Localization of the chemoreceptive properties of the surface membrane of *Paramecium tetraurelia*

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**Summary.** The plasma membrane of *Paramecium tetraurelia* comprises two morphologically distinct components; a membrane that encloses the cell body and a ciliary membrane. In order to investigate the relative contributions of the two membranes to attractant-induced membrane potential changes, cells were deciliated with ethanol and their subsequent responses to attractants examined.

Deciliation did not significantly affect the magnitude of the hyperpolarizations evoked by acetic or lactic acids, and had no effect on the concentration dependence of responses to folic acid. We conclude that the components necessary for detection and response to attractants are not exclusive to the ciliary membrane of *P. tetraurelia*. Deciliation of *Paramecium* concomitantly permits localized chemical stimuli to be applied directly to the cell surface in the absence of strong fluid currents that are generated by the activity of the locomatory organelles. By systematically applying $K_2$ folate to a number of sites on the cell surface, it has been possible to demonstrate an anterior-posterior gradient of chemosensitivity on the cell body of *P. tetraurelia*.

**Introduction**

*Paramecium tetraurelia* exhibits locomotory responses to a diverse range of chemicals (Dryl 1974; Van Houten et al. 1981). While some of these may be effected by non-specific interactions with the cell surface, attraction to organic acids such as acetate, lactate, and folate is specific, suggesting that each acts on the cell via separate chemoreceptor sites (Van Houten 1976; Schulz et al. 1984). It has recently been suggested that although the ciliary membrane comprises 50% of the total surface area of *Paramecium* (Dunlap 1977), these organelles are not involved in stimulus detection (Van Houten et al. 1983). There are a number of ways of producing viable cells that lack cilia with which to test this hypothesis, but since deciliated paramecia lack their locomotory organelles, it is not possible to test their behavioural responses to attractants. However, since the locomotory activity of *P. tetraurelia* reflects changes in membrane potential (Machemer 1974), it is possible to examine the electrophysiological effects of attractants on deciliated cells and predict how such cells would respond behaviourally to the same stimuli (Van Houten 1979).

In the present study we report that the membrane responses of deciliated *P. tetraurelia* to acetate, lactate, and folate are indistinguishable from those of normal, ciliated cells, and provide evidence of an anterior-posterior gradient of chemosensitivity in the cell body membrane of *Paramecium tetraurelia*.

**Materials and methods**

*Cells.* *Paramecium tetraurelia*, stock 51 S was cultured on a rye grass seed infusion, inoculated with *Klebsiella pneumoniae* immediately prior to use. Cultures were maintained at 22 °C.

*Solutions.* All solutions contained 1 mM Ca(OH)$_2$, 1 mM Citric acid, 1.3 mM Tris (hydroxymethyl) aminomethane (Tris) base, pH 7.0. Potassium salts of acetate, lactate, and folate were included in the basic buffer (above) as required, and were balanced by an equivalent concentration of KCl in the control solution. — All chemicals were of analytical grade and were obtained from either Sigma Chemical Co., J.T. Baker Chemicals, or Fisher Scientific Products.

*Deciliation.* Deciliated specimens of *P. tetraurelia* were prepared by the method described by Machemer and Ogura (1979) for
**P. caudatum**. A 100 μl aliquot of absolute ethanol was added to 1.9 ml of cells in 5 mM KCI buffer to give a final ethanol concentration of 5% (v/v). Deciliation was effected by rapidly agitating the cell suspension for 2 min; the cells were then washed in 5 mM KCl buffer.

**Intracellular recording.** Intracellular recording techniques were similar to those described in a previous report (Preston and Van Houten 1987). 2-3 cells from a logarithmic growth phase culture of **P. tetraurelia** were placed in an experimental bath mounted on the stage of an inverted microscope. The tip of a glass capillary microelectrode was then placed in a selected cell and the bath flooded with 5 mM KCl buffer. Electrodes were filled with 0.5 M KCl and had tip resistances of 60-80 MΩ. The bath was perfused with buffer throughout the recording period, test solutions being introduced to the bath as required. Membrane potential measurements in test solutions were taken 6 min after their introduction to the cells.

**Local perfusion.** 6-10 deciliated specimens were pipetted into the experimental bath containing 5 mM KCl buffer. A specimen lying perpendicular to the flow of bathing medium was selected for intracellular recording and the tip of a glass capillary microelectrode was inserted into the approximate midpoint of either the ventral or dorsal surface. The chemosensory properties of the cortex of **P. tetraurelia** were tested by local perfusion of 2.5 mM KFolate. A glass capillary micropipette containing KFolate was placed within 25-30 μm of the deciliated cell surface at one of eight positions along the length of the cell. A 3 s pressure pulse was then applied to the pipette from an air-filled syringe, causing the test solution to flow out over the cell from the 10-20 μm pipette tip. The continual flow of 5 mM KCl buffer in the experimental bath (2 ml/min) prevented a concentration build-up during repeated folate applications, and ensured that the outflow from the pipette tip was confined to a relatively small area of the cell surface.

