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Molecule Dependent Chemotactic Responses of *Tetrahymena pyriformis* Elicited by Volatile Oils

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Summary. Chemotactic potency of five volatile oils was studied on *Tetrahymena pyriformis* cells. The linalool-acyclic monoterpene alcohol from *Ocimum basilicum* was the only chemoattractant substance. The monocyclic and bicyclic monoterpene rich volatiles from *Mentha piperita* and *Salvia officinalis* - elicited very similar, dose dependent repellent effect. The citronellal - acyclic monoterpene alhedyde - from *Eucalyptus citriodora* had the strongest repellent character of the volatiles tested, but acyclic and monocyclic monoterpene alcohols (citronellol as well as isopulegol) are also present in it. The phenyl propane derivate from *Cinnamomum aromaticum* had also significant repellent effects. In contrast the tested non-volatile oil, Oleum helianthi presented a moderate repellent effect independent to the concentration applied. Our results indicate that effect of signal molecules derived from plants have specific, recognition-dependent effects on the unicellular model cell, *Tetrahymena*.

Key words. Chemotaxis, volatile oils, *Tetrahymena*, signalling.

INTRODUCTION

Volatile oils possessing characteristic scent are obtained by water stream distillation from leaves and/or flowers of several plants. Their role in plants is very obscure, as they are stored like inclusions of different tissues and the very scent character (they are volatile at low temperatures) provides them a signal role in nature. Mono- and sesquiterpenes (C_{10} and C_{15}), and their alcohols, aldehydes, ketones etc. are the main components of the distilled, purified products of volatile oils (Petri 1991). Some of their physiological effects (Schilcher 1984) e.g. hyperemic effect in the skin or spasmolytic, stomachic or carminative effects in the intestinal tract were well-known in the ancient ages of medicine. Recent investigations demonstrated their antibacterial effect in Gram negative (*E. coli, Pseudomonas aeruginosa*), and in Gram positive (*Bacillus subtilis, Staphylococcus aeruginosa*) bacteria, and in *Candida albicans* (Jansen et al. 1986). Their fungicide character in *Trichophyton rubrum*, *Trichophyton equizum* was also demonstrated (Dikshit and Husain 1984), and there are references to their antihelminthic (Jain and Jain 1972) and atherosclerosis protective potency (Nikolaevskii et al. 1990).

Chemotaxis is an essential physiological response of animals of all phyla. In vertebrates several basic immunological responses, e.g. inflammation, are thought to be chemotaxis dependent processes (Roitt et al. 1985).

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The origin and character of corresponding chemoattractants is very diverse. In unicellular organisms chemoattraction is more fundamental in nutrition (Levandowsky et al. 1984) and mating (Nobili et al. 1987). In addition unicellular organisms, e.g. *Tetrahymena* respond to hormone-like substances, e.g. insulin, ACTH, T2 (Kőhidai et al. 1994a) and other signal molecules, eg. N-formyl-Noeleucine-Phenylalanine, of vertebrates (Kőhidai et al. 1994b).

Tetrahymena is a popular model cell of molecular and cell biology alike (Csaba 1985). These ciliated protozoans possess almost all pathways of signal transduction e.g. cAMP (Csaba and Lantos 1976, Kovács and Csaba 1987), IP_3 (Kovács and Csaba 1990), Ca^{2+}-calmodulin systems (Kovács and Csaba 1987) which are thought to be essential in higher ranks of evolution. The effect of a great number of signal molecules, e.g. hormones, drugs, inorganic substances was characterized on this cell and the responses elicited were evaluated on different levels of action, e.g. receptor binding (Hegyesi and Csaba 1992), membrane structure (Kőhidai et al. 1986a) and potential (Kőhidai et al. 1986b), metabolic pathways (Kőhidai and Csaba 1985), nuclear level changes (Kőhidai et al. 1985).

The purpose of our present study was to evaluate the chemotactic potential of some volatile oils obtained from different plants on *Tetrahymena* cells. There were two questions to be answered: (1) is there any concentration dependent chemoatractant or repellent potential for *Tetrahymena* of volatile oils from plants? (2) Is there any relation between the supposed chemotactic response evoked and the molecular structure of the substance?

MATERIALS AND METHODS

Tetrahymena pyriformis GL cells, maintained in 0.1% yeast extract containing 1% Bacto tryptone (Difco, Michigan, USA) medium at 28°C were used in the logarithmic phase of growth.

