

## Effect of Oxytocin and its Analogues on the Phagocytosis of *Tetrahymena*: Outstanding Impact of Isotocin

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**Summary.** The influence on the phagocytosis of *Tetrahymena pyriformis* of oxytocin and its analogues (derivatives) tocinoic acid, isotocin and the tyrosin supplemented tail part (Tyr-Pro-Leu-Gly=Tyr) as well, as the impact of oxytocin and its analogues on the phagocytosis of the populations of *Tetrahymena* selected to itself and to the three latter molecules were studied in the experiments. The molecules tested did not influence phagocytosis in the random populations. However, populations selected to isotocin have a higher phagocytotic activity (without further stimuli) and also reacted to oxytocin or isotocin with an increased phagocytosis. Also isotocin was the only molecule the selection of which resulted in a "size-altered" population (smaller cells), and produced minimal number of non-phagocytizing "0" cells. Populations selected to tocinoic acid or Tyr reacted with a decrease of phagocytosis to oxytocin treatment. The experiments calls attention to the possible evolutionary role of (chemotactic) selection to signal molecules, to the differentiating ability of *Tetrahymena* between signal molecules and to the advantage of phylogenetically older molecules from this point of view.

**Key words:** chemotactic selection, evolution, oxytocin analogues, phagocytosis, *Tetrahymena*

### INTRODUCTION

Hormone receptors, signal transduction pathways and hormones, characteristic to higher vertebrates are also present at unicellular level (LeRoith *et al.* 1983; Csaba 1985, 2000; Christopher and Sundermann 1995). *Tetrahymena* can react to histamine and serotonin with increased phagocytosis (Csaba and Kovács 1994, Hegyesi *et al.* 1998), to thyroxin and its precursors with enhanced growth (Csaba and Németh 1980), to insulin, epinephrine and glucagon with altered sugar metabo-

lism etc (Csaba and Lantos 1975, 1976; Csaba 1994). However, in addition to the identical response, these hormones can develop other physiological reactions of protozoa. At the same time, the binding sites (receptors) sometimes are very sensitive to differences in the hormone molecule given. Precursors of hormones are more effective in provoking response than the vertebrate hormone itself and some amino acids of peptides are preferred in a signal molecule - binding site connection, which could have some importance in the evolution of signalization (Csaba 1994, 2000).

In earlier experiments, when the effect of the 9 amino acid containing peptide hormones, oxytocin and vasopressin were studied to the function of contractile vacuole, the phylogenetically older oxytocin showed the more prominent influence, in contrast to the fact that in higher

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animals the regulator of water “management” is the vasopressin (Csaba and Kovács 1992). In an other experiment, studying the effect of oxytocin and its five analogues to a basic index, chemotaxis, the consistent, repellent response was developed only by the two “matured” hormone, oxytocin and vasopressin (Csaba *et al.* 2000). This made reasonable to study the effect of oxytocin and its analogues to an other basic physiological index, the phagocytosis of *Tetrahymena*. The study has been combined with chemotactic selection of *Tetrahymena* by previous encounter with the hormone or hormone like molecule, and the study of the selected populations.

## MATERIALS AND METHODS

**Cells and Culturing.** Cultures of *Tetrahymena pyriformis* GL were used in the logarithmic phase of growth. The cells were sustained in a medium containing 1% tryptone (Difco, Michigan, USA) and 0.1% yeast extract (Difco, Michigan, USA) at 28 °C.

**Chemicals.** Oxytocin and its derivatives were obtained from Sigma Ltd. (St. Louis, USA). Substances were diluted in fresh culture medium immediately before the experiments.

**Chemotactic selection.** For this purpose chemotaxis assays were carried out according to Kóhidai *et al.* (1995), the test containing two-chambers: the outer chamber was filled with the cells to be tested, the inner one contained the test substance. In this setup tips of multi-8-channel micropipette served as inner chambers, while wells of 96-well microtitration plate were the outer chambers. Capillaries of tips served as connecting junctions between the inner and outer chambers. The concentrations of the oxytocin derivatives for these assays were chosen according to their most effective concentration of the concentration course (Csaba *et al.* 2000). In this set-up, cells of the outer chamber were considered as “mixed population”, and the drive of chemotactic substances, filled into the inner chamber, were applied to select populations on the basis of their chemotactic preference. In control groups fresh culture medium was applied as chemoattractant. Experiments were done under sterile air-flow, the time of incubation was 20 min.

