The Journal of Experimental Biology **198**, 2537–2545 (1995)
Printed in Great Britain © The Company of Biologists Limited 1995

VOLTAGE-DEPENDENCE OF CILIARY ACTIVITY IN THE CILIATE *DIDINIUM NASUTUM*

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Accepted 26 July 1995

Summary

In the gymnostome ciliate *Didinium nasutum*, swimming behaviour depends upon the cyclic activity of about 3000 cilia. The normal beating mode, resulting in forward swimming of the cell, is characterized by a posteriad effective beat (18° left of the longitudinal axis) at a frequency of approximately 15 Hz. Activation of depolarization-sensitive ciliary Ca^{2+} channels leads to an increase in intracellular Ca^{2+} concentration and a change in the beating mode. Following rapid reorientation, the effective stroke is anteriad (24° right of the longitudinal axis) and the beating frequency is about 26 Hz, resulting in fast backward swimming of the cell. In response to minor depolarizations, and hence small increases in cytoplasmic Ca^{2+} concentration, the cilia inactivate. Frequency increase and reversal in beat orientation share a single threshold level of membrane potential, since both changes of the beating mode occur simultaneously.

Key words: cell locomotion, ciliary beating, electromotor coupling, Ca^{2+} gradient, Protozoa, voltage-clamp, *Didinium nasutum*.

Introduction

Ciliary organelles serve a multiplicity of physiological functions in widely different phylogenetic systems. Besides their function in feeding (Ciliata: Fenchel, 1980; Placozoa: Wenderoth, 1990; Mollusca: Stommel and Stephens, 1988), sensory perception (Vertebrata: Frings and Lindemann, 1991) and transport (Vertebrata: Sanderson *et al.* 1992), locomotion is the main function of ciliary performance (Ciliata: Machemer, 1974; Ctenophora: Mogami *et al.* 1991; sperm cells: Okuno and Brokaw, 1981).

Ciliary activity is due to microtubular sliding of the axonemal complex (Satir, 1989). The axoneme consists of the highly conserved 9+2 pattern of microtubules (Witman, 1990) with dynein as the force-generating ATPase (Warner, 1989). Relative sliding between peripheral microtubular doublets produces cyclical bending of the axoneme (Holwill, 1989). This movement generates the propulsive force for locomotion and is the cause of water streaming around the cell (Sleigh, 1991).

In ciliates, axonemal activity and, therefore, swimming behaviour are controlled by the membrane potential (Machemer, 1989, 1990). Electromotor coupling of cilia is also found in ctenophores (Moss and Tamm, 1986), molluscs (Murakami, 1968) and vertebrates (Boitano and Omoto, 1991). Basic experiments concerning electromotor coupling in ciliates were performed using *Paramecium caudatum* (Kinosita *et al.* 1964) and *Euplotes* sp. (Naitoh and Eckert, 1969) and, more recently, these have been extended to other ciliates such as *Stylonychia mytilus* (Deitmer *et al.* 1984) and *Blepharisma japonicum* (Matsuoka *et al.* 1991).

The free Ca^{2+} concentration plays a crucial part as an intracellular messenger in electromotor coupling (Mogami *et al.* 1990). Extracellular Ca^{2+} enters the cell because of changes in the conductance of voltage-activated Ca^{2+} channels (Pernberg and Machemer, 1989), thus increasing the intracellular Ca^{2+} concentration (J. Pernberg and H. Machemer, in preparation).

Didinium nasutum offers considerable advantages for analyzing ciliary activity since the restriction of the cilia to two circular bands (Lipscombe and Riordan, 1992) and good metachronal coordination aid identification of the beating mode of cilia.

After extensive electrophysiological characterization of *Didinium nasutum* (Pape and Machemer, 1986; Pernberg and Machemer, 1989), this work analyzes the voltage-dependent ciliary activity in relation to inactivation. Compared with *Paramecium caudatum* and other ciliates, *Didinium nasutum* exhibits some interesting differences in ciliary beating mode. Our results enable us to illuminate further the role of free Ca^{2+} as an intracellular messenger in electromotor coupling.

Materials and methods

Cell culture

Didinium nasutum was cultured in Pringsheim solution at

18 °C and fed with *Parameciumcaudatum* every second day. Egg-shaped *Didinium nasutum* had a mean length of 127 μm (93–154 μm) with a maximum diameter of 80 μm (57–95 μm). Cells were ready for experimentation 20 h after feeding.

Videomicroscopy

For recording of ciliary beating, a high-frequency video system (NAC, Tokyo) was used. Image frequency was 200 Hz and a single flash of stroboscobic illumination lasted 15 μs. During the experiments, a specimen was voltage-clamped at a holding potential equal to the resting potential (approximately −35 mV). The cell was under optical control using a standard microscope equipped with a differential interference contrast device (Zeiss, Oberkochen).

