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CHEMOTAXIS: THE PROPER PHYSIOLOGICAL RESPONSE TO EVALUATE PHYLOGENY OF SIGNAL MOLECULES*

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In this review we summarize our results gained on the investigations focused to characterize ligand and signaling mechanisms required for the chemotaxis in the unicellular model *Tetrahymena.* Our data show that short chain signal molecules (amino acids, oligopeptides) are distinguished upon their physicochemical characteristics – lipophylicity, residual volumes and statistical distribution of side-chain distances (e.g. in proline containing dipeptides), while the vertebrate hormones have also specific attractant or repellent effects in the model (FSH vs. TSH). Hormonal imprinting developed by pretreatments has also special, signal molecule dependent effect (histamine vs. serotonin). It is shown that "chemotactic selection" of cells, by the new probe developed by us is a suitable tool to provide subpopulations possessing enhanced chemotactic receptor–effector mechanisms with respect to the selector signal molecules (IL-8, TNF-α).

Keywords: Chemotaxis – signalling – hormonal imprinting – chemotactic selection – *Tetrahymena*

INTRODUCTION

The ability to recognize and select different molecules had a significant role in the early evolution of molecular development in living organisms. Because the detection of chemical signals was essential to both uni- and multicellular organisms, the development of ligands and their proper receptors represented an intercellular communication dependent and molecule-dependent event. According to Lenhoff's theory, molecular selection played a key role in this process [47]. Although the number of candidate molecules (e.g. short and long peptides) was high, most of them were consumed simply as nourishment, and had no special physiological effect on the target cells. Only those molecules "selected" as efficient signal molecules which were able to induce metabolic or other pathways. This mechanism is rather complex, involving physicochemical characteristics (stereo- and electrochemical properties) as well as

* Dedicated to Professor György Csaba for his 70th birthday.

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structural matching between ligand and receptor which influence selection. The present day ligand–receptor complex interactions are thought to be the results of the above mentioned processes [9, 11].

A wide rage of different experiments proved the feasibility of the unicellular ciliated *Tetrahymena pyriformis* GL as a good model for evaluation and characterization of the phylogeny of signaling [10]. Homologies were described on different levels: these cells possess binding sites/receptors in the surface membrane for vertebrate type hormones e.g. insulin [8], ACTH [20], ET-1 [32]; presence and adequate functioning of second messenger systems like cAMP [18], cGMP [29], IP3 [26] and Ca^{2+}-calmodulin [28] show also homologies with the higher ranked models and essential metabolic activities like carbohydrate metabolism, are modulated also similarly as in the higher ranked animals [30]. The complexity of the unicellular signaling is more underlined since on the basis of pioneering works of Csaba [19] several endogenous vertebrate hormones were described e.g. insulin [49], relaxin [58], ACTH [48], ET-1 [32] in *Tetrahymena.* The biological role of these molecules is still obscure, possibly they have a role in autocrine and paracrine regulation or they represent phylogenetic ancestors of the vertebrate isoforms.

One of the most "physiological" response of these motile model cells is the chemotaxis. Although these cells have different swimming behaviours (e.g. swimming, creeping, sliding, coiling) [54, 55] their frequent trajectory swimming makes possible to detect the required range of concentrations for the chemotactic response. Previous data showed that similarly to bacterial tumbling, in this movement there are also "stop" phases, due to the ciliary reversal. This interrupted way of swimming makes possible to develop a short-term memory by consecutive shifts from adaptation into deadaptation phase [46]. Scanning of environment via the above-mentioned motions makes possible to detect a variety of different chemoattractant or chemorepellent signal molecules. Inorganic salts [61], amino acids [3, 50], short and longer peptides [44] were reported as specific chemotactic factors to *Tetrahymena,* the effectiveness of these molecules is dependent both on the molecular structure and concentration. The high selectivity and sensitivity of these cells to chemoattractant or chemorepellent substances explains our decision, to consider chemotactic response as an index to evaluate the character of signal molecules or effect of different treatments on the basis of the chemotactic responsiveness of the cells.

In the last decade most of our projects focused on the characterization of phylogeny of signal molecules. Our goal was to describe the preferred and proper molecular structures possessing chemotactic character to answer a general problem: what makes signal molecule suitable for signaling in chemotaxis? In the majority of these works axenic cultures were investigated in the logarithmic phase of growth, but the effect of inorganic environment was also tested. The chemotactic activity of cells was determined in capillary assays [45] modified by us [40]. In the study of chemotactic selection the optimal chemotactic concentrations were applied according to the former concentration course experiments.

We approached the general problem from three main aspects: the ligand; receptors (binding sites) and pretreatments; receptors (binding sites) and selection.

On the basis of the above mentioned facts our present work attempts to summarize our results in four fileds of problems:

1. Do amino acids and short chain peptides have characteristic, signal-molecule like chemotactic effects on the phylogenetically lower level eukaryote *Tetrahymena?*
2. Do vertebrate hormones influence also chemotactic effects at this level of phylogeny?
3. Does hormonal imprinting have any molecule dependent influence on chemotactic response in the cellular progeny?
4. Will cellular progeny of signal molecule selected subpopulations via chemotaxis have a higher response to the selector substance?

