J. Membrane Biol. 60, 257–264 (1981)

The Journal of
Membrane Biology

Evidence for a Ca-Channel Mutation in the K^+-Resistant Mutants of *Paramecium*

Helen G. Hansma
Department of Biological Sciences, University of California, Santa Barbara, California 93106

Summary. The K^+-resistant mutants of *Paramecium tetraurelia* were isolated for their ability to survive high concentrations of K^+ that kill wild type (Shusterman et al. (1978) *Proc. Natl. Acad. Sci. USA* **75**:5645). These mutants have a normal turnover of the K-analog ^{86}Rb and do not appear to be defective in their K-regulation. Instead, the following evidence suggests that these mutants have a defective Ca channel that carries a lower-than-normal current: (1) Two K-resistant mutants survive longer than wild type in Ba^{++}, which enters *Paramecium* through the Ca channel during an action potential. (2) The most resistant mutant swims backward longer in Ba than wild type and has a much slower uptake of ^{133}Ba per second backward swimming. (3) Only the influx of Ba^{++} is altered in this mutant; the efflux of Ba^{++} is normal. All of the phenotypic differences of these mutants, including their lack of "adaptation" to high K^+, can be explained by the postulated Ca-channel defect.

Paramecium has an excitable membrane that controls its swimming behavior. By simply looking through a dissecting microscope, one can tell whether a paramecium is swimming forward or backward. When the paramecium is swimming forward, its membrane is at the resting potential level; when it is swimming backward, its membrane is depolarized. A Ca^{++} or Ba^{++} influx depolarizes the membrane, while a K^+ efflux repolarizes the membrane (Eckert, 1972; Eckert & Brehm, 1979). Since one can easily "see" this membrane excitation through a microscope, *Paramecium* is a convenient microorganism for directly measuring the ion fluxes occurring during membrane excitation. A further advantage is that Kung and co-workers have been carrying out a "genetic dissection" of the excitable membrane of *Paramecium tetraurelia* and have isolated many mutants with corresponding defects in both swimming behavior and membrane excitation (Kung, et al., 1975; Nelson & Kung, 1978).

Shusterman et al. (1978) have described a class of K^+-resistant mutants whose phenotype cannot be explained using the present knowledge about membrane excitation in *Paramecium*. Many of these mutants map at 2 gene loci. Mutants at one locus are the most resistant to K^+ and survive up to 80 mM KCl; the mutant d4–521 is in this class. Mutants at the other locus show intermediate resistance to K^+ and can survive in 35 mM KCl, which kills wild type within one day; the mutant d4–524 is in this intermediate class.

All of the K-resistant mutants also show less "adaptation" to K^+ than wild type. When the K^+ concentration of the culture medium is raised to 15–20 mM, wild type paramecia gradually lose their normal avoiding response to Ba^{++} solutions and show forward swimming instead. This loss of excitability was first reported by Hildebrand and Dryl (1976) who named it "adaptation", although Shusterman et al. (1978) have suggested that "desensitization" might be more correct. The K^+-resistant mutants do not "adapt" normally and continue to show backward swimming and "avoiding reactions" (brief backward jerks) even after 2 days in 15–20 mM K^+. The lack of "adaptation" in the mutants parallels their degree of K resistance. The most resistant mutant, d4-521, shows almost no "adaptation". The intermediate mutant, d4-524, shows some "adaptation" and loses the most extreme response to Ba, which is backward swimming; but it continues to show frequent avoiding reactions to Ba even after 2 days in 15–20 mM K-medium (Shusterman et al., 1978).

We have studied many aspects of K regulation in *Paramecium* and have concluded that the K-resistant mutants are probably normal with respect to their K turnover. Instead, they appear to have an altered Ca channel, which carries a lower-than-normal current. These results are presented below.

0022-2631/81/0060-0257 $01.60
© 1981 Springer-Verlag New York Inc.

Materials and Methods

Supplies

Barium-133, rubidium-86 and calcium-45 were obtained from New England Nuclear; RbCl was "Suprapur" from EM Reagents. HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) was obtained from Sigma.

Cell Stocks

The strains used were *Paramecium tetraurelia* (formerly *P. aurelia* syngen 4). The wild-type stock was 51s, from which all mutants were derived. The K-resistant mutants were d4-521 and d4-524. All strains are homozygous at all loci and were kindly supplied by Ching Kung.

