACTH) have a positive chemoattractant effect on *Tetrahymena*. Non-hormone protein-sulphate provoked negative chemoattraction. Of the amino acid type hormones, serotonin produced negative, and the histamine and di-iodotyrosine induced positive chemotactic effects. The first encounter with these molecules may modify the chemotactic behaviour of the progeny cells. In a significant number of cases there was a different response after repeated encounters of cells and hormones.

Introduction

Unicellular and multicellular organisms possess the ability to detect chemical changes in the environment (Roth *et al.*, 1983). This phenomenon embodied in negative or positive chemotaxis may play a role in the development of several diseases. The 'lazy leucocyte' (Miller *et al.*, 1971) or the Chediak-Higashi (Boxer, 1976) syndromes involving extremely high immunoglobulin concentration of serum or abnormally large lysosomes can inhibit chemotaxis. In cases of psoriasis or rheumatoid arthritis (Wardle, 1985) there is enhanced chemotactic activity which is presumably induced by leucotrienes formed locally.

The amino acids and peptide type hormones belong to a group of biologically active molecules, inducing chemotaxis (Levandowsky *et al.*, 1984). The opportunity is presented by this reaction to investigate the function of hormone receptors of certain model cells *in vivo*. After special pretreatments the changes of receptors or the operation of the intracellular receptor-effector complex can be followed.

The first encounter with a hormone leads to the development of hormonal imprinting, that is the fixation of the memory at receptor level (Csaba, 1980, 1981, 1986). Applying the hormone repeatedly results (in general) in an increased reaction, *e.g.* phagocytosis (Darvas and Csaba, 1990), division (Darvas *et al.* 1987), and binding of hormones (Köhidai *et al.*, 1986). The problem is how and in what degree can the receptor-level alterations develop whilst hormonal imprinting influences chemotaxis.

To solve this problem it is essential to clarify hormonal imprinting and, by selection of a suitable model, it may help to answer particular phylogenetical questions on hormone evolution. It is possible that in the pathways for the development of hormone molecules differences in chemotactic activity of the molecules may play an important role in selection.

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In our present experiment the frequently applied eukaryotic model cells of *Tetrahymena pyriformis* GL strain (Csaba, 1985) were investigated. The chemoattractant hormones were selected according to differences in their size and biological effects. The concentration dependence and the chemotaxis influencing effect of hormonal imprinting were tested using five hormones (ACTH, insulin, histamine, serotonin and di-iodotyrosine).

Materials and methods

Cell cultures

Tetrahymena pyriformis GL cells were used in the logarithmic phase of growth. The cells were grown in culture medium containing 1% Bacto trypton (Difco, Michigan, U.S.A.) and 0.1% yeast extract at 28°C.

Hormones and buffers

The hormones used were adrenocorticotropic hormone (ACTH; Richter G. R. T. Budapest, Hungary); insulin (INS; Semilente MC, Novo, Copenhagen, Denmark); serotonin (SER; Reanal, Budapest, Hungary); histamine (HIS; Reanal, Budapest, Hungary); di-iodotyrosin (T2; Fluka Chemicals A. G., Buchs, Switzerland); and protamine sulphate (PS; Hoffmann La-Roche and Co., Basel, Switzerland) as non-hormone material. NaCl-phospate buffer (PBS) (0.05 M phospate buffer containing 0.9% NaCl at pH 7.2) was used throughout.

Experimental schedule

Initially chemotactic activity was measured as a function of cells induced by 10^{-12} to 10^{-6} M concentrations of the hormones investigated. Fresh culture medium served as a control. Then the most effective hormone concentration was applied. Following 1 h pretreatments with the hormones the cells were cultured in fresh culture medium for 24 h. The chemotactic activity of pretreated and repeatedly treated cells was analysed and the following experimental groups were set up. (1) The control/control group was of cells which had never been in contact with the hormone. (2) The control/hormone group was of cells which had met the hormone only in the chemotactic chamber. (3) The hormone/control group consisted of cells which had been in contact with the hormone for 24 h before measurement, but in the chamber they met the control medium. (4) The hormone/hormone group of cells was in contact with the hormone twice, during pretreatment and in the chamber.

Measurement of chemotaxis

The Leick and Helle (1983) method was adapted to measure the chemotactic activity of the cells. Briefly a tube filled with chemoattractant was dipped into a hole containing test-cells. The tube had capillary gaps and this facilitated communication between the spaces containing the chemoattractant and the test cells. During incubation the result of a positive chemical signal was that the cells moved to the inner chamber containing the solution of attractant

molecules in the control medium. Thus the number of cells which entered the inner chamber were determined by sampling.