CHEMOTACTIC EFFECTS OF AMINO ACIDS AND SHORT CHAIN PEPTIDES

Among other organic substances [7] amino acids and short chain peptides are the primary candidates to be consumed as nourishment [13]. In contrast the "simple" usage of these molecules, it was demonstrated in prokaryotes that amino acids e.g. Asp or di- and tripeptides have the ability to induce chemotactic responses and some significant receptors are well characterized [6, 53]. The protein circuit based on phosphorylation-dephosphorylation of "Che" proteins, mediating chemotactic stimuli from the receptor to the effector system is almost the only completely known biochemical information process in intracellular signaling [2, 52]. On the other hand, bacteria are not only migratory units of the environment, but some of the bacterial short peptides e.g. formyl Met-Leu-Phe are potent chemoattractants of vertebrate granulocytes [57]. All these findings were not surprising as both amino acids and oligopeptides have characteristic physicochemical characteristics, they are different in the size of side chains, hydrophobicity or solvent accessible areas. The high variance of these and other factors explains why we focused a significant part of our experiments onto this group of molecules. A selection of physicochemically divergent five amino acids representing structures with non-polar aliphatic R groups (Pro); molecules with positively (His) or negatively (Glu) charged R groups; polar amino acids with uncharged R groups (Met) or with an aromatic character (Phe) were studied, on the basis of their chemotactic potency. In these works the significance of environmental conditions and the optimal concentrations were evaluated since previous data of the literature were focused only to the higher concentration range (10^{-6} –10^{-3} M), and no environmental effect was analysed in parallel assays [3, 50]. Chemotaxis was tested in normal axenic culture medium and in Losina Losinsky solution, the physiological starving medium of *Tetrahymena* containing only inorganic ions. Our results showed in concert with previous data of literature [62] (Fig. 1) that *Tetrahymena* is really a very good and selective chemotactic sensor-organism. These introductory results showed us that there are strong chemorepellent molecules e.g. the aromatic Phe where neither the applied concentrations nor the environment could not modify this character.

The applied *concentration* was also decisive in other cases, e.g. Met could induce significant chemotactic effects in the low concentration regardless the composition of the environment. Similar characters were observed in the case of Pro, however, in this type of amino acid the higher concentrations made the chemotaxis reciprocal in the different media: in the normal medium Pro could work as a chemoattractant but in the inorganic environment its character turned into a strong repellent. The opposite tendency was observed in the case of Glu bearing negatively charged R groups. This molecule, or chemotaxis induced by it seems to be very sensitive to the *environment,* as both in low and high concentrations of Glu works as a strong repellent in the medium, however, in Losina solution it works as a chemoattractant. Histidine showed also high sensitivity to the composition of the environment. This amino acid could induce the most intensive chemotactic response, but only in medium and in the low concentration study.

Since the results reviewed above confirmed our basic concept that these relatively small organic molecules are still able to work not only as simple food substances but as efficient signal molecules in our *Tetrahymena* model. In the next step chemotactic effect of "mirror" variants of proline containing dipeptides was analysed. Building this special component as reference unit into peptides was promising for several reasons. Structure of proline differs sharply from that of other amino acids in that its side chain is bonded to the nitrogen as well as to the α-carbon of the central compound. This amino acid is very rare in alpha helical structures, as intramolecular

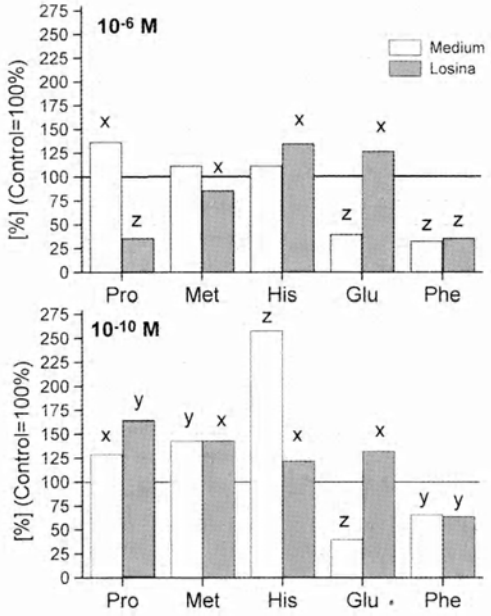

Fig. 1. Chemotactic effect of amino acids applied in 10^{-6} M and 10^{-10} M concentrations to *Tetrahymena* cells (x – $p < 0.05$; y – $p < 0.01$; z – $p < 0.001$)

rotations of N-Cα bound are limited by the rigid pyrrolidine ring, on the other hand, this is the responsible amino acid for reverse turns in proteins [43].

In these experiments [42] Pro was on the carboxy- or aminoterminal part of the molecules. Our results showed that presence of Pro makes the majority of the 12 tested substances chemoattractant. In the case of carboxy-terminal Pro some physicochemical characteristics of the partner amino acid like the residual volume or lypophilicity of the molecule had a good correlation with the chemoattractant character of the molecule (Fig. 2), in this way the small aminoterminal amino acids or the low lipophilicity was preferred like Gly-Pro and Ala-Pro, while chemotactic behaviour of Val-Pro – due to other, still obscure reasons – did not follow the trends described above. It is worth to mention that optimal separation distances between two sidechains in the dipeptides seem to be essential in the case of dipeptides (Fig. 3) which fact varifies our previous hypothesis that a well defined, optimal matching e.g. Gly-Pro and Ala-Pro is required between the ligand and its receptor for maximal chemotactic responses, while significant shifts or diversities of this profile (Phe-Pro or Val-Pro) results inadequate stimulation. All these data strengthen our concept that – although in the background there are complex interactions –, changes in the intramolecular polarity might be responsible for the modification of the chemotactic character in relatively small organic compounds.

