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PRESSURE-INDUCED CHANGES IN Ca^{2+}-CHANNEL EXCITABILITY IN *PARAMECIUM*

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SUMMARY

The behaviour of swimming *Paramecium* is markedly affected by hydrostatic pressure (50–200 atm, 1 atm = 101 325 Pa). To investigate whether pressure might alter behaviour by acting directly on specific ion channels that mediate the behavioural responses, we examined the effects of K^+, Na^+ and Ba^{2+} ions on swimming speed and the reversal response during pressurization and decompression. If pressure acted on the channels that transport these ions, then the pressure-induced responses of swimming *Paramecium* should be exaggerated or diminished, according to which ions were present in the experimental buffer. Pressurization to 100 atm in standard buffer inhibited the brief reversal of swimming direction that occurred at atmospheric pressure when a paramecium encountered the wall of the pressure chamber. To determine whether pressure impaired mechanoreceptor function or directly blocked the Ca^{2+}-channels that control ciliary reversal, we added Ba^{2+} or Na^+ to standard buffer to induce multiple spontaneous reversals. Pressurization blocked these reversals, suggesting that channel opening is directly inhibited by pressure. Decompression in standard buffer elicited momentary ciliary reversal and backward swimming. Buffers with a high ratio of K^+ to Ca^{2+} suppressed this response, and the decompression-induced reversal was exaggerated in the presence of Ba^{2+} or Na^+, consistent with the effects that these ions are known to have on *Paramecium*'s reversal response. These data imply that, upon decompression, the Ca^{2+}-channels that mediate ciliary reversal open transiently. In addition to blocking the reversal response, pressurization slowed forward swimming. By examining the response to pressurization of *Paramecium* immobilized by Ni^{2+}, we found that hydrostatic pressure apparently slows swimming by reorientating the direction of ciliary beat.

INTRODUCTION

Many types of excitable cells are sensitive to changes in hydrostatic pressure (reviewed by Wann & MacDonald, 1980). Kitching has described, in detail, the responses of a variety of ciliates and flagellates, including

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Paramecium and *Spirostomum*, to pressurization and decompression (Kitching, 1957, 1969; MacDonald & Kitching, 1976). His results demonstrated that the sensory behaviour of ciliates is impaired by pressurization. More recently, studies of voltage-clamped neurones under pressure have indicated that pressures of 200 atm or less can alter neuronal function and nerve excitability (Conti, Fioravanti, Segal & Stuhmer, 1982; Harper, MacDonald & Wann, 1981; Parmentier & Bennett, 1980; Wann, MacDonald & Harper, 1979).

We have used hydrostatic pressure to study the sensory and motor behaviour of *Paramecium*. Hydrostatic pressure is particularly well-suited for these studies because pressurization and decompression are rapid and the effects of pressure changes appear completely reversible up to about 280 atm (Otter & Salmon, 1979). Within this pressure range there are no obvious changes in *Paramecium*'s structure, and the cells respond instantaneously to changes in pressure. The pressure-sensitive components of *Paramecium*'s motility reside primarily in its electrically excitable plasma membrane (Otter & Salmon, 1979). In particular, the Ca^{2+}-channels that mediate ciliary reversal appeared to be sensitive to changes in hydrostatic pressure.

Sensory behaviour in the ciliate *Paramecium* is linked to the cell's motor response by its electrically excitable plasma membrane. Normally, a *Paramecium* swims forward because its cilia beat with their effective strokes directed toward the cell's posterior. Reversal of ciliary beat direction and backward swimming result from depolarization of the surface membrane. An inward flow of calcium ions through voltage-sensitive channels, located in the plasma membrane covering the cilia, depolarizes the cell, elevates the Ca^{2+} concentration around the ciliary axonemes and triggers reversed beating of the cilia. When the surface membrane re-establishes its resting electrical potential and the calcium concentration surrounding the axonemes falls, normal forward swimming resumes. This scenario of sensory-motor coupling in *Paramecium* has been deduced from a variety of experiments using genetic, biochemical, electrophysiological and microscopical techniques (Dunlap, 1977; Eckert & Brehm, 1979; Kung *et al.* 1975; Kung & Saimi, 1982; Machemer & Ogura, 1979; Naitoh & Kaneko, 1972). Nevertheless, relatively little is known about the molecular basis of *Paramecium*'s membrane excitation or the Ca^{2+}-dependent pathway in the cytoplasm that leads to reversal of ciliary beat direction.

In this paper, we describe the motility of *Paramecium* during pressurization and decompression in buffers of different ionic composition made by systematically varying the concentrations of Ca^{2+}, K^+, Na^+ and Ba^{2+} ions. Because these cations are known to induce alterations in the behaviour of *Paramecium* swimming at atmospheric pressure, we can make specific predictions regarding how substitution of cations might alter the responses of *Paramecium* to changes in hydrostatic pressure (Kung & Saimi, 1982). We have analysed the pressure-induced responses of *Paramecium* according to these predictions in order to understand how pressure alters the physiology of the excitable plasma membrane. In order to study ciliary movements under pressure, we have examined pressure-induced changes in orientation of cilia paralysed by nickel ions. Finally, we describe a new, enlarged microscope pressure chamber for studying cell movements under pressure. Portions of this work have been published in abstract form (Otter, 1980; Otter & Salmon, 1982).

