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LONG-TERM ADAPTATION OF Ca^{2+}-DEPENDENT BEHAVIOUR IN *PARAMECIUM TETRAURELIA*

ROBIN R. PRESTON* AND JOCELYN A. HAMMOND

Department of Physiology, M.C.P. Hahnemann School of Medicine, Allegheny University of the Health Sciences, 2900 Queen Lane, Philadelphia, PA 19129, USA

*e-mail: prestoR@wpo.auhs.edu

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Summary

Prolonged exposure to KCl has long been recognized to modify swimming behaviour in *Paramecium tetraurelia*, a phenomenon known as 'adaptation'. In this study, we have investigated behavioural adaptation systematically. A 24 h exposure to 30 mmol l^{-1} KCl deprived cells of the ability to respond behaviourally to two established chemoeffectors. We also explored the effects of 30 mmol l^{-1} KCl on the duration of backward swimming induced by Ba^{2+} and Mg^{2+}. A brief (60 min) exposure prevented cells from swimming backwards in response to either cation, but recovery was rapid (<60 min) following a return to control medium. Prolonged (48 h) exposure caused a more persistent loss of response to Ba^{2+}, so that several hours was now required for recovery. Surprisingly, responses to Mg^{2+} reappeared during 6–8 h in KCl, with backward swimming durations increasing to more than 300 % of control values after 26 h. Thus, we can distinguish two phases to adaptation. The short-term phase is characterized by an inability to respond behaviourally to most stimuli and might be adequately explained in terms of Ca^{2+} channel inactivation and K^+-induced shifts in membrane potential. The long-term phase is characterized by enhanced responses to Mg^{2+} (and also to Na^+), suggesting that a more extensive reprogramming of membrane excitability may occur during chronic K^+-induced depolarization.

Key words: *Paramecium tetraurelia*, adaptation, behaviour, Ca^{2+} current, Ba^{2+} response, Mg^{2+} response.

Introduction

The phenomenon of adaptation in *Paramecium caudatum* was first described by Dryl (1952, 1959) more than four decades ago. *Paramecium* is a motile, freshwater protozoan that responds behaviourally to a variety of environmental stimuli, including chemicals (for a review, see Van Houten, 1992), heat (Nakaoka and Oosawa, 1977; Tominaga and Naitoh, 1992), touch (Eckert *et al.* 1972), gravity (see Machemer *et al.* 1991) and light (Iwatsuki and Naitoh, 1983). Dryl (1959) was curious to know what would happen to the cells' ability to respond to various stimuli if they were maintained for 24 h in high (20–40 mmol l^{-1}) concentrations of salts such as KCl, NaCl, $MgCl_2$ or $CaCl_2$. Interestingly, KCl rendered the cells unresponsive to quinine and ethanol, chemicals that are noxious and strongly repellent under control conditions. The consequences of this sensory deprivation were often lethal.

In the years since Dryl's (1952, 1959) original observations, the mechanisms that control swimming behaviour in *Paramecium* have been dissected (for a review, see Preston and Saimi, 1990) and we have a better understanding of what happens to the cell during prolonged exposure to KCl (for a review, see Machemer, 1989). In *Paramecium*, motility is determined by the activity of some 5000 cilia that cover the cell body. Ciliary activity is, in turn, under the exclusive control of the membrane potential. Encountering a noxious chemical or colliding with an object in the cell's swimming path triggers a depolarizing receptor potential. If the stimulus is sufficiently strong and the receptor potential sufficiently large, it activates a voltage-sensitive Ca^{2+} current (I_{Ca}) associated with the ciliary membrane. The resultant increase in intraciliary Ca^{2+} concentration causes the cilia to reverse their beating direction and the cell swims backwards. Weak stimuli cause a brief Ca^{2+} influx and a turn, whereas strong stimuli can cause backward swimming for tens of seconds or minutes. The membrane potential in *Paramecium*, as in most excitable cells, is very sensitive to extracellular K^+ concentration such that adding KCl extracellularly causes depolarization. This places the cell in a predicament, because as long as the membrane remains depolarized by KCl, it is 'blind' to other (potentially lethal) stimuli. To protect against such sensory deprivation, the cell modulates its 'resting' ion conductances to bring the membrane potential slowly back towards pre-stimulus levels (Oka *et al.* 1986; Machemer-Röhnisch and Machemer, 1989). Resetting membrane excitability in this manner allows the cell to respond to new stimuli, despite the continued presence of KCl, and the cell is said to have 'adapted'. Once adapted, however, the background stimulus must be maintained for normal responsiveness, because returning cells to a non-

adapting medium causes the membrane potential to deepen. This means that the receptor potentials generated by noxious and depolarizing stimuli (such as quinine and ethanol) are insufficient to cross the threshold required for activation of I_{Ca} and the cell fails to swim backwards.

The phenomenon of adaptation has been revisited many times since Dryl's (1952, 1959) original observations (Shusterman *et al.* 1978; Shusterman, 1981; Hansma, 1981; Thomson *et al.* 1981; Oka *et al.* 1986; Oka and Nakaoka, 1989) yet the underlying molecular mechanisms remain unknown. In fact, while the concept of adaptation is simple and easy to grasp, it is mechanistically far from trivial. What are the pathways that modulate 'resting' ion conductances during sustained depolarization? Are other conductances affected? Similar questions have yet to be answered with respect to the excitable cells of higher organisms. Here, we begin studies of the mechanisms of membrane adaptation in *Paramecium tetraurelia* with a systematic investigation of its basic properties.

Materials and methods

Cell stocks and culture conditions

The present studies were performed using *Paramecium tetraurelia,* stock 51 (sensitive). Cells were cultured either in wheat-grass medium (Sonneborn, 1970) or in 'C7', a semi-defined, artificial medium similar to that devised originally by Y. Saimi (University of Wisconsin-Madison; Preston *et al.* 1990). C7 contained 3 mmol l^{-1} NaOH, 0.5 mmol l^{-1} K_2HPO_4, 0.5 mmol l^{-1} $CaCl_2$, 0.2 mmol l^{-1} $MgSO_4$, 0.2 g l^{-1} glucose, 0.1 g l^{-1} ammonium acetate, 0.5 mg l^{-1} stigmasterol, 5 mmol l^{-1} Hepes and 7.5 mg l^{-1} Phenol Red. Both types of medium were inoculated with *Enterobacter aerogenes* (to provide a source of food) 16–24 h before adding *Paramecium*.

Solutions

Unless stated otherwise, all solutions contained 0.75 mmol l^{-1} $CaCl_2$, 0.25 mmol l^{-1} $Ca(OH)_2$, 0.01 mmol l^{-1} EDTA, 1 mmol l^{-1} Hepes buffer, pH 7.2. Other ions were added to this solution as required and at the concentrations stated. Mg^{2+} responses were assessed in 'Mg^{2+} solution', containing 5 mmol l^{-1} $MgCl_2$ and 10 mmol l^{-1} tetraethylammonium (TEA^+) chloride; Na^+ responses were determined in 'Na^+ solution', containing 5 mmol l^{-1} NaCl and 5 mmol l^{-1} TEA^+, or in 20 mmol l^{-1} NaCl (no TEA^+); Ba^{2+} responses were determined in 'Ba^{2+} solution', containing 8 mmol l^{-1} $BaCl_2$. 'K^+ solution' contained 30 mmol l^{-1} KCl, whereas 'control solution' contained 1 mmol l^{-1} KCl. To determine the effects of prolonged exposure to KCl in the absence of nutrients, *Paramecium* were maintained in a saline solution of similar ionic composition to C7. 'C7 saline' contained 3 mmol l^{-1} NaCl, 1 mmol l^{-1} KCl, 0.4 mmol l^{-1} $CaCl_2$ and 0.2 mmol l^{-1} $MgCl_2$.