Culturing and Harvesting Cells

Cells were grown in Cerophyl medium inoculated with *Enterobacter aerogenes* (Sonneborn, 1950; Hansma, 1979). This medium contained 5 mM K^+ and 0.2 mM Ca^{++}. Cells were harvested by centrifugation (Hansma, 1979) and were kept at room temperature for all experiments.

Measuring ^{133}Ba Uptake

Concentrated cells were washed in buffer (10 mM HEPES, 6 mM Tris, 0.1 mM $CaCl_2$, 0.02 mM EDTA, pH 7) and resuspended at densities of $2–5 \times 10^4$ cells/ml. At "zero time" ^{133}Ba and 0.8 mM $BaCl_2$ were added. Duplicate 1-ml samples of the cell suspension were layered over wash solution (buffer with 1% sucrose added) in centrifuge tubes made by flaming shut the tips of six-inch Pasteur pipettes. After centrifugation the tips of the centrifuge tubes containing the cell pellets were cut off, and their radioactivity was measured in a gamma counter (Hansma & Kung, 1976).

Measuring ^{133}Ba Efflux

Concentrated cells in buffer were incubated 30 min in 0.6 mM $BaCl_2$ with ^{133}Ba. The cells were then centrifuged and resuspended in fresh buffer. Ba efflux was measured by centrifugation as described above, and the radioactivity was measured in the cell pellets and/or supernatants.

Measuring ^{86}Rb Efflux

Cells were concentrated 15-fold and incubated overnight in culture medium containing ^{86}Rb, RbCl and KCl at concentrations stated in figure captions. Cells used for measuring steady-state turnover were centrifuged and resuspended in nonradioactive culture medium containing the same concentrations of RbCl and KCl. ^{86}Rb efflux was measured by centrifuging cells through a wash solution (containing culture medium, 1% sucrose, and the same concentrations of RbCl and KCl as the efflux medium). The data were plotted as $\log (D_t - D_\infty)/D_0 - D_\infty)$ *vs.* time, where D_t and D_0 are the specific activities of ^{86}Rb at a time "t" and time zero, respectively, and D_∞ is the specific activity of ^{86}Rb in cells plus efflux medium, i.e., the minimum level to which the cellular specific activity will drop.

Cells used for measuring the nonequilibrium efflux of ^{86}Rb were centrifuged and resuspended in buffer. Duplicate 1-ml aliquots were centrifuged to obtain the "time zero" data; then the other salts were added as indicated in Fig. 1, and duplicate aliquots were centrifuged at subsequent times. Radioactivity in crushed cell pellets and/or supernatants was measured in a liquid scintillation counter.

Measuring Backward Swimming

Concentrated cells in buffer were used for simultaneous measurements of Ba uptake and backward swimming. Single cells in buffer were transferred into 0.8 mM Ba-buffer and observed under a dissecting microscope. The swimming behavior was recorded with an event marker for 60 sec during each of the time intervals shown in Table 1. The speed of backward swimming usually decreased with time until the cell was spinning in place. The end of "backward swimming" was taken to be the time when the cell began to swim forward again.

Statistical Analyses

Ba-uptake data are presented as means ± standard deviations; $n=4$ to 6. Student's t-test (Campbell, 1974) was used to determine the statistical significance of differences between means.

The distribution of data on the duration of backward swimming is not Gaussian, since many cells swam backward for the entire 60 sec. Therefore, the Mann-Whitney U test (Campbell, 1974) was used to determine the significance at the 5% confidence limit of differences between groups; $n=16$ to 24.

Results

K-Turnover Is Normal in K-Resistant Mutants

Steady-state turnover of the K analog ^{86}Rb was measured in cells equilibrated with either 6 or 25 mM Rb+K (Fig. 1). The following results were obtained: (1) The efflux of ^{86}Rb shows approximately first-order kinetics, suggesting that there is only one pool of Rb+K in the cell. The deviations from first-order kinetics suggest either that there are two or more pools of Rb+K with slightly different kinetics or that there is a small artifact in the data. These possibilities have been discussed previously (Hansma, 1981). (2) There is no significant difference in the steady-state turnover of ^{86}Rb between wild type and the two K-resistant mutants. This conclusion is supported by the results of other experiments similar to those shown in Fig. 1. (3) There is no significant difference in steady-state turnover between cells in 6 mM Rb+K and cells in 25 mM Rb+K at a constant ratio of Rb to K. A further discussion of Rb+K turnover is presented elsewhere (Hansma, 1981); the main conclusion from Fig. 1 is that wild type and the K-resistant mutants are not different.

