CHEMOTACTIC RESPONSE OF UNICELLULAR TETRAHYMENA TO A LEUKOCYTE ATTRACTANT PEPTIDE AND ITS REPELLENT DERIVATE: EVOLUTIONARY CONCLUSIONS.

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ABSTRACT

Chemotactic properties of the leukocyte chemoattractant N-formyl-Norleucine-Leucine-Phenylalanine (NLP) and its antagonist N-t-BOC-Norleucine-Leucine-Phenylalanine (BOC-NLP) were investigated in unicellular Tetrahymena pyriformis cells. NLP express its attractant character in a two-peak profile of concentration course (maximum at 10^{-8}-10^{-7} M and 10^{-11} M) while BOC-NLP has a constant repellent character (maximum at 10^{-12} M). The observed homology of action concurs with our theory about the wide evolutionary background of signal molecules and receptors.

INTRODUCTION

Chemotaxis is a fundamental physiological response in prokaryotic and eukaryotic unicellular organisms and in multicellular organisms alike. Nutrients (Bassler et al., 1991, Levandowsky et al., 1984), exogenous substances of bacteria (Schiffman et al., 1975, Stevens et al., 1987), hormones (Kõhidai et al., in press), bioactive molecules (Ueda and Kobatake, 1977) and inorganic molecules (Aiuchi et al., 1980) all have potency to induce chemotactic responses. A large number of molecules are reported to influence chemotactic character for neutrophil leucocytes (Cybulsky et al., 1988, Snyderman et al., 1970, Turner and Lynn, 1978, Wright et al., 1988) and among these molecules some synthetic peptides are well characterized (Freer et al., 1979, Gressi et al., 1986, Laskin et al., 1986, Showell et al., 1976, Smith et al., 1979). In the case of derivates of synthetic tripeptides, previous work demonstrated the essential role of i) formyl group, ii) length of side chain, iii) sulphur in the side chain and iv) the presence of phenylalanine in position 3 (Freer et al., 1979). Some other variants like BOC (butyloxycarbonyl) derivates or molecules with branched side chains proved to elicit depressed chemotactic response in leukocytes (Freer et al., 1980, Laskin et al. 1986, Wallace et al., 1984). Chemosensory function is essential to the free living unicellular eukaryotic cell Tetrahymena, too. Chemotaxis promotes the basic activities like phagotrophy or swimming behaviour by the help of selective structures (receptors) of the surface membrane. Receptorial binding of chemoattractant peptides also has been demonstrated in Tetrahymena (Leick, 1992).

The aim of our present work was to study the chemotactic character of two synthetic2 tripeptide derivates (N-formyl-Norleucine-Leucine-Phenylalanine and N-t-BOC-Norleucine-Leucine-Phenylalanine) in unicellular Tetrahymena cells.

MATERIALS AND METHODS

Cells and culturing: Tetrahymena pyriformis GL cells were grown in axenic cultures containing 1% Tryptone and 0.1% yeast extract (Difco, Michigan, USA) at 28 °C. Cells of logarithmic phase of growth were used, cell density was 10^4 cell/ml.

The concentration course of the two tripeptides N-formyl-Norleucine-Leucine-Phenylalanine (NLP) and N-t-BOC-Norleucine-Leucine-Phenylalanine (BOC-NLP) (Sigma, St. Louis, USA) was set between 10^{-12} M - 10^{-6} M. In control group fresh culture medium was applied.

Assay of chemotaxis: The chemotaxis-assay was an 8-channel-micro-pipette modification of Leicks

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method (Leick and Helle, 1983). Incubation time was 15 min. Cells responding positively to the attractant were fixed in phosphate buffer (PBS, pH 7.4) containing 4% formaldehyde. The number of cells was determined by Neubauer hemocytometer. All experiments were done in five parallels. The data were analysed with the statistical programs, Quatro-Pro and Excel 4.0.