Adaptation and de-adaptation

When studying the time course of the effects of prolonged exposure to KCl on behaviour, cells were maintained in C7 ('control medium') or C7 + 30 mmol l^{-1} KCl ('adapting medium') at room temperature. The time course of de-adaptation was also determined at room temperature (22–25 °C). When examining the effects of a series of KCl concentrations on behaviour, cells were adapted for 24 h at 28 °C (cell generation time at 28 °C was 5.3±0.3 h, mean ± S.D., N=3, compared with 7.9 h at room temperature; see Results). When comparing the effects of KCl exposure with the effects of exposure to other ions, cells were maintained in C7 for at least a week. When comparing the effects of KCl with the effects of stimuli that can be metabolized by bacteria (choline, glutamate, sorbitol), cells were twice concentrated by centrifugation, resuspended in C7 saline and then exposed to adaptation stimuli for 24 h at 28 °C.

Behavioural assays

Responses to organic chemoeffectors were quantified using a T-maze assay (Van Houten *et al.* 1982). Cells were washed and concentrated by centrifugation to a final density of approximately 500 cells ml^{-1} in 5 mmol l^{-1} KCl and placed in the centre arm of a modified three-way stopcock. The control arm of the maze and the stopcock bore contained 5 mmol l^{-1} KCl, while the test arm contained 5 mmol l^{-1} potassium acetate or 0.1 mmol l^{-1} quinidine (+5 mmol l^{-1} KCl). When responses were assayed under depolarizing conditions, all three arms of the maze and the tap bore contained an additional 25 mmol l^{-1} KCl. The maze was opened for 5 min, the tap was then closed and the number of cells in each arm was counted under low-power magnification. Indices of chemokinesis (In_{che}) were determined from the number of cells in the test arm divided by the total number of cells in the test and control arms. In_{che} values below 0.5 indicate repulsion from the test solution, values greater than 0.5 indicate attraction. Responses to GTP were determined in a similar manner, but all three arms of the maze contained 5 mmol l^{-1} NaCl and 1 mmol l^{-1} $MgCl_2$ in place of KCl.

Responses to inorganic ions (Mg^{2+}, Na^+, Ba^{2+}, K^+) were determined by transferring individual cells briefly (<20 s) to control solution and then forcing them from a micropipette into a test solution. The cells' responses were noted under low-power magnification, and the duration of backward swimming was recorded with a stopwatch.

The dependence of adaptation on protein synthesis was assessed using G-418 (Geneticin; Gibco BRL). The inhibitor was added to the cells at a concentration of 40 μg ml^{-1}, 90 min prior to KCl exposure. Previous studies have shown 20 μg ml^{-1} G-418 to be more than 95 % effective in preventing the incorporation of labelled amino acids into *Paramecium* (Haga *et al.* 1984).

Paramecium tetraurelia showed considerable variability in the duration of responses to ionic test solutions from day to day and from cell culture to cell culture. This variability was normal and common and did not prevent identification of statistically significant differences between the responses of control and test populations. To help emphasise changes in the

cells' responses to test solutions following exposure to adaptation stimuli, backward-swimming durations were normalized to control values. Tests were typically conducted on five cells and were repeated on four different occasions over the course of several weeks.

All data are presented as means ± S.D., with significance of differences between means being determined using a Student's *t*-test.

Results

Effects of adaptation on chemoresponses

Dryl's (1959) original studies of adaptation in *Paramecium caudatum* focused on chemoresponses, so we began our investigation of similar phenomena in *P. tetraurelia* by examining the effects of KCl on responses to an established attractant (acetate) and a repellent (quinidine; Van Houten, 1978). Under control (non-adapting) conditions, *P. tetraurelia* were attracted to 5 $mmol\,l^{-1}$ potassium acetate with an index of chemotaxis (In_{che}) of 0.69±0.03 (N=6). After 24 h of growth in wheat-grass medium supplemented with 30 $mmol\,l^{-1}$ KCl, acetate was no longer an attractant (In_{che}=0.51±0.11, N=6). Similarly, 0.1 $mmol\,l^{-1}$ quinidine was strongly repellent to *P. tetraurelia* under control conditions (In_{che}=0.32±0.09, N=6) but not following adaptation to 30 $mmol\,l^{-1}$ KCl (In_{che}=0.51±0.07, N=6). Responses to both chemoeffectors depend on the cells being able to turn at the border between the test and control solutions (Jennings, 1906). Turning is dependent, in part, on resting membrane potential. Previous work on *P. caudatum* suggested that prolonged exposure to a depolarizing stimulus such as KCl causes a compensatory shift in membrane potential that might account for the loss of chemosensitivity (see Discussion). Thus, we tested the ability of cells to respond to acetate and quinidine when the depolarizing stimulus (30 $mmol\,l^{-1}$ KCl) was maintained during the assay. *P. tetraurelia* were now attracted to acetate (In_{che}=0.59±0.09, N=6) and repelled by quinidine (In_{che}=0.40±0.09, N=6), although the acetate response was significantly weaker than under non-adapting, control conditions (see above).

We also examined the effects of adaptation on responses to 0.1 $\mu mol\,l^{-1}$ GTP. GTP is also repellent (Clark *et al.* 1993), but the underlying mechanisms are different from those involved in responses to quinidine (see Discussion). A 24 h exposure to 30 $mmol\,l^{-1}$ KCl had no effect on repulsion from GTP (In_{che}=0.28±0.07, N=6, in control cells and 0.28± 0.09, N=7, following adaptation).

Effects of adaptation on responses to inorganic ions

We next examined the effects of chronic exposure to KCl on the responses to various ions. These responses are used routinely to test for the presence and normal functioning of several key ion conductances in *P. tetraurelia*.

Ba^{2+} solution caused cells to swim backwards for 23±3 s (N=20) under control conditions. The Ba^{2+} response is a direct reflection of the duration of Ca^{2+} entry into the cilium

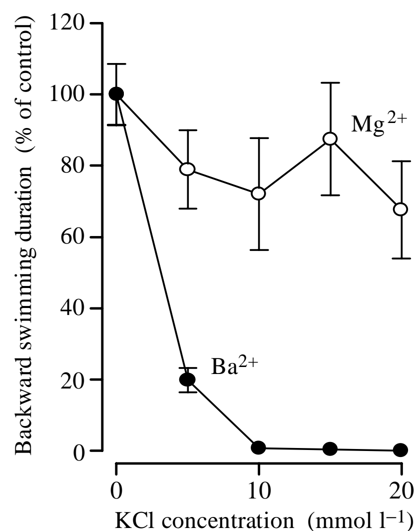

Fig. 1. Effects of prolonged exposure to KCl on cell behaviour. Cells were maintained in wheat-grass medium with increasing concentrations of KCl for 24 h at 28 °C and then tested for the duration of backward swimming in Ba^{2+} solution (filled circles) and Mg^{2+} solution (open circles). Points are mean (±S.D.) responses from 20 cells, and durations are given as a percentage of control durations.

via a voltage-sensitive current, I_{Ca}. Prolonged (24 h) exposure to 5 $mmol\,l^{-1}$ KCl significantly reduced the duration of this response (Fig. 1, filled circles), while cells that had been maintained in 10 $mmol\,l^{-1}$ K^{+} or above failed to respond.