MATERIALS AND METHODS

Growth media and harvesting cells

Paramecium caudatum (Carolina Biological Supply, Burlington, NC) were grown at room temperature in bacterized Cerophyl medium (Cerophyl Corp., Kansas City, MO) as described by Sonneborn (1970). *Paramecium* were transferred from culture medium to an experimental buffer (see below) by washing them three times at room temperature by gentle hand centrifugation (30–60 s, 150 × ***g***). Experiments described here concern swimming behaviour after an initial period of adjustment to the ions in the buffer. We allowed an equilibration period of at least 30 min before beginning experiments. *Paramecium* observed during a transfer from culture medium to buffer often swam backwards or forwards rapidly, but after several minutes of exposure this behaviour subsided.

Chemicals and experimental buffers

Our standard buffer (TECK) contained (in mmol l^{-1}): Tris, pH 7·2, 10·0; EDTA, 0·1; $CaCl_2$, 1·0; KCl, 4·0. Salts were purchased from Sigma Chemical Company, St. Louis, MO. To this buffer we added either: 10 mmol l^{-1} KCl (K^+-TECK), 10 mmol l^{-1} NaCl (Na^+-TECK) or 20 mmol l^{-1} KCl. To induce 'barium dancing' we added 4 mmol l^{-1} $BaCl_2$ to TECK, K^+-TECK or TEC buffer. TEC buffer is standard buffer without KCl.

Paralysis by nickel ions

The effective concentration of $NiCl_2$ for paralysis of cilia was determined by transferring about 100 μl of TECK containing about 50 *Paramecium* to a dish of TECK plus $NiCl_2$ at various concentrations. We chose a concentration of nickel that immobilized 90 % of the *Paramecium* within 10 min without obvious physical distortion of the cells. For *Paramecium* that had been preincubated in TECK, 0·2 mmol l^{-1} $NiCl_2$ proved effective; other concentrations were not suitable. Higher concentrations (0·5–1 mmol l^{-1} $NiCl_2$) lysed many cells and at 0·1 mmol l^{-1} $NiCl_2$ paralysis was complete in 2 h.

Hydrostatic pressure chamber for microscopy

We have designed and built a pressure chamber for observing *Paramecium* at pressures of 350 atm or less. The chamber is a larger, modified version of a miniature pressure chamber for microscopy described by Salmon & Ellis (1975; Fig. 1).

The microscope chosen for this study was a Leitz Diavert inverted microscope (Otter & Salmon, 1979; Otter, 1981). In order to record the orientation of paralysed cilia during pressurization and decompression, we fitted the Diavert with a Zeiss Model I long working distance phase contrast condensor and a Nikon 40× DML phase contrast objective with correction collar; paralysed specimens were pressurized in the miniature chamber (Salmon & Ellis, 1975). Illumination was routinely a tungsten filament bulb. For flash photography we used a Strobex model 136 power supply equipped with a Xenon arc flash tube (model 35 s). All triggering circuitry for driving the Strobex power supply was home-made.