All the volatile oils tested were obtained from the suitable part of the plant by water steam distillation.

Salvia officinalis L. (Lamiaceae): "Folium salviae" is official in Hungarian Pharmacopoeaia (Ph.Hg. VII.) (Végh 1986). Its volatile oil content is min. 1.5 ml/100 g. The oil was obtained from the leaves by us. Characteristic compounds of the oil are eucalyptol 10%, thujone 26% and camphor 15%.

Ocimum basilicum L. (Lamiaceae) (Basil oil): this oil was obtained by us from the flowering herb of *Ocimum basilicum* cultivated in the Botanical Research Institute, Vácrátót (Hungary). The main component of the oil is linalool 40-60% (Lemberkovics et al. 1993).

Mentha piperitha L. (Lamiaceae): Aetheroleum menthae piperitae is official in the Ph.Hg. VII. It is obtained from the herb of the plant; it contains 3-9% of ester in methylacetate, min. 50% of menthol (free and bounded).

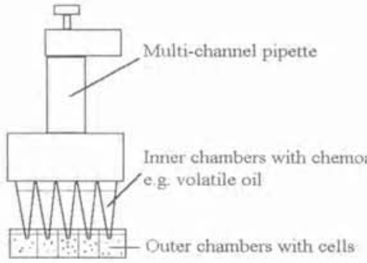


Fig. 1. Setup of capillary chemotaxis assay with a multi-channel pipette

Cinnamomum aromaticum Nees (Lauraceae): Aetheroleum cinnamomi is official in the Ph.Hg. VII. It is obtained from the leaves and branches of the tree. It contains min. 80% of aldehydes in cinnamic aldehyde.

Eucalyptus citriodora, Hook (Myrtaceae): It is originated from Pakistan. The oil is obtained from the leaves of the tree. Characteristic compounds of the oil are: citronellal 60-80%, citronellol and isopulegol (Masada 1976).

Oleum helianthi was applied as negative referent, non-volatile fatty oil. Each oil was tested in a concentration course in range of 10^{-6} - 1 vol%.

The assay of chemotaxis was a modified test of Leick (Leick and Helle 1983). According to this there are two chambers: the outer one is filled with the cells to be tested, the inner one contains the test substance with the chemoattractant or repellent volatile oil. Small capillaries serve as connecting junctions between the two chambers. In our setting tips of a multi-8-channel automatic pipette served as inner chambers (Fig. 1), this way we could minimize the standard error of sampling and the parallels gained were in good correlation. After 15 min incubation with the different concentrations of volatile oils the samples were fixed in 4% formaldehyde containing PBS, (0.05 M phosphate buffer, pH 7.2; 0,9 M NaCl).

The samples were evaluated by Neubauer hemocytometer. Each volatile oil was tested in five replica assays, the figures demonstrate the averages of these results. The statistical analysis was done by SigmaPlot 4.0 and Origin 2.8.

RESULTS

The five tested volatile oils elicited different, concentration dependent chemotactic responses in *Tetrahymena pyriformis* (hereafter we use the plant name which the volatile was obtained from to label the substance origin).

Salvia officinalis and *Mentha piperitha* had very similar profiles in respect of chemotaxis (Fig. 2). Both volatile oils had no chemoattractant character, they were only neutral at 10^{-3} vol% concentration. At the lower ranges the two volatile oils had moderate repel-

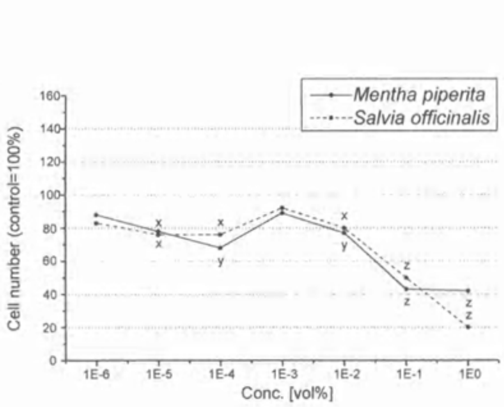


Fig. 2. Chemotactic activity of volatile oils prepared from *Mentha piperita and Salvia officinalis* in *Tetrahymena pyriformis* GL. (x $p<0.05$, y $p<0.01$, z $p<0.001$)