P. tetraurelia also swim backwards in Mg^{2+} solution, but this behaviour depends on Ca^{2+} entry *via* the ciliary I_{Ca} and Mg^{2+} entry *via* a Mg^{2+}-specific current, I_{Mg}. Under control conditions, *P. tetraurelia* swam backwards for 6±1 s (N=20) in Mg^{2+} solution. Cells that had been maintained for 24 h in medium supplemented with 5–20 $mmol\,l^{-1}$ KCl also swam backwards in Mg^{2+} solution for times that were not significantly different from those of controls (Fig. 1, open circles).

Membrane adaptation under controlled conditions

The wheat-grass medium used to raise cells for the experiments described above was based on an infusion of a natural product and its composition was largely unknown. This made it difficult to control in any systematic fashion. Thus, we next investigated the progress of adaptation in a synthetic medium with a known and readily manipulated ionic composition. An additional advantage of growing cells in this medium is that they are far less susceptible to the deleterious effects of high K^{+} concentrations compared with cells grown in wheat-grass medium. This allowed us to examine the effects of strong and saturating adaptation stimuli.

Fig. 2 shows the effects of a 24 h exposure to a range of KCl concentrations (0–40 $mmol\,l^{-1}$) on responses to Ba^{2+} solution and Mg^{2+} solution. As seen previously (Fig. 1), the duration of backward swimming in Ba^{2+} solution was reduced

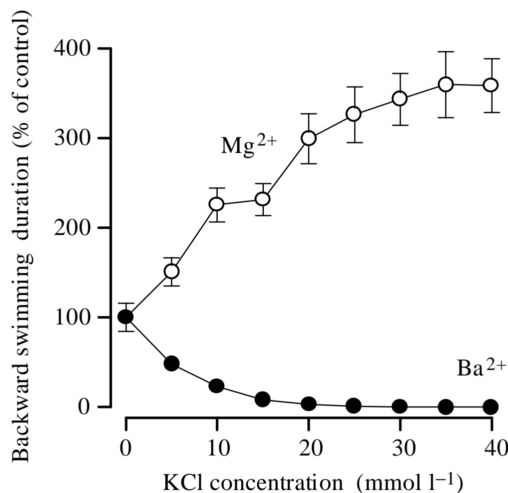

Fig. 2. Effects of prolonged exposure to KCl in a semi-defined growth medium. Cells were maintained in C7 medium with increasing concentrations of KCl for 24 h at 28 °C and then tested for the duration of backward swimming in Ba^{2+} solution (filled circles) or Mg^{2+} solution (open circles). Points are mean (±S.D.) responses from 16 cells, and durations are given as a percentage of control durations.

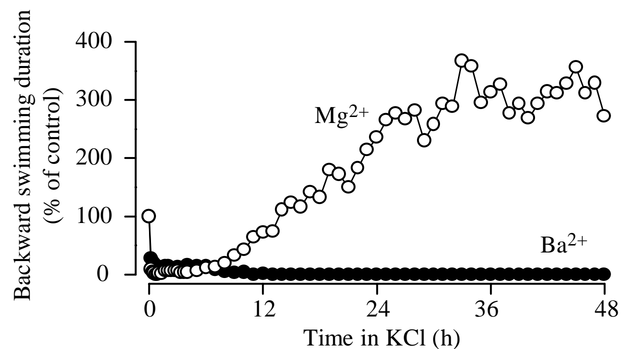

Fig. 3. Time course of the effects of KCl on cell behaviour. Cells were transferred to a C7 growth medium supplemented with 30 mmol l^{-1} KCl at time zero and then tested at regular intervals for the duration of backward swimming in Ba^{2+} solution (filled circles) or Mg^{2+} solution (open circles). Cell cultures were maintained at room temperature. Points represent mean responses from 8–40 cells. Error bars have been omitted for clarity: variance was similar to that shown in Figs 1 and 2. Durations are given as a percentage of control durations.

significantly by prolonged exposure to 5 mmol l^{-1} KCl and was suppressed fully by 20 mmol l^{-1} and above (Fig. 2, filled circles). In contrast, backward swimming in Mg^{2+} solution was significantly enhanced by KCl, with response durations increasing by more than 300 % at 20–40 mmol l^{-1} KCl (Fig. 2, open circles).

Time course of adaptation

To explore the time course of adaptation, cells were transferred to growth medium containing 30 mmol l^{-1} KCl and then examined at frequent intervals over a period of 48 h for responses to Ba^{2+} and Mg^{2+} (Fig. 3). The duration of backward swimming in Ba^{2+} solution decreased to less than 30 % of control values within seconds of transferring the cells to KCl-supplemented medium (Fig. 3, filled circles) and then declined further in the ensuing 8 h until it was no longer possible to elicit a response. Backward swimming in Ba^{2+} solution was not regained in the continued presence of KCl.

Responses to Mg^{2+} showed a similarly rapid decline during the initial seconds of exposure to KCl and by 30 min had been suppressed fully (Fig. 3, open circles). After 6–8 h, however, the cells regained the ability to swim backwards in Mg^{2+} solution and response durations increased slowly towards and above control values. The duration of backward swimming eventually reached a plateau of approximately 300 % (approximately 22 s) at 26 h. No further increases in sensitivity were observed between 26 and 48 h.

Thus, there appeared to be two phases to adaptation as manifested in the duration of backward swimming in Mg^{2+}: an initial, rapid loss of responsiveness, followed by (or coincident with) a slower enhancement of swimming duration.

Time course of de-adaptation

The initial phase of adaptation that is manifest as a loss of backward swimming in both Ba^{2+} and Mg^{2+} solution is sufficiently rapid that it might be explained in terms of Ca^{2+} current inactivation and activation of mechanisms that compensate for the K^{+}-induced depolarization (see Discussion). The time required for the reappearance and ultimate enhancement of the Mg^{2+} response represented more than three cell generations, however (generation time in 30 mmol l^{-1} KCl is 7.9±0.7 h, N=3, at room temperature), suggesting that cell growth and protein synthesis might be required. If so, the time course of de-adaptation upon removal of KCl might also be expected to show two distinct phases that correspond to an initial, rapid recovery from channel inactivation and a slower degradation or replacement of the newly synthesized adaptation factors.

To determine the time course of de-adaptation, cells were exposed to 30 mmol l^{-1} KCl for 1, 2, 4, 8, 12, 16 or 48 h and then returned to control growth medium. The duration of backward swimming in Ba^{2+} solution or Mg^{2+} solution was determined immediately before removal from KCl and at regular intervals thereafter (Fig. 4).