The direction of the cilium at the end of the effective stroke with respect to the longitudinal axis of the cell was defined as the beating angle. At this position, cilia showed a typical straight configuration. To analyze the beating angle, ciliary activity was documented as viewed from above. With the cell soma in the background, the contrast of the cilia decreased when compared with the profile view used for the acquisition of frequency data. For this reason, only a few video sequences could be analyzed in terms of beating angle owing to the critical dimensions of a single cilium (diameter approximately 0.25 μm).

Experimental procedures

Cells were equilibrated for more than 1 h in the experimental solution of 1 mmol l^{-1} KCl, 1 mmol l^{-1} $CaCl_2$ and 1 mmol l^{-1} Tris buffer at pH 7.2–7.4. Ciliary beating patterns were studied during the application of 450 ms voltage steps to different step potentials. Room and bath temperatures were kept constant at 18–20 °C. For electrophysiological details, see Pernberg and Machemer (1989).

Results

Morphology and behaviour of the equilibrated cell

Each of the approximately 3000 cilia (Pape and Machemer, 1986; estimated after Wessenberg and Antipa, 1968) is 17 μm long and has a diameter of 0.25 μm. They are densely packed in the anterior and equatorial belts. Beating frequency and the direction of the effective stroke are conventional parameters with which to describe the cyclic ciliary activity.

Didinium nasutum swam in a right-handed helix with a velocity of 1.6±0.1 mm s^{-1} (continuous forward swimming; mean ± S.D.; *N*=20 cells). Spontaneous reversals were observed at mean intervals of 2.9±1.0 s (*N*=38). The duration of backward swimming was very short (175±61 ms; *N*=26). This value corresponds to the duration of spontaneous ciliary reversals (177±80 ms; *N*=9).

Resting ciliary activity

In a tip-to-base view, the cilium moved in a counterclockwise direction for all observed beating patterns.

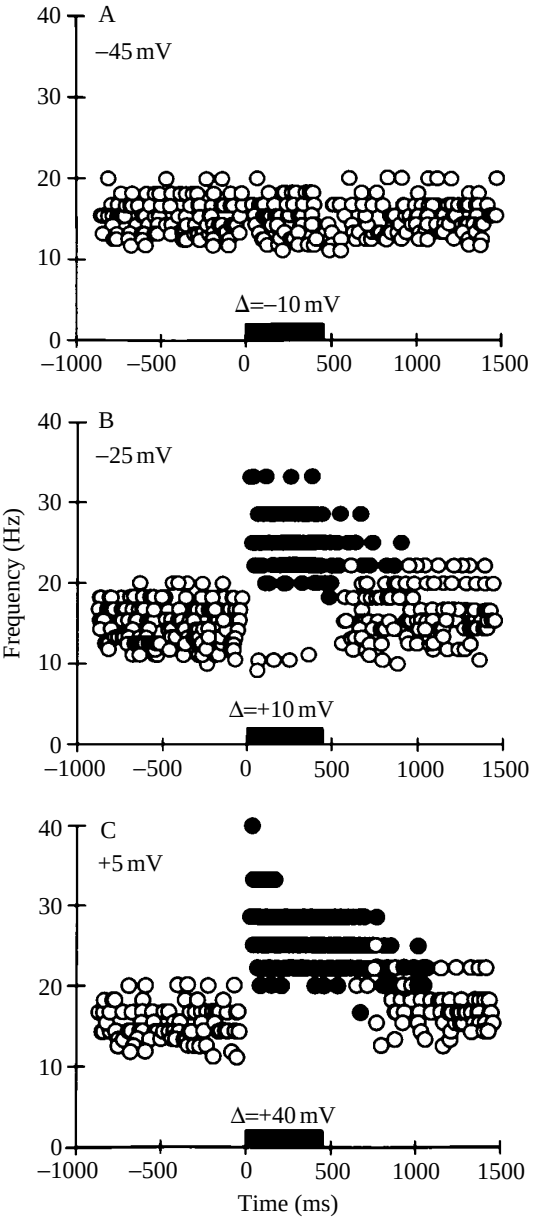

Fig. 1. Ciliary beating frequency following voltage steps applied from the resting potential of −35 mV. At time *t*=0, the membrane potential was changed for 450 ms (black bar on the abscissa, the amplitude of the voltage change is given). The absolute membrane potential during the voltage step is shown next to the ordinate. Open circles, normal beating (the direction of the effective stroke is posteriad); filled circles, reversed beating (the direction of the effective stroke is anteriad). (A) Hyperpolarization of 10 mV. Neither the frequency nor the direction of ciliary beating was influenced by the voltage step (*N*=10 cells). (B) Depolarization of 10 mV. 16 out of 17 cells tested showed an increase in beating frequency and a change in beating direction. In only one cell was the beating behaviour unchanged. (C) Depolarization of 40 mV. All cells (*N*=11) showed a depolarization-induced ciliary response (DCA), which persisted for longer beyond the end of the voltage step than in B.

Each ciliary cycle was seen to have a clear polarity. During the force-generating effective stroke, the cilium was bent at its

base and the tip shifted at the maximum distance from the cell surface. During the return stroke, the entire cilium moved close to the cell surface and was bent more or less throughout.