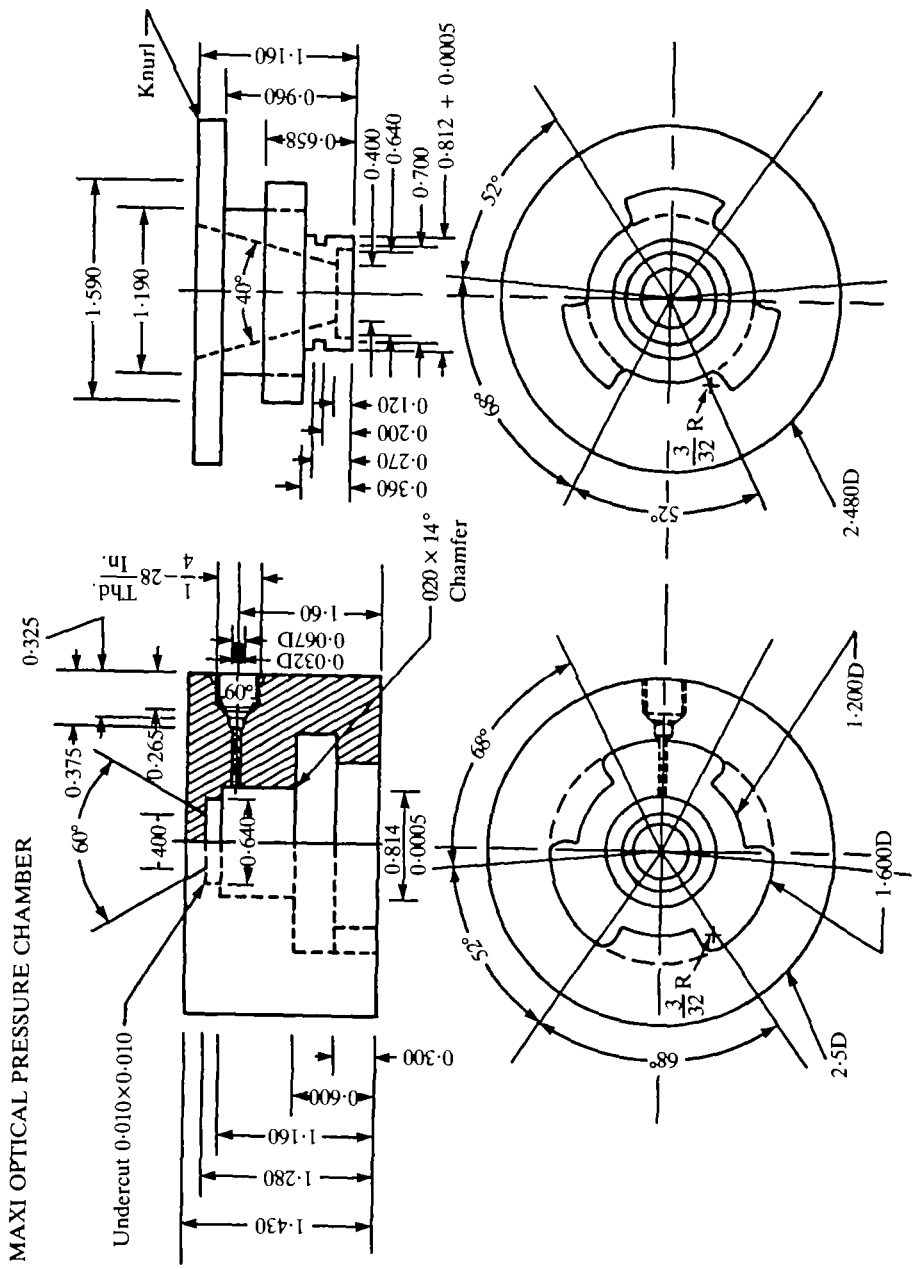

Fig. 1. Detailed drawings of large microscope pressure chamber. The material is 316 stainless steel. All dimensions are ± 0.001 inches unless otherwise noted. All sharp corners are broken. All surfaces machine finished with no burrs. The window seats should be turned flat within 0.0002 inches. The windows are 3 mm-thick optical glass. Substituting sapphire windows for glass would increase the working pressure range to 1000 atm.

Recording swimming behaviour

Qualitative observations on the responses of swimming *Paramecium* to step changes in pressure and counts of reversals were obtained by directly viewing *Paramecium* in the chamber. All measurements of swimming speed and ciliary orientation were taken from photographic records (Otter & Salmon, 1979). Swimming direction was deduced from the experimenter's written and mental records that a cell was swimming backward or forward and from the clearly-defined body shape of the *Paramecium* recorded on the film. Because the *Paramecium* moved about rapidly in the chamber, it was important to have a simple, highly-automated method to record swimming tracks. The strobe delivered a pre-set number of flashes at a predetermined interval, τ, usually 0·3 s. After the final flash, the trigger circuit closed the shutter, reset itself and advanced the film.

RESULTS

Pressurization in culture medium

Paramecium were highly sensitive to pressure when they were swimming in culture medium (Table 1). As little as 30 atm produced a noticeable decrease in both forward speed and in reversal frequency; at 70 atm many *Paramecium* came to rest against the lower cover-glass surface. On release of pressure, *Paramecium* backed up quickly and then swam actively.

Pressurization in standard buffer (TECK)

The pressure-induced responses of *Paramecium* swimming in TECK buffer were described briefly elsewhere (Otter & Salmon, 1979). At atmospheric pressure, *Paramecium* in TECK buffer swam in a series of relatively straight paths interrupted by brief reversals or 'avoiding reactions'. Normally, a *Paramecium* swam at about 1·4 mm s^{-1} and backed up about 7 times per minute (Table 2). Pressurization immediately slowed their forward speed; the higher the pressure the lower the average speed of swimming *Paramecium* (Figs 2,4,5). At 100 atm, they swam at about one-

Table 1. *Responses to pressurization and decompression*

	Pressurization		Decompression
Buffer	Slowing	Fewer reversals	Transient reversal
TECK	+	+	+
TEC	+	+	+
K^+-TECK	++	++	−−
Na^+-TECK	+	−	++
Ba^{2+}-TECK	+	−−	+++
Cerophyl	++	++	++

Responses to changes in hydrostatic pressure ranged from very weak (−−) to very strong (+++). The 'average' responses (+) were defined as those seen in TECK.

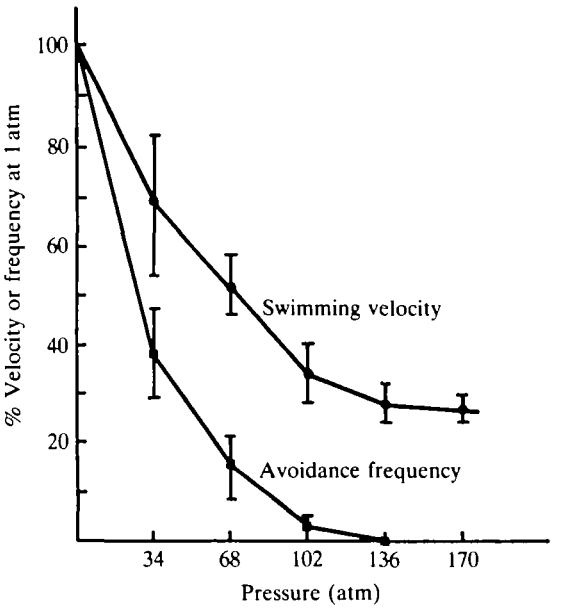

Fig. 2. Decrease in velocity of forward swimming (●) and frequency of avoiding reactions (■) with increasing pressure. Pressure was raised from atmospheric in steps of 34 atm at 5-min intervals. At each pressure, swimming speeds of 5–10 organisms were determined from photographs taken during each 5-min interval. Data from four such experiments were pooled and averaged for each pressure level. In five separate experiments, visual counts of the number of jerks, hesitations or reversals of four or five organisms were recorded at each pressure. Values plotted are the mean ± S.E. normalized to the initial value at atmospheric pressure.

Table 2. *Ion effects on swimming behaviour*

	Avoidance frequency Reversals ($cell^{-1} min^{-1}$)	Forward swimming speed ($mm\ s^{-1}$)
TECK	6·75 ± 0·76 (7)	1·37 ± 0·25 (102)
Na^+–TECK	11·48 ± 1·05 (7)	1·36 ± 0·16 (73)
K^+–TECK	2·51 ± 0·45 (4)	0·93 ± 0·09 (183)
TEC	9·4 ± 2·7 (7)	1·59 ± 0·38 (112)

Values are means ± S.D. (*N*).

third their initial speed, and many *Paramecium* were completely immobilized by this pressure. When the pressure was increased further, more *Paramecium* came to rest at the edge of the specimen enclosure, and the remaining cells slowed down. Pressurization also inhibited reversal of swimming direction that was observed at atmospheric pressure. Occasionally at 100 atm *Paramecium* hesitated briefly; above 100 atm, some continued to move forward slowly, but they did not back up.

Rapid decompression (within 2 s) to 13.6 atm elicited a transient reversal response similar to an avoiding reaction (Figs 3A, 5). Even if the pressure was reduced slowly ($\simeq 1{\cdot}5\ atm\ s^{-1}$), the *Paramecium* jerked backwards when the pressure reached 68 atm. The effects of pressurization and decompression appeared to be completely reversible up to about 300 atm. After the brief reversal upon decompression, the *Paramecium* swam in a manner characteristic for the lower pressure.

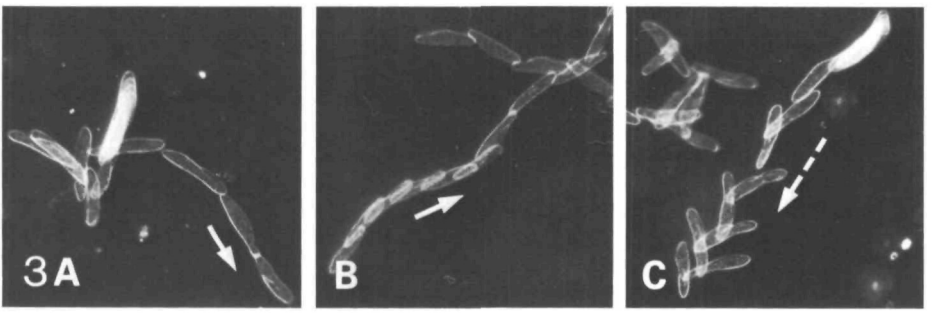

Fig. 3. Responses to abrupt decompression. Pressure was held at 170 atm during the first four strobe flashes and then quickly lowered to 13·6 atm. In TECK buffer (A), the *Paramecium* jerked backwards upon decompression and then swam forwards (arrow). Upon decompression in K^+-rich buffer (B), the *Paramecium* simply accelerated forwards (arrow). The response to decompression in Ba^{2+}-TECK (C) was prolonged backward swimming (dashed arrow). $\tau = 0{\cdot}3$ s. Magnification, ×40.

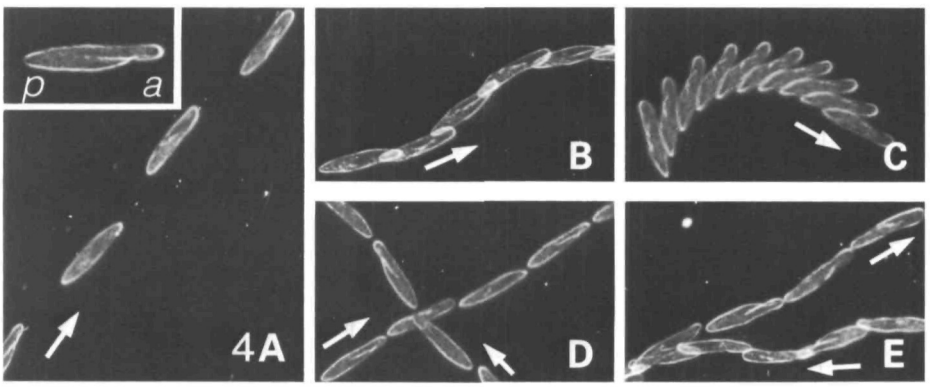

Fig. 4. Slowing of forward swimming induced by increasing pressure (A,B,C) or increasing [KCl] at atmospheric pressure (A,D,E). (A) TECK buffer at atmospheric pressure; (B) 68 atm in TECK: (C) 136 atm in TECK; (D) 10 mmol l^{-1} KCl added to TECK at atmospheric pressure; (E) 20 mmol l^{-1} KCl-TECK at atmospheric pressure. Inset (A) Posterior (*p*) and anterior (*a*) are easily distinguished. In all photographs, arrows indicate the direction of forward movement and $\tau = 0{\cdot}3$ s. Magnification, ×40.

Effect of pressure in K^+-rich buffer (K^+-TECK)

At atmospheric pressure, increasing the KCl concentration from 4 mmol l^{-1} (TECK buffer) to 10 or 20 mmol l^{-1} slowed swimming *Paramecium* (Table 2; Fig. 4). In 10 mmol l^{-1} K^+ buffer (K^+-TECK), the average speed of *Paramecium* was reduced by about 40 %, so that they travelled approximately one body length per flash interval; in 20 mmol l^{-1} K^+ buffer successive images of swimming *Paramecium* appeared slightly overlapped. At either KCl concentration the swimming paths of *Paramecium* appeared wavy or corkscrew-shaped compared to paths in TECK buffer, and the forward speed of individual *Paramecium* was more monotonous. They swam slowly from one side of the enclosure to the other, often without spontaneous hesitation or acceleration. When they reached the edge, they backed up weakly, turned and swam slowly in a new direction. The frequency of avoiding reactions was reduced in K^+-TECK buffer (Table 2). Many cells appeared noticeably flattened by

exosmosis in 20 mmol l^{-1} K^+ buffer, so we did not use this buffer for further experiments with hydrostatic pressure.

We compared the swimming behaviour of *Paramecium* in TECK buffer and in K^+-TECK during pressurization to 204 atm in 68-atm steps and during decompression (Fig. 5). In either buffer, after a step increase in pressure, the forward speed of an individual decreased immediately. In K^+-TECK at 68 atm nearly all *Paramecium* had stopped moving forwards. A few individuals continued to swim forward slowly, and they never backed up. In TECK buffer a pressure of 136 atm or above was required to produce the same effect. At a given pressure, *Paramecium* swimming in TECK changed speed more quickly and more often than *Paramecium* in K^+-TECK buffer (Fig. 5).

Paramecium in K^+-TECK did not reverse their ciliary beat during decompression to 13.6 atm; they hesitated briefly and then simply accelerated forward (Fig. 5). Reducing the Ca^{2+}-concentration from 1 mmol l^{-1} (in TECK) to 0·1 mmol l^{-1} produced the same result: no reversal occurred on decompression (Fig. 3B).

Removing K^+-ions from the external medium (TEC) changed the swimming behaviour of *Paramecium* only slightly compared to their behaviour in TECK. At atmospheric pressure, the forward speed and frequency of reversals increased, but the responses of *Paramecium* in TEC to changes in pressure were essentially identical to those in TECK (Tables 1,2).

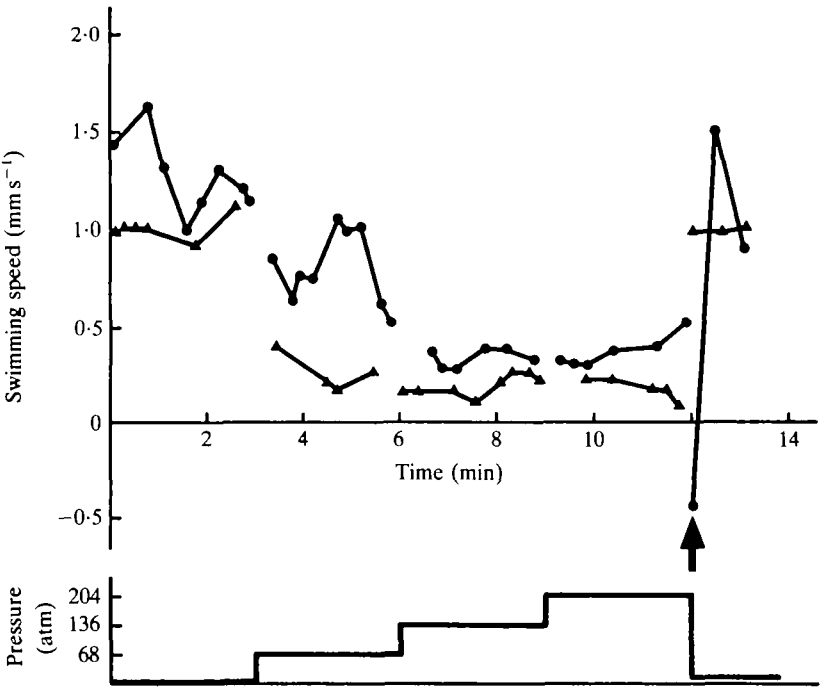

Fig. 5. Instantaneous velocity of typical individual *Paramecium* during stepwise pressurization in TECK (●) or K^+-TECK (▲). Pressure was increased from atmospheric in steps of 68 atm at 3-min intervals to 204 atm and then returned abruptly to 13·6 atm. Swimming velocity was determined from photographs taken during each interval. Decompression elicited an avoiding reaction (arrow) in TECK buffer, but not in K^+-TECK.

Effect of pressure in Na^+- or Ba^{2+}-rich buffer (Na^+-TECK or Ba^{2+}-TECK)

We examined the responses of swimming *Paramecium* to pressurization and decompression in Na^+-TECK containing 10 mmol l^{-1} NaCl (Table 1). At atmospheric pressure, adding NaCl slowed their forward speed slightly, but it increased their frequency of avoiding reactions 1·7-fold (Table 2). Occasionally, prolonged reversals (> 3 s duration) occurred in Na^+-TECK. Pressurization of *Paramecium* in Na^+-TECK to 68 atm slowed their forward movement. This pressure did not completely block reversals, but 136 atm did. Nearly all *Paramecium* were stopped at 136 atm. Decompression caused an exaggerated reversal, followed by rapid forward swimming punctuated by frequent reversals. This latter result contrasted strikingly with the weak hesitation that accompanied decompression in K^+-TECK.

Addition of 4 mmol l^{-1} $BaCl_2$ to TECK, TEC or K^+-TECK induced frequent repetitive avoiding reactions, a behaviour known as the 'barium dance' (Fig. 6). Many cells jerked backwards repeatedly and made no forward progress through the medium. For example, in TECK buffer the *Paramecium* backed up about three times as often with barium present. Additionally, most of the reversals occurred spontaneously, when no obvious physical stimulus was present.