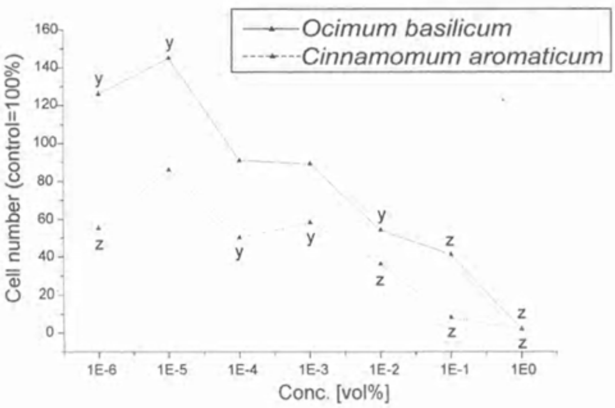


Fig. 3. Chemotactic activity of volatile oils prepared from *Ocimum basilicum* and *Cinnamomum aromaticum* in *Tetrahymena pyriformis* GL. (x $p<0.05$, y $p<0.01$, z $p<0.001$)

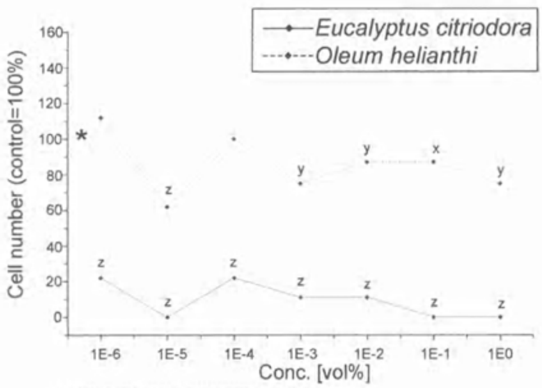


Fig. 4. Chemotactic activity of volatile oils prepared from *Eucalyptus citriodora* and Oleum helianthi in *Tetrahymena pyriformis* GL. (x $p<0.05$, y $p<0.01$, z $p<0.001$)

lent effect while in the higher concentration range (10^{-2}-1 vol%) they had a concentration dependent strong repellent effect.

Cinamomum aromaticum had the second strongest repellent character and it was significant in all concentrations tested. However the higher concentrations had the predominance (Fig. 3).

Ocimum basilicum was the only volatile which could work as an attractant. This positive chemotactic character was present in the low concentrations (10^{-6} - 10^{-5} vol%). The higher concentrations of this oil were also repellent to *Tetrahymena*.

Eucalyptus citriodora had the strongest repellent effect (Fig. 4). In the tested range of concentrations the response was between 11-22%. The well expressed repellent character urge us to find the threshold concentration which was 10^{-7} vol%.

The reference substance Oleum helianthi had significantly different effect compared to volatile oils. While the chemotactic profile of the signal molecules was always concentration dependent, the effect of Oleum helianthi was although slightly negative but roughly independent at the high concentrations (10^{-5} - 1 vol%). This non-signal molecule could induce a marked chemotactic effect only at the lowest (10^{-6} vol%) concentration.

DISCUSSION

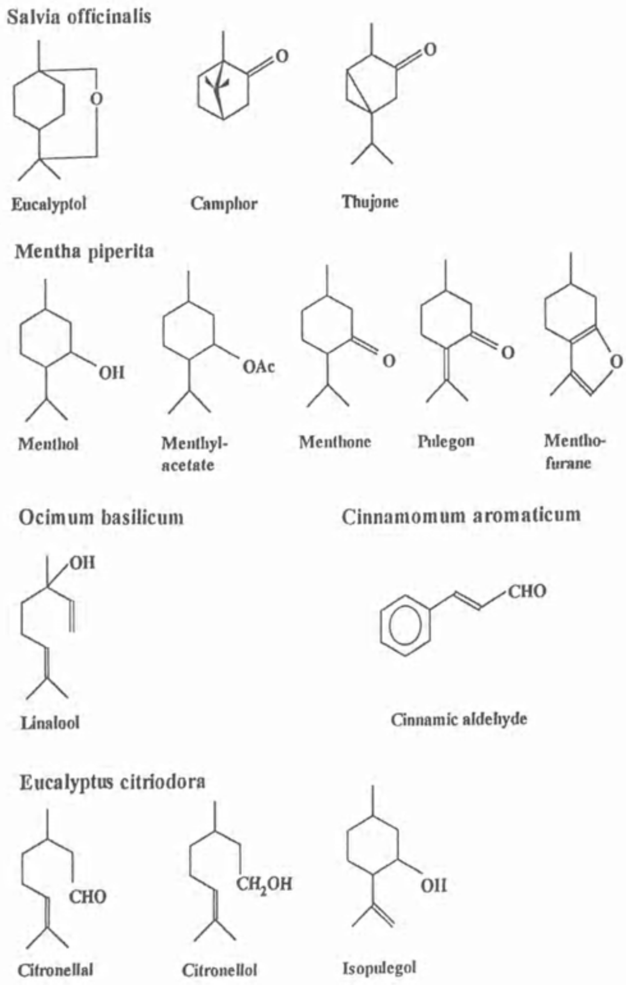
Numerous assays demonstrated that the chemotactic responses elicited and the molecular structure of the attractant molecules, e.g. bioactive peptides, insulin, histamine, serotonin have close relationship (Freer et al. 1979, Kőhidai et al. 1994a). These observations indicate that in the backgrounds of the chemotactic actions of the cell there are well defined interactions between the attractant molecule and the responsible receptor pool of the cell.