We define ciliary activity under resting conditions as the ciliary movement observed at a membrane holding potential (=resting potential) of −35±3 mV (*N*=20 cells). The resting frequency of the cilia was 14.7±2.6 Hz. This value was calculated for each cell within an 800 ms prestimulus time. As long as the holding potential was unchanged, the beating frequency was constant (Fig. 1) and varied from 10 to 20 Hz in individual cells. The direction of the effective stroke pointed posteriorly and to the left at an angle of 18° to the longitudinal cell axis (see below). Metachronism was dexioplectic; that is, with the effective stroke pointing posteriorly and to the left, the metachronal wave moved in a 90° counterclockwise direction (see Fig. 4C, inset top).

Ciliary beating mode depends on the membrane potential

During hyperpolarizing voltage steps with a maximal amplitude of −35 mV, no change of the beating mode was detected (Figs 1A, 2A). Depolarizations of more than 5 mV increased the beating frequency from the resting value (14.7 Hz) to 26.0±2.4 Hz (*N*=22 cells). The directions of both the effective stroke and the metachronal wave reversed (see Fig. 4C, insets). The dexioplectic metachronal coordination persisted. After repolarization to the resting potential, ciliary beating frequency returned to the resting value. With increased voltage steps, the duration of reversed ciliary beating after the end of the voltage step increased, but rarely exceeded 0.5 s (Fig. 1B,C).

To analyze the voltage-dependence of ciliary beating, the change in frequency was plotted against the membrane potential. The change in frequency (Δfrequency) was calculated by subtracting the resting frequency from the mean frequency during the step potential (Fig. 2A). We defined as the 'physiological voltage range' all membrane potentials between −70 mV and 0 mV. Potentials measured in the 'free', unclamped cell are within this range when measured, for instance, during spontaneous action potentials (Pernberg and Machemer, 1989).

Increasing the negativity of the membrane potential changed neither the frequency nor the direction of ciliary beating (Fig. 2A). Thus, in contrast to *Paramecium caudatum* (Machemer, 1988) and *Stylonychia mytilus* (Mogami and Machemer, 1991), a hyperpolarization-induced ciliary activation (HCA) is missing in *Didinium nasutum*. At membrane potentials more positive than −25 mV (Δ=+10 mV), reversed beating occurred (depolarization-induced ciliary activation, DCA). Within the voltage range −25 mV to 0 mV, the frequency and direction of reversed beating were unchanged.

Ciliary inactivation occurs with transitions between normal and reversed beating

Near the 'transition potential' of −30 mV (Δ=+5 mV), cilia on some cells still showed normal beating, cilia on others beat in the reverse direction, while a third group of cilia was inactivated (Fig. 2). For each cell, inactivation was seen within a voltage range of a few millivolts (Fig. 2B). With decreasing negativity of the membrane, the proportion of cells that had undergone inactivation before changing to reversed beating increased. During the phase of inactivation, the cilia assumed a more or less straight conformation virtually perpendicular to the cell surface.

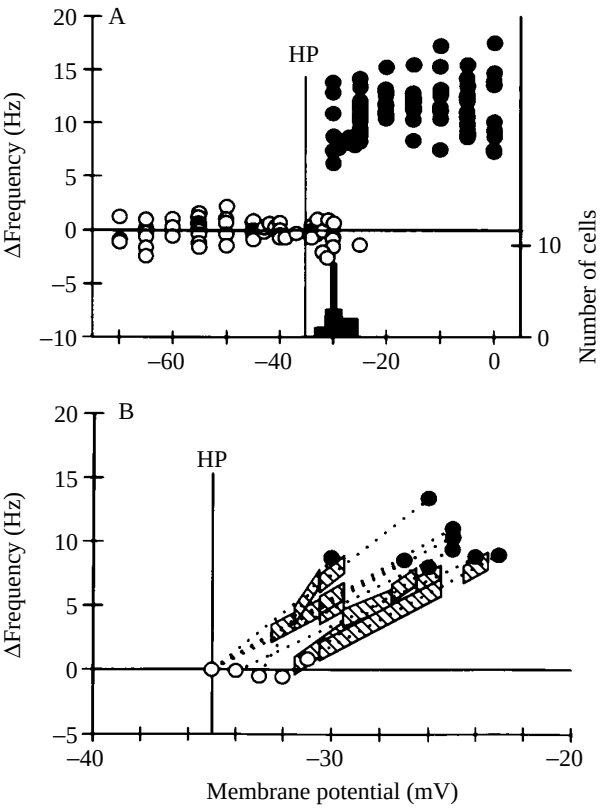

Fig. 2. Voltage-dependence of the change in beating frequency and direction within the physiological response range. Open circles, the effective stroke is posteriad (normal beating); filled circles, the effective stroke is anteriad (reversed beating). Black columns on the abscissa indicate the number of cells showing ciliary inactivation (right ordinate). HP, holding potential. (A) Between the potential of transition from normal to reversed beating (transition potential; −30 mV) and 0 mV, a depolarization-induced ciliary response (DCA) occurred at maximal frequency increase. No hyperpolarization-induced ciliary activity (HCA) was seen. Data from 22 cells. (B) The transition from normal to reversed beating with increasing membrane potential is shown in detail for nine cells. The two frequency data points for each cell are connected by dotted lines. Measured inactivation potentials are indicated by hatched fields. Inactivation was always identified at potentials between those associated with normal beating (open circles) and reversed beating (filled circles). Five open circles are superimposed at the origin.