The cells were investigated in culture medium and accordingly, as previously mentioned, the hormones were also diluted in the culture medium. The working dilutions of hormones were prepared immediately before all experiments. The incubation time was 15 min and after this the samples were fixed in 4% formaldehyde diluted in PBS.

Evaluation of data

Determination of the number of cells in the samples was carried out using a Neubauer haemocytometer. For statistical analysis the NCSS and Quattro statistical programs were used.

Results

In this experiment our aim was to characterize the chemotactic activity of *T. pyriformis* GL cells induced by peptide and amino acid type hormones and to study the chemotactic response induced by different hormonal pretreatments. Cells in log growth phase responded differently to the chemoattractant hormones and this difference was observed in responses according to the concentration (Figures 1 to 4).

Two ACTH concentrations, 10^{-9} M and 10^{-6} M, showed a positive, significantly different effect from controls, while at 10^{-12} M concentration the induced effect was significantly negative to the control group (Figure 1). Insulin presented a significantly positive effect. The elevation in the range of 10^{-10} M to 10^{-8} M concentration was striking (Figure 2). In the case of histamine there were very strong, positive chemotactic responses. The maximal effect was at 10^{-10} M and only one concentration (10^{-9} M) induced chemotactic activity at the control level (Figure 3). The histamine result was inexplicable producing the highest standard deviation of the group.

Serotonin was the only hormone which did not induce chemotaxis in a positive significant way at any concentration. In contrast the range of 10^{-10} M to 10^{-9} M concentration had a significantly negative, repulsive effect in relation to the control (Figure 4). Only the higher ranges of 10^{-9} M to 10^{-6} M di-iodotyrosine concentrations induced positive chemotaxis with the maximal effect at 10^{-8} M (118%). A significant decrease occurred in relation to the control (Figure 5). The effects of the non-hormone molecule of protamine sulphate were observed as reference data. There was a negative effect at all concentrations and this was significantly lower at 10^{-10} M and 10^{-8} M concentrations (Figure 6).

In the second part of the experiments the effects of pretreatments with adequate hormone was studied. Results of ACTH pretreatment are shown in Figure 7. In this case those cells had the highest chemotactic affinity which had already met ACTH 24 h previously (ACTH/ACTH). The increased

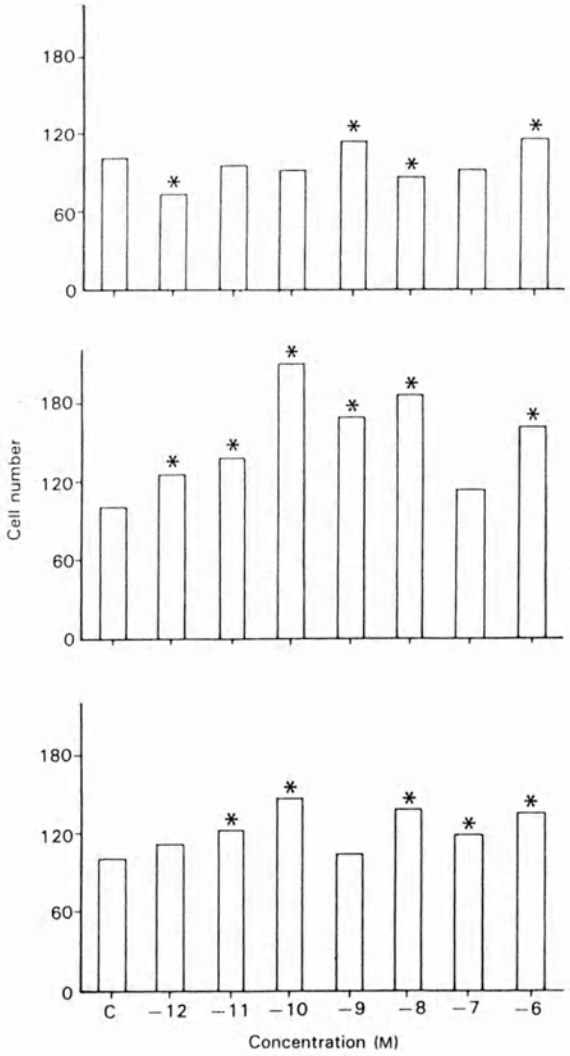

Figure 1 (top) Concentration dependence of chemotaxis evoked by ACTH. C, control = 100; *p <0.01.
Figure 2 (centre) Concentration dependence of chemotaxis evoked by insulin (INS). C, control = 100; *p <0.01.
Figure 3 (bottom) Concentration dependence of chemotaxis evoked by histamine (HIS). C, control = 100; *p <0.01.

affinity found in the group control/ACTH (C/ACTH) was analogous to the result obtained in the first part of our experiments. In the group ACTH/control (ACTH/C) an increase was also typical.