The following groups were formed (see Table 1).

After each run, selected cells (of the inner chamber) were transferred into fresh culture media. The cultures formed this way were transferred every third day.

**Phagocytosis assay.** After a week of the chemotactic selections the phagocytotic activity of cultures were tested. Three hours prior to the assay the cells were transferred to Losina-Losinsky solution (hereafter LL solution - containing 1% NaCl, 0.1% MgCl<sub>2</sub>, 0.1% CaCl<sub>2</sub>, 0.1% KCl and 0.2% NaHCO<sub>3</sub>) in the aim to have starved model cells with particle free cytoplasm.

Volumes of starved cultures, suspension of Chinese ink and agonists were mixed (v/v/v=1:1:1) After 5 min incubation the cells were fixed with 4% formaldehyde containing LL solutions. The phagocytosis assays were done in the following groups: cultures provided by

**Table 1.** Chemoattractants used. The first word indicates the type of selection / the second indicates the one applied week later

<b>Oxytocin:</b> Control/Control Control/Ox1-9 Ox1-9/Control Ox1-9/Ox1-9	<b>Tocinoic acid:</b> Control/Control Control/Toc Toc/Control Toc/Toc
<b>Isotocin:</b> Control/Control Control/Iso Iso/Control Iso/Iso	<b>Tyr-pro-leu-Gly:</b> Control/Control Control/Tyr Tyr/Control Tyr/Tyr

chemotactic selection were treated (i) with the solvent (fresh culture medium); (ii) with oxytocin and (iii) with the identical “selector” oxytocin derivative. The applied concentrations were: oxytocin 10<sup>-12</sup> M; tocinoic acid 10<sup>-10</sup> M, isotocin 10<sup>-7</sup> M, Tyr-Pro-Leu-Gly 10<sup>-7</sup> M. The test particle number was determined by light microscope in 200 cells/group.

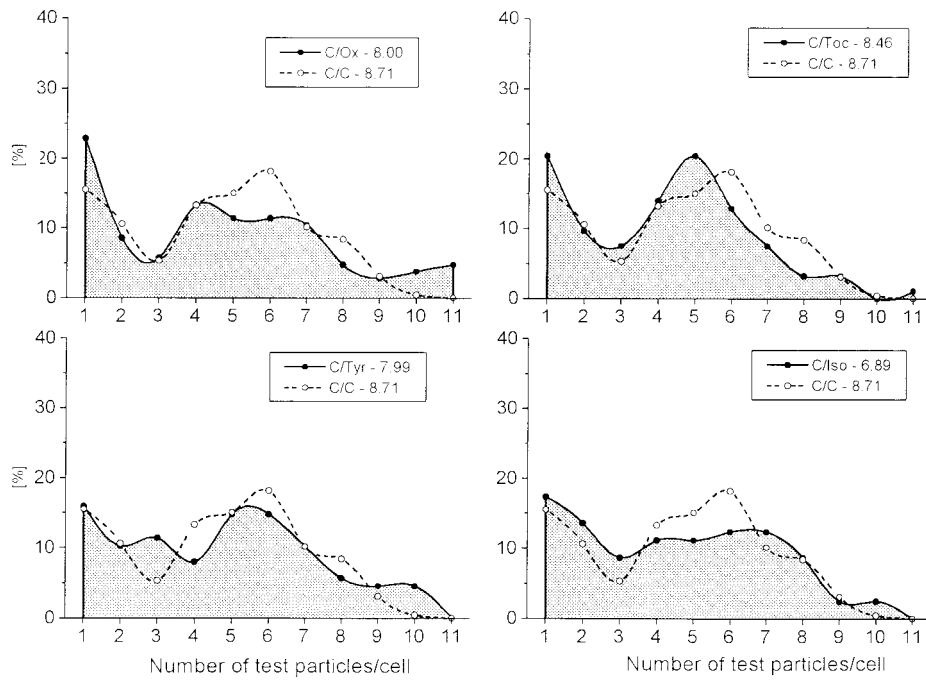
The distribution of particle quantity in the cells was also determined (Figs 1-4). Fig. 5 shows the number of cells containing no test particle (“0 cells”).

