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DIFFERENT AND SELECTIVE CHEMOTACTIC RESPONSES OF *TETRAHYMENA PYRIFORMIS* TO TWO FAMILIES OF SIGNAL MOLECULES: LECTINS AND PEPTIDE HORMONES

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Tetrahymena chemotactically responds to the presence of different signal molecules. The response elicited is different to peptide hormones and to lectins however the cell distinguishes the individual members of the two groups. The negative chemotactic effect is characteristic of the hormones tested and it is especially true for oxytocin and vasopressin, however *Tetrahymena* distinguishes also these two hormones. While the effect of hormones was mainly independent to the concentration tested, lectins elicit an increasing chemoattractant effect above 10^{-9} M concentration. Below 10^{-9} M the sugar specific, receptor level effect of lectins is probable. The experiments draw attention to the perceptional selectivity of *Tetrahymena* embodied in chemotaxis.

Lectins, the mainly plant origin proteins are carbohydrate binding molecules and their sugar-specificity has a close relation to their receptor-specificity. Mostly two or more subunits compose the lectin molecule and this character is responsible for e.g. haemagglulatination, the characteristic function of the firstly described lectins, like ricin [1]. The capacity to recognize carbohydrates of the cell surface provides a very significant function of lectins in intervertebrates. However, in these organisms immunoglobulins are absent, they have a variety of agglutinins which molecules help in fine-tuning of immune recognition system based on carbohydrate specificities. This kind of role of lectins was demonstrated in molluscs [2] and tunicates [3] where lectins participate in recognition of particles to be phagocytized. Some evolutionary conclusions were also drawn as there are common determinants of lectins isolated from tunicates and immunoglobulins while mollusc lectins have no such homology [3]. The membrane associated glycosaminoglycanes of sponges are also suggested to have a role in immune recognition [4] and some endogenous lectins were detected in human peripheral mononuclear leukocytes [5] as well as in the unicellular *Tetrahymena* [6]. This way we can say that lectins are significant signal molecules at lower and higher ranks of evolution alike. Moreover this function, lectins have intracellular functions, too. Certain lectins e.g. PHA,

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Con A, Glycine max have mitogenic potency [7] and some other lectins are toxic e.g. a subunit of *Ricinus communis* penetrates the surface membrane and inhibits the protein synthesis at a ribosomal level [8]. Many of these functions are closely related to effects on different enzymes, however some lectins themselves have an enzymatic character, too [9].

Over these significant actions of lectins there are several unsolved problems. Among them the physiological actions of lectins are inspiring as these actions are also related to signal recognition but they are not well described.

In vertebrate and invertebrate systems hormones and hormone like substances represent an other significant and well-known group of signal molecules [10]. However, the hormone-receptor mediated signaling requires a highly specific recognition both at membrane or intracellular levels, the glycosyl groups of the receptor complexes – in our present knowledge – are not essential elements of this form in signal recognition.

Considering the action of peptide hormones not only the binding itself but the consecutive steps of action: action through the receptor linked systems (in the membrane and in the second messenger level in the cytoplasm) and the internalization of the receptor are also prerequisites of specific way of action [11]. It is worth to be mentioned that some second messengers e.g. cAMP, inositol phosphates or the internalization is involved in way of action of some lectins, too [12].

Chemotaxis is one of the most fundamental physiological reactions of the cell. This activity of cell is thought to be important in evolution of cell signalling. Finding and selection of the appropriate food – especially at unicellular level – is a chemotaxis related mechanism and some theories suggest that membrane components responsible for food recognition were the origin of membrane localized hormone-receptors [13].