**Results and discussion**

Ethanol treatment provides a rapid, efficient, and non-lethal means of removing the somatic ciliation of **P. tetraurelia**. In common with other methods of artificial deciliation, ethanol apparently weakens the ciliary base so that the mechanical forces exerted on the cilia during agitation are sufficient to cause their shearing from the cell body (Blum 1971; Ogura 1981). The oral cilia are protected from these forces by the buccal organelle and hence are retained during deciliation procedures.

Upon transferring deciliated paramecia to ethanol-free media, ciliary regeneration proceeds rapidly, particularly in well-fed cells. Regrowing cilia are readily apparent on the cell surface 25-30 min following deciliation. After 45 min, many cells can generate Ca++-action potentials, and by 90 min, the cells are again capable of slow forward movement (Preston 1983). Thus, all experiments were conducted within 30 min of deciliation. During this period, the cells were continually monitored for signs of ciliary regrowth or calcium channel function (by testing membrane responses to depolarizing current pulse injection). Specimens showing either indication of ciliary regeneration within 30 min were discarded, ensuring that the observations reported below were obtained in the absence of ciliary membrane.

The resting potential of deciliated **P. tetraurelia** in 5 mM KCl buffer does not differ significantly from that of untreated ciliares, both being of the order of -31 mV (Table 1). This observation confirms previous reports of the effects of deciliation on **P. caudatum** (Machemer and Ogura 1979), and suggests that the resting K+ and Ca++ conductances that maintain the resting potential of **P. tetraurelia** are resident in the soma membrane and are not affected by ethanol or ciliary removal.

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<th>Table 1. Effects of attractants on the membrane potential of ciliated and deciliated <strong>P. tetraurelia</strong></th>
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<td><em>E</em>&lt;sub&gt;m&lt;/sub&gt;, mV</td>
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<td><strong>Ciliated</strong></td>
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* Data from Van Houten (1979)

Membrane potentials (*E*<sub>m</sub>) in 5 mM acetate, 5 mM lactate, or 2.5 mM KFolate were determined 6 min after replacing the control solution (5 mM KCl) with test solutions. Membrane potential changes (Δ*E*<sub>m</sub>) are the differences in membrane potential measured immediately before and 6 min following replacement of the bath solution with a test solution. Data are the means ± SD of (n) determinations.
tential response to acetate, lactate, and folate are not exclusively resident in the ciliary membrane of *P. tetraurelia*. Previous radiolabelled folate binding studies would further extend this hypothesis to suggest that cilia are not essential to chemoreception in *Paramecium*. Paramaecia specifically bind $^3$H folate with a $K_D$ of 29 $\mu$M (Schulz et al. 1984). Since cells that have been selected for their inability to respond behaviourally to folate are coincidentally unable to bind the radiolabelled ligand, this binding site is presumed to be a receptor that transduces chemostimulation to produce a behavioural response. Cilia isolated from wild-type cells similarly show only small amounts of non-specific $^3$H folate binding, suggesting that these organelles are not involved in reception of this stimulus (Van Houten et al. 1983). Complementary data showing that the amount of radiolabel binding to cell bodies is not significantly reduced by ciliary removal awaits an efficient, non-lethal means of deciliating paramecia in large quantities.

These observations contrast with reports of radiolabelled amino acid binding to the cilia of *P. tetraurelia* (Preston 1983) and the demonstration of a highly specific L-($^3$H) glutamic acid binding site on isolated cilia (Preston 1984). Electrophysiological studies further show that deciliation modifies the concentration:response characteristics of transient hyperpolarization to L-glutamate, suggesting that the locomotory organelles do indeed have a chemosensory role in this uncell (Preston 1983).

In a systematic investigation of the chemosensitive properties of the cell body membrane of *P. tetraurelia*, 2.5 mM K$_2$ folate was pressure perfused onto selected areas of the surface of deciliated cells. Test solutions are expelled from the pipette tip with considerable force, however, raising the possibility that any observed responses may contain a component that results from hydromechanical stimulation of the cell surface. As a control, 5 mM KCl buffer was pressure perfused onto cells, yet despite the well documented mechanoreceptive properties of *Paramecium* (Eckert et al. 1972; Naitoh and Eckert 1973), there was no effect on membrane potential.