The results of insulin pretreatments are shown in Figure 8. It is clear that all three protocols resulted in increased chemotactic activity. Following pretreatments the chemotactic activity was positive in both cases. The activity

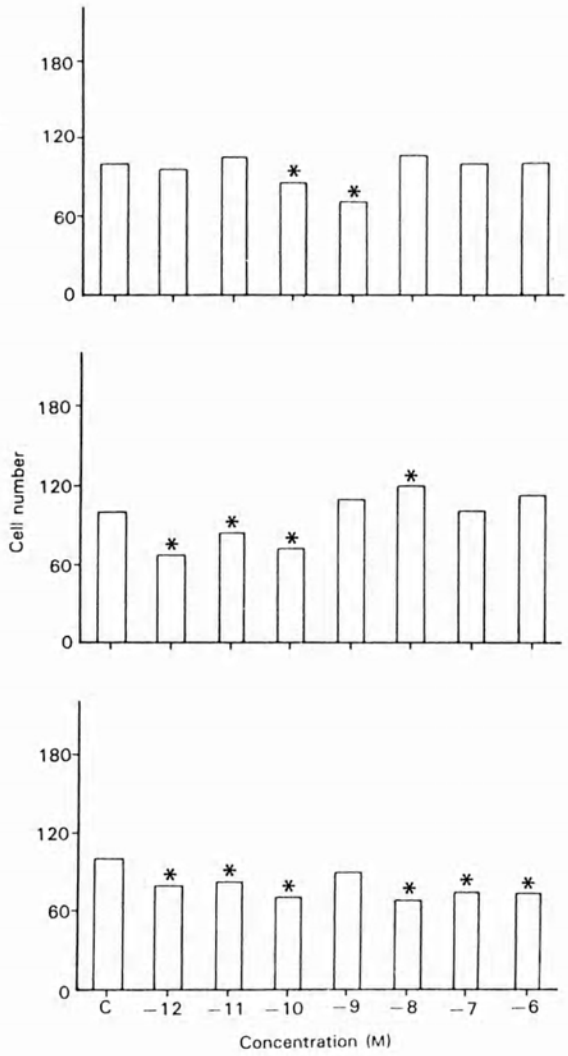

Figure 4 (top) Concentration dependence of chemotaxis evoked by serotonin (SER). C, control = 100; *p <0.01.
Figure 5 (centre) Concentration dependence of chemotaxis evoked by di-iodotyrosine. C, control = 100; *p <0.01.
Figure 6 (bottom) Concentration dependence of chemotaxis evoked by protamine sulphate (PS). C, control = 100; *p <0.01.

of those cells which met the hormone twice (INS/INS) was significantly higher than those of the insulin/control (INS/C) group. Insulin was clearly the most intensive chemoattractant of these cells at first encounter.

Following histamine pretreatment there was no difference between the control/control (C/C) group and those cells which met the histamine only at pretreatment (HIS/C). However, there was significantly increased chemo-