In further experiments more mirror variants of dipeptides and some tripeptides composed by Ala, Ser, Leu and Gly were also tested. In these model peptides the

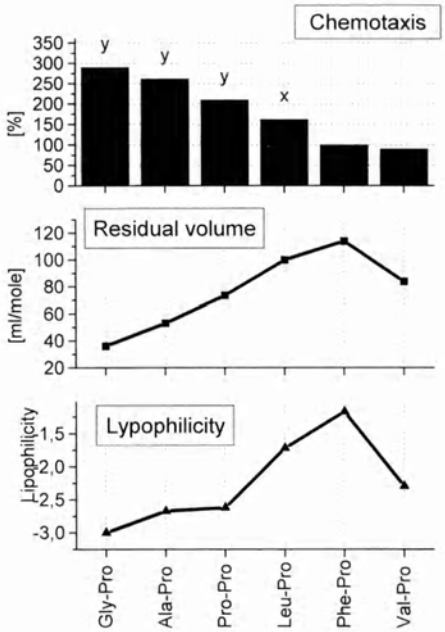

Fig. 2. Chemotaxis induced with dipeptides containing carboxy-terminal Pro. Relations of residual volumes or lipophilicity to the chemotactic activities elicited

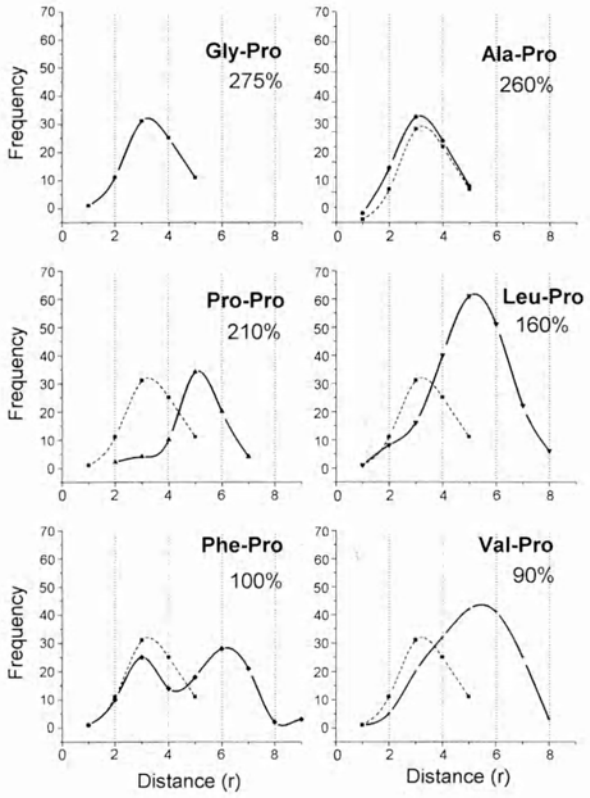

Fig. 3. Distribution of separation distances between the two sidechains of six Pro containing dipeptides. Percents represent the chemotactic activities of dipeptides compared to the identical controls

unique characteristics of proline, e.g. pKa values reaching the limiting ranges, were not expressed. Chemotactic assays with these molecules showed clearly that the size of amino acid has slight effect on the chemotactic behaviour of the molecule. The Leu-Leu dipeptide composed by big and the Ala-Ala dipeptide composed by small amino acids could induce chemotaxis on the same scale, while di- or tripeptides of the also small amino acid Gly were chemorepellent (Fig. 4). Other results suggested that the hydrophobic character is advantaged on the carboxy-terminal part of the molecule (Ser-Ala, Ser-Phe), however, there might be correlation between the hydrophobicity and pKa values of both the carboxyterminal and aminoterminal parts in identical peptides (Ser-Phe, Ala-Ser) with a preference for the higher values on both terminus (Fig. 4b). It was concluded from evaluation and comparison of data gained by tripeptides that increasing the length of the peptide chain with a single amino acid could result significant changes on the level of biological responses, but it is not regular. In the case of tripeptides, the modification of the Gly-Gly-Gly into

Ala-Gly-Gly results a more potent molecule (with a weak hydrophobic aminoterminal part), but the intramolecular modification of the molecule resulting Ala-Pro-Gly, has almost no further effect, while its mirror variant Gly-Pro-Ala proved to be the most chemoattractant among the peptides tested (Fig. 4a). This change of the molecular character is hard to be explained, nevertheless we have to remember that the activity of this molecule resembles its precursor dipeptide Gly-Pro and in this relation it is not neglectable the relatively small and close to neutral Ala probably has no potency to modify the strong chemoattractant character of Gly-Pro.

The problem of longer peptide chains mentioned above was tested more with three types of model oligopeptides composed by 1 to 5 units of the same amino acids (Gly,

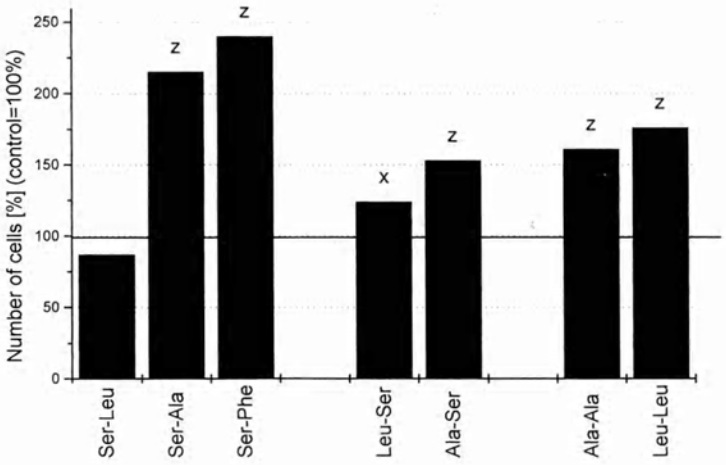

Fig. 4a. Chemotactic potency of 10^{-6} M di- and tripeptides composed by Gly, Ala and Pro

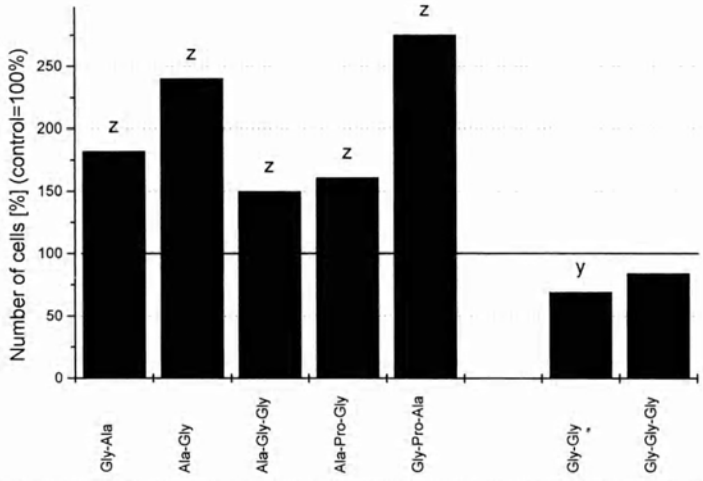

Fig. 4b. Chemotactic potency of 10^{-6} M di- and tripeptides composed by Ser, Leu and Ala