Wild type cells in 25 mM Rb+K have "adapted"; i.e., they have lost their normal avoiding reactions to Ba solution. Wild type cells in 6 mM Rb+K and mutants in both solutions all show strong avoiding reactions to Ba. Thus, the phenomenon of "adaptation" is not reflected in the kinetics of steady-state turnover of Rb.

The total cellular concentrations of Rb+K were

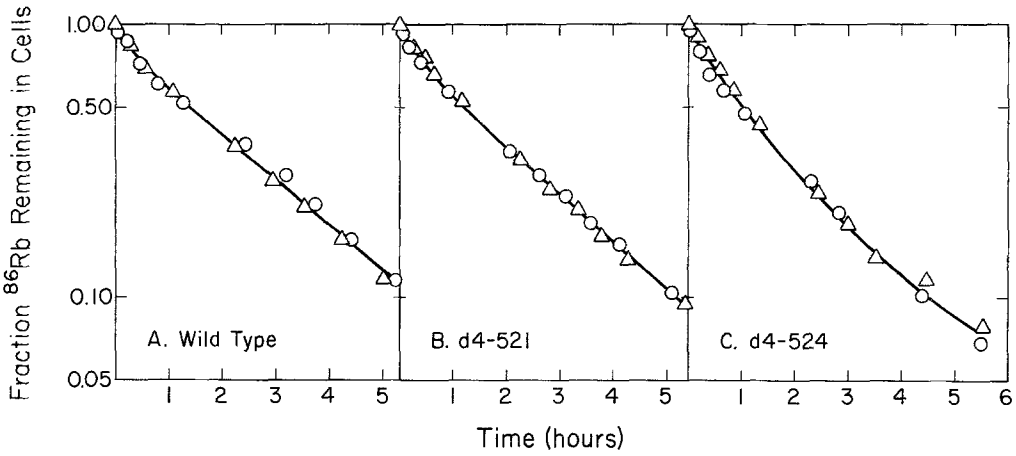

Fig. 1. Steady-state turnover of ^{86}Rb in wild type *Paramecium* and two K-resistant mutants. Extracellular medium contains (in mM): 1 Rb^+ and 5 K^+ (triangles); 4.2 Rb^+ and 20.8 K^+ (circles). Ratio of Rb to K=0.2 in both media. Data points are means of duplicate determinations

measured from the radioactivity of cells incubated overnight in culture medium containing ^{86}Rb. In 6 mM Rb+K medium the total cellular concentrations of Rb+K are similar for wild type (2.2 ± 0.6 nmol Rb+K/10^3 cells), d4-521 (2.7 ± 1.1), and d4-524 (3.1 ± 1.1). The mutants' Rb+K concentrations are not significantly higher than wild type. The Rb+K concentrations of cells in 25 mM Rb+K are similar or slightly higher. This is consistent with our earlier data on the total K content of wild type *Paramecium* as measured by flame photometry (Hansma & Kung, 1976; Satow, Hansma & Kung 1975).

Cells equilibrated overnight with ^{86}Rb were also transferred to buffer with or without added cations to determine the effect of different cations on the efflux of ^{86}Rb. Wild type and both mutants all show an exchange of ^{86}Rb for either nonradioactive Rb or K (Fig. 2*b*). This exchange is reasonably specific, since it is not stimulated by Na or Ca (Fig. 2*c*). There is very little net efflux of ^{86}Rb into buffer without added cations, which contains about 30–50 μM ^{86}Rb+K contamination (Fig. 2*a*). These results are consistent with the evidence of Browning (1976) that *Paramecium* has one or more high affinity systems for the uptake of Rb and K. Again, there are no significant differences between wild type and either of the mutants.

When wild type and mutant cells equilibrated with ^{86}Rb were transferred to buffer containing Ba, they showed prolonged backward swimming and large net effluxes of ^{86}Rb (Fig. 2*d*). This Rb efflux associated with backward swimming and membrane excitation is probably the voltage-dependent K-efflux or "delayed rectification" that repolarizes the membrane after an action potential (Eckert & Brehm, 1979), since it is much smaller in a pawn mutant of *Paramecium* that shows very little membrane excitation (Hansma, 1981).

In the first 5 min of exposure to Ba, wild type and both mutants lose about 20% of their total cellular Rb. At later times, there is a difference between wild type and mutants: the wild type cells die after 15–30 min in 1 mM Ba-buffer and lose most of their ^{86}Rb. The most resistant mutant d4-521 survives the longest in this Ba-buffer (more than 4 hr), and the intermediate mutant d4-524 survives about 1 hr. Thus these mutants are not only K resistant but also Ba resistant. (None of the wild type cells died, however, during the experiments described below in lower concentrations of Ba.)