Exposing cells to 30 mmol l^{-1} KCl for 1 h caused Ba^{2+}-induced backward swimming durations to decrease to approximately 35 % of control values. These cells recovered rapidly when returned to control medium, with response times approaching 80 % of control values during the initial 20 min (Fig. 4A, filled circles). Cells that had been exposed to KCl for longer periods required correspondingly longer to recover. A 16 h exposure suppressed backward swimming in Ba^{2+} solution completely (Fig. 4A, open squares), and this inhibition persisted for approximately 1 h after a return to control medium. Once recovery began, however, its time course approximated that observed following shorter-term

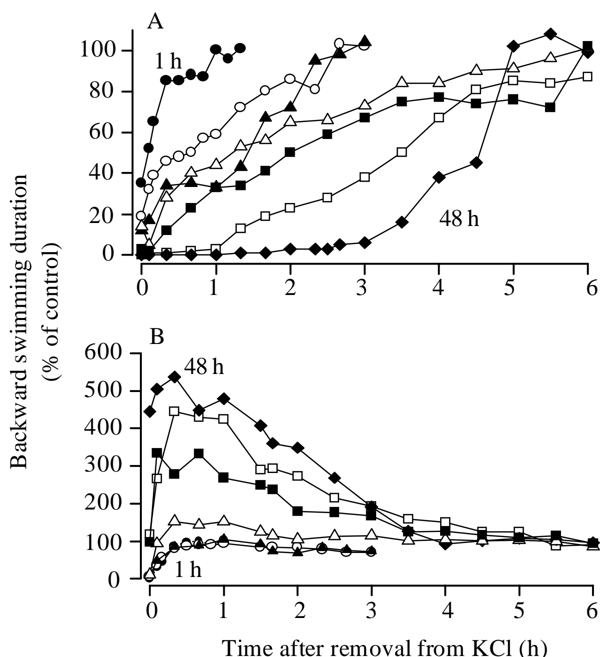

Fig. 4. Time course of recovery of normal behaviour following removal from KCl. Cells were exposed to 30 mmol l^{-1} KCl for 1 h (filled circles), 2 h (open circles), 4 h (filled triangles), 8 h (open triangles), 12 h (filled squares), 16 h (open squares) or 48 h (diamonds) and then washed into control growth medium. Cells were tested at regular intervals thereafter for the duration of backward swimming in (A) Ba^{2+} solution or (B) Mg^{2+} solution. Points represent means from 16–24 cells. Error bars have been omitted for clarity: variance was similar to that shown in Figs 1 and 2. Durations are given as a percentage of control durations.

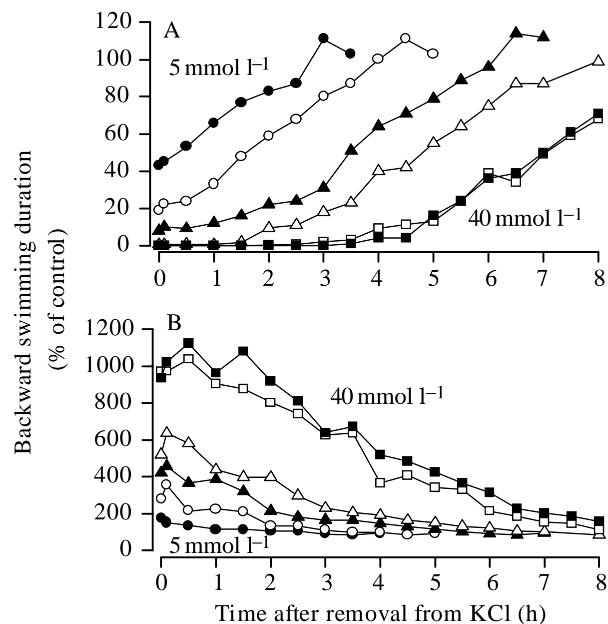

Fig. 5. Recovery of normal behaviour following adaptation to various KCl concentrations. Cells were maintained for approximately 1 month in growth medium with various concentrations of KCl. At time zero, cells were transferred to control growth medium, and the duration of backward swimming in (A) Ba^{2+} solution or (B) Mg^{2+} solution was tested at regular intervals thereafter. Concentrations of KCl used to adapt cells were 5 mmol l^{-1} (filled circles), 10 mmol l^{-1} (open circles), 15 mmol l^{-1} (filled triangles), 20 mmol l^{-1} (open triangles), 30 mmol l^{-1} (filled squares) or 40 mmol l^{-1} (open squares). Points are mean responses of 16 cells. Error bars have been omitted for clarity: variance was similar to that shown in Figs 1 and 2. Durations are given as a percentage of control durations.

exposure. Cells that had been adapted for 48 h required more than 2 h in normal medium before restoration of backward swimming in Ba^{2+} solution could begin. Fig. 4B shows the time course of de-adaptation as reflected in responses to Mg^{2+}. Exposing cells to KCl for 1, 2 or 4 h suppressed backward swimming in Mg^{2+} solution almost fully (Fig. 3), but recovery was complete within approximately 30 min after removal from KCl (Fig. 4B, circles, triangles). Cells that had been incubated with KCl for 8 h or more showed a biphasic recovery. Returning these cells to control medium caused a rapid increase in the backward-swimming duration during the initial 30 min, followed by a gradual decline towards control values during the subsequent 4 h (Fig. 4B, squares, diamonds).

We also examined recovery time courses following adaptation to various concentrations of KCl. Cells were exposed to KCl for at least a month to ensure that the changes that occur during adaptation were complete (Fig. 5). Exposure to 5 mmol l^{-1} KCl reduced backward swimming times in Ba^{2+} solution by approximately 60 % (Fig. 5A, filled circles), but recovery following a return to control medium was complete within approximately 3 h. 10 mmol l^{-1} KCl further suppressed the Ba^{2+} response, but recovery was again rapid once the cells had been removed from KCl. At 15 mmol l^{-1} KCl or more, recovery was delayed following a return to normal medium (for up to 3 h at 30–40 mmol l^{-1} KCl; Fig. 5A, squares), but proceeded with a similar time course once backward swimming in Ba^{2+} had resumed.

Prolonged exposure to KCl enhanced backward-swimming times in Mg^{2+} solution (Fig. 5B), as seen previously. Once removed from KCl, however, recovery of the Mg^{2+} response proceeded with a similar time course regardless of the strength of the adaptation stimulus.

There are several points of interest in these data. First, cells that had been exposed to KCl for short periods recovered responsiveness to both Ba^{2+} and Mg^{2+} rapidly, whereas recovery from prolonged KCl exposure required several hours (Fig. 4). Thus, we can distinguish two phases to adaptation on the basis of onset and offset rates: a short-term phase (complete within 4–6 h) and a long-term phase (requiring 24–48 h). A second point of interest is the rapid increase in the duration of the Mg^{2+} swimming response over the first 30 min following removal from KCl (e.g. Fig. 4B, squares; Fig. 5B, squares). We might interpret these observations as indicating that cells recovered rapidly from the effects of short-term adaptation (i.e.

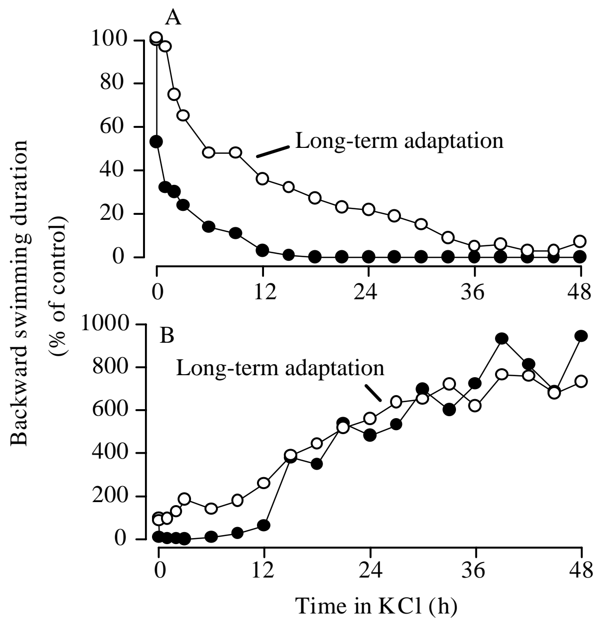

Fig. 6. Time course of long-term adaptation. Cells were transferred to growth medium supplemented with 30 mmol l^{-1} KCl at time zero. At regular intervals thereafter, cell samples were removed and split into two groups. The first was tested immediately (filled circles) for the duration of backward swimming in (A) Ba^{2+} solution or (B) Mg^{2+} solution, the second was incubated in control medium for 30 min and then tested behaviourally (open circles). Note that control values for both Ba^{2+} and Mg^{2+} responses change during a 30 min incubation in control medium so that the two curves in B appear to superimpose. Points are means from 12–28 cells. Error bars have been omitted for clarity: variance was similar to that shown in Figs 1 and 2. Durations are given as a percentage of control durations.

from KCl-induced suppression of backward swimming in Mg^{2+} solution) to reveal the full consequences of long-term adaptation (enhanced backward swimming).