Our unicellular model cell the eukaryote *Tetrahymena pyriformis* is a good tool to evaluate chemotaxis related effects [14]. Not only hormones [15] but other signal molecules released during immunological responses [16], volatile oils [17], some characteristic inorganic substances [18] are also able to induce specific chemotactic activity of this cell. Beside this the cell is well characterized, it possesses almost all of the essential biochemical pathways of intracellular signal transport (cAMP, cGMP, calmodulin-Ca^{2+}, inositol phospholipids) and hormone- and lectin-binding assays [18, 19] verified that there are specific binding structures in the surface membrane. Moreover the physiological responses (growth, phagocytosis, contractile vacuolar activity etc.) [20–22] are also pointing to that *Tetrahymena* is a suitable model for characterization of chemotactic activity to lectins and hormones.

In the present work there were two problems to be answered:

(i) According to concentration course study which are the chemotactic and repellent lectins and pituitary hormones acting at unicellular level?

(ii) Considering ligand specificity, is there any similarity of lectins and hormones which are eliciting chemotactic response?

Materials and methods

Cells and culturing. Logarithmic phase cultures of *Tetrahymena pyriformis* GL were applied grown in 1% Tryptone (Difco, Michigan, USA) medium, containing 0.1% yeast extract at 28 °C.

Hormones and lectins applied. The concentration course of lectin and peptide hormone induced chemotaxis was tested between 10^{-12}–10^{-6} M concentration. For final dilution of the substances we used always fresh culture medium, identical to the medium for culturing *Tetrahymena.* The following lectins were tested: Concanavalin A (Serva, Germany); Lens culinaris* [23]; Glycine max (Fluka, Swiss); Helix pomatia* [24]; Phytohemagglutinin M* [25]. Substances marked with asterisk were purified by us according the technique cited.

Peptide hormones tested were: Follicle stimulating hormone (FSH) (Peninsula, USA); Luteinizing hormone + Follicle stimulating hormone (HCG) (Serono, Italy); Thyroid stimulating hormone (TSH) (Organon, Oss, Holland); Growth hormone (STH) (Kio Vitrum, Denmark).

Assay of chemotaxis. To assay chemotaxis we applied a double-chamber test [26] modified by us [27] where the outer chamber was filled with the suspension of cells (cell density 10^4 cells/ml) while the inner chamber contained the test substance. A capillary served as connecting link between the two chambers. The assays were carried out at room temperature (22 °C), the incubation time was 15 min. The short time of incubation was chosen as our goal was to determine the unidirectional chemotactic movement of cells towards the attractant applied. Following incubation samples were taken from the inner chamber, the cells were fixed in 4% formaldehyde containing phosphate buffer (PBS). Determination of cell density of samples was done by Neubauer cytometer. Each experiment was repeated five times.

Statistical evaluation. For statistical evaluation of data SigmaPlot 4.0 and Origin 2.8 were used.

Results and discussion

Previous experiments demonstrated that the unicellular *Tetrahymena* possesses receptors for the endogenous signal molecules of its own or such signal molecules which are characteristic of higher evolutionary levels [28]. These receptors make possible the selection among the signal molecules in the binding of these substances. This activity of the cell induces a response which is specific at many times. Our previous binding studies demonstrated file presence of Con-A-insulin receptor overlap in *Tetrahymena,* which has been known in higher animals [18]. Chemotaxis experiments also pointed to the chemotactic or repellent effect of a wide range of hormones, e.g. ACTH, insulin, serotonin, histamine [15]. The present experiments support our previous observations as they show that *Tetrahymena* is able to recognize the signal family (lectin, hormone) specific moieties and selects the applied peptide molecules.

It seems to be group specific to lectins – except Con A – that the elicited chemotactic effect is dose dependent and increasing above 10^{-9} M concentration (Figs 1a and 1b). Below 10^{-9} M the effect (curve) is unbalanced and there are some peaks, e.g. PHA at 10^{-11} M or Glycine max at 10^{-10} M (Fig. 1a). Over these differences it is obvious that all of the lectins tested had positive chemoattractant effect, We explain this positive chemoattractant effect as the significant sugar specificity of the lectins applied considering that the plasma membrane contains high numbers of sugars and amino sugars in association with or independent to the receptors. The 50 000–120 000 dalton molecular mass proteins can serve as nourishment which fact could give an other possible

explanation. However we cannot reject the respect that sugar specificity dominates at the low concentrations and the protein character (consumption as a food) dominates at the higher concentration range.