Ciliary inactivation always occurred during the relatively slow transition from normal to reversed beating (or *vice versa*, Fig. 3). A small depolarizing stimulus, which was insufficient to evoke reversed beating, tended to inactivate the cilia (Fig. 3A). With a larger step amplitude, reversed beating

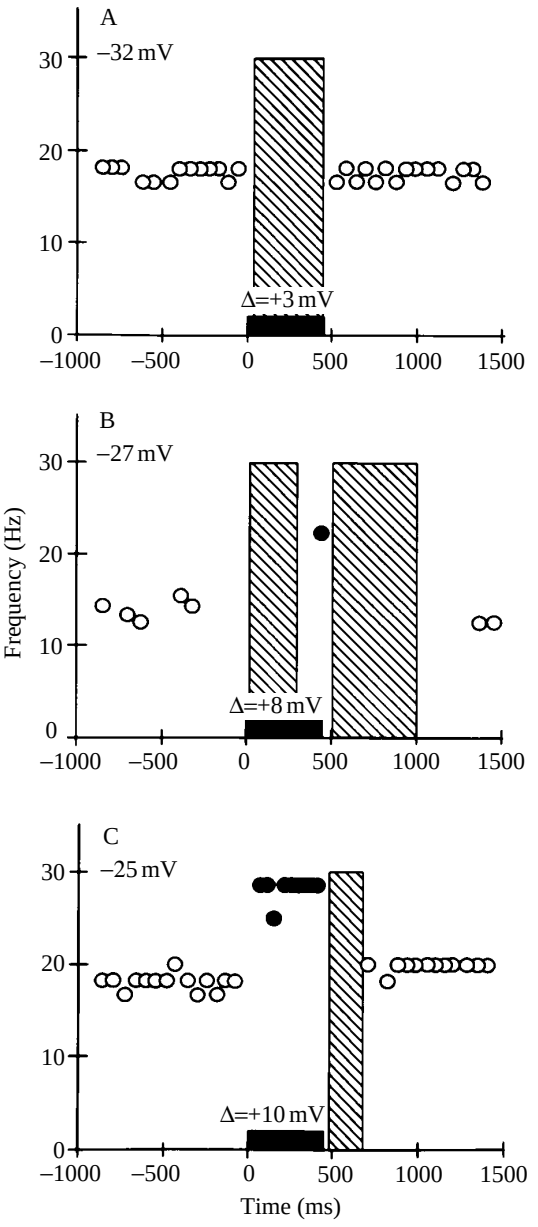

Fig. 3. Voltage-dependence of DCA with particular regard to ciliary inactivation. Values of the beating frequency in A–C were within the typical variability. Each diagram is from a different cell. Hatched areas show observed ciliary inactivation; for details, see legend to Fig. 1. (A) Depolarization of 3 mV induced ciliary inactivation. No reversed beating of cilia was observed. (B) Depolarization of 8 mV; inactivation was followed by a brief period of reversed beating. After the end of the voltage stimulus, a second phase of inactivation occurred. (C) Depolarization of 10 mV; induction of reversed beating only, followed by inactivation and normal beating.

occurred at the end of the voltage step after a period of ciliary inactivation (Fig. 3B). A second ciliary inactivation preceded the restoration of normal beating. At depolarizations positive to the transition potential of −30 mV, inactivation typically lasted 200–500 ms during the transition from reversed to normal beating (Fig. 3C). At these potentials, the transition from normal to reversed beating at the onset of the depolarization was rapid, suggesting the total absence of a transient state of ciliary inactivation.

Change in beating mode at extreme membrane potentials

Extreme membrane potentials, beyond the physiological voltage range, were applied to manipulate the driving force for Ca^{2+} (Fig. 4). A second transition potential was seen at positive voltage steps to +65 mV. Near this potential, ciliary reversal tended to transform to normal beating of the cilia. With even more positive voltages, no reversed beating occurred; that is, neither the frequency nor the direction of beating was changed (Fig. 4A). A brief reversal was seen, however, after the end of the voltage step (an off-response, Fig. 4B).

During negative potentials between about −100 mV and −200 mV, normal beating, reversed beating and ciliary inactivation were observed. With even more negative potentials, the proportion of events of reversed beating increased. In the reversed cilia, the increment in frequency rose as a linear function of voltage (with a slope of approximately 7 Hz per 100 mV; line in Fig. 4A). The frequency saturated at more negative potentials. The maximal frequency corresponded to that seen during depolarizations.