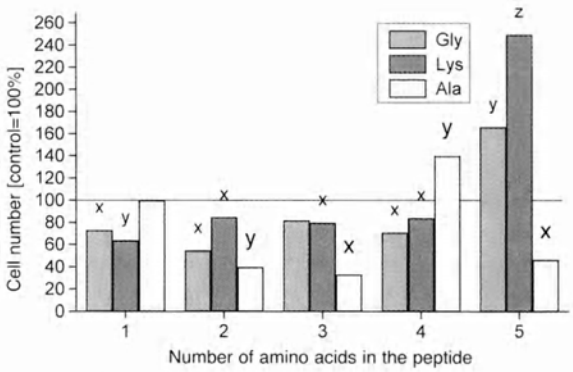

Fig. 5. Chemotactic activity of 10^{-6} M Gly, Lys and Ala and their oligopeptides composed by the same amino acid in *Tetrahymena*

Ala, Lys), in inorganic environment [39]. The chemotactic effect of these peptides (Fig. 5) showed that only the polar, hydrophilic oligopeptides, composed by 5 Gly or 5 Lys could work as potent chemoattractants, the shorter chains were chemorepellent or neutral. Comparison of the dipeptide results – gained in normal medium – with these data, underlined again the individual sensitivity of amino acids or peptides to the environment [17]. In these conditions 2-Ala (Ala-Ala) could not express its previously described chemoattractant character, however, the 4-Ala proved to be optimal with its significant chemoattractant character.

In summary of the experiments mentioned above, it can be concluded that these short molecules have the selective ability to work as chemoattractants or chemorepellents in our model system. Our results support the almost 20 year old findings of Tanabe et al. [62] that hydrophobicity is the determinant in chemotaxis as it has the proper depolarizing effect to the *Tetrahymena* membrane. On the basis of our investigations we suggest to comprise into previous theory that some other characteristics of the molecules like pKa values of the carboxyterminal amino acids or the intramolecular hydrophilic-hydrophobic axis seems to be essential, optimal matching of these factors – and other still unknown effects – is required for the induction of chemotactic process. The way of induction is not known but interaction with these molecules or incorporation of them into some compartments of the cell fulfills Leick's hypothesis [44], that an anterior location of small vesicles with a hydrophobic microenvironment in their membrane is important for the directed migration during chemotaxis.

CHEMOTACTIC EFFECTS OF VERTEBRATE HORMONES

On the basis of the specific chemotactic effects of amino acids and model peptides it was worth to evaluate the signal character of such larger oligo- or polypeptide hormones which possess well-defined biological effects in vertebrates. Detection of