Pressurization to 68 atm reduced the frequency of barium-induced reversals. In Ba^{2+}-TECK buffer or Ba^{2+}, K^+-TECK buffer, 204 atm blocked the barium-induced reversals completely, but in Ba^{2+}-TEC buffer, barium-induced reversals continued

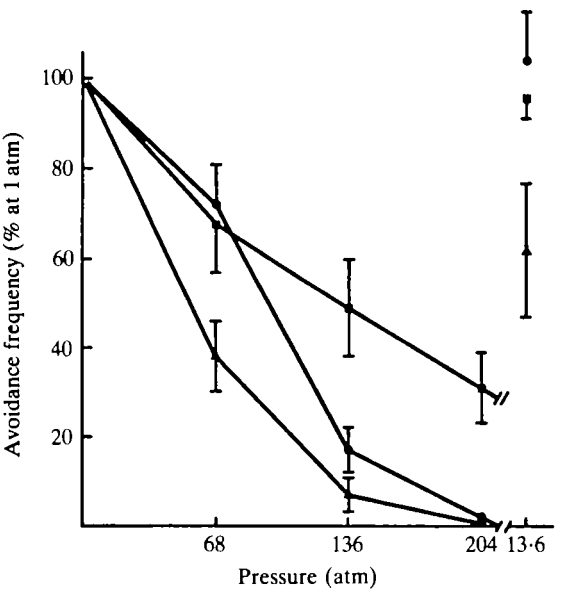

Fig. 6. Decreasing frequency of spontaneous reversals in Ba^{2+}-rich media with increasing pressure. (●) Ba^{2+}-TECK; (▲) Ba^{2+}, K^+-TECK; (■) Ba^{2+}-TEC. Pressure was increased from atmospheric in steps of 68 atm to 204 atm and then reduced in a single step to 13·6 atm. At each pressure, reversals of four or five *Paramecium* were counted for 3 min. The experiment was repeated four times for each buffer and the data for each pressure level were pooled and normalized to the initial value at atmospheric pressure. Values are the mean ± S.E. At atmospheric pressure, (●) = 15·8 ± 1·8; (▲) = 18·2 ± 2·9; (■) = 26·4 ± 1·3 reversals (cell^{-1} min^{-1}).

even at 204 atm. Reversals were more violent in Ba^{2+}-TEC than in the other two buffers at any pressure. *Paramecium* jerked backwards rhythmically and rotated rapidly about their long body axis. Even though the *Paramecium* made little or no forward progress at 204 atm, they still occasionally jerked backwards.

These decreases in Ba^{2+}-induced reversals under pressure appeared to be completely reversible within the pressure range studied. Upon decompression in any medium containing barium, many *Paramecium* reversed vigorously and then danced wildly for the next several minutes (Fig. 3C). Often during the few minutes following decompression in barium-containing media, the frequency of reversals was higher than it had been before pressurization (Fig. 6).

Pressure-induced changes in ciliary orientation and movement

In order to examine specifically if pressure induced changes in the orientation of cilary beat, we observed the behaviour of *Paramecium* immobilized by nickel ions during step pressurization and decompression. Nickel ions paralyse cilia so that they do not beat, but they retain the ability to reorientate in response to chemical, physical or electrical stimuli (Naitoh, 1966; Eckert & Naitoh, 1970). The orientation of cilia immobilized by nickel reflects the direction of the effective stroke that a normal beating cilium would have. To minimize the toxic effects of nickel, we performed all experiments within the first 40 min following incubation in 0·2 mmol l^{-1} $NiCl_2$-TECK. Gradually, over the next 30 min, the cilia assumed a resting position that was more or less at a right angle to the cell surface.

During the first 30 min of paralysis, the cilia appeared gently curved. They 'vibrated' and occasionally reversed spontaneously. When the nickel-paralysed *Paramecium* were transferred from TECK buffer plus nickel to 20 mmol l^{-1} K^+-TECK, their cilia reversed immediately and continued to point towards the anterior for tens of seconds, similar to K^+-induced reversal of swimming direction in unparalysed *Paramecium* (Fig. 7D; Naitoh, 1968). To interpret the pressure-induced changes in ciliary orientation (discussed below), we designated the normal orientation and the reversed orientation in 20 mmol l^{-1} K^+-TECK as two extremes.

Pressurization to 68 atm caused cilia paralysed by nickel ions to move from their normal, posteriorly-directed posture to nearly perpendicular to the *Paramecium*'s surface (Fig. 7). Increasing the pressure to 136 atm caused the cilia to point slightly toward the cell's anterior (Fig. 7C). Above 70 atm pressure, the paralysed cilia never reversed or pointed towards the posterior; they stuck out straight and vibrated weakly. As noted above, cells that had been incubated in nickel medium for over 30 min had many of their cilia pointing at a right angle to the cell surface at atmospheric pressure. These cells responded to pressure by shifting their cilia slightly towards the anterior, but this change was not nearly as striking as the pressure-induced reorientation in a freshly immobilized *Paramecium*.

Decompression caused a *Paramecium*'s paralysed cilia to swing towards the anterior transiently, similar to a spontaneous or K^+-induced reversal (Fig. 7E). In standard buffer containing nickel, the reversal was momentary and a recovery period of confused orientations of cilia followed (Fig. 7F). During recovery many of the cilia appeared to lie flat against the cell surface. The first recognizable position assumed by the cilia after decompression-induced reversal was directed towards the