At the resting membrane potential (−35 mV), the power stroke of the cilium was posteriad; that is, 18 ° to the left of the longitudinal axis of the cell (198 ° in Fig. 4C). Within the physiological range of hyperpolarizing voltage, no change in beating angle was detected. Whenever, under extreme negative and positive potential steps, the direction of beating changed from normal to reversed, the resulting power stroke was anteriad 24 ° to the right of the longitudinal axis (Fig. 4C). This corresponds to a ciliary reversal in the counterclockwise direction of 174 °.

Discussion

The three-dimensional ciliary cycle of *Didinium nasutum*, as well as that in *Paramecium caudatum* (Machemer, 1972) and *Stylonychia mytilus* (Machemer and Sugino, 1986), describes an imaginary cone. The effective stroke and the return stroke are distinct both temporally and spatially (Sugino and Naitoh, 1982). The dexioplectic metachronism seen in *Didinium nasutum* (Fig. 4C) is common to all ciliates so far investigated (Machemer, 1974) and has also been found in the Metazoa (Knight-Jones, 1951).

With a mean swimming speed of 1.6 mm s^{-1}, *Didinium nasutum* moves faster than *Paramecium caudatum* (0.8–1.2 mm s^{-1}, Machemer, 1989). Both the duration (100 ms, Machemer, 1989) and the frequency (one reversal per 5–10 s; Becker, 1983) of spontaneous reversals of *Paramecium* cilia are smaller than those in *Didinium nasutum* (175 ms, one reversal per 2.9 s).

Spontaneous action potentials, inducing reversals in forward swimming, are caused by fluctuations in the conductance of ion channels within the cell membrane (Martinac *et al.* 1986). They have been observed in various ciliates (Naitoh, 1966;

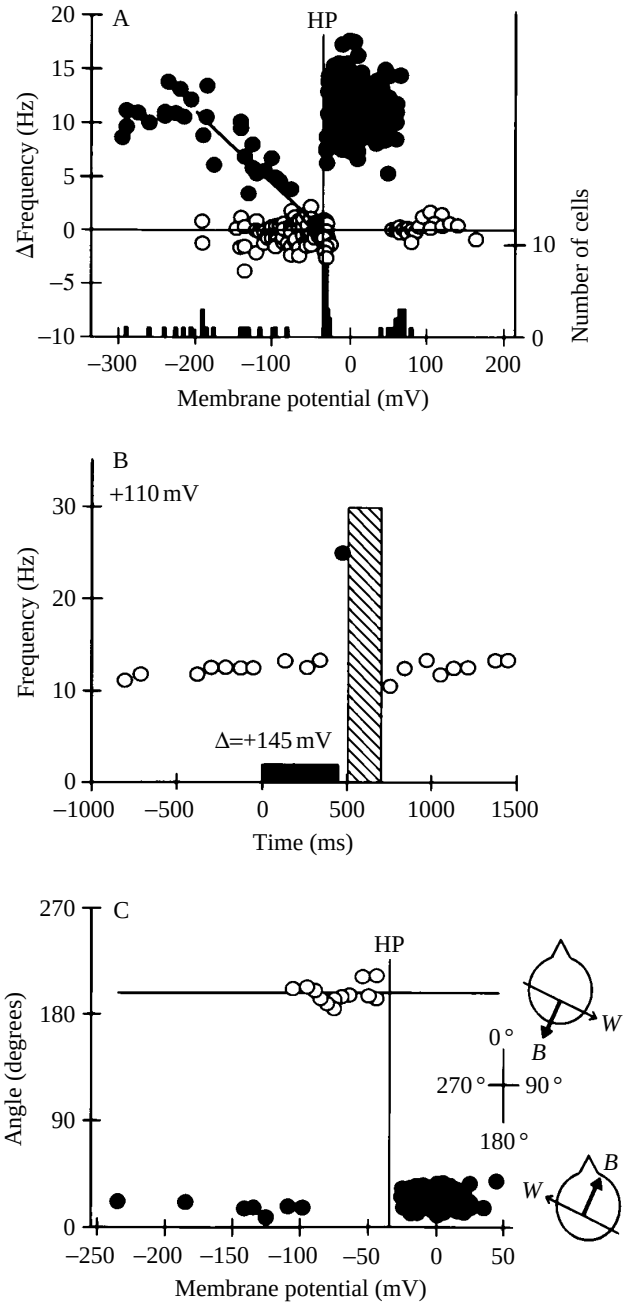

Fig. 4. Voltage-dependence of the change in beating frequency and beat direction. For explanation see Fig. 2A. (A) Apart from the transition potential at −30 mV, a second sharp transition potential was seen at extreme positive potentials near +65 mV. A wide transition field between about −100 mV and −200 mV occurred during extreme hyperpolarizations. The line indicates the relationship between reversed beating frequency and membrane potential within this range (approximately 7 Hz per 100 mV). The maximal frequency change at extreme hyperpolarizations corresponds to that seen during depolarizing voltage steps. (B) During a positive step to +110 mV, no change in beating mode was observed. After termination of the voltage step, a brief reversed beating (an off-response) was seen; ciliary inactivation and normal beating followed. (C) Dependence of the angle of the ciliary effective stroke on the membrane potential. Right: schematic drawings of the normal (top) and reversed (bottom) beating direction (*B*) and the direction of the metachronal wave (*W*). Normal beating was found within a voltage range of −30 mV to about −100 mV. The horizontal line gives the mean beat angle under resting conditions. HP, holding potential (−35 mV); *N*=4 cells.