RESULTS AND DISCUSSION

The concentration course experiment of NLP presents a two-peak profile curve (Figure 1a). This shows that there is one biologically active range in "high" concentrations between 10^{-8}-10^{-7} M (134% and 125% respectively) and there is another peak at 10^{-11} M (141%). The other applied concentrations are similar to the control responses except 10^{-10} M where significant depression was present.

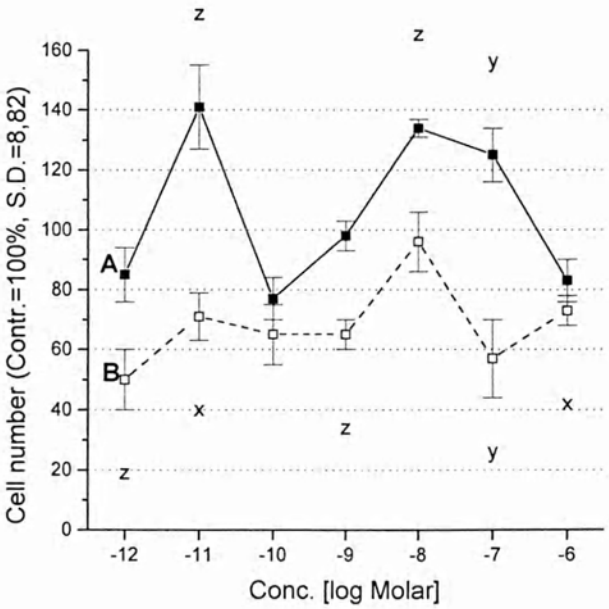

Figure 1. Chemotaxis of Tetrahymena.
A) concentration course of NLP, B) concentration course of BOC-NLP.
(x - $p<0.05$, y - $p<0.01$, z - $p<0.001$).

The observed two-peak profile is not surprising as other hormones or hormone-like substances also have this bi-phase profile in chemotaxis and other physiological responses (e.g. growth) also present this one. One possible interpretation is that the response to high concentration is non-specific (e.g. in the case of hormones in this range not the hormone feature but the food feature is what is able to induce the positive response). Another possibility is that there are different binding sites for the same ligand and differences in binding capacity might be responsible for the special response.

The chemotaxis profile induced by the BOC-NLP derivate is very different from the previous one (Figure 1b). The molecule display a wide concentration range (10^{-7}-10^{-6} M 57%-73% and 10^{-12}-10^{-9} M 50%-71%) and repelled Tetrahymena. The only control level peak was observed at 10^{-8} M (96%).

Comparing the concentrations of maximal responses we can find that there are also some differences. Apart from the fact that BOC-NLP has similarities in action in both Tetrahymena and vertebrates the observed highest response (10^{-12} M 50%) differs from the maximal of vertebrate system (10^{-7} M). In vertebrates the maximal response of NLP was observed at (10^{-9} M) while in Tetrahymena there is a shift to the lower concentrations (10^{-11} M). Perhaps this difference results from the free living organisms possesing a slightly more sensitive system for detection of surroundings. This seems to be logical, since the medium around Tetrahymena is a more dilute solution than the blood. It seems to be important to mention the discriminative capacity of Tetrahymena, expressed in the response to the two substances studied. This ability is a characteristic of the membrane receptors present also at this low level of phylogeny to peptides in general (Csaba, 1987) and to chemotactic peptides used in this experiment (Leick, 1992). In this latter case the substances in natural conditions are produced by bacteria which provide for the nutrition of Tetrahymena (Schiffmann et al., 1975). This means that a substance which acts as an attractant at a low level of phylogeny has the same property at a higher level.

The results clearly demonstrate that chemotactic character of NLP tripeptide is not a vertebrate-specific character, likewise its antagonist (repellent) derivate BOC-NLP also develops its well-characterized negative response in the unicellular Tetrahymena. In the light of this experiment the chemotactic effects are similar to the twi types of cells (leukocytes and Tetrahymena) that are widely separated phylogenetically.

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