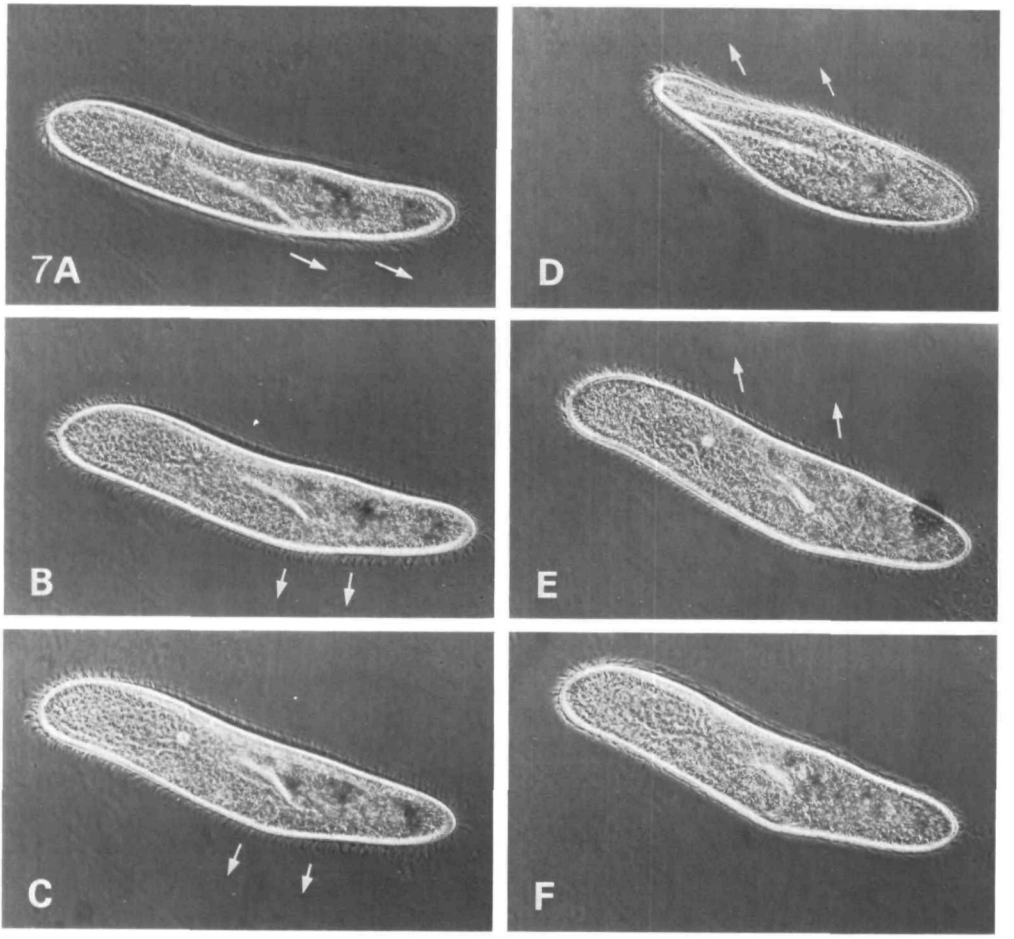

Fig. 7. Reorientation of nickel-paralysed cilia during pressurization and decompression. Orientation of lateral body cilia of an individual *Paramecium* (arrows) is shown at atmospheric pressure (A), 68 atm (B), 136 atm (C), during decompression (E) and immediately after decompression to 13·6 atm (F). Pressure was increased stepwise and held for several minutes at each pressure. For comparison, 'reversal' of paralysed cilia induced by 20 mmol l^{-1} KCl-TECK is shown (D). Magnification, ×220.

posterior, as in normal forward-swimming cells at the end of their effective stroke.

DISCUSSION

Pressure effects on the reversal response

The cilium and its Ca^{2+}-channels appeared to be specific targets of pressure action. Decompression-induced reversal apparently did not result from a global influx of Ca^{2+} across *Paramecium*'s surface; if this were the case, then 'pawn' mutants should have reversed during decompression, but they did not (Kung & Naitoh, 1973; Otter & Salmon, 1979). Rather, the response of *Paramecium* to decompression depended on the functional state of the Ca^{2+}-channels in their plasma membrane.

The excitability of the Ca^+-channels that mediate ciliary reversal can be altered by mutation or by changing the ionic composition of the medium surrounding the *Paramecium*. For example, barium ions facilitate Ca^{2+}-entry through the channels by inducing spontaneous and prolonged channel opening and so barium changes *Paramecium*'s Ca^{2+}-response from one graded with stimulus intensity to an all-or-none response (Naitoh & Eckert, 1968). 'Pawn' mutants have genetically altered Ca^{2+}-channels that do not open when electrically stimulated (Kung *et al.* 1975; Satow & Kung, 1980). As one might have predicted, the transient reversal upon decompression was absent in 'pawn' mutants (Otter & Salmon, 1979) and was exaggerated in media containing Ba^{2+} ions (Fig. 3C; Table 1). At atmospheric pressure, Na^+ induces spontaneous membrane depolarizations and frequent, brief reversals of ciliary beat in wild-type *Paramecium*, and elevated Na^+ causes prolonged reversals in 'paranoiac' mutants of *Paramecium* (Satow, Hansma & Kung, 1976; Table 2). Decompression elicited an exaggerated reversal response in the presence of Na^+, suggesting that Na^+ acts during decompression to prolong backward swimming. While the mechanism by which Na^+ alters *Paramecium*'s membrane excitation is unclear, our results are consistent with the observation that high Na^+ levels induce hyperexcitable behaviour in *Paramecium* (Kung & Saimi, 1982). On the other hand, buffers with high K^+ concentrations relative to Ca^{2+} inhibit ciliary reversal and attenuate the membrane's response to mechanical or electrical stimulation (Eckert, Naitoh & Friedman, 1972; Naitoh, 1968; Naitoh, Eckert & Friedman, 1972). It is not surprising, then, that the decompression-induced reversal response was weak or lacking in K^+-rich buffer (Fig. 3B; Table 1).

The above results suggest that decompression specifically opened the Ca^+-channels that normally mediate ciliary reversal and elicited a response physiologically similar to an avoiding reaction.

