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Short Communication

Effect of Oxytocin and its Analogues on the Chemotaxis of *Tetrahymena*: Evolutionary Conclusions

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Summary. Oxytocin and its five analogues (arginin vasopressin included) were studied in chemotaxis assays using *Tetrahymena* as unicellular model cells. Exclusively the two real hormones (oxytocin and vasopressin) were consequently repellent, other analogues (with exchanged or missed amino acids) were neutral, attractant or unbalanced. *Tetrahymena* was not able to differentiate qualitatively between oxytocin and vasopressin, however it sharply discriminated the real hormones from the analogues. Considering that in earlier experiments oxytocin and vasopressin also equally influenced the function of the contractile vacuole, it is supposed that the uniformity of the effect of these hormones is not a chance event and they had to be diverged (in function) later, during the evolution.

Key words: chemotaxis, evolution, neurohypophyseal hormones, *Tetrahymena*.

INTRODUCTION

The unicellular organisms can react to the hormones of multicellular animals and can select between them (Josefsson and Johansson 1979; Csaba 1980, 1985, 1994; O'Neill *et al.* 1988; Renaud *et al.* 1991; Christopher and Sundermann 1995). Histamine, which has a phagocytosis stimulating effect in mammals, stimulates phagocytosis also in *Tetrahymena* (Csaba and Lantos 1973). Serotonin does the same and *Tetrahymena* can differentiate the animal hormone, serotonin (5HT) from the plant hormone indoleacetic acid, which are chemically related molecules. Thyroxin and its precursors (monoiodotyrosine, diiodotyrosine, triiodothyronin) enhance the growth of *Tetrahymena* with the advantage of the phylogenetically older molecules (Csaba and Németh 1980). Using oligopeptides and dipeptides as chemoattractant or repellent molecules, the *Tetrahymena* can differentiate between them thereby showing preference of some amino acids (Kőhidai *et al.* 1997, Kőhidai 1999).

Oxytocin and vasopressin are amino acid type (related) hormones, containing 9 amino acid residues (Frieden 1976). In *Tetrahymena* these exogeneously given hormones influence the function of the contractile vacuole, with the priority of the phylogenetically older oxytocin

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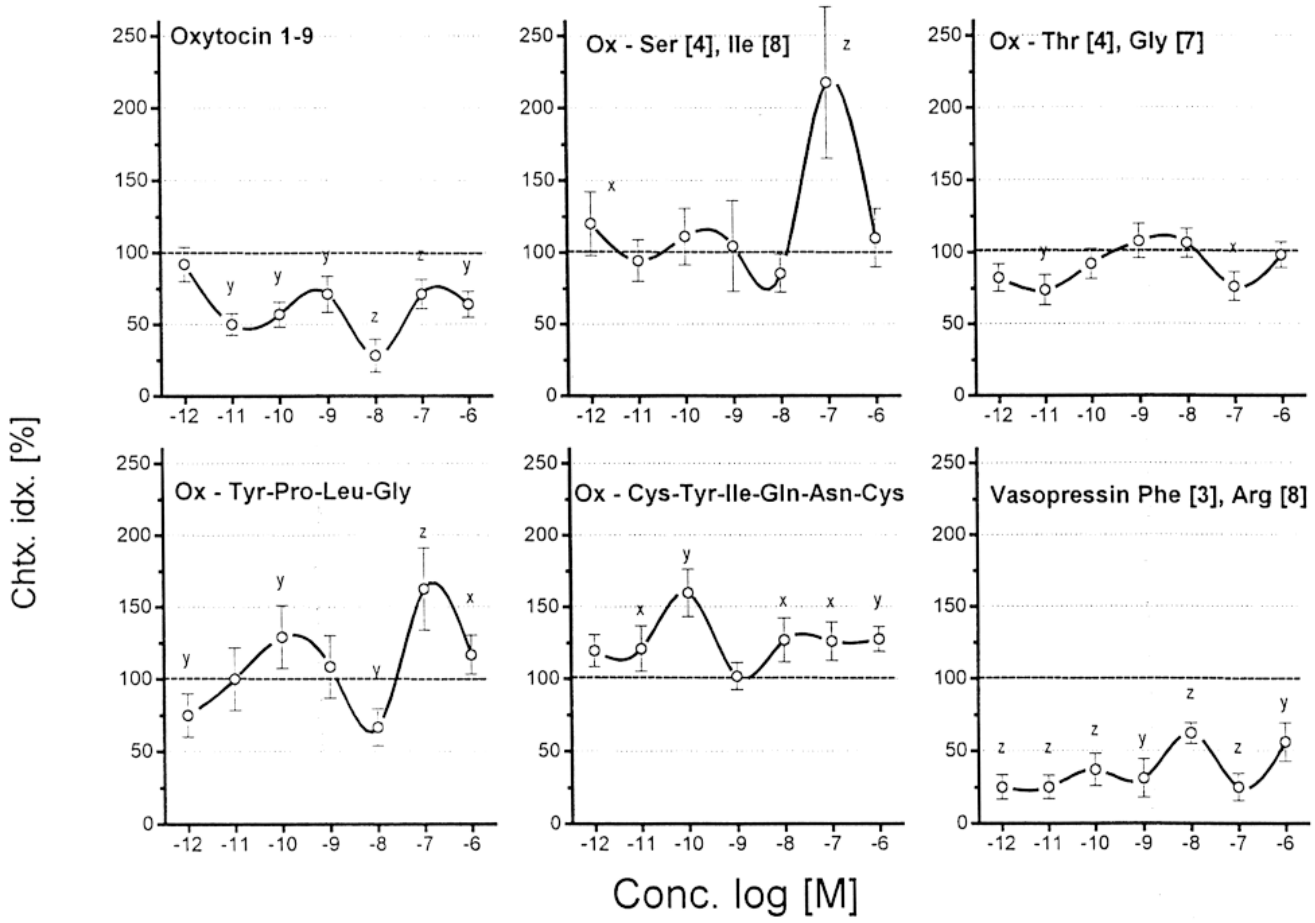