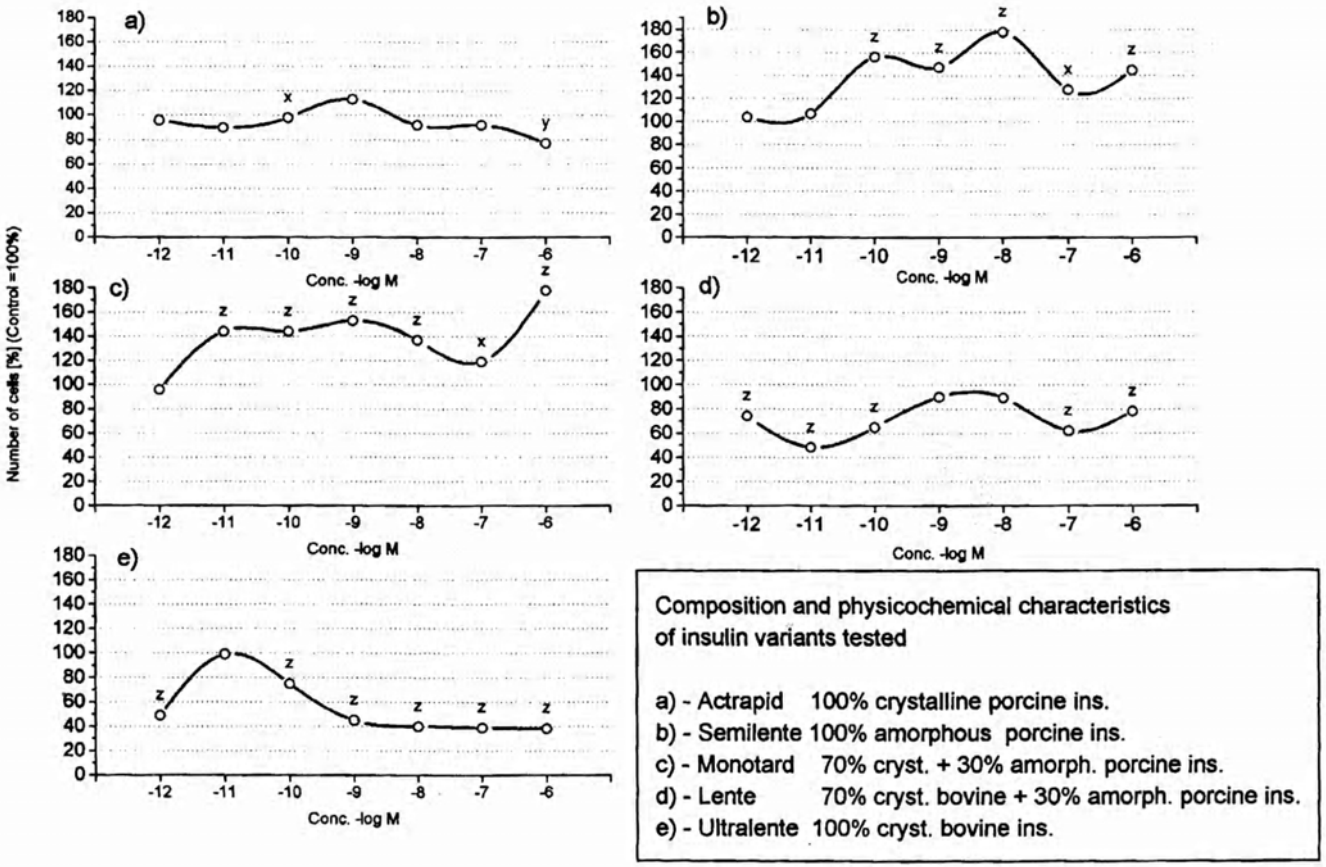

Fig. 6. Concentration course of chemotaxis induced with variants of insulin in *Tetrahymena*

their chemotactic nature in *Tetrahymena* was promising, even when they are not chemotactic in the higher ranks of phylogeny, as some of these hormones were already detected as endogenous substances of this ciliate.

Investigations of peptides present in different fluids of vertebrates showed that several growth factors proved to be effective chemoattractant to normal and malignant cells [65], some of them e.g. PDGF were also reported as chemoattractants for *Tetrahymena* [4].The frequently studied hormone, insulin belongs into this group as its chemotactic character and the requirement of selective induction of its receptor mediated signaling was demonstrated in CHO cells [64]. All the facts mentioned supported our purpose to test whether the system of vertebrate chemoreception works in ciliates. At first concentration dependence of chemotaxis induced by bovine and porcine isoforms was tested [14]. Results of these experiments point to that *Tetrahymena* is also able to distinguish relatively slight differences in insulin (Fig. 6) (they differ in their A chains as in the bovine form Ala and Val, in the porcine form Thr and Ile are present in the identical and functionally important positions) and prefers the porcine isoforms, while bovine insulins have repellent effects. Its nanomolar effectiveness has also good correlation to the chemotactic activity of porcine insulin to human T lymphocytes [5]. Over the above-mentioned observations, the chemoreception of our model seemed to be also sensitive to physicochem-

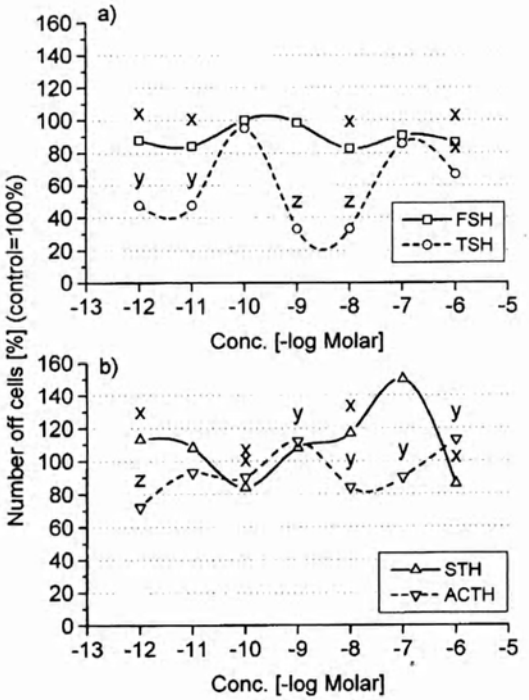

Fig. 7. Concentration course of chemotaxis induced with four characteristic hormones of the hypophysis

ical characters of the applied insulins as amorphous insulins were more effective than the crystalline isoforms.

Hormones of the hypothalamo-hypophyseal system were also good models in chemotaxis studies [33]. Although their molecular mass is ranging from 1000 to 40,000 and their size makes them ideal food substances, the size itself and differences in it had almost no influence on the chemotaxis which indicates to that only appropriate sequences or parts of the molecules are responsible for their action in this ciliate. Concentration course studies proved (Fig. 7) that two structurally homologous glycoprotein hormones TSH and FSH – their α subunits are identical and the β subunits have also homologous sequences – have diverse effects: the repellent effect in TSH was much more expressed than in FSH. Chemoattractant character was observed in the case of other two hormones STH and ACTH (Fig. 7). STH had a significant chemoattractant effect at 10^{-7} M but it was effective in the 10^{-9}–10^{-8} M range too, while ACTH had only one and weak peak at 10^{-9} M. Although the primary effects of these hormones is not to induce chemotaxis in vertebrates it is worth to note that serum concentrations of the two hormones are in a close, 10^{-11}–10^{-10} M range, which foreshadows the presence of a phylogenetical continuity of these signaling systems.

The selectivity of chemotaxis was also proved by two other members of the family mentioned above (Fig. 8). The sequence of the two nanopeptides vasopressin and oxytocin vary in position 3 (Phe-Vas.; Ile-Ox.) and position 8 (Arg-Vas.; Leu-Ox.) but these differences are so significant that these hormones have not only diverse physiological effects in mammals, but the chemotactic potency – repellent effect – of vasopressin in the low concentration range (10^{-12}–10^{-9} M) significantly differ from oxytocin. The chemorepellent effect itself in *Tetrahymena* is surprising as oxytocin was already referred to be chemoattarctant to other highly motile cells, the mouse spermatozoa [59], however, the presence of oligopeptides in the ovarian granulosa