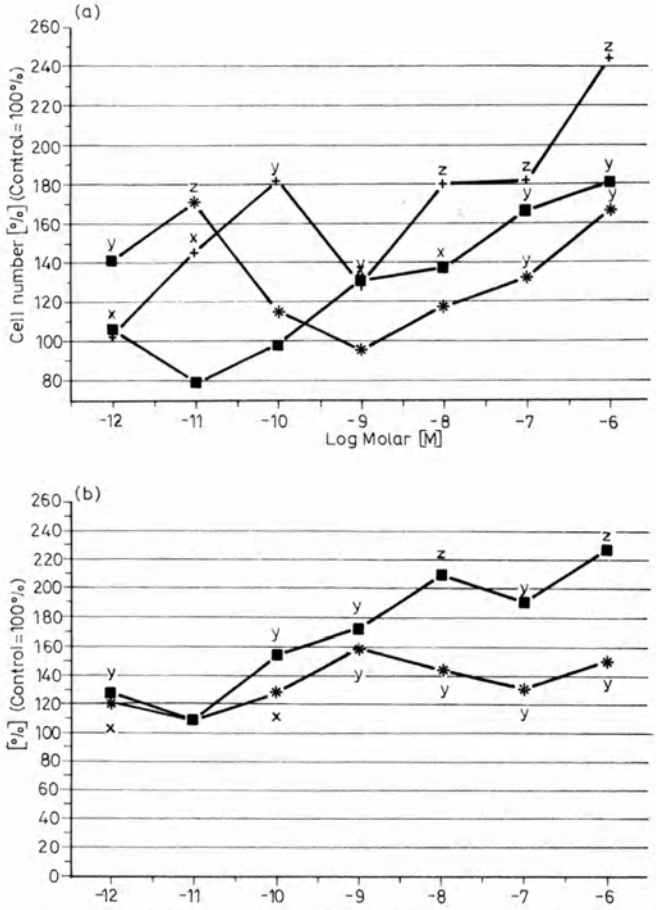

Fig. 1. Concentration course of chemotaxis elicited by (a) *Helix pomatia* (■), phytohemagglulinin (*) and Glycine max (+) and by (b) Lens culinaris (■) and Concanavalin A (*) lectins in *Tetrahymena.* (x – p>0.05; y – p>0.01; z – p>0.001)