**Morphometry.** Effect of chemotactic selection upon formation new subpopulations was investigated in respect of morphological characteristics of cells. For this purpose cells of the chemotactically selected cultures (Ox, Toc, Iso, Tyr) without any further treatment were investigated by fluorescent activated cell sorter (FACS-Calibur, Becton-Dickinson). The number of evaluated cells was 10000/ sample. The FSC-H values provided us to describe the subpopulations (Fig. 6).

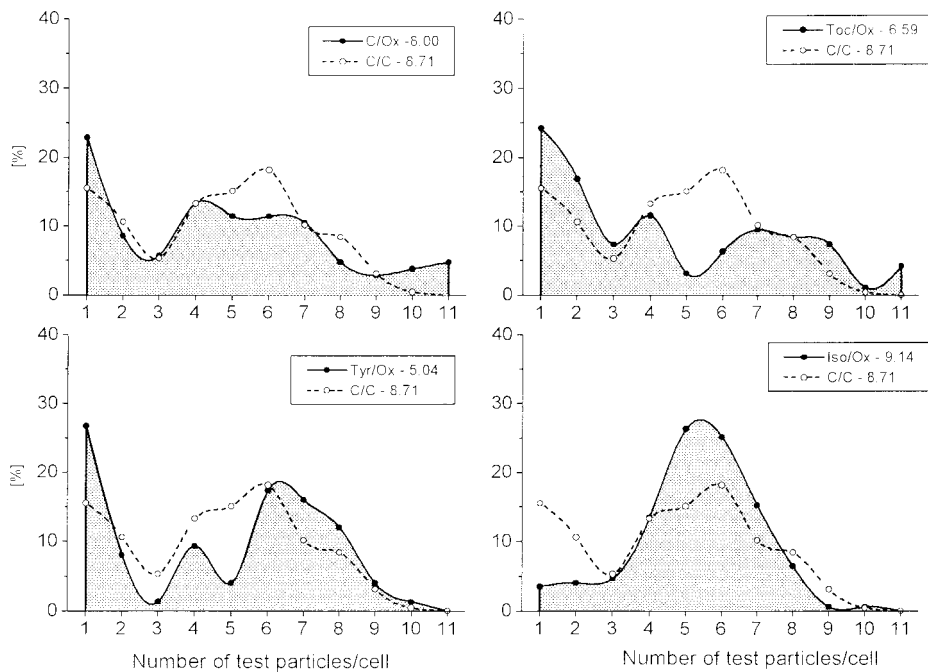
**Statistical evaluation of data.** Each assay was repeated in five independent experiments, in three replica of each. Groups treated with culture medium or groups selected with culture medium and tested with plain medium were considered as “absolute control” groups. In phagocytosis assay for each group/experiment mean values of number of particles were calculated. Data-points of figures were calculated from the mean values of identical groups. In phagocytosis assays and morphometry values of geo-mean are also given. Other data were evaluated by using statistical tests (ANOVA and two tailed t-test) of Microcal Origin 4.0.

## RESULTS AND DISCUSSION

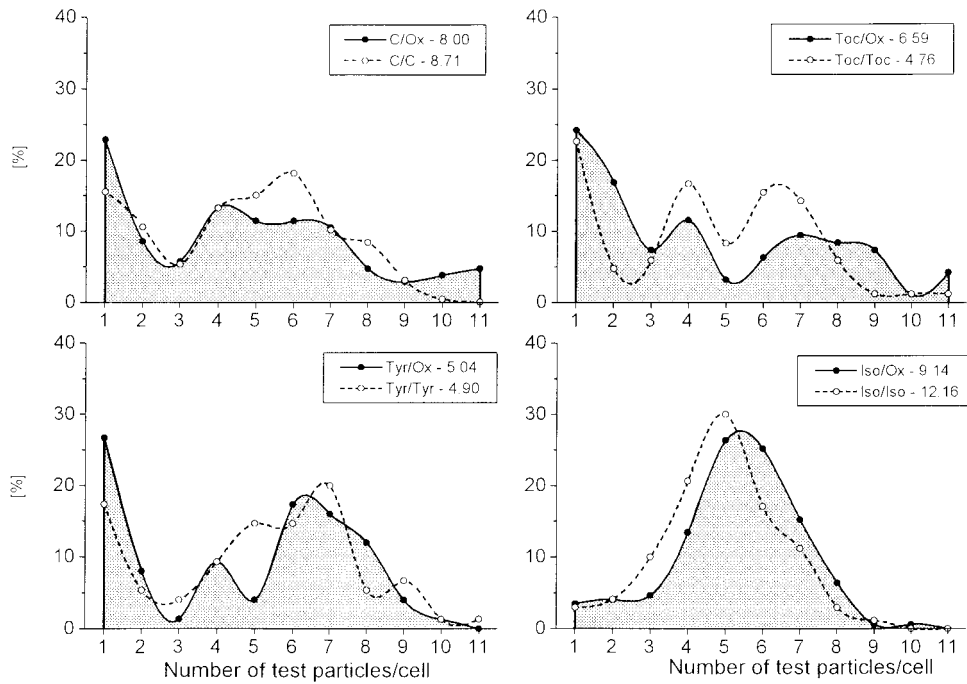
Of the four molecules studied, oxytocin is the “real” hormone which is present also in higher vertebrates regulating smooth muscle contraction and maternal behavior (Pedersen *et al.* 1982). Isotocin can be found only in bony fishes as a hormone, influencing a variety of physiological functions (Hausmann *et al.* 1995). Tocinoic acid, which contains the first six amino acids (the ring) of oxytocin is known as a molecule, which can inhibit