^{133}Ba Uptake and Backward Swimming Are Different in K-Resistant Mutants

The Ba resistance of these mutants was investigated further by comparing the uptake of ^{133}Ba in wild type and mutants (Fig. 3). There are three reproducible differences between d4-521 and wild type in the time course of uptake of ^{133}Ba. First and most obvious, the rates of uptake of ^{133}Ba after 10 min are slower for the mutant than for wild type. Between 10 and 20 min, d4-521 shows a mean Ba uptake of 0.15 nmol/10^3 cells, while wild type shown mean Ba uptake of 0.27. Similar results have been obtained by Ling and Kung (K.-Y. Ling & C. Kung, *personal communication*). Second, the time course of ^{133}Ba uptake during the first 5 min is different for wild type and d4-521 (Fig. 3, Table 1). The mutant d4-521 always shows a nearly linear increase in ^{133}Ba over the first three time points. Wild type, however, shows a large increase in ^{133}Ba between the first and second

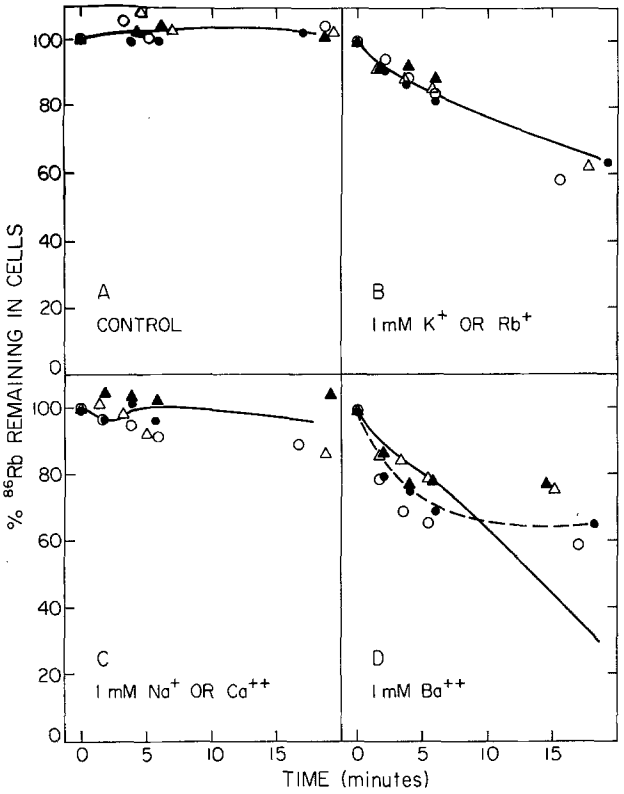

Fig. 2. Effects of cations on the efflux of ^{86}Rb from *Paramecium*. Cells were equilibrated by overnight incubation in culture medium containing ^{86}Rb and 1 mM RbCl and were then transferred to buffer or buffer with chloride salts added. Open symbols are data for the most K-resistant mutant, d4-521. Closed symbols are data for the intermediate mutant, d4-524. Solid lines are drawn from the data for wild type (Hansma, 1981). Each symbol is the mean of duplicate determinations. (*A*): Cells do not lose ^{86}Rb when suspended in buffer. (*B*): Cells lose ^{86}Rb when suspended in Rb^+ (open triangles) or K^+ (all other symbols). (*C*): Cells show little loss of ^{86}Rb when suspended in Na^+ (triangles) or Ca^{++} (circles). (*D*): Cells lose ^{86}Rb when suspended in Ba^{++}