Kokina, 1970; Moolenaar *et al.* 1976; Deitmer *et al.* 1986) and in *Opalina ranarum* (Kokina, 1964).

Ciliary inactivation is an intermediate mode between normal and reversed ciliary beating

Within the physiological voltage range, a hyperpolarization-induced ciliary activation (HCA) was missing in *Didinium nasutum* which, therefore, differs from *Paramecium caudatum* and *Stylonychia mytilus* (De Peyer and Machemer, 1982*b*; Machemer, 1986). Depolarization-induced ciliary activation (DCA), in contrast, resembles the DCA seen in these two ciliates (Machemer and De Peyer, 1982; Mogami *et al.* 1990). The intraciliary Ca^{2+} concentration is believed to be the messenger regulating DCA, as documented by *in vitro* reactivation experiments in *Paramecium caudatum* (Naitoh and Kaneko, 1972; Nakaoka *et al.* 1984). The elevation of the ciliary Ca^{2+} concentration results from a voltage-gated influx of Ca^{2+} from the extracellular milieu.

The gradual decrease in beating frequency after the end of the depolarizing voltage step (Fig. 1B) corresponds to the kinetics by which the elevated Ca^{2+} concentration is believed to return towards the resting value. Removal of Ca^{2+} is mediated by an active transport mechanism (Hildebrand, 1978), presumably an ATPase which pumps Ca^{2+} from the ciliary cytoplasm (Noguchi *et al.* 1979; Martinac and Hildebrand, 1981). This assumption is indirectly supported by experiments showing that a reduction in temperature results in a counterclockwise shift of the effective ciliary beat in *Paramecium caudatum* corresponding to an increase of the intraciliary Ca^{2+} concentration (Machemer, 1972). Moreover, an inhibition of the presumed active transport system extended the duration of DCA (Doughty, 1978; Hildebrand, 1978).

Following the same line of reasoning, the extension of DCA with increasingly depolarizing voltage steps (Fig. 1B,C) results from a more elevated Ca^{2+} concentration and a longer period of Ca^{2+} pumping (De Peyer and Machemer, 1982*a*; Mogami and Machemer, 1991).

Ciliary inactivation is seen in various ciliates when a small increase in the ciliary Ca^{2+} concentration takes place. In *Paramecium caudatum*, transient ciliary inactivation was observed after a depolarization during the transition from reversed to normal beating (Machemer, 1974, 1975). In *Stylonychia mytilus*, the marginal cirri are inactive at the resting potential. This intermediate mode between normal and reversed beating is associated with the resting potential (De Peyer and Machemer, 1983).

In conclusion, the electromotor coupling and cell reactivation data from different ciliate species show that, within a narrow range of slightly raised intraciliary Ca^{2+} concentrations, cyclic ciliary beating is suppressed. Although

the mechanism is unknown, inactivation may be important for ciliary reorientation during the change in beating direction. A raised intraciliary free Ca^{2+} concentration during inactivation and reversed beating may affect the axonemal complex directly or *via* calmodulin (see Preston and Saimi, 1990). Rates of active sliding and sliding translocation between microtubule doublets (Sugino and Naitoh, 1982; Sugino and Machemer, 1988) and/or the dynein mechanochemical cycle (see Omoto, 1991) could be changed. Mogami and Machemer (1990) presented a quantitative model that included a modulatory function for Mg^{2+}, which competes with Ca^{2+} for binding to an axonemal protein.

Narrow Ca^{2+} concentration range of gradual frequency regulation

Ca^{2+} influx through the ciliary membrane depends on (1) the conductance for Ca^{2+} and (2) the driving force for Ca^{2+}. Augmentation of each of these leads to an elevated Ca^{2+} concentration. Depolarizing voltage steps increase the Ca^{2+} conductance; very large positive steps depress, in addition, the driving force for Ca^{2+}. Extreme hyperpolarizations, however, increase the driving force.

With the ciliary resting conductance unchanged during hyperpolarizations, Ca^{2+} influx is slightly augmented as a result of the increased driving force for Ca^{2+}. The gradual augmentation in inward leakage of Ca^{2+}, and hence of the intraciliary Ca^{2+} concentration, explains the gradual increase in the frequency of reversed beating (line in Fig. 4A). Thus, it is possible that there is a graded ciliary frequency response in *Didinium nasutum*, but it is not seen under physiological stimulus conditions because the corresponding range of voltage-regulated conductance is very narrow. The gradual frequency increase was documented for hyperpolarizations up to -200 mV, corresponding to a doubling of the driving force and Ca^{2+} entry [Ca^{2+} equilibrium potential (E_{Ca})=+107 mV; J. Pernberg and H. Machemer, in preparation]. From this, it can be inferred that, if depolarizations in 1 mV increments induce a transition from normal to reversed beating at maximal frequency, the ciliary Ca^{2+} conductance was at least doubled.