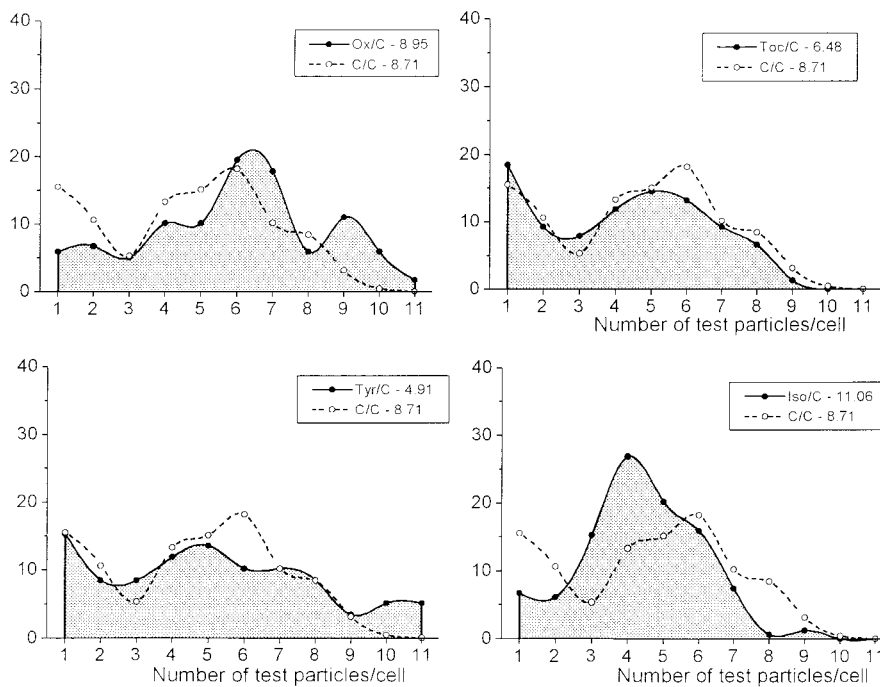
**Fig. 1.** Phagocytotic activity induced with  $10^{-12}$  M oxytocin (C/Ox);  $10^{-10}$  M tocinoic acid (C/Toc);  $10^{-7}$  M Tyr-Pro-Leu-Gly (C/Tyr) and  $10^{-7}$  M isotocin (C/Iso) of *Tetrahymena pyriformis* cultures selected with control culture medium. Dotted line represents the histogram of absolute control (C/C). Geo-means of histograms are given after the abbreviations in the boxes



**Fig. 2.** Phagocytotic activity induced with  $10^{-12}$  M oxytocin in *Tetrahymena* cultures selected with culture medium (C/Ox);  $10^{-10}$  M tocinoic acid (Toc/Ox);  $10^{-7}$  M Tyr-Pro-Leu-Gly (Tyr/Ox) and  $10^{-7}$  M isotocin (Iso/Ox). Dotted line represents the histogram of absolute control (C/C). Geo-means of histograms are given after the abbreviations in the boxes



**Fig. 3.** Comparison of phagocytotic responsiveness to identical selector and oxytocin in cultures selected by oxytocin derivatives. Selections were made as it was described above. Solid lines represent effect of  $10^{-12}$  M oxytocin; dotted lines represent histograms of identical derivative. Geo-means of histograms are given after the abbreviations in the boxes