Fig.1. Chemotactic index (Chtx. idx) in percents (ordinate) related to the control as 100%. In the abscissa the concentrations of the materials are shown

(Csaba and Kovács 1992). In our present experiments these two hormones and their analogues were chosen for studying the effect of the alteration of molecules' amino acid composition in a chemotactic model system. Chemotaxis is an ancient and vital reaction of cells and single-celled animals, as a result of interaction between receptors and ligands (the chemotaxis provoking molecules - Kőhidai 1999). So the chemotactic index can give information on the interaction mentioned above neglecting the molecules' functional specificity at higher evolutionary level.

MATERIALS AND METHODS

Cells and culturing: *Tetrahymena pyriformis* GL cells were cultured in axenic cultures containing 1% Tryptone and 0.1% yeast extract (Difco, Michigan, USA). Cells of logarithmic growth phase (48 h) were assayed. Cell density was 10^4 cell/ml.

Hormones and buffers: The hormones and analogues used were: Oxytocin (OX) 1-9; OX Ser [4], Ile [8] (isotocin); OX Thr [4], Gly [7]; OX 6-9; OX 1-6 (tocinoic acid); vasopressin: OX Phe [3], Arg [8]. NaCl-phosphate buffer (PBS) 0.05 M phosphate buffer containing 0.9% NaCl at pH 7.2 was also used.

Chemotaxis assay: The chemotactic activity of *Tetrahymena* cells was evaluated with a two-chamber, capillary chemotaxis assay (Leick and Hell 1983, Levandowsky *et al.* 1984) modified by one of us (Köhidai 1999). In this set-up, an 8-channel-micropipette served as the inner chamber of the system filled with the test substances (buffers containing different concentrations of the actual oxytocin), while the outer chamber, a microtitration plate was filled with the cells. The incubation time was 20 min., this relatively short time was necessary to measure the pure chemotactic responses and prevented the contamination of our samples by chemokinetic responder cells. In the concentration course study the chemotactic responses were tested in the range 10^{-12} - 10^{-6} M. Fresh culture medium served as control substance in the simultaneous runs. After incubation the samples of the inner chamber were fixed in 4% formaldehyde diluted in PBS. The number of cells was determined using Neubauer haemocytometer.

The experiments were repeated five times in two parallels. Statistica and Origin 4.0 were used to statistically analyze data.

Values of t-probe are shown on the figures: x = $p<0.05$; y = $p<0.01$; z = $p<0.001$.

RESULTS AND DISCUSSION

Of the six molecules studied, only the two real hormones (oxytocin and arginine vasopressin) exhibited repellent activity in each concentration. The effect of vasopressin (OX Phe [3], Arg [8]) was more expressed. The change of glycine and proline to threonin and glycine, respectively (OX Thr [4], Gly [7]) neutralized the repellent effect in each concentration. Similar, however less balanced effect was produced by OX 6-9 and OX Ser [4], Ile [8]. These two molecules had a strong chemoattractant effect at 10^{-7} M concentration. OX 1-6 produced a mild, nevertheless constant attractant activity.

Repellence means that the cells recognize the surrounding molecules and escape. The two hormones-oxytocin and vasopressin- were uniformly recognized and a very low concentration (10^{-11}, 10^{-12} M) was enough for provoking running away. The effect of these hormones in higher organism is different, mainly influencing smooth muscle contraction and water resorption in mammals, respectively (Sawyer and Pang 1979). However, in earlier experiments (Csaba and Kovács 1992), in *Tetrahymena* both hormones influenced the function of contractile vacuole similarly, as was done also in the present case. This means that the *Tetrahymena* does not differentiate qualitatively between the two hormones, however it sharply discriminate the non-hormone analogues from the hormones. In addition it can distinguish between the non-hormone analogues, however this distinction is not so sharp.

The ring of oxytocin composed by six amino acids [1-6] alone has an attractant effect. The influence of the „tail" [7-9] combined with Tyr [6] is unbalanced and mostly attractant (in this case the aromatic amino acid, Tyr also could have a role). However, it seems to be likely that the ring and tail together (OX 1-9 or vasopressin) is needed for the repellent effect expressed by the real hormones.

It can be concluded that uniform and characteristic response was provoked only by the two molecules, which are hormones at higher level of evolution (Sawyer and Pang 1979). This means that selection of molecules for being hormones is not a chance event and this selection is started at a very low level of phylogeny. In the higher steps of evolution a functional refinement will happen according to the actual requirements (divergence of oxytocin and vasopressin).

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