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 locomotory 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 KCl 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 K$_2$folate. A glass capillary micropipette containing K$_2$folate was placed within 25–50 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 ciliature 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 depolariz-

Table 1. Effects of attractants on the membrane potential of ciliated and deciliated *P. tetraurelia*

	E_m, mV	ΔE_m, mV
Ciliated		
5 mM KCl	-31.5 ± 2.9 (43)	
5 mM acetate	-40.3 ± 1.7 (8)	-7.4 ± 0.7 (8)
5 mM lactate	-37.4 ± 1.4 (16)[a]	-7.9 ± 1.3 (13)[a]
2.5 mM K$_2$folate	-42.8 ± 3.5 (18)	-11.4 ± 1.6 (22)
Deciliated		
5 mM KCl	-31.0 ± 3.5 (42)	
5 mM acetate	-39.6 ± 3.2 (10)	-7.9 ± 0.3 (10)
5 mM lactate	-37.8 ± 3.4 (9)	-6.9 ± 0.6 (10)
2.5 mM K$_2$folate	-44.1 ± 4.6 (9)	-11.4 ± 0.7 (9)

[a] Data from Van Houten (1979)

Membrane potentials (E_m) in 5 mM acetate, 5 mM lactate, or 2.5 mM K$_2$folate were determined 6 min after replacing the control solution (5 mM KCl) with test solutions. Membrane potential changes (ΔE_m) 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 $\pm$ SD of (n) determinations

ing 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 ciliates, 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.

Untreated, ciliated paramecia hyperpolarize in response to 5 mM K-acetate, 5 mM K-lactate, and 2.5 mM K$_2$folate relative to the membrane potential in 5 mM KCl. Table 1 shows that deciliation affects neither the magnitude of the hyperpolarizations induced by acetate or lactate, nor the 6 min membrane potential values in either compound relative to the membrane potential in 5 mM KCl buffer. Similarly, there are no significant effects of deciliation on the membrane potential changes induced by K$_2$folate (relative to twice the concentration of KCl), and there are no effects on the concentration dependence of the membrane responses to folate (Fig. 1). These observations permit the following conclusions: a) since the voltage-dependent channels that mediate Ca^{++}-action po-
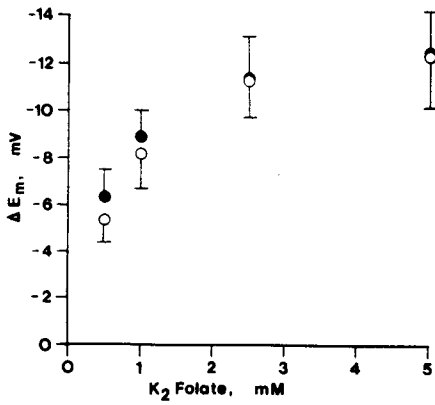
Fig. 1. Concentration dependence of the membrane responses of ciliated and deciliated paramecia to folate. Data points are the mean (±SD) membrane potential changes induced by increasing concentrations of K_2folate, relative to 2 × the concentration of KCl. Open circles represent responses of untreated, ciliated cells, closed circles represent those of ethanol treated deciliates. $n = 3$–29
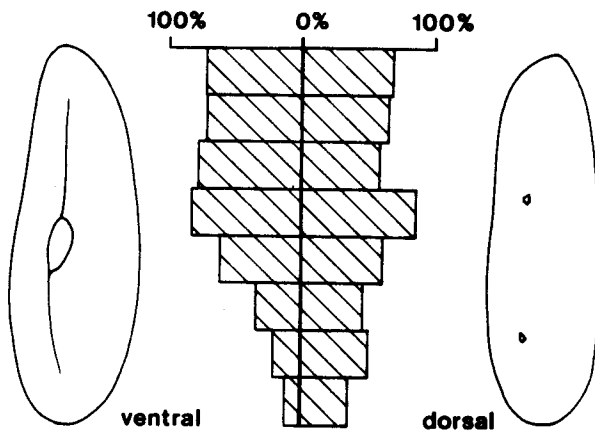

Fig. 2. Localization of chemosensitivity in the soma membrane of deciliated *Paramecium tetraurelia*. 2.5 mM K_2folate was locally applied by pressure perfusion to 8 sites along the ventral and dorsal surfaces of cells. The resultant membrane hyperpolarizations are expressed as a percentage of the maximum response obtained from each cell; the Fig. represents mean responses of 20 deciliated specimens

tentials (or some factors essential for Ca^{++} channel function) are resident in the ciliary membrane of *Paramecium* (Ogura and Takahashi 1976; Dunlap 1977), the channel cannot be involved in the mechanism of attractant-induced hyperpolarization. This finding concurs with the normal membrane potential responses to folate and acetate shown by Pawns (Van Houten 1980; Preston and Van Houten 1987), despite this mutant's lack of functional calcium channels (Kung and Eckert 1972). b) The putative chemoreceptors and membrane components necessary for detection and po-

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*. Paramecia specifically bind ^3H folate with a K_D of 29 μM (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 ^3H 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-(^3H) 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 unicell (Preston 1983).

In a systematic investigation of the chemosensitive properties of the cell body membrane of *P. tetraurelia*, 2.5 mM K_2folate 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_2folate 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 (Alverdes 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 ($pI = 3.7$) 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 anterioventral surfaces of paramecia. Although deciliation eliminates the currents in the bathing medium that are generated by the somatic ciliature, 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 anterio-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 *Stylonychia* (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 fluoroscein-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*.

References

Alverdes F (1922) Zur Lokalisation des chemischen und thermischen Sinnes bei *Paramaecium* und *Stentor*. Zool Anz 55:19–28

Blum JJ (1971) Existence of a breakage point in cilia and flagella. J Theor Biol 33:257–263

Doroszewski M (1961) Reception areas and polarization of ciliary movement in ciliate *Dileptus*. Acta Biol Exp 21:15–34

Doroszewski M, Dryl S (1978) Contribution to studies on localization of chemoreceptor properties of cell surface in *Dileptus cygnus*. Acta Protozool 31:561–565

Dryl S (1969) Motor response of protozoa to external stimuli. Proc 3rd Int Conf Protozool, Leningrad, pp 124–126

Dryl S (1974) Behavior and motor response of *Paramecium*. In: Van Wagtendonk J (ed) *Paramecium*: a current survey. Elsevier, Amsterdam, pp 165–218

Dunlap K (1977) Localization of calcium channels in *Paramecium caudatum*. J Physiol (Lond) 271:119–133

Eckert R, Naitoh Y, Friedman K (1972) Sensory mechanisms in *Paramecium*. I. Two components of the electric response to mechanical stimulation of the anterior surface. J Exp Biol 56:683–694

Jennings HS (1906) Behavior of the lower organisms. Columbia University Press, New York

Kung C, Eckert R (1972) Genetic modification of electrical properties of an excitable membrane. Proc Natl Acad Sci USA 69:93–97

Machemer H (1974) Frequency and directional responses of cilia to membrane potential changes in *Paramecium*. J Comp Physiol 92:293–316

Machemer H, Ogura A (1979) Ionic conductances of membranes in ciliated and deciliated *Paramecium*. J Physiol (Lond) 296:49–60

Matsuoka T (1983) Distribution of photoreceptors inducing ciliary reversal and swimming acceleration in *Blepharisma japonicum*. J Exp Zool 225:337–340

Naitoh Y (1961) Local stimulation of *Opalina*. I. The mode of action of K-ions to induce ciliary reversal. Zool Mag Tokyo 70:435–446

Naitoh Y, Eckert R (1973) Sensory mechanisms in *Paramecium*. II. Ionic basis of the hyperpolarizing mechanoreceptor potential. J Exp Biol 59:53–65

Ogura A (1981) Deciliation and reciliation of *Paramecium* after treatment with ethanol. Cell Struct Funct 6:43–50

Ogura A, Machemer H (1980) Distribution of mechanoreceptor channels in the *Paramecium* surface membrane. J Comp Physiol 135:233–242

Ogura A, Takahashi K (1976) Artificial deciliation causes loss of calcium dependent responses in *Paramecium*. Nature 264:170–172

Preston RR (1983) Studies on the responses of *Paramecium tetraurelia* to amino acids. PhD Thesis, University of Nottingham

Preston RR (1984) A specific L-^{3}H glutamic acid binding site on cilia isolated from *Paramecium tetraurelia*. J Cell Biol 99:187a

Preston RR, Van Houten JL (1987) Chemoreception in *Paramecium tetraurelia*: acetate and folate-induced hyperpolarization. J Comp Physiol A 160:525–535

Schulz S, Denaro M, Xypolyta-Bulloch A, Van Houten J (1984) Relationship of folate binding to chemoreception in *Paramecium*. J Comp Physiol A 155:113–119

Totwen-Nowakowska I, Dryl S (1976) Motor response of fragments of *Stylonychia mytilus* (O.F.M.) to treatment with potassium ions. Acta Protozool 15:77–84

Van Houten J (1976) Chemokinesis in *Paramecium*: a genetic approach. PhD dissertation, University of California, Santa Barbara

Van Houten J (1979) Membrane potential changes during chemotaxis in *Paramecium*. Science 204:1100–1103

Van Houten J (1980) Chemosensory transduction in *Paramecium*: the role of membrane potential. In: Starre H van der (ed) Olfaction and taste VII. IRL Press Ltd, London, pp 53–56

Van Houten J, Hauser DCR, Levandowsky M (1981) Chemosensory behavior in Protozoa. In: Levandowsky M, Hutner SH (eds) Biochemistry and physiology of Protozoa, vol 4. Academic Press, London, 2nd edn, pp 67–124

Van Houten J, Schulz S, Denaro M (1983) Characterization and location of folate binding sites involved in *Paramecium* chemoreception. J Cell Biol 97:1797a

Van Houten J, Smith R, Wymer J, Palmer B, Denaro M (1985) Fluorescein-conjugated folate as an indicator of specific folate binding to *Paramecium*. J Protozool 32:613–616

Watanabe T (1978) A scanning electron-microscopic study of the local degeneration of cilia during sexual reproduction in *Paramecium*. J Cell Sci 32:55–66