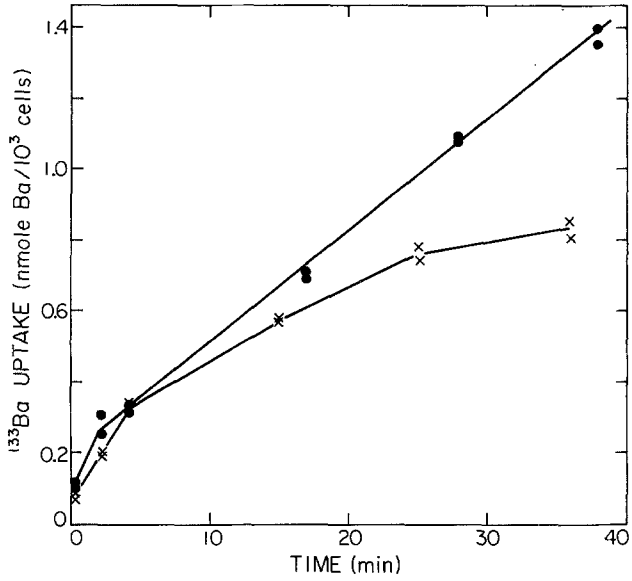

Fig. 3. Uptake of ^{133}Ba by wild type Paramecium (circles) and the K-resistant mutant d4-521 (x) in buffer containing 0.8 mM Ba^{++}, 0.1 mM Ca^{++} and 0.02 mM EDTA. Data show duplicate samples in a single experiment. Wild type and mutant were at the same growth stage in this experiment. The time after feeding affects the absolute levels of Ba uptake but not the shape of the curves

point, followed by a smaller increase only about half as large between the second and third points. The third, related, difference between wild type and d4-521 is that the maximum rate of Ba uptake seen during the first 2 min is more than twice as large for wild type as for d4-521 (Table 1). The intermediate mutant, d4-524, is intermediate between wild type and d4-521 in its Ba uptake.

Ling and Kung (1980) have good evidence that ^{133}Ba goes through the voltage-dependent Ca channel in *Paramecium*. Therefore, the Ba influx would be much larger when the membrane is depolarized and the cells are swimming backward than when the membrane is at rest and the cells are swimming forward. To measure Ba influx during backward swimming, a series of experiments was done in which backward swimming and Ba uptake were measured on the same groups of washed cells during the first three time intervals after Ba addition (Table 1). Backward swimming is much longer for the most resistant mutant d4-521 than for wild type, although this mutant has a slower rate of Ba uptake. Therefore, the rate of

Table 1. Ba^{++} Uptake and Backward Swimming

Cell type and phenotype	Time after Ba^{++} addition		
	0–2 min	2–4 min	4–6 min
	Backward swimming (sec/min)[a]		
Wild type	33±16	23±15	28±19
d4-521, most resistant	54±14	49±17	47±14
d4-524, intermediate	53±10	26±16	23±24
	Ba^{++} Uptake (nmol/10^6 cells·sec)		
Wild type	2.46±1.50	1.07±0.81	1.23±0.55
d4-521, most resistant	0.98±0.28	0.94±0.28	0.60±0.13
d4-524, intermediate	2.17±0.72	0.91±0.44	0.54±0.28
	Ba^{++} Uptake during backward swimming (nmol/10^6 cells·sec backward swimming)		
Wild type	5.32±2.46[b]	2.49±0.94	2.54±0.78[c]
d4-521, most resistant	1.26±0.44[b]	1.31±0.10	0.76±0.09[c]
d4-524, intermediate	2.89±0.95	2.54±1.62	1.42±0.51

[a] Significant differences between groups:
at 0–2 min: wild type and d4-521, wild type and d4-524
at 2–4 and at 4–6 min: d4-521 and wild type, d4-521 and d4-524.
[b] Significant differences between groups, $P<0.01$
[c] Significant differences between groups, $P<0.02$.

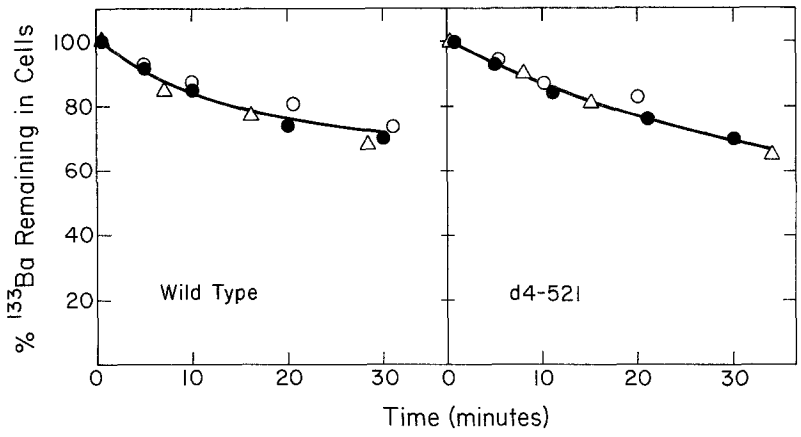

Fig. 4. Efflux of ^{133}Ba from wild type Paