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Effects of L-Alanine and L-Alanine Peptides on the Chemotaxis of Tetrahymena: Evolutionary Conclusions

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L-alanine and its peptides (L-Ala-2-6) do not attract or repulse Tetrahymena in a 10^{-8} M concentration. In 10^{-10} M concentration there is a consistent repellent effect. Twenty four hours after L-alanine or L-alanine-peptides' pretreatment (imprinting) the progeny generation of the cells react differently to the same materials. L-Alanine, L-alanine penta- and hexapeptide in both concentrations are chemoattractant, while L-alanine tetrapeptide is repellent. L-Alanine dipeptide is inert in 10^{-10} M and repellent at 10^{-8} M concentrations, while L-alanine tripeptide is strongly repellent at 10^{-10} M and attractant at 10^{-8} M concentrations. This means, that the first encounter (imprinting) with an exogeneous amino acid or peptide is decisive to the later reaction of the protozoan cell. The chain length is important in the imprinting, however the reaction is not consistent. The experiments call the attention to the significance of imprinting in the receptor and hormone evolution.

KEY WORDS: L-alanine; evolution; chemosensory response; peptides; imprinting.

INTRODUCTION

Living in water and surrounded by different useful and noxious molecules the recognition capacity is absolutely essential for Protozoa. It was experimentally demonstrated that the unicellular ciliate Tetrahymena has binding sites (similar to vertebrate receptors) to vertebrate hormones and responds to them (1–4, 24). These receptors are further specified in the first encounter with a hormone by a process named hormonal imprinting (1, 4). The presence of recognition and imprinting capacity was demonstrated in the case of amino acids and peptides, too. Tetrahymena could distinguish between the different amino acids and peptides with disparate binding and physiological responses (5–7, 9). The imprinter capacity of some amino acids and peptide combinations is outstanding (8).

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According to Lenhoff (19), hormone receptors originated from food receptors. Indeed, appropriate amino acids and amino acid type hormones can influence—stimulate and inhibit—the response to each other (5). This makes likely that they use the same receptors. Considering these facts it seemed to be meaningful to study systematically the effect of amino acids and their oligopeptides having different chain lengths on the reaction of Tetrahymena, and to study the effect of imprinting on this response. At present the chemotactic response was studied—having precedents in experiments of others—and L-alanine and peptides composed from it were chosen as test substances.

MATERIALS AND METHODS

Cells and Culturing

Populations of Tetrahymena pyriformis GL in the logarithmic phase of growth were cultured in 0.1% yeast extract containing tryptone medium (Difco, Michigan, USA) at 28°C.

Chemicals

The applied alanine (L-Ala-1) and its peptides (L-Ala-2, Ala-3, Ala-4, Ala-5 and Ala-6) were obtained from Sigma Chemicals (St. Louis, MO, USA). The applied concentrations were 10^{-10} and 10^{-8} M. For pretreatments 10^{-6} M L-Ala and its peptides were used.

Pretreatment

Cells were pretreated with 10^{-6} M L-Ala or its peptides. After the treatments the cells were washed and transferred to fresh culture medium for 24 hours. Non-treated cells served as controls. In the assay of chemotaxis the identical Ala peptide served as test substance.

Assay of Chemotaxis

Capillary chemotaxis assay of Leick and Helle (16) was used, as modified by us (14). In this assay an outer and an inner chamber are connected with a capillary. The cells are placed into the outer chamber, while the inner chamber is filled with the test substance (L-Ala or its peptides dissolved in culture medium or fresh culture medium alone). In our setup the tips of a multi-8-channel

micropipette served as inner chambers. The incubation time was 15 min. After this the cells were fixed with 4% formaldehyde. The number of cells was determined in Hausser cytometer with light microscope.

Statistical Evaluation

Each experiment was repeated three times. Sigma Plot 40 and Origin 2.8 were used for statistical evaluation of data.

RESULTS AND DISCUSSION

Amino acids could be attractant or repellent for a unicellular organism. In bacteria alanine is known to be an attractant (10, 12, 13, 21–23). In Tetrahymena L-alanine, according to previous experiments was inert in a high (10^{-3} M) concentration (20). In the present experiment alanine was repellent when Tetrahymena met it at first in a very low (10^{-10}) concentration. It is worth mentioning that a higher (10^{-8} M) concentration of L-alanine was already neutral. L-Alanine peptides were mostly inert in the higher concentrations and mostly repellent in the lower concentrations (Fig. 1).

The repellent effect in lower concentrations and ineffectiveness in higher ones shows that L-alanine and its peptides worked as a signal and not as a simple nourishment or toxic substance. This is very important from the point of view of further discussion.

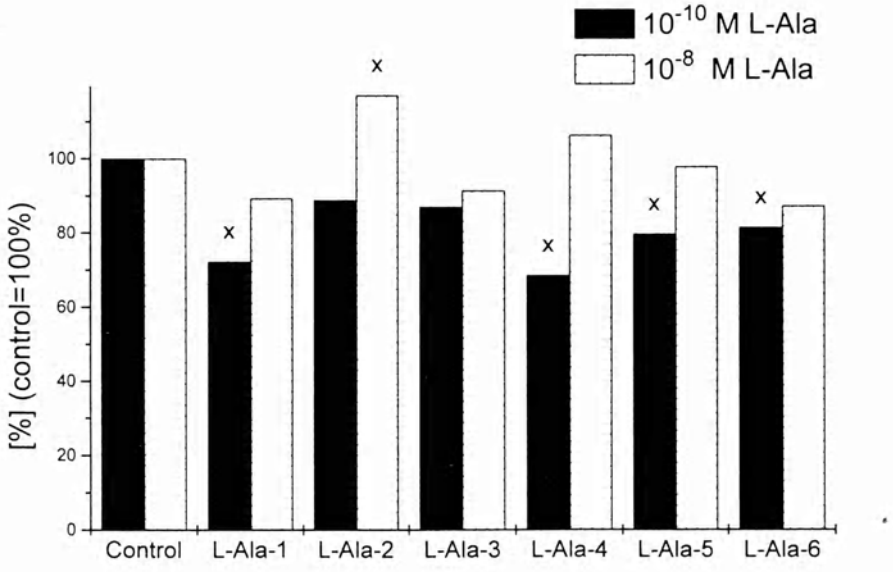

Fig. 1. Chemosensory response of Tetrahymena to the medium (control) and to the medium containing L-alanine (L-Ala-1) and different alanine peptides (L-Ala-2-6) in 10^{-8} M or 10^{-10} M concentrations ($x = p < 0.05$).

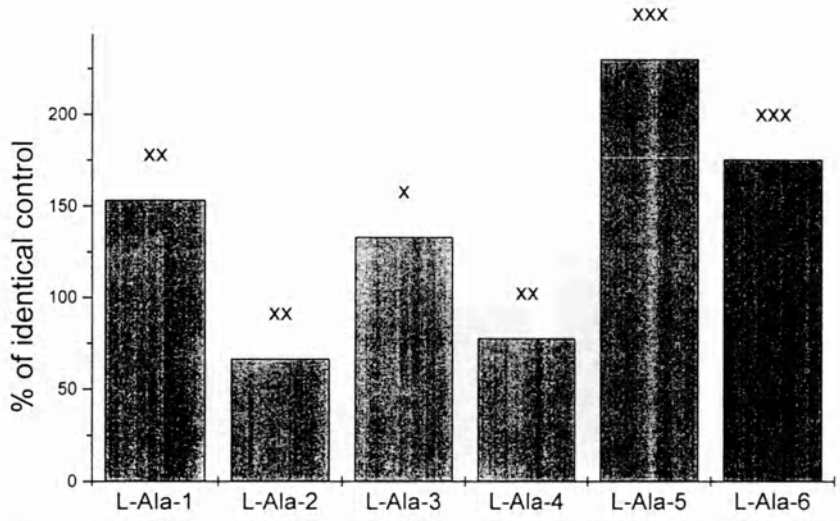

Fig. 2. Chemosensory response of alanine or alanine peptides pretreated (L-Ala-1-6) Tetrahymena to the medium containing the appropriate L-alanine or L-alanine peptide in 10^{-8} M concentration, related to the non-pretreated control as 100% ($x = p < 0.05$; $xx = p < 0.01$; $xxx = p < 0.001$).

Pretreatment (imprinting) with L-alanine or the peptides completely changec the chemosensory reaction of Tetrahymena after 24 h to the materials used in the pretreatment. The previously (at the first encounter) inert cells reacted to the materials present in 10^{-8} M concentration with attraction or repulsion depending on the materials' nature. L-Ala 1, 3, 5 and 6 provoked attraction, while L-Ala 2 and 4 repulsion. The peak of attraction was produced by L-Ala 5 (Fig. 2).

In the case of 10^{-10} M concentration the cells were repulsed by L-Ala and L-Ala-peptides in general at the second encounter producing as heterogeneous a behaviour as in the case of 10^{-8} M concentration. Only the reaction to L-Ala 2 was changed from attractant to repellent, the other molecules provoked simila reactions (Fig. 3). This shows that:

1. the first encounter with an amino acid or its peptides transforms th chemosensory reaction of the protozoan for later encounters

2. the imprinting eliminates the uniformity of reaction to peptides havin different chain lengths

3. chain length of peptides composed of the same amino acids is no indifferent in provoking imprinting, however the reaction is not consister considering the growth of the chain.

It is known that peptone or tryptone media are chemoattractants fc Tetrahymena (11, 15, 17, 18). In our experiments one day after treatment wit L-alanine or its peptides the chemosensory behaviour of Tetrahymena to th medium, changes. From L-alanine to the L-alanine tripeptide (L-Ala 3) there is continuous increase in chemoattraction which is followed by a decrease (relate to the top value) and a repellent effect in the case of L-Ala 5 and L-Ala 6 (Fig. 4 Considering that the cells had been treated 24 h before, in the experiments th

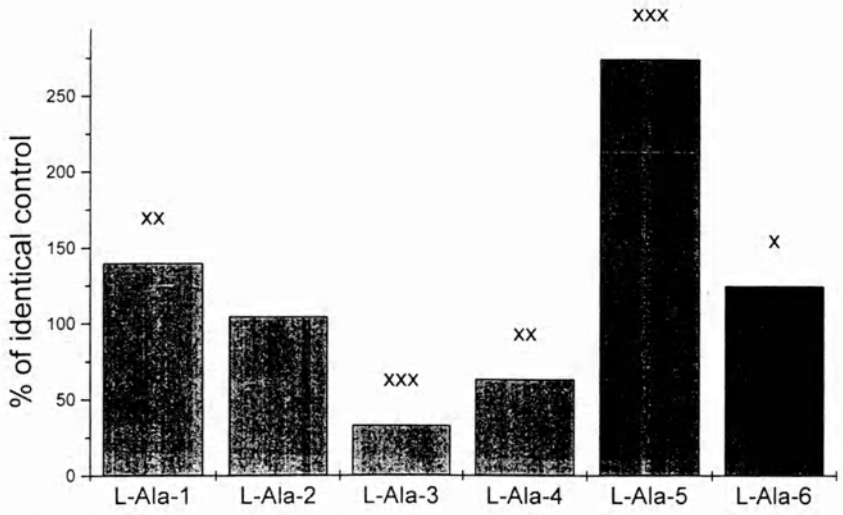

Fig. 3. Chemosensory response of L-alanine or L-alanine peptide pretreated (L-Ala-1-6) Tetrahymena to the medium containing the appropriate L-alanine or L-alanine peptide in 10^{-10} M concentration, related to the non-pretreated control as 100% ($x = p < 0.05$; $xx = p < 0.01$; $xxx = p < 0.001$).

8th-10th progeny generations were studied. This means that the treatment with different L-Ala peptides increased or spoiled the "appetite" of Tetrahymena for generations. The reaction provoked by the materials studied was different with positive peak by L-Ala 3 and with negative one by L-Ala 6. Although the reaction was regular and convincing, it is difficult to explain the reason for it.

How a common amino acid can provoke imprinting requires explanation. It

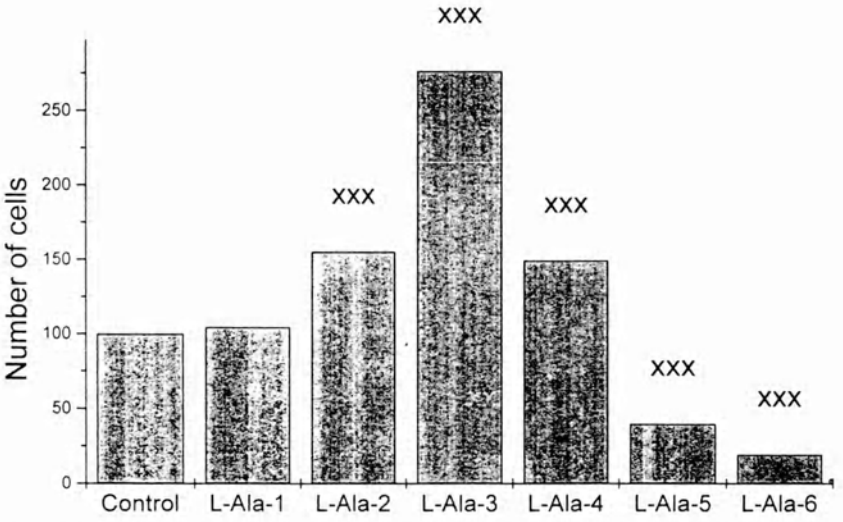

Fig. 4. Chemosensory response of L-alanine or L-alanine peptide pretreated (L-Ala-1-6) Tetrahymena to the tryptone medium, related to the non-pretreated control as 100% ($xxx = p < 0.001$).

is supposed that at a protozoan level the amino acid is bound to surface recognition structures (receptors) and the excitation of them provokes the imprinting. Nevertheless, this does not explain why the imprinting was provoked just now and not earlier when L-alanine would have been present as a component of the media. However the facts remain facts and there were similar situations in mammals, too, when exogeneous insulin or other hormones (present also in the developing organism) provoked life long imprinting (4).

The imprinting potential of a molecule would have been decisive during the evolution of receptors and hormones. If a molecule could provoke imprinting, this has been fixed and helped the progeny generations in the prompt recognition, and because of this helped survival. Those molecules which could provoke imprinting or "better" imprinting could be selected for signals. The imprinting could influence the selection of optimal amino acid composition of peptides as well as the optimal chain length. The peptides which could provoke imprinting in lower concentration were more suitable for signals, too. In the study of these problems the method of chemotaxis is a suitable tool, as it points to a very important characteristic of unicellular organisms in an inevitable connection with survival.

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