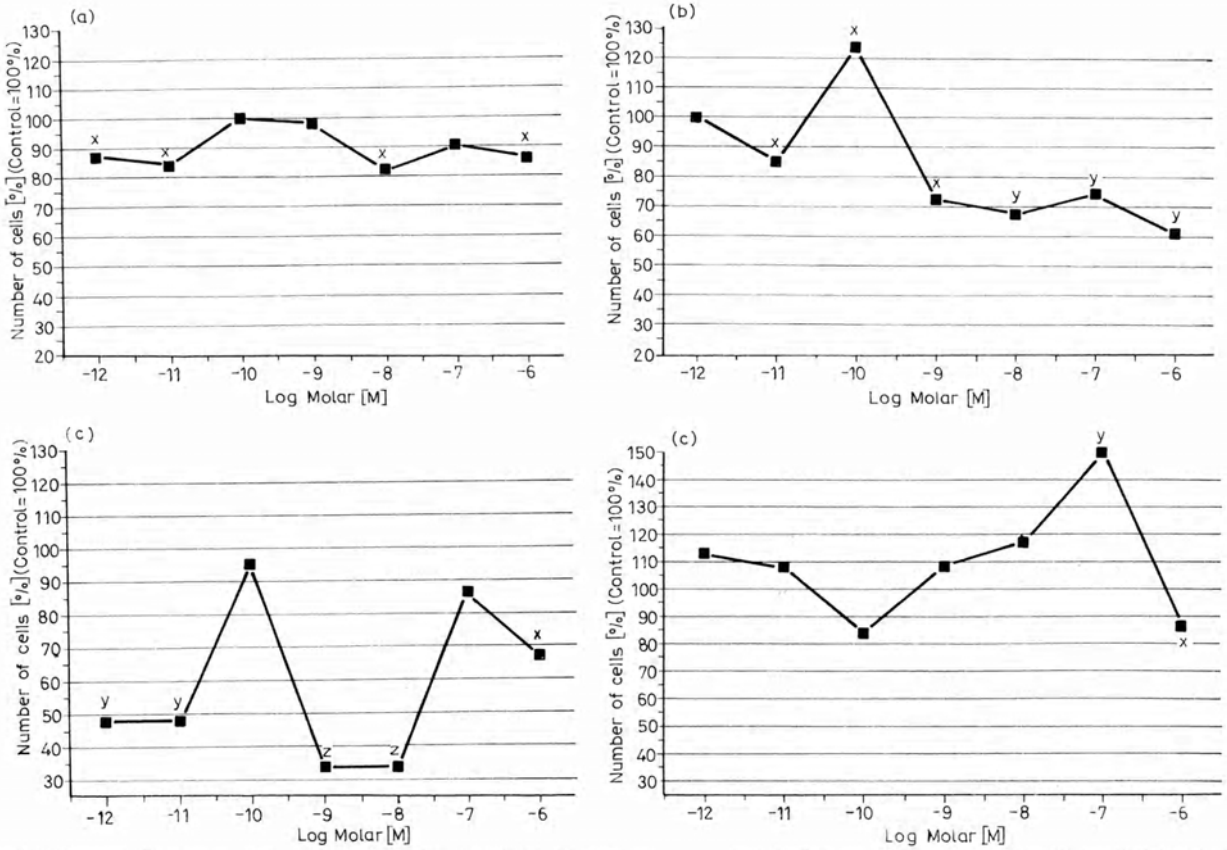

Fig. 2. Concentration course of chemotaxis elicited by (a) follicle stimulating hormone (FSH); (b) human chorion-gonadotropic hormone (HCG=FSH+LH); (c) thyrotropic hormone (TSH), and (d) somatotropic hormone (STH) in *Tetrahymena* (x – p>0.05; y – p>0.01; z – p>0.001)

Of the peptide hormones tested, oxytocin, vasopressin, follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH) had unambiguously a characteristic, negative chemotactic effect (Figs 2 and 3). The only fact that the chorionic gonadotropin (HCG = a combination of FSH and LH) had positive effects at 10^{-10} M does not modify the above-mentioned general conclusion. (Fig. 2b). The somatotropic hormone (STH) had positive chemoattractant effects at 10^{-8} and 10^{-7} M concentration (Fig. 2d).

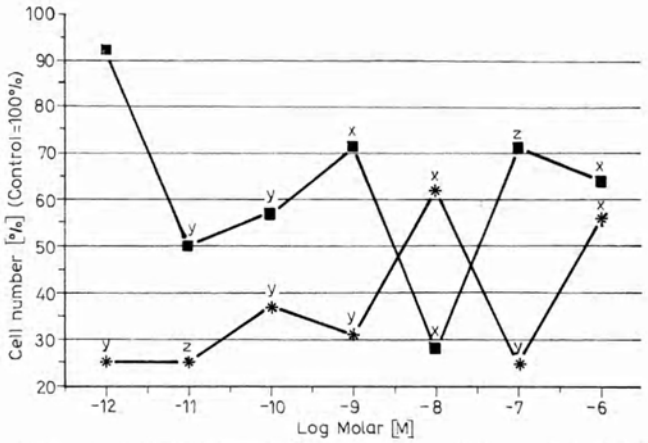

Fig. 3. Concentration course of chemotaxis elicited by oxytocin and vasopressin in *Tetrahymena* (x – p>0.05; y – p>0.01; z – p>0.001)

At high concentration (10^{-6} M) of lectins the chemoattractant effect was always considerable and values over 200% of the control were not rare. In the case of hormones the elicited chemotaxis was unambiguously negative (repellent effect) at the high concentration. This calls the attention that *Tetrahymena* does not deal with peptide hormones like peptide type nourishments. *Tetrahymena* has the potency to select between highly related hormones like oxytocin and vasopressin; the negative chemotactic effect of the two hormones is definite but it is more expressed in the case of vasopressin.