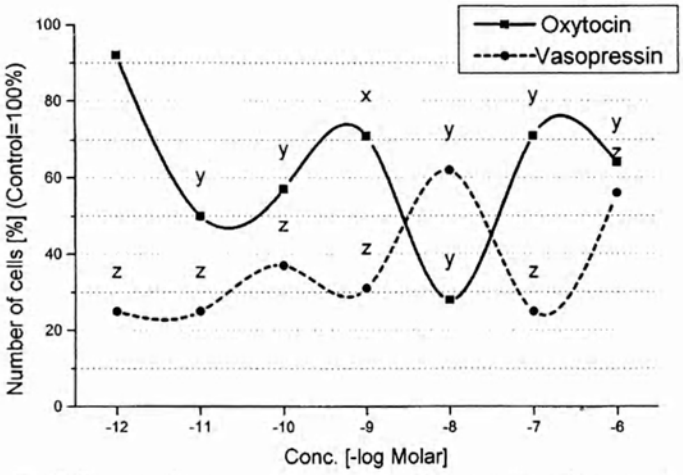

Fig. 8. Concentration course of two nanopeptides, oxytocin and vasopressin

cells and the fluid around, explains the attractant character. In addition, this the difference between the effects of the two relative peptides should be discussed. It is important to note that oxytocin is phylogenetically more ancestral [22] than vasopressin and it is presumable that because of this ancestral character of oxytocin it is considered as less "foreign" molecule than the strong repellent vasopressin. The more intensive induction of contractile vacuoles by oxytocin was also deduced from the above mentioned phylogenetical difference of the two molecules [48].

Finally we review the effects of two hormones thought to be specific to the circulatory system: endothelin-1 (ET-1), a representative of potent mediators of vasoconstriction in mammals and atrial natriuretic peptide (ANP), which complex physiological effects are portrayed as induction of natriuresis, inhibition of aldosterone, vasopressin and renin secretion and relaxation of smooth muscles. Both hormones are polypeptides, the effective form of ET-1 is composed by 21 amino acids, while the length of ANP varies from 21 to 73 amino acids in various vertebrate tissues. Though some references pointed to the chemotactic effect of ET-1 and ANP in PMN [21, 63] and monocytes [1]; our chemotaxis assays proved for the first time in the literature that these hormones can act selectively at the level of protozoa (Fig. 9). Comparison of the two hormones showed that, although *Tetrahymena* considers both hormones as chemoattractants even at very low concentrattions (10^{-15} M), the ANP-response is more significant. Here we have to remind of that ET-1 like endogenous substances were also detected in *Tetrahymena* which provides the possibility of auto- and paracrine regulatory mechanisms in contrast the "foreign" and therefore more inducing ANP. Problem of phylogenetical continuity is again propounded as we can find again overlapping ranges of the effective concentrations and the serum levels of these hormones even in human.

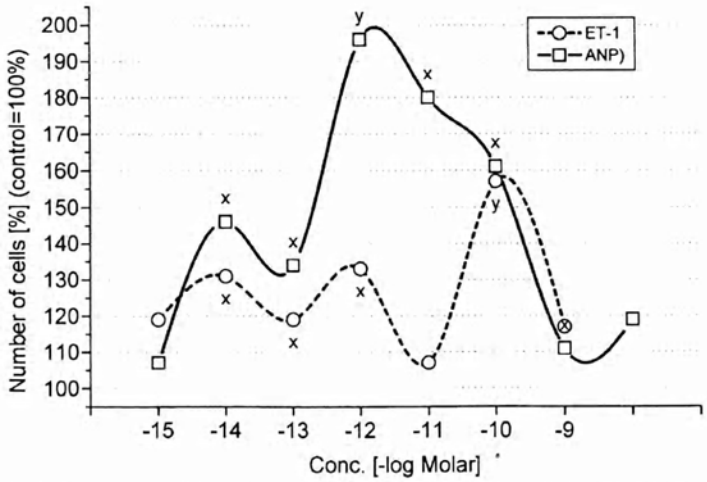

Fig. 9. Concentration course study of two cardiovascular peptides – endothelin-1 (ET-1) and atrial natriuretic peptide (ANP) in *Tetrahymena*

The conclusion of our results reviewed in this part show that not only small molecules – amino acids or short oligopeptides – have the potency to induce chemotaxis in our model cell. Even relatively small structural divergences e.g. insulin variants, TSH-FSH, oxytocin-vasopressin are selectively detected via chemotactic responses. These results point to that the *Tetrahymena* model is still a proper object for the ligand – receptor studies focused to characterize the optimal – big or small – signal molecule for chemotaxis or interactions between chemotactic signal molecules and the target cell.

RELATION OF HORMONAL IMPRINTING AND CHEMOTACTIC RESPONSE IN THE CELLULAR OFFSPINGS

Following characterization the main types of ligands required for chemotactic responses, essential part of our work was to get information about dynamics of the inducible mechanisms. In this case, to study the development of hormonal imprinting was very advantageous. Hormonal imprinting develops by pretreatments of cells or whole organisms, being in sensitive period, with signal molecules [12] (Fig. 10). The result of the pretreatments is a "tuned" cell or organism which has a altered responsiveness to the "imprintor" – the signal molecule treated with. In many cases this means that a special memory develops in the "imprinted" cells and at a second encounter with the imprintor molecule the cell or the offsprings of it usually express a changed, often an enhanced response for hundreds of generations [36]. There are several levels of which we can influence this process: the membrane and its receptors [41, 51], the second messenger mechanisms [24] and other enzyme systems [27]