However the applied volatile oils are mixtures of some related terpenes (first monoterpenes) - as in general - in each case we can characterize the substance with the 1-3 major components (Table 1).

In the group of monocyclic monoterpenes the essential oil of *Mentha piperita* contains menthol (50-70%) further menthylacetate, menton, pulegon, menthofuran; *Salvia officinalis* has three main components: thuyon (30-40%), cineol (15%) and camphor (8%) (Végh 1986). In both essential oils the non-aromatic ring, the mono- and bicyclic monoterpene skeletons are predominant and this might result similarities in

Table 1

Characteristic components of essential oils investigated



chemotactic capacity. However monocyclic monoterpene alcohol, e.g. isopulegol are also present in *Eucalyptus citriodora* but there are two acyclic monoterpenes (citronellal an acyclic monoterpene aldehyde, and citronellol) (Masada 1976) presented the highest repellent activity.

Not only the two above mentioned monoterpenes provided repellent effect. *Cinnamomum aromaticum* had a more moderate repellent effect. In this volatile oil the predominant component is a phenyl propanederivatecinnamic aldehyde (76%) which has an aromatic structure (Végh 1986).

Ocimum basilicum was the only volatile oil which could serve as a chemoattractant substance. Its main component is linalool, an acyclic monoterpene alcohol (Lemberkovics et al. 1993). All of its predominant components, e.g. citronellol, citronellal, citral have a significantly different structure, they are acyclic monoterpenes.

The different but negative effect of non-volatile oil control Oleum helianthi contains an α-linolic acid (35%), β-linolic acid (13%) and oleic acid (35%). Although these molecules have long chains they did not induce chemotaxis. This negative result indicates that the plasticity of the non-ring structure itself without ketone or alcohol groups is not enough to induce positive chemotactic responses.

The components of the oils used have chemotactic effects in other organisms, too. Citronellol, component of *Ocimum basilicum* is repellent to mole rats (Heth et al. 1992) and larvae of *Aedes aegypti*. Eucalyptol also repellent to *Anopheles* (Schreck and Leonhardt 1991). In other aspect, the effect of volatile oils is always inhibitory, inhibiting enzymes - eudesmol, component of *Eucalyptus globulus* - (Satoh et al. 1992, Nojima et al. 1992); growth of bacteria and yeasts - cinnamic aldehyde, component of *Cinnamomum aromaticum* (Raharivelomanana et al. 1989, Saeki et al. 1989); citral, component of *Melissa* or *Citrus* oils (Végh 1986). This seems to be logical as these negative effects can defend the plants from the attacks of their enemies. However, in the case of *Tetrahymena* both repellent and attractant effects were observed. More repellent effect was observed, however the attractant effect of *Ocimum* was expressed in low concentrations. This demonstrates that *Tetrahymena* is very sensitive to chemical signals and can differentiate between the volatile oils. This differentiation is independent on the general aversion (of other organisms) to these oils.

The structure-dependence of chemotaxis suggests that the elicited responses were realized through a receptor mediated signalling pathway. The sensitivity of receptors is concentration-dependent. The observed negative and positive effects to a physiological response, chemotaxis draws our attention to the evolutionary accord of signalling mechanisms of plants and animals.

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