We cannot explain differences in the positive chemoattractant effect of lectins only with their sugar specificity. Con-A and Lens lectins are highly similar in their sugar specificity, in spite of this the chemoattractant effect of Lens lectin reaches 230% while Con-A only 150% at 10^{-6} M. It is possible that we can explain this difference with the difference in the size of the molecule and not with the sugar specificity. The molecular mass of Lens lectin is less than the half of the Con-A and this way the Lens lectin has the capacity to diffuse more fast and develop a more expressed effect on this way. Nevertheless at 10^{-12}–10^{-11} M range, which is the supposed effective concentration for the

receptors, the result of the two lectins is very similar. In the case of lectins (Glycine max, Helix and PHA) which are able to bind amino sugars the deviation of the curves is bigger. The binding itself to the amino sugars is only a group character of these substances, and there are great differences in the specificity of the individual lectins.

The concentration dependence of hormones was relatively low. Perhaps it is due to the low number of receptors of *Tetrahymena* which could be saturated by the lowest concentration applied. An other given explanation is that the low concentration has already the potency to elicit the maximal effect. It is known that oxytocin and vasopressin can influence the contractile vacuole of *Tetrahymena* therefore it is conceivable that the negative chemotaxis serves to avoid it. The physiologic response to gonadotropic hormones is less known. These hormones influence the RNA synthesis of *Tetrahymena*, too. However it is far from sure that the chemotactic response has connections to the mentioned effect.

We have to note the significant difference of chemotaxis elicited by follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH) (Figs 2a and c). While FSH was slightly repellent, the repellent effect of TSH was much more expressed. The two glycoprotein hormones structural homology is expressed in the identical structure of their alpha subunits and also with homologous sequences in the beta subunits (Frieden [28, 29]). This homology and the significant differences in the chemotactic potency point to a very sensitive receptor pool functioning during the chemotaxis in *Tetrahymena*.

When we consider the similar tendencies of the negative chemotactic effects elicited by oxytocin and vasopressin (Fig. 3) it is important to note that oxytocin is phylogenetically more ancestral than vasopressin [29]. The more intensive effect of oxytocin to the contractile vacuole was deducted from the above-mentioned character of the molecule, previously [30]. Nevertheless it is presumable that because of the ancestral character of oxytocin it is less foreign substance to *Tetrahymena* in such a general function like influencing of chemotaxis. This ancestral character might be responsible for the ineffectiveness of the 10^{-12} M oxytocin while vasopressin has a repellent effect at this concentration.

It is worth to mention that the molecular mass of oxytocin and vasopressin is around 1000, the gonadotropic hormones, thyroid stimulating hormone and somatotropine reach the range of 36 000, while the molecular mass of lectins is between 50 and 120 000 dalton (as it was mentioned before) [28]. If we compare the differences of chemotactic effect to the molecular mass it is provable that the role of molecular mass might be only exceptional in chemotaxis.

Hormone receptors are regularly containing glycoproteins and the sugar recognizing molecules, lectins are available to link to these residues. According to our previous experiments this lectin binding is enough to prevent binding of the hormone [31] but it is not enough to elicit a hormone like chemotactic response.

Our present experiments suggest that hormones and lectins are chemoattractant or repellent for the unicellular *Tetrahymena*. The protozoan able to distinguish the different groups of molecules (hormones and lectins) just like it has the capacity to differentiate between the individual molecules of the above mentioned two groups. The results obtained by us at 10^{-12} M concentration, point to the high sensitivity of our model cell.

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