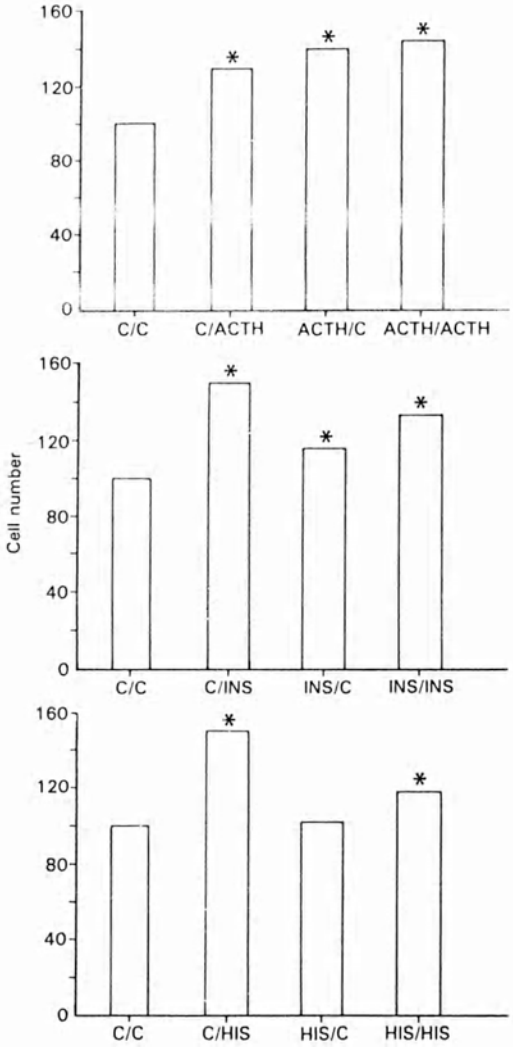

Figure 7 (top) Effects of pretreatment with ACTH on chemotactic activity. C, control = 100; *p <0.01.
Figure 8 (centre) Effects of pretreatment with insulin (INS) on chemotactic activity. C, control = 100; *p <0.01.
Figure 9 (bottom) Effects of pretreatment with histamine (HIS) on chemotactic activity. C, control = 100; *p <0.01.

tactic activity when cells met the histamine molecules again, after pretreatment (HIS/HIS). The amount of this chemotactic activity was less than the activity of those cells which met the hormone only once (C/HIS) (Figure 9).

Treatments in groups SER/C and SER/SER resulted in a distinct negative response (Figure 10). The di-iodotyrosine had a positive effect in the first part of our experiments. Here it depressed the chemotactic activity when

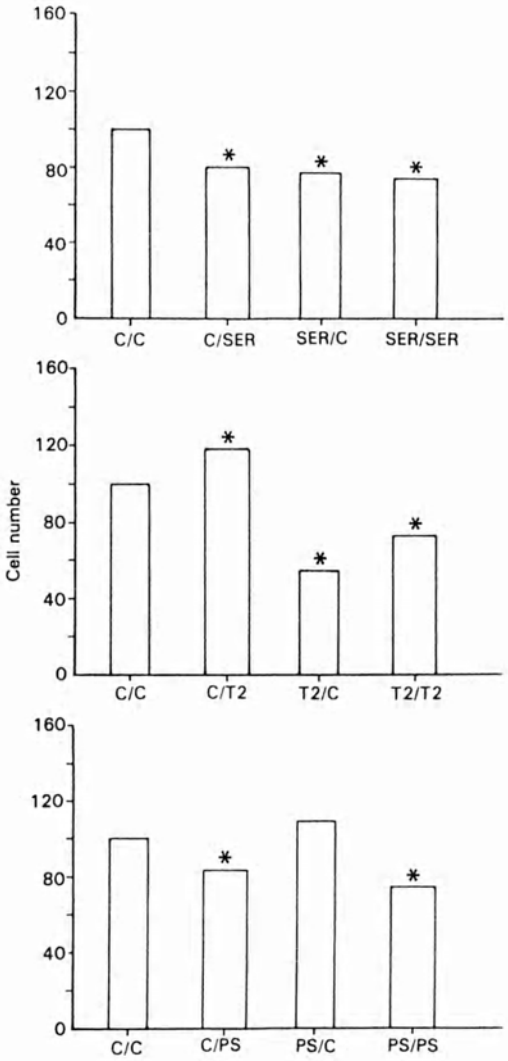

Figure 10 (top) Effects of pretreatment with serotonin (SER) on chemotactic activity. C, control = 100; *p <0.01.
Figure 11 (centre) Effects of pretreatment with di-iodotyrosine (T2) on chemotactic activity. C, control = 100; *p <0.01.
Figure 12 (bottom) Effects of pretreatment with protamine sulphate (PS) on chemotactic activity. C, control = 100; *p <0.01.

there was only a single pretreatment (T2/C) or a second treatment 24 h later (T2/T2) (Figure 11).

Following the pretreatment schedule protamine sulphate (PS) showed identical results to the first part of our experiments. The single C/PS group activity was significantly decreased in accordance with the concentration. The chemotactic activity of pretreated cells (PS/C) was at the control level. Chemotaxis

observed in double-treated cells (PS/PS) was more depressed compared with the single-treated ones, but this difference was not significant (Figure 12).

Discussion

The phylogeny and ontogeny of hormone receptors and the biological role of their molecules which influence chemotactic activity (positively or negatively) is important. In the evolution of peptide and amino acid type hormones this is especially true, and during the evolution of these molecules the former roles were defined by biological and biochemical characteristics (Ueda and Kobatake, 1977). During evolution, the signal evoked by a molecule may be a signal of nutrition and subsequently other intracellular biochemical reactions may be activated after initiation of the cell-ligand interaction (Kovács and Csaba, 1987). The appearance of hormones and bioactive materials may be regarded as products of such molecular level selection. The bases of this selection process may vary, and the structural characteristics and their chemical aspects have an important role, e.g. the capacity of the molecule to react with the surface structures of the cell.