**Fig. 4.** Basic phagocytotic activity of *Tetrahymena* cultures selected with  $10^{-12}$  M oxytocin (Ox/C);  $10^{-10}$  M tocinoic acid (Toc/C);  $10^{-7}$  M Tyr-Pro-Leu-Gly (Tyr/C) and  $10^{-7}$  M isotocin (Iso/C). Dotted line represents the histogram of absolute control (C/C). Geo-means of histograms are given after the abbreviations in the boxes

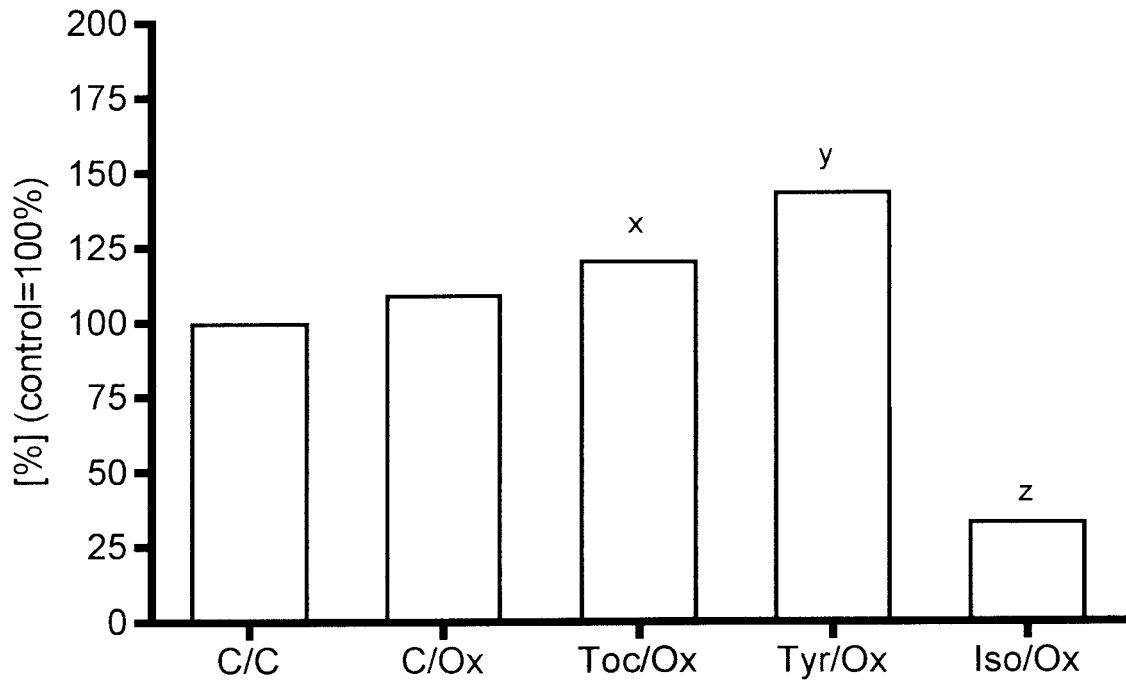


Fig. 5. Number of non-phagocytotic, „0-cells” in response of  $10^{-12}$  M oxytocin treatment in *Tetrahymena* populations selected with control medium (C/C and C/Ox) and oxytocin derivatives (Toc/Ox; Tyr/Ox; Iso/Ox)