Pressurization immediately blocked avoiding reactions of swimming *Paramecium* (Fig. 2; Table 1); above 68 atm, *Paramecium* did not back up. As postulated earlier, pressure may have inhibited the anterior mechanoreceptors and so reduced the frequency of avoiding reactions that occurred when a *Paramecium* contacted the wall of the pressure chamber (Kitching, 1969; Otter & Salmon, 1979). However, our experiments in barium medium suggest that increased pressure directly inhibited the calcium channels from opening (Fig. 6). Barium-induced reversals are independent of contact with the chamber wall and their frequency decreased with increasing hydrostatic pressure. Substantially higher pressure was required to block barium-induced reversals (approx. 200 atm), so impairment of mechanoreceptor and channel function by pressurization may be additive.

Pressurization does not appear to block ciliary reversal by directly inhibiting the events in the ciliary axoneme or basal apparatus that cause reversed beating. Above 68 atm in TECK buffer *Paramecium* did not back up (Fig. 2), but if the buffer contained Na^+ or Ba^{2+}-ions, *Paramecium* occasionally backed up at a pressure of over 200 atm (Fig. 6). Abrupt decompression of 40 atm elicited a reversal response, even at 200 atm, and Triton-extracted *Paramecium* swam backwards under pressure when reactivated in 50 μmol l^{-1} $CaCl_2$ (Otter & Salmon, 1979). Similarly, Kitching (1969) reported that *Spirostomum* backed up in response to strong electrical, ionic or mechanical stimulation at 300 atm, a pressure sufficient to block all spontaneous

reversals. These observations suggest that cilia are able to reverse their direction of beating at a pressure that inhibits normal avoiding reactions.

Pressure effects on swimming speed

Pressurization altered the movement of cilia so that they were not effective for swimming. At a pressure of 68 atm or more, *Paramecium* slowed down or stopped, even though their cilia continued to beat rapidly (Figs 2,4,5). Pressure-induced reorientation of the direction of ciliary beat appears to be a major cause of slowing (Fig. 7). The laterally-directed position of cilia under pressure resembles 'ciliary inactivation' described by Machemer (1974).

A direct causal link between resting membrane potential (V_{rest}) and swimming speed has not been established, but slight membrane depolarization correlates strongly with a counterclockwise shift in beat direction (Machemer, 1974), anteriorly-directed reorientation of non-beating cilia (de Peyer & Machemer, 1983) and slowed forward swimming (Van Houten, 1979). Increased potassium concentrations depolarize V_{rest} (Machemer & Ogura, 1979) and slow swimming *Paramecium* at atmospheric pressure (Fig. 4; Table 2). Of the ions that we tested, only potassium acted synergistically with pressurization (Fig. 5). Thus, pressure-induced slowing of *Paramecium* closely resembles their response to small membrane depolarization (approx. 10 mV). Direct electrophysiological measurements on pressurized *Paramecium* would clarify whether V_{rest} changes under pressure.

Molecular basis of pressure action

In general, pressure effects on cellular processes can be understood qualitatively in terms of the Le Chatelier Principle. If a process is inhibited by pressure, then it proceeds with an increase in molecular volume; pressurization favours the low-volume state (Johnson, Eyring & Polissar, 1954). When ionizable groups are present, application of hydrostatic pressure favours dissociation of the ions, due to a volume decrease that accompanies 'electroconstriction' of water molecules around the dissociated species (Distèche, 1972). In *Paramecium*, for example, pressure-induced dissociation of ions bound to specific lipids near the mouth of the Ca^{2+}-channel might prevent channel 'gating' (Conti *et al.* 1982; Saimi & Kung, 1982).

Neither the molecular events of Ca^{2+}-channel opening nor the mechanism for transducing the Ca^{2+} influx into a change in ciliary beat direction is well-understood, so we cannot easily identify a particular biological reaction as pressure-sensitive. However, closing *Paramecium*'s Ca^{2+}-channels, reorientating the direction of ciliary beating and slowing of forward swimming are all Ca^{2+}-dependent processes: the calcium channels inactivate when intracellular Ca^{2+} rises, and an increase from 0·1 μmol l^{-1} Ca^{2+} to 1 μmol l^{-1} Ca^{2+} at the ciliary axoneme slows forward swimming (Brehm & Eckert, 1978; Mogami & Takahashi, 1982; Naitoh & Kaneko, 1972). Thus, the pressure-induced changes that we observed in *Paramecium*'s behaviour could be explained by alterations that resulted from a small rise in intracellular Ca^{2+} when the cells were pressurized. Since 'pawn' mutants slowed down under pressure and Ca^{2+} entry through the voltage-sensitive Ca^{2+}-channels is genetically blocked in 'pawn' mutants, it is unlikely that an inward leak of extracellular calcium through these channels causes pressure-induced slowing (Otter & Salmon, 1979;

Satow & Kung, 1980). Alternatively, pressurization may cause cellular Ca^{2+} receptor proteins to assume a 'Ca^{2+}-bound' configuration and thus produce a physiological state that mimics a rise in intracellular Ca^{2+}.

Our results are particularly striking because relatively low pressures produce drastic changes in *Paramecium*'s sensory-motor function. Calcium concentrations in cells are normally carefully regulated to low levels, and a small increment in cell calcium can markedly alter its physiology (Rose & Lowenstein, 1975; Baker, 1976). Further studies on excitable cells such as *Paramecium* may help to clarify whether cellular regulation of calcium ions is an important target of low levels of hydrostatic pressure.

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