Application of 2.5 mM K$_2$ folate to deciliated cells produced immediate hyperpolarizations that were sustained for as long as the stimulus was present. The magnitude of the responses showed considerable variation from cell to cell and according to the position of the pipette tip on the cell surface. In order to normalize these responses, the maximum hyperpolarization obtained from the cell under investigation (usually of the order of 5 mV)
was taken as 100% and subsequent responses are expressed as a percentage of the maximum. The resultant data are shown in Fig. 2. It should be noted that while it is possible to show statistically significant regional differences in response magnitude (using the Mann-Whitney U-test), the Figure is presented to show trends in responsiveness rather than absolute values. Both ventral and dorsal surfaces of *P. tetraurelia* are chemosensitive; maximal hyperpolarizations are evoked by stimuli applied within or anterior to the buccal organelle while the posterior pole of the cell is least responsive to folate. These data confirm the contentions of previous workers (Alander 1922; Dryl 1969) that the anterior of *Paramecium* shows enhanced sensitivity to chemicals and, further, provides evidence for an anterior-posterior gradient of chemosensitivity in the cell body membrane of *P. tetraurelia*. The reported observations yield less information about the location of putative folate chemoreceptors, however.

Ideally, it would be possible to prepare an exact and detailed map of cortical chemosensitivity, using iontophoretic techniques to systematically apply small, localized chemical stimuli to the cell surface. Such an approach has not proved possible however; repeated iontophoretic application of folate to deciliated cell surfaces failed to elicit a membrane response (Preston, unpublished observations). At pH 7, folate should be completely dissociated (pK = 3.7) and should therefore be amenable to iontophoretic application, but the limited solubility of the compound restricts the concentration that can be placed in the iontophoretic pipette. As a result, it is likely that the concentration of chemoeffector at the pipette tip never reaches that required to evoke membrane hyperpolarization. It was therefore necessary to employ a pressure perfusion technique to apply chemoeffector to the cell surface, but the resolution afforded by such a technique is poor. Even when the spread of perfusate is limited by imposing a continual flow of bathing medium over the cell surface, at least 1/8th the cell length still comes in contact with the stimulus. Resolution is further limited by the size of the maximum potential change elicited by perfusion. The 5 mV hyperpolarization is considerably less than the −12 mV membrane potential change resulting from bath application of 2.5 mM K$_2$folate to deciliated cells: the difference is taken to mean that dilution of the perfusate prevents the cells from experiencing more than 0.5 mM K$_2$folate (Fig. 1) or that the number of receptors bound by ligand is insufficient to elicit a full response.

The problem of confining the stimulus to a small area is particularly acute when trying to examine the chemosensory properties of the anterior-ventral surfaces of paramecia. Although deciliation eliminates the currents in the bathing medium that are generated by the somatic cilia, ethanol treated cells retain active buccal cilia and, as demonstrated by Jennings (1906), the activity of these organelles creates vigorous 'feeding currents' which suck the surrounding medium into the buccal cavity. Thus there is a strong possibility that anterior-ventrally applied stimuli are being detected either by receptors on the buccal cilia, or by the food vacuole-forming membrane within the buccal cavity. Very little is known of the chemoreceptive properties of either organelle, and there is no known means of removing the buccal ciliature. Scanning electron microscopic studies of *P. caudatum* have shown that conjugating cells resorb cilia within the buccal cavity prior to intercellular fusion (Watanabe 1978), but the deciliation process is incomplete; data resulting from local perfusion of such cells would still be open to interpretation.

When stimuli are applied to the dorsal surfaces of deciliated cells, the ventral surfaces are pressed against the bottom of the experimental bath and the perfusate is not subject to interference by feeding currents. Thus the gradient of chemosensitivity observed along the dorsal surfaces may be taken as evidence for a gradient of putative folate chemoreceptors in the surface membrane.

There is a well established precedent for such gradients in a number of protozoa, including *Paramecium*. Enhanced chemosensitivity of the anterior of the cell body has been demonstrated in *Dileptus* (Doroszewski and Dryl 1978), *Opalina* (Naitoh 1961), and *Styloynchia* (Totwen-Nowakowska and Dryl 1976). *Blepharisma* (Matsuoka 1983) and *Dileptus* (Doroszewski 1961) swim backwards if the anterior of the cell is illuminated, while there is a gradation of mechanosensitivity in the surface of *P. caudatum* (Ogura and Machemer 1980).

The anterior of the cell is the most plausible position for a chemoreceptor since it is this region that first contacts the bathing medium ahead of its swimming path. Such a location would be particularly important for fast-swimming cells such as *Paramecium*; in an environment filled with microgradients of chemicals, a ciliate could pass through an area of attractant unless it were capable of rapid detection and response to the gradient. Van Houten et al. (1985) recently developed a fluorescein-folate conjugate that labels the surfaces of *P. tetraurelia* in a folate-specific manner. It is anticipated that studies involving the fluorescent label will provide confirmation of the data pre-
sented in this report, and will yield a more detailed description of chemoreceptor localization in the surface membranes of *Paramecium tetraurelia*.

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