Stepwise rising depolarizations did not result in graded ciliary responses (Fig. 2A). Near the transition potential of -30 mV, reversed beating was associated with a maximal frequency increase. In order to identify a possible graded ciliary motor response in this voltage range, depolarization step intervals should be smaller than 1 mV. This kind of experiment has not yet been performed.

Differences between the Ca^{2+} equilibrium potential and the observed transition potential

With extreme depolarizing membrane potentials of +65 mV or greater, DCA was no longer elicited. This suggests that, beyond this transition potential of +65 mV, the entrance of Ca^{2+} through voltage-activated Ca^{2+} channels was insufficient to elevate the intraciliary Ca^{2+} concentration above the threshold for DCA. After stepping back to the holding potential (-35 mV), Ca^{2+} influx through still open Ca^{2+} channels elicited a ciliary 'off-response' (Fig. 4B).

A voltage-dependent suppression of DCA at very positive membrane potentials has been reported in other species of ciliates (*Paramecium caudatum*, +70 mV, Machemer and Eckert, 1975; *Stylonychia mytilus*, +100 mV, De Peyer and Machemer, 1982*a*; +104 mV, Mogami and Machemer, 1991). In *Didinium nasutum*, the Ca^{2+} equilibrium potential is near +107 mV (external [Ca^{2+}], 10^{-3} mol l^{-1}; internal [Ca^{2+}], 2×10^{-7} mol l^{-1}, J. Pernberg and H. Machemer, in preparation). A marked discrepancy (about 40 mV) exists between the observed transition potential (+65 mV) and the calculated value for E_{Ca}. A possible explanation is that the Ca^{2+} influx is too small to increase the Ca^{2+} concentration above the threshold for DCA. This could only be true for a minor conductance of Ca^{2+}, which is unlikely because the open probability of voltage-dependent Ca^{2+} channels increases to a maximum as the membrane potential becomes more positive (Chen and Hess, 1990; Pietrobon and Hess, 1990).

The reversal potential for the early inward current (+45 mV; Pernberg and Machemer, 1989) also differs from the value of E_{Ca}. It might, therefore, be argued that an equilibrium potential of +107 mV does not apply to the Ca^{2+} channels in the ciliary membrane. This paradox may be explained by assuming a screening effect on the ciliary Ca^{2+} channel. Positive charges next to the external mouth of the channel could repel Ca^{2+} electrostatically, and such screening could be due to the presence of free or bound cations.

Screening could explain why a reduction of the early Ca^{2+} influx is found when the total concentration of extracellular cations is increased (Oka and Nakaoka, 1989). With the hypothetical assumption of an effective Ca^{2+} equilibrium potential of +65 mV across the Ca^{2+} channel, the concentration of free Ca^{2+} at the outer face of the channel would be 3.5×10^{-5} mol l^{-1} (3 % of the nominal value of 1 mmol l^{-1}).

Electrical properties of the ciliary membrane

At a depolarization of 10 mV, reversed beating was seen on the first video frame after the onset of the voltage step; that is, after less than 5 ms. During this time, the maximal Ca^{2+} influx is 10^{-17} mol (Pernberg and Machemer, 1989). The total volume of all cilia in *Didinium nasutum* is 2.5×10^{-12} l. Assuming that the volume of the ciliary liquid space is 50 % of the geometric volume (Machemer, 1986), the Ca^{2+} influx results in an increase in intraciliary Ca^{2+} concentration of up to 8.5×10^{-6} mol l^{-1}. Using this concentration together with the latency time and the driving force for Ca^{2+}, a minimal estimate of the resting resistance of the ciliary membrane can be obtained. Applying Ohm's law, the quotient of the difference between driving force and membrane potential (voltage), on the one hand, and the charge influx per time (current), on the other hand, gives the resistance. Data from extreme hyperpolarizations in 13 cells suggest a minimal resting resistance of 10 GΩ. Using a total ciliary surface of 4×10^{-4} cm^2 (Pape and Machemer, 1986), the minimal specific resting resistance is 4×10^6 Ω cm^2.

The limited temporal resolution of the video system (5 ms, see Materials and methods) means that the calculated value for the intracellular [Ca^{2+}] threshold of ciliary reversal (8.5×10^{-6} mol l^{-1}) is an overestimate. Thus, the estimate of the resting resistance of the ciliary membrane of 10 GΩ is on the safe side. Assuming an intracellular [Ca^{2+}] threshold for ciliary reversal of 6×10^{-7} mol l^{-1}, as determined from reactivated models of *Paramecium caudatum* (Nakaoka *et al.* 1984; Noguchi *et al.* 1991), the ciliary resting resistance is 200 GΩ (specific resistance 8×10^{7} Ω cm^2). In agreement with this, deciliation experiments in *Paramecium caudatum* (Machemer and Ogura, 1979) and *Didinium nasutum* (Pape and Machemer, 1986) suggested an extremely high ciliary membrane resistance because deciliation, that is removal of 50 % of the total cell surface, did not raise the input resistance of the cells.

Voltage-dependent Ca^{2+} channels from the ciliary membrane of *Paramecium caudatum* placed into lipid bilayers gave a single-channel conductance of 1.5–2.0 pS (Ehrlich *et al.* 1984). This value is smaller than that for mammalian cells (3–25 pS, Carbone and Lux, 1987; Fox *et al.* 1987; Droogmans and Nilius, 1989). Assuming that *Didinium nasutum* has a similiar channel conductance to the ciliary Ca^{2+} channel suggested for *Paramecium caudatum*, a ciliary resting resistance of 200 GΩ would result from the opening of 2–3 Ca^{2+} channels. At 0 mV, the ciliary resistance is minimally 21 MΩ (Pernberg and Machemer, 1989). This corresponds to a ciliary conductance of 4.8×10^{-8} S or 3×10^{4} open Ca^{2+} channels. If all voltage-dependent ciliary Ca^{2+} channels are open at this potential and if they are equally distributed over all 3000 cilia, a ciliary membrane would incorporate 10 fast-activating Ca^{2+} channels. Similar values for the resistance of the depolarized ciliary membrane have been calculated in *Paramecium caudatum* (2.5×10^{-8} S, Schein *et al.* 1976; 2.7×10^{-8} S, Oertel *et al.* 1977). In *Didinium nasutum*, an activated ciliary membrane conductance of 1.6×10^{-11} S corresponds to previous estimations for *Paramecium caudatum* (1.7×10^{-11} S, Eckert and Brehm, 1979). With an intraciliary resting Ca^{2+} concentration of 2×10^{-7} mol l^{-1} (J. Pernberg and H. Machemer, in preparation), the number of calcium ions within a single cilium must rise by 1.7×10^{-22} mol (approximately 100 ions) to reach the threshold for reversed beating.

The 'two-threshold' Ca^{2+} hypothesis is to be rejected for Didinium nasutum

According to the 'two-threshold' Ca^{2+} hypothesis (Eckert, 1972), an increase in the driving force during membrane hyperpolarization increases the inward Ca^{2+} leakage. From this, the intraciliary Ca^{2+} concentration can reach the threshold for augmentation of the beating frequency seen during hyperpolarization in *Paramecium caudatum*. Moreover, a reversal in beating direction should occur when the Ca^{2+} concentration passes a second, even higher, Ca^{2+} concentration threshold as a result of further elevation of the driving force for Ca^{2+}. In *Didinium nasutum*, a hyperpolarization-induced increase of the Ca^{2+} leakage current and intraciliary Ca^{2+} concentration results in a frequency augmentation of only those cilia beating in reverse. A voltage-dependent, gradual increase of the beating frequency was seen exclusively in these cilia. Therefore, it has to be concluded that there is a single Ca^{2+} threshold for both frequency increase and reversal of beat direction. Individual differences in threshold voltage for reversed beating can be explained by assuming a divergence in resting Ca^{2+} conductances.

Electromotor coupling of cilia in Didinium nasutum

How do Ca^{2+} conductances (Pernberg and Machemer, 1989) regulate the Ca^{2+}-dependent ciliary movement and, consequently, the locomotion of *Didinium nasutum*? The fast transient Ca^{2+} conductance generates the regenerative depolarization, a rapid rise in the Ca^{2+} concentration and ciliary reorientation (reversed beating) together with an increased beating frequency. This relationship corresponds to data from other ciliates (Machemer and Eckert, 1975; De Peyer and Machemer, 1982*a*; Machemer and Sugino, 1986).

A later-activating, persistent Ca^{2+} conductance supports continued ciliary beating in reverse. As discussed for *Paramecium caudatum* (Machemer and Eckert, 1975), a slowly inactivating Ca^{2+} influx is responsible for intraciliary Ca^{2+} concentrations above the threshold for ciliary reversal. A late increase in K^+ conductance supports membrane repolarization and, therefore, deactivation of the Ca^{2+} influx. After substitution of Ba^{2+} for Ca^{2+}, the inactivation of the early inward current and the late K^+ efflux were inhibited, resulting in a widening of the action potentials (*Didinium nasutum*, Pernberg and Machemer, 1989; *Paramecium caudatum*, Brehm *et al.* 1978).

In *Didinium nasutum*, maintenance of the resting membrane potential at a constant level allows steady ciliary beating and forward swimming of the cell. Transient backward swimming results primarily from spontaneous action potentials. The repeated reorientations that follow such transient reversals may increase the probability of encounters with prey cells (such as *Paramecium caudatum*).

The levels of membrane depolarization normally achieved fall because of the Ca^{2+}- and voltage-dependent inactivation of the Ca^{2+} channels. Only when more positive membrane potentials are reached does the late-activating K^+ conductance repolarize the membrane. The major function of this conductance is, therefore, the homoeostasis of the cell membrane.

We are grateful to Dr G. Schweigart for helpful discussion. This work was supported by the Deutsche Forschungsgemeinschaft, Forschergruppe Konzell 3.

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