Time course of long-term adaptation

We re-examined the effects of 30 mmol l^{-1} KCl on behaviour in Ba^{2+} and Mg^{2+} over time but, in addition to testing cells immediately after removal from KCl, we also tested their responses 30 min after returning them to the control medium. This 30 min respite was to allow the cells to recover from short-term adaptation so that their behaviour would reveal the progress of long-term adaptation alone. The results are shown in Fig. 6. As found previously (Fig. 3), exposure to KCl caused a rapid decrease in the duration of backward swimming in Ba^{2+} solution, with complete inhibition being observed by approximately 13 h (Fig. 6A, filled circles). This response reflected the net effect of combined short-term and long-term adaptation mechanisms. Stripping out the contribution of short-term adaptation from the total revealed that long-term adaptation also reduced backward swimming duration in Ba^{2+} solution, but that the effects were more gradual than short-term adaptation, requiring approximately 36 h to reach a maximum (Fig. 6A, open circles). The combined effects of short-term and long-term adaptation on responses to Mg^{2+} are shown in Fig. 6B (filled circles). After removing the inhibition of backward swimming caused by short-term adaptation, long-term enhancement of the Mg^{2+} response was evident after approximately 6 h in KCl and required between 24 and 36 h for maximal effect (Fig. 6B, open circles).

Dependence of long-term adaptation on KCl concentration

We used a similar strategy to examine the dependence of long-term adaptation on KCl concentration, recognizing that allowing time for recovery from short-term adaptation may cause us to underestimate long-term effects at low KCl concentrations ($\leqslant$10 mmol l^{-1}: see Fig. 5B). Cells were exposed to 0–40 mmol l^{-1} KCl and then tested for the duration of backward swimming in Ba^{2+} and Mg^{2+} solutions and also in Na^{+} and K^{+} solutions. Cells were tested both immediately after removal from adaptation medium and also 30 min after they had been returned to the control medium (Fig. 7). KCl exposure suppressed backward swimming in Ba^{2+} solution in a concentration-dependent manner (Fig. 7A, circles). KCl also suppressed backward swimming in K^{+} solution (Fig. 7A, triangles). K^{+} solution is traditionally used to collapse membrane potential and to examine the effects of I_{Ca} activation in the absence of a repolarizing K^{+} efflux. A 30 min return to control medium allowed the cells to regain partial sensitivity to both Ba^{2+} and K^{+} solutions (Fig. 7C). KCl exposure enhanced the duration of backward swimming in Mg^{2+} solution (Fig. 7B, circles), with maximal effects being observed at 30 mmol l^{-1}. The percentage increase in backward-swimming times in Mg^{2+} solution was greater than that observed in Fig. 2, but the dependence on KCl concentration was similar. Fig. 7B (squares) shows that responses in Na^{+} solution were also enhanced by KCl with a similar concentration-dependence to that seen for Mg^{2+} responses. Whereas the Mg^{2+} responses reflect activation of I_{Ca} and the Ca^{2+}-dependent I_{Mg}, the responses to Na^{+} solution reflect activation of I_{Ca} and a Ca^{2+}-dependent I_{Na}. Fig. 7D shows that the responses to both solutions were still enhanced following a 30 min respite in control medium.

Effects of KCl on cells under non-growth conditions

The protracted time course of long-term adaptation suggested that cell growth might be required. If so, cells that have been maintained in KCl in the absence of nutrients should exhibit behavioural changes that reflect short-term adaptation alone. To test this hypothesis, cells were washed into a non-nutrient saline solution of similar ionic composition to culture medium (see Materials and methods). They were then exposed to KCl for 24 h and tested for the duration of backward swimming in Ba^{2+} and Mg^{2+} solution. The results are shown in Fig. 8. Ba^{2+} responses were suppressed by KCl at concentrations of 10 mmol l^{-1} and above (Fig. 8A, filled circles). A 30 min incubation in control solution did not relieve this inhibition fully, however, suggesting that the cells had indeed undergone long-term adaptation (Fig. 8A, open circles). The effects of nutrient deprivation on backward swimming in

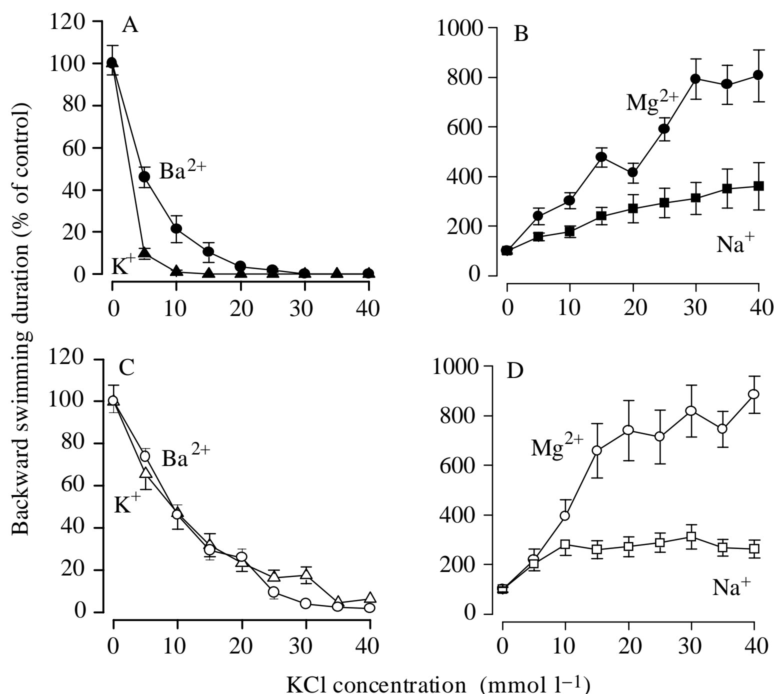

Fig. 7. Effects of KCl exposure on behavioural responses to various test solutions. Cells were exposed to 0–40 mmol l^{-1} KCl for 24 h at 28 °C and then tested for the duration of backward swimming in (A) Ba^{2+} solution (circles) and K^+ solution (triangles) or (B) Mg^{2+} solution (circles) and Na^+ solution (squares). (C) Responses to Ba^{2+} (circles) and K^+ (triangles) solution after a 30 min incubation in control medium. (D) Responses to Mg^{2+} (circles) and Na^+ (squares) solution after a subsequent 30 min in control medium. Points are means (±S.D.) from 16 cells. Durations are given as a percentage of control durations.

response to Mg^{2+} solution are shown in Fig. 8B. While KCl did not appear to have any effect on backward-swimming times in cells taken directly from adapting saline, a 30 min return to control solution produced a substantial increase in backward swimming duration that was characteristic of the effects of long-term adaptation seen previously (Fig. 7D). Similar results were obtained with cells that had been maintained in a nutrient medium until they had exhausted their food supply before adding KCl (results not shown).