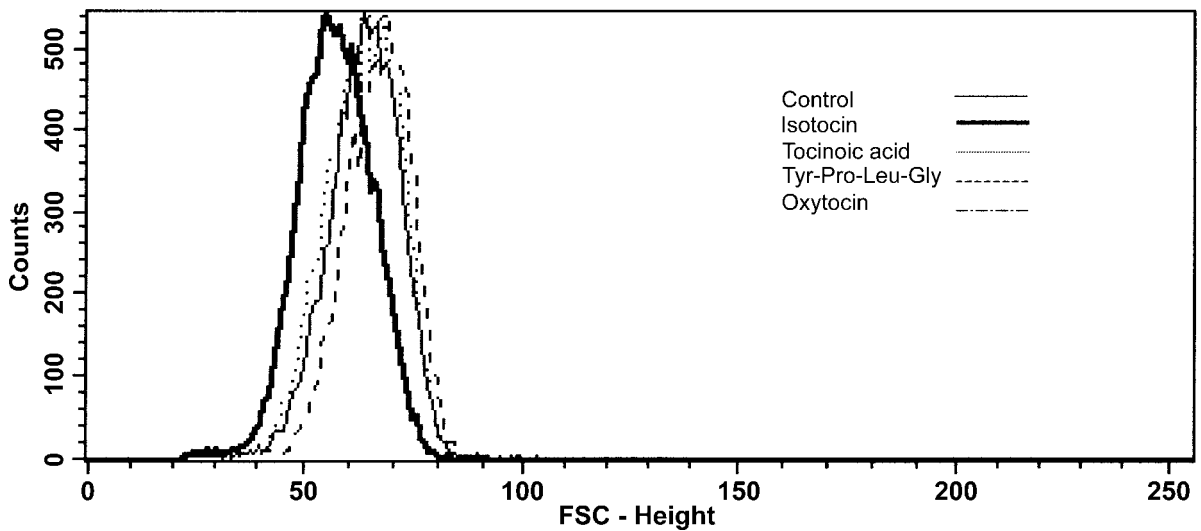


Fig. 6. Flow-cytometric results on the effect of selection with oxytocin derivatives on the morphometric properties of subpopulations

melanocyte stimulating hormone (MSH) release from the (rat) pituitary and also induces maternal behavior (Pedersen *et al.* 1982). The fourth molecule is a tyrosine containing variant of the "tail" of oxytocin (Tyr), the last three amino acids. There are no data in the literature on the effect of three of these four molecules influencing phagocytosis, however there are scarce data on the effect of vasopressin and oxytocin to the phagocytosis of macrophages (Block *et al.* 1981, Fernandez-Repollet *et al.* 1983), in higher animals.

There was no significant effect of molecules studied on the phagocytosis of *Tetrahymena* in case of random cell population (Fig.1). Though some non significant differences were observed in the number of test particles per cell, the mean values were near to each other. This means that oxytocin and its analogues are indifferent to phagocytosis in *Tetrahymena*. However selection of the cells changed the picture. Subpopulations gained by selection with Tyr or tocinoic acid the phagocytotic responsiveness to oxytocin was reduced (Tyr/Ox, Toc/Ox; Fig. 2), while it seemed to be indifferent after isotocin selection. This means that selection is working and the selected population is different -depending on the selector molecule- from the random population and because of this, hormonal effects are also differently manifested.

Comparing the phagocytic activity to oxytocin in the selected populations with the reaction of selected populations to the selector hormone itself, isotocin and tocinoic acid showed a considerable difference. In this case isotocin treatment of isotocin-selected population (Iso/Iso) significantly surpassed the value of isotocin-treated in control population (C/Iso) (Fig. 1 compared to 3) and also oxytocin treatment in isotocin selected population (Iso/Ox) (Fig. 3). In addition, isotocin selection alone considerably elevated phagocytosis (Fig. 4), while tocinoic acid- or Tyr-selection did not do the same (Fig. 4). If we also consider that Iso/Ox was significantly higher than C/Ox, which was similar to C/C, in addition only isotocin selection produced a "size-altered" population (Fig. 6), and produced the less "0" cells (Fig. 5), these facts point to the outstanding and special influence of isotocin. However, selection to tocinoic acid or Tyr also influenced (reduced) the sensitivity to oxytocin or to the selectors themselves, their effect confined to this single act (Fig. 3).

Isotocin has a hormonal function in teleosts (Hausmann *et al.* 1995) and, however its effect is manifested also in higher animals on oxytocin or vasopressin receptors, this effect is much lower than that of oxytocin (Meidan and

Hsueh 1985). Nevertheless, isotocin preference by *Tetrahymena* is not surprising, in earlier experiments also the phylogenetically older signal molecules were more effective in oxytocin-vasopressin relation as well (Csaba and Kovács 1992), as in case of the thyroxin series (Csaba and Németh 1980). From this aspect the higher effectiveness of the "real" hormones (oxytocin, vasopressin in case of chemotaxis) seems to be the exception.

From the results of the experiments it can be concluded that 1) *Tetrahymena* can differentiate between related signal molecules; 2) selection to a signal molecule can change the functional state of the cells and this could have an evolutionary role; 3) of the molecules studied isotocin has a prominent role influencing phagocytosis in selected cell populations; 4) phylogenetically older (more ancient) signal molecules are preferred by *Tetrahymena*.

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