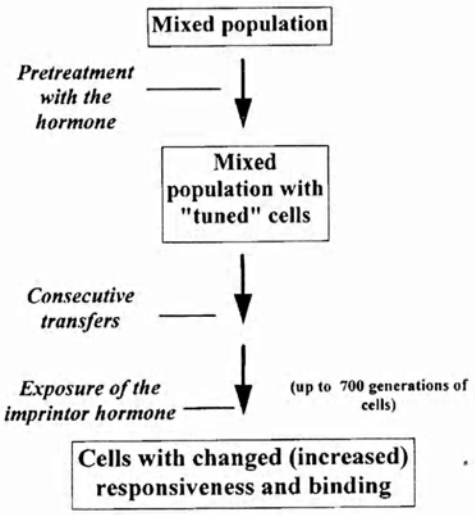

Fig. 10. Development of hormonal imprinting – general scheme

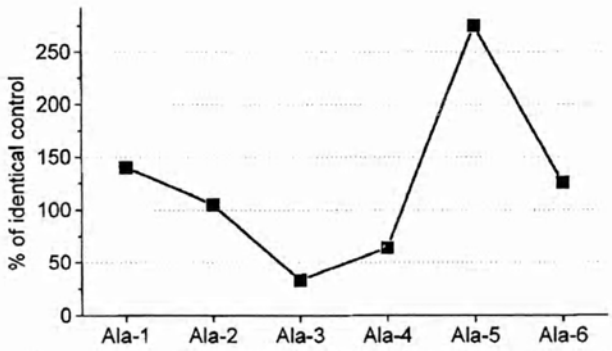

Fig. 11. Effects of hormonal imprinting with 10^{-10} M Ala and its oligopeptides on the chemotaxis to the imprintor Ala variants in *Tetrahymena*

or some nuclear mechanisms [38], all were proved to be responsible for development of the mechanism, but the size or other chemical characters of the acting ligand [31], the applied concentrations [16] the time courses and down-regulation [15] also influence the process, however, the whole pathway of saving the character of the first signal is still obscure.

In this relation study of chemotaxis in imprinted and in control *Tetrahymena* cultures provides a more complex understanding the ligand-receptor interactions of chemical signaling. At first the nonhormone Ala amino acid and its oligopeptides were studied [17] (Fig. 11). Following the pretreatments with these substances the cells were tested again in the 70th offspring generation. Results suggest that the length of chain is important in the imprinting, as the 5 alanine unit containing oligopeptide proved to be an excellent imprintor and chemoattractant, however, there is no consistent relation between the length and its imprintor capacity. Among others, four hormones – ACTH, insulin, histamine and serotonin –, were also tested as imprintors [37] (Fig. 12) and their ability to induce long-lasting memory was clearly different. Insulin and ACTH, the two moderately large hormones (Mol. weights: insulin – 5700, ACTH – 4500) worked in a diverse way, pretreatment with ACTH resulted a significantly enhanced chemotactic response, the insulin pretreated cells expressed also higher chemotactic activity then the absolute (C/C) or relative controls (INS/C), but this activity was lower than the response to insulin at the first encounter. The contradiction between the results cited above and our binding site studies points to that the high number of binding sites for insulin following pretreatments [25] is not unambiguous guarantee of enhanced physiological response e.g. chemotaxis in a ciliate.

Chemotactic potencies of the two structurally related biogenic amines – serotonin and histamine – were also different following pretreatments (Fig. 12). Although histamine and insulin differ not only in molecular characters but their signaling mechanisms are also diverse, the profiles of their chemotactic responses following the pretreatments are similar, the first encounter was more successful then the second. These

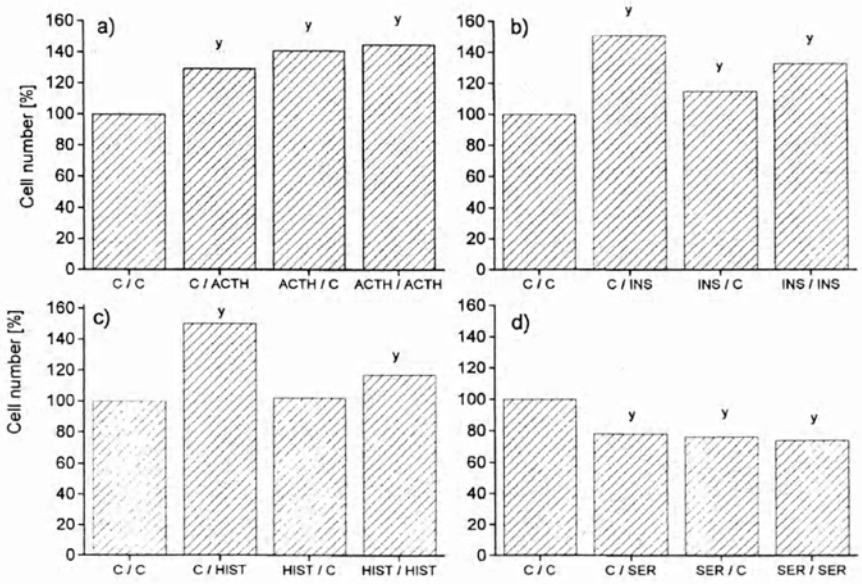

Fig. 12. Hormonal imprinting with four hormones (a – 10^{-9} M ACTH; b – 10^{-10} M insulin; c – 10^{-10} M histamine; d – 10^{-8} M serotonin)

experiments proved again our observation concerning the repellent effect of aromatic ring as serotonin, which molecule is different from histamine in its aromatic ring has adverse, chemorepellent character, and pretreatments with serotonin expressed the signal receiver mechanism to this effect.

EFFECT OF CHEMOTACTIC SELECTION ON THE RESPONSIVENESS OF SUBPOPULATIONS

Besides hormonal imprinting a new technique the "chemotactic selection" was developed by us [34] (Fig. 13). Since the hormonal imprinting is acting on mixed populations bearing receptors in different functional phases, there might be factors, (e.g. being in different phases of the cell cycle) which can influence the sensitivity of cells. Therefore our goal was to get "homogeneous" subpopulations concerning their responsiveness (receptors). In the basic hypothesis the chemotactic response is used as a receptor mediated process and positive responder cells are considered as a subpopulation possessing the appropriate receptor of chemotaxis and/or its signaling mechanism in a higher volume. Application of this technique made possible to evaluate different signal molecules whether the chemotactic responses induced by them is a dynamic, short-term character of the cells or it is a long-lasting and permanent quality of the subpopulation.

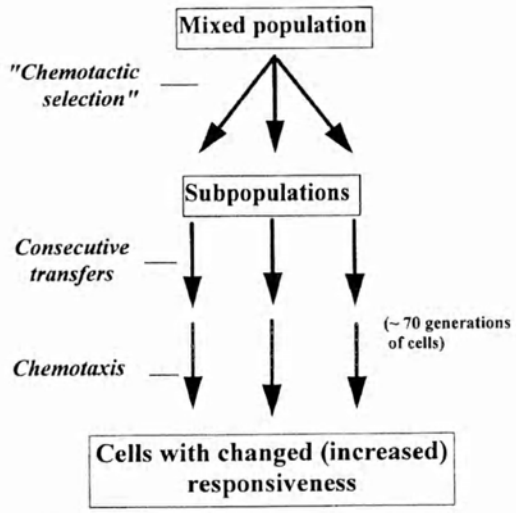

Fig. 13. Chemotactic selection – general scheme

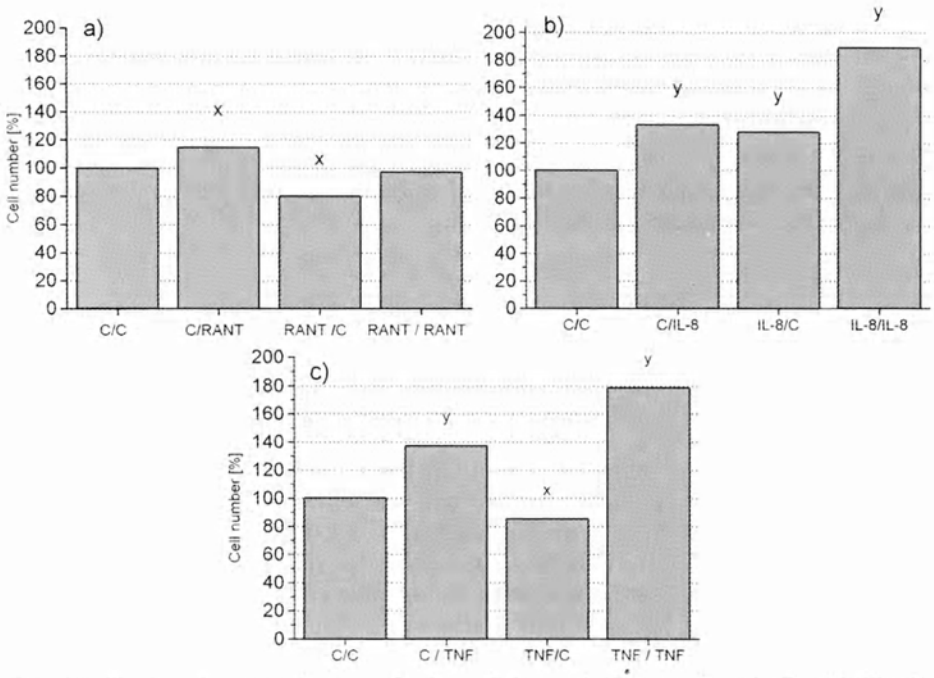

Fig. 14. Chemotactic responsiveness of subpopulations selected via chemotactic selection to 75 ng/ml RANTES (a), 1 ng/ml IL-8 (b) and 1 ng/ml TNF-α (c)

In this relation a the CC chemokine IL-8, the CXC chemokine RANTES and TNF-α were tested. The two chemokines are *per se* chemotactic in different target cells of mammals [23, 56], but the third cytokine TNF-α is also chemoattractant to special cells [60]. Concentration course studies documented that all the three cytokines can induce chemotaxis of *Tetrahymena,* too [34], and the sensitivity to IL-8 was higher than in the mammal reference, while RANTES worked in the same range, which shows that cytokines have also deep phylogenetical backgrounds in their receptor mediated functions.

On the basis of previous results chemotactic selections were done with the optimal concentrations of the cytokines and following consecutive transfers the chemotactic responses were analysed again towards the selector cytokine in the 70th generation. The differences of chemotactic responsiveness (Fig. 14) show that the two structurally divergent chemokines (IL-8 and RANTES) have various potencies in chemotactic selection: the CC chemokine IL-8 and TNF-α are good selectors as subpopulations of the chemotactically selected with them have significantly enhanced response, while the CXC chemokine RANTES cannot select via chemotaxis, its chemotactic character proved to be uncertain in long-lasting experiments.

Other still unpublished data also suggest that the mechanism mentioned above – to select high-responder subpopulations via chemotaxis – is a ligand specific process and chemotactic selections with amino acids, oligopeptides or peptide hormones are linked to specific signaling mechanisms.

In the future, the major purpose of our work will be to clarify the afferent or efferent mechanisms determining the succesful selection via chemotaxis, therefore our investigations are essentially focused to different points of the interactions between receptor and ligand.

CONCLUSION

In our works reviewed above the chemotaxis was used as a functional test of mostly surface membrane receptor mediated processes. The aim of our work was/is to characterize the ligands required for chemotactic responses and to get information about dynamics of signaling mechanisms making possible these reactions. Using *Tetrahymena* the survey proved that our model is as sensitive object for these experiments as the other cells applied previously in higher ranked organisms. Concerning the types of ligands we conclude that amino acids and oligopeptides are distinguished upon their physicochemical characteristics (e.g. in proline containing dipeptides – lipophylicity, residual volumes, statistical distribution of sidechain distances). The sensitivity of the model is not strictly dependent upon the size of the interacting molecule as small or large vertebrate hormones could work as attractant (e.g. histamine, STH) or repellent (e.g. serotonin, TSH) signals as well, but it perceives slight changes in the interacting signal sequences of the molecule (e.g. insulin variants). Studies of hormonal imprinting suggest that chemotactic responses following the pretreatments are also molecule dependent and the phylogenetically selected signal

molecules have a unique potency to modify chemotactic responsiveness even in the offspring (e.g. ACTH). Finally our chemotactic selection studies open a new field in investigations of ligand–signaling relations as it makes possible to evaluate the durability of chemotactic characters of subpopulations selected with chemotactic, however, different molecules (e.g. chemokines).

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