It is important to understand how a certain molecule can influence membrane fluidity (Tanabe *et al.*, 1980; Kovács *et al.*, 1984), membrane potential (Tanabe *et al.*, 1980; Köhidai *et al.*, 1986), and the permeability of ion channels of the membrane (Köhidai *et al.*, 1987). The manner in which the molecule is transported to different parts of the cell, or which enzyme systems are modulated by the molecule itself or by liberated second messengers (Csaba *et al.*, 1976; Köhidai *et al.* 1992) are of vital significance.

Chemoattraction is another biological characteristic of such molecules. In unicellular or multicellular organisms the molecule may or may not induce a positive effect on the target cell. In this way the number of targets in the optimal zone of the chemoattractant concentration may increase. Indeed, it is conceivable that special biological functions may be developed only by molecules selected according to their positive chemoattractant character.

In our present work we investigated the effects of polypeptide and amino acid type hormones (acting in uni- and multicellular systems), as chemoattractant signals. Such effects were compared with non-hormone molecules in *T. pyriformis* model cells representing the early stage of eukaryote evolution.

The results of our experiments show unambiguously that *Tetrahymena* can detect the presence of various hormone-type molecules. The reactions manifested in chemotaxis vary in the case of hormones having an amino acid or polypeptide structure. At the same time there are significant differences within the peptide and amino acid groups. It is unlikely that hormones or hormone-type molecules are regarded as single food molecules by *Tetrahymena*. This is because the molecules having chemically similar structures can evoke dissimilar responses. Thus, the reaction is individual to such hormone-type molecule.

The two polypeptide hormones tested, namely insulin and ACTH, both at the first and the second encounter with the cells had a positive, attractant effect. Insulin in each concentration had a positive effect and this emphasizes the importance of the molecule for *Tetrahymena*. The reason is unknown and insulin synthesis in *Tetrahymena* (LeRoith *et al.*, 1980) does not provide a satisfactory interpretation. At the same time the protamine sulphate molecules, which are non-hormone, alkaline molecules, had a negative, repulsive effect in all concentrations tested. These show that hormone and non-hormone molecules can act as chemoattractants but the individual character of the molecule is responsible for the direction of the action.

The polypeptide hormones produced different results. ACTH provoked positive imprinting in which the effect was distinct, while insulin did not initiate any response. This emphasizes the difference between the two polypeptide hormones and directs attention to possible differences in insulin binding and effect. The imprinting with insulin always increases insulin binding of *T. pyriformis* cells (Köhidai *et al.*, 1990).

The amino acid type hormone serotonin had a significant repulsive effect on *Tetrahymena* cells. This was detectable at the first treatment and the cells retained the negative trend in their 'memory'. As a consequence, 1 day after treatment was initiated the activity of migration toward the test chamber was reduced. The second treatment with serotonin did not increase the repulsive activity of the molecule, but subsequently a decrease in chemotaxis was evident. This consistency, with positive signs, was detectable in the case of ACTH. An increased chemotactic reaction occurred at the first treatment and this was maintained on the next day. There was no significant increase following the new ACTH treatment.

The profiles of di-iodotyrosine and histamine were similar. Histamine had a very strong effect following the first treatment but by the next day the cells had 'forgotten' and showed a significantly lower increase at the second encounter (both treatments had an attractant character). Di-iodotyrosine showed a positive, attractive effect at the first treatment and 24 h later this became negative. Thus, repeated treatment can induce a significant elevation related to the 24-h-old pretreated cells, but it is still significantly below the control level.

Serotonin and histamine belong to the group of biogenic amines, and their chemoattractant effects and formation of functional imprinting are different. Di-iodotyrosine represents a precursor of a true hormone which appeared at a later phase of evolution. According to previous results di-iodotyrosine has a more marked effect on *Tetrahymena* proliferation than the more complex molecules developed from it. The chemoattractant effects of the three amino acid type hormone-like molecules show that this effect depends on the individual structure of the molecules.

The number of the substances investigated is insufficient to draw conclusions on the role of chemoattractant effects in hormone evolution. Nevertheless,

it seems clear that independent of the chemical character (amino acid, polypeptide), the structure of the molecules can influence considerably both the chemoattractant effects and the imprinting.

On the basis of these observations it is evident that a unicellular organism is able to select among the signal molecules and the indifferent molecules and this is manifest in the direction of chemotaxis. In many cases the encounter with the signal molecule can change the behaviour and receptive features of *Tetrahymena*. This is apparent in the retention of the effect of hormone (memory) and in altered chemotaxis when comparing the first and subsequent repeated hormonal treatments.

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