While *P. tetraurelia* appeared to be capable of adapting to KCl in the absence of growth, the experiments described above do not address a need for protein synthesis. To examine this directly, cells were exposed to KCl in the presence of G-418. G-418 is used routinely to block protein synthesis in *Paramecium* sp. (Haga *et al.* 1984; Haynes *et al.* 1995), but it failed to prevent the inhibition of backward swimming in Ba^{2+} following a 24 h exposure to 30 mmol l^{-1} KCl (Table 1). It also failed to prevent an increase in the long-term Mg^{2+} response (Table 1), suggesting that protein synthesis was not required for either short-term or long-term adaptation.

Adaptation to other ions

A priori, behavioural adaptation could represent a cellular response to KCl as an osmotic agent or to the charge carried by K^+ and Cl^-. To investigate these possibilities, cells were exposed to sorbitol or various ions in place of KCl and then tested in Ba^{2+}, K^+, Mg^{2+} and Na^+ solutions. Behaviour was assessed immediately upon taking cells from adaptation medium and after a 30 min respite in control medium (Table 1). Prolonged (24 h) exposure to 60 mmol l^{-1} sorbitol decreased backward swimming durations in most of our test solutions compared with control values (Table 1). A 30 min

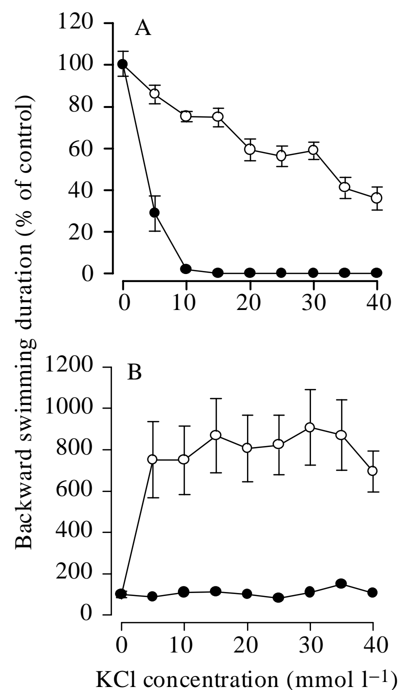

Fig. 8. Behavioural adaptation in a non-nutrient saline solution (see Materials and methods). Samples of cells were exposed to 0–40 mmol l^{-1} KCl in a non-nutrient saline solution for 24 h and then tested behaviourally in (A) Ba^{2+} solution or (B) Mg^{2+} solution. Filled symbols show responses of cells immediately upon removal from KCl-supplemented medium, open symbols show responses following a 30 min incubation in control solution. Points are mean responses (±S.D.) from 16 cells. Durations are given as a percentage of control durations.

Table 1. *Specificity of behavioural adaptation of* Paramecium tetraurelia

Group	Adaptation stimulus	Concentration (mmol l^{-1})	Test solution			
			Ba^{2+}	K^+	Mg^{2+}	Na^+
Immediate responses						
(i)	Control	0	100±5		100±16	
	G-418	0	59±11*		178±26	
	KCl	30	0±0*		44±14*	
	G-418+KCl	30	0±0*		45±12*	
(ii)	Control	0	100±5	100±4	100±24	100±34
	KCl	30	0±5*	0±0*	104±48	5±2*
	Sorbitol	60	83±5*	73±5*	49±25	36±17*
	Potassium glutamate	30	0±0*	0±0*	4±4*	0±0*
	Choline chloride	30	0±0*	0±0*	0±0*	0±0*
(iii)	Control	0	100±7	100±6	100±6	100±7
	KCl	20	0±0*	0±0*	528±71*	482±89*
	LiCl	20	0±0*	53±6*	53±13*	40±10*
(iv)	Control	0	100±7	100±4	100±6	100±12
	KCl	30	0±0*	0±0*	769±67*	1029±143*
	NaCl	30	2±2*	94±9	155±27*	321±71*
	$MgCl_2$	15	21±3*	31±8*	145±31	393±66*
	$SrCl_2$	15	0±0*	0±0*	0±0*	0±0*
	$CaCl_2$	15	195±25*	167±17*	247±44*	342±78*
Responses after 30 min in control saline solution						
(i)	Control	0	100±3		100±13	
	G-418	0	62±10*		122±22	
	KCl	30	60±7*		340±25*	
	G-418+KCl	30	68±7*		337±27*	
(ii)	Control	0	100±5	100±3	100±16	100±11
	KCl	30	59±4*	36±3*	290±24*	185±25*
	Sorbitol	60	75±5*	83±6	135±20	73±10
	Potassium glutamate	30	42±5*	41±6*	278±26*	157±20*
	Choline chloride	30	0±0*	35±15*	0±0*	7±5*
(iii)	Control	0	100±3	100±5	100±11	100±7
	KCl	20	25±2*	49±4*	315±39*	131±12
	LiCl	20	98±9	59±6*	110±17	170±23
(iv)	Control	0	100±8	100±4	100±12	100±8
	KCl	30	27±4*	37±3*	701±93*	475±142*
	NaCl	30	122±12	110±6	177±37	123±40
	$MgCl_2$	15	67±4*	57±3*	81±12	100±31
	$SrCl_2$	15	92±14	39±6*	51±10*	0±0*
	$CaCl_2$	15	224±15*	123±7*	236±24*	102±16

Responses of cells to Ba^{2+}, K^+, Mg^{2+} or Na^+ solutions tested immediately upon removal from adaptation medium (immediate responses) or after 30 min in control solution.

Four separate groups of cells were used in compiling these data. The first two groups (i and ii) were maintained in C7 saline during a 24 h exposure to the adaptation stimuli listed. The second two groups (iii and iv) were maintained in C7 growth medium with the cations listed for at least 1 week during adaptation.

Values are means ± S.D. for normalized backward swimming durations (as a percentage of the control value) from 20 cells (except for group i, in which only 12 cells were tested). Asterisks indicate that values are significantly different from controls, $P<0.05$.

return to control saline solution did not produce an increase in response durations over controls (Table 1). Comparing the effects of 24 h of exposure to KCl and potassium glutamate (glutamate does not permeate Cl^- channels in *Paramecium* and thus does not produce any sustained change in membrane potential; Preston and Usherwood, 1988) showed that they produced equivalent behavioural changes (Table 1), suggesting that K^+ rather than Cl^- is the effector of both short-

and long-term adaptation. To determine the specificity of the actions of K^+, cells were exposed to RbCl, LiCl and NaCl. RbCl was lethal at 10 mmol l^{-1} or above, so was excluded from the study. LiCl was also lethal at high concentrations (30 mmol l^{-1}), but cells readily survived prolonged exposure at 20 mmol l^{-1}. Cells tested immediately following removal from LiCl showed no response to Ba^{2+}, while responses to K^+, Mg^{2+} and Na^+ were all significantly reduced (Table 1). A 30 min return to LiCl-free medium permitted recovery of responses to all of our test solutions with the exception of K^+ (Table 1). NaCl suppressed backward swimming duration in Ba^{2+} and significantly enhanced backward swimming durations in Mg^{2+} and Na^+ solutions (Table 1). Cells that had been returned to control medium for 30 min exhibited responses that were slightly elevated compared with controls, but these differences were not significant (Table 1). Cells were also exposed to various divalent cations (Table 1). $MgCl_2$, like KCl, suppressed backward swimming in Ba^{2+} and K^+ solution and enhanced responses to Na^+. Interestingly, however, the latter effect did not persist when the cells were returned to control medium for 30 min. $SrCl_2$ suppressed backward swimming in all of our test solutions, but partial recovery of all responses except to Na^+ was evident following a return to control medium. $CaCl_2$ was notable in that it increased backward-swimming times in all test solutions (Table 1), and responses to Ba^{2+}, K^+ and Mg^{2+} remained elevated after a return to control solution (Table 1). We also incubated cells with choline, a non-permeant cation. Choline suppressed responses to all test solutions and this inhibition persisted following a 30 min respite in choline-free saline solution.

Finally, we re-examined the effects of KCl on cell behaviour, but we used 20 mmol l^{-1} Na^+ as a test solution in place of 5 mmol l^{-1} Na^+ and 5 mmol l^{-1} TEA^+. The purpose was to determine whether the behavioural changes reported above persisted when normal membrane recovery mechanisms were not suppressed by K^+ channel inhibitors. KCl enhanced the duration of backward swimming in 20 mmol l^{-1} Na^+ with a concentration-dependence that was similar to that reported above (Fig. 7; data not shown). The time course of onset of these changes followed that described for Mg^{2+} (Fig. 6B). Cells that had been incubated with 30 mmol l^{-1} KCl for 2 h showed an almost complete inability to swim backwards in 20 mmol l^{-1} Na^+ compared with controls (6±4 %, N=20), but a 30 min respite in control solution allowed almost complete recovery of the response (71±7 %, N=20). Long-term adapted cells (more than 48 h in 30 mmol l^{-1} KCl) swam backwards for 1020±75 % (N=20) longer than non-adapted controls, an effect that persisted following a 30 min return to control solution (720±64 %, N=20).

Discussion

We have investigated behavioural adaptation in *P. tetraurelia*, a phenomenon first described by Dryl (1952, 1959). Adaptation was manifest as changes in the duration of backward swimming stimulated by various ions during prolonged exposure to KCl and occurred in two phases. During an initial, short-term phase (0–4 h), the cells rapidly (<30 min) lost the ability to swim backwards in both Ba^{2+} and Mg^{2+} solutions. Long-term adaptation was manifest as a more persistent loss of response in Ba^{2+} solution and a gradual return and ultimate enhancement of backward swimming duration in Mg^{2+} solution over a period of 24–36 h. The two phases were also evident in the time course of recovery: short-term-adapted cells recovered fully in less than an hour, whereas long-term adaptation was accompanied by behavioural changes that persisted for 5–6 h. While the slow time course of long-term adaptation might suggest that cell growth is required, the behavioural changes proceeded in the absence of nutrients and in the presence of a protein synthesis inhibitor.

Electrophysiological basis for behaviour in P. tetraurelia

Ejecting *P. tetraurelia* from a micropipette into Ba^{2+} or Mg^{2+} test solutions activates voltage-sensitive Ca^{2+} channels in the ciliary membrane and allows Ca^{2+} influx. The rising intraciliary Ca^{2+} concentration reverses the direction of the ciliary power stroke, and the cell swims backwards. Recovery from backward swimming requires inactivation of the ciliary Ca^{2+} current, an event triggered by Ca^{2+} influx itself (Brehm *et al.* 1980). Membrane repolarization is facilitated by K^+ efflux *via* separate voltage-dependent and Ca^{2+}-dependent K^+ conductances (Oertel *et al.* 1977; Brehm *et al.* 1978; Satow, 1978; Saimi *et al.* 1983), while recovery of forward swimming requires that intraciliary Ca^{2+} concentration fall, presumably through sequestration or extrusion. These events are usually transient, but Ba^{2+} permeates the Ca^{2+} channel and interferes with inactivation (Brehm *et al.* 1980). This prolongs Ca^{2+} influx to produce prolonged backward swimming. Ba^{2+} also inhibits K^+ conductances to further prolong the reversal response. Mg^{2+} similary activates the ciliary Ca^{2+} conductance, but the rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) also elicits Mg^{2+} influx *via* a Mg^{2+}-specific conductance (Preston, 1990). Mg^{2+} entry further depolarizes the cell and prolongs ciliary reversal. The Mg^{2+} test solution additionally contains TEA^+ to block K^+ efflux and repolarization, and this further potentiates the response. Thus, while both Ba^{2+} and Mg^{2+} responses reflect activation I_{Ca}, Mg^{2+} responses also reflect $[Ca^{2+}]_i$ and an inward Mg^{2+} current (I_{Mg}).

Chronic KCl exposure is associated with changes in Ca^{2+} excitability

Although we have not examined the effects of prolonged KCl exposure on I_{Ca} *per se*, previous findings on the electrophysiological and behavioural consequences of depolarization in *Paramecium* allow us to discuss our results in terms of its effects on this current.

The membrane potential in *Paramecium*, as in most excitable cells, is maintained in part by a ‘resting’ K^+ conductance. Thus, adding KCl extracellularly depolarizes the cell and thereby causes backward swimming through activation of the ciliary I_{Ca}. Several compensatory mechanisms

are triggered to help restore normal excitability. First, I_{Ca} inactivates through the actions of the rapid (within milliseconds) Ca^{2+}-dependent pathway discussed above and a slower (within tens of seconds, Hennessey and Kung, 1985) Ca^{2+}-independent mechanism. These pathways retard Ca^{2+} influx and aid recovery of forward swimming. The drawback to I_{Ca} inactivation is that it also desensitizes the cell to other stimuli. This not only prevents avoidance of noxious and repellent conditions, it also prevents responses to attractants, such as food (Van Houten, 1978). The cell escapes from this dilemma through modulation of 'resting' membrane conductances that allow slow (over a period of 2–3 h) recovery of membrane potential towards levels that approximate those recorded before adding KCl (Oka *et al.* 1986). For a discussion of the theoretical consequences of increasing extracellular K^+ concentration and the cell's response to this insult, interested readers are referred to an excellent review by Machemer (1989). This cell and membrane 'adaptation' enable I_{Ca} to reset and excitability to be restored. Once adapted, however, the background levels of KCl have to be maintained for normal cell responsiveness. Transferring cells to KCl-free medium (such as the Ba^{2+} and Mg^{2+} test solutions) causes the membrane potential to hyperpolarize away from the newly established membrane potential, and a depolarizing mechanoreceptor potential is now insufficient to activate I_{Ca}. The cells thus fail to swim backwards.

Effect of KCl exposure on chemoresponses

The effects of adaptation on responses to acetate and quinidine are consistent with this interpretation of the effects of KCl exposure on cell excitability. Quinine and quinidine repel by causing cells to turn on contact (Jennings, 1906). The turn results from a depolarizing receptor potential that then triggers I_{Ca} (Van Houten, 1979; Oami, 1996). Cells were no longer repelled from quinidine after KCl exposure (see Results), consistent with the idea that the chemoreceptor potential was now inadequate to trigger I_{Ca}. If the background concentration of KCl was maintained during the cells' encounter with quinidine, however, they retained their ability to respond, as predicted. Similarly, cells were no longer attracted to acetate after exposure to KCl unless the background stimulus persisted during the assay (see Results). Attraction to acetate depends on the cell being able to turn upon leaving the area of stimulation (Van Houten, 1978).

Chronic exposure to KCl had no effect on behavioural responses to GTP, a nucleotide that elicited strong repulsion through repeated bouts of prolonged backward swimming (Clark *et al.* 1993). Since KCl prevented repulsion from quinidine, one might expect GTP responses to have been affected similarly, but recent studies show that the two repellents act by fundamentally different mechanisms. Applying GTP to cells under voltage clamp was found to cause periodic increases in $[Ca^{2+}]_i$ that occurred independently of the ciliary I_{Ca} (Clark *et al.* 1997). The Ca^{2+} oscillations triggered Ca^{2+}-dependent Mg^{2+} and Na^+ fluxes and resultant depolarizations that were greatly in excess of that required to activate I_{Ca}, even accounting for any KCl-induced shifts in excitability. The resultant Ca^{2+} flux caused ciliary reversal, and the cells thus swam backwards normally.

Adaptation is a response to chronic membrane depolarization

Adding KCl to the extracellular medium challenges cells ionically and osmotically. Cronkite *et al.* (1985, 1993) and Cronkite and Pierce (1989) showed that hyperosmotic stress of *Paramecium* sp. caused the accumulation of free amino acids, principally proline and alanine. Similar osmoprotective mechanisms occur in bacteria and yeast (for a review, see Serrano, 1996). While such changes might also affect membrane excitability, the data in Table 1 suggest that adaptation was not a response to increased osmolarity. If osmotic stress were a factor, inorganic ions should produce equivalent behavioural changes regardless of ionic species, but this was clearly not the case (Table 1). Further, challenging the cells with a non-ionic osmoticant (sorbitol) failed to elicit long-term adaptation, suggesting that charge was important. Sorbitol did produce a general suppression of backward swimming that persisted to some extent for at least 30 min following a return to control medium, but this may have reflected non-specific effects on cell viability (cells died after 48 h in a non-nutrient saline solution when stressed osmotically).

KCl also challenges cells ionically. Substituting a large impermeant anion (glutamate) for Cl^- had no effect on short- or long-term adaptation (Table 1), suggesting that K^+ was the effector. K^+ depolarizes cells in part through a reduction in the transmembrane gradient and also through interaction with the strong negative surface potential that *Paramecium* develops as a result of life in a medium of low ionic strength (fresh water). How this affects membrane potential is complex and controversial (Eckert and Brehm, 1979; Machemer-Röhnisch and Machemer, 1989; Genet and Cohen, 1996; for a review, see Machemer, 1989), but surface-potential theory predicts that other cations should act similarly to depolarize the cell. An exception is Ca^{2+} which, for reasons that are detailed elsewhere (Machemer, 1989), is predicted to hyperpolarize rather than depolarize the membrane. An examination of the ability of various cations to substitute for KCl in eliciting adaptation reveals that all (with the exception of $CaCl_2$) suppressed backward swimming in Ba^{2+} solution (Table 1; immediate responses). This included choline, a large organic cation that was chosen to separate the extracellular effects of cations from those associated with permeation. None was able to reproduce the long-term effects of KCl or potassium glutamate on the Mg^{2+} response (Table 1), suggesting that the enhanced backward swimming duration may have reflected the cells' attempt to restore membrane potential when a primary means of repolarization (K^+ efflux) had been perturbed by the reduced transmembrane K^+ gradient.

Mechanism of short-term and long-term adaptation

The effects of KCl exposure on responses to Ba^{2+} and Mg^{2+}

suggest that adaptation is a much more complex phenomenon than suggested previously (Oka *et al.* 1986; Oka and Nakaoka, 1989; Machemer, 1989; Machemer-Röhnisch and Machemer, 1989). During the initial minutes in KCl, *P. tetraurelia* rapidly lost the ability to swim backwards in Ba^{2+} and Mg^{2+} solution (Fig. 3). This insensitivity can readily be explained in terms of I_{Ca} inactivation (Brehm and Eckert, 1978; Hennessey and Kung, 1985) and the cells' attempts to restore membrane potential in the continued presence of K^+ (Oka *et al.* 1986). This short-term phase of adaptation has also been referred to as membrane 'accommodation' (Machemer and De Peyer, 1977; Machemer-Röhnisch and Machemer, 1989).

Long-term adaptation is clearly distinct from the short-term effect and cannot be explained simply in terms of Ca^{2+} channel inactivation and membrane accommodation. Its onset was slow, requiring approximately 36 h to cause full depression of backward swimming in Ba^{2+} solution and enhancement of Mg^{2+} responses (Fig. 6, open symbols). The effects were also persistent, such that recovery of a Ba^{2+} response did not begin until 3–4 h after removing cells from 30–40 $mmol\,l^{-1}$ KCl and recovery was still not complete after 8 h in control medium (Fig. 5A, squares). The nature of the effect of long-term adaptation on the responses to Mg^{2+} (Figs 3, 6B) and Na^+ solutions may indicate a substantial change in the properties of the ciliary Ca^{2+} conductance itself. The behaviours in response to Mg^{2+} and Na^+ solutions are dependent on I_{Mg} and I_{Na}, respectively, currents that both require an increase in $[Ca^{2+}]_i$ to activate (Saimi, 1986; Preston, 1990). Thus, the simultaneous enhancement of both behaviours could indicate a common, adaptation-induced increase in I_{Ca}. An accompanying shift in the voltage sensitivity of I_{Ca} might also explain the observed loss of Ba^{2+} sensitivity. Alternatively, the enhanced Mg^{2+} and Na^+ responses could reflect direct effects on I_{Mg} and I_{Na}. Oka and Nakaoka (1989) noted that an unidentified outward current was enhanced during exposure to 8 $mmol\,l^{-1}$ KCl, suggesting that adaptation may involve a fairly extensive reprogramming of membrane excitability. If adaptation were associated with changes in the Ca^{2+} sensitivities of I_{Mg} and I_{Na}, it might explain how we were able to elicit Mg^{2+} and Na^+ behaviour when Ba^{2+} responses had been suppressed.

Unlike *Paramecium*, the excitable cells of higher organisms are protected from drastic swings in extracellular ion concentration that result from drought or rainfall, for example, but sustained activity can produce periods of prolonged depolarization equivalent to that produced by KCl. Several authors have noted that prolonged (3–4 day) KCl exposure caused a substantial decrease in neuronal Ca^{2+} channel density, presumably in response to the threat of Ca^{2+}-induced excitotoxicity (DeLorme and McGee, 1986; DeLorme *et al.* 1988; Franklin *et al.* 1992; Liu *et al.* 1994, 1995). Franklin *et al.* (1992) showed that this down-regulation could be prevented by inhibitors of protein synthesis and suggested that depolarization stimulates the production of a factor that degrades the channels.

Neither short-term nor long-term adaptation was affected by a protein synthesis inhibitor (Table 1), suggesting that the observed behavioural changes occurred without the synthesis of new, 'adapted' Ca^{2+} channels or of a channel modifier. If protein synthesis is not required, then what accounts for the slow time course and persistence of the long-term effects? One possibility is that the cells synthesize a negatively charged molecule to compensate for the altered transmembrane K^+ gradient, and this molecule then influences the conductances and/or ionic equilibria that determine motile behaviour. De-adaptation upon removal from KCl might then involve degradation or dumping of this molecule. A second possibility is that KCl exposure causes the ciliary Ca^{2+} channels or their lipid surrounds to be modified by pre-existing regulatory enzymes. The purpose of this modification would presumably be to 'reprogramme' the electrical output of the cells to optimize responsiveness in the new environment.

Regardless of mechanism, these behavioural studies have revealed an important and unsuspected process by which one excitable cell can modify its output in response to chronic stimulation. Studies of *Paramecium* behaviour over the past two decades have uncovered several fundamental truths about the function of excitable membranes, so it will be interesting to see whether neuronal cells of higher animals possess similar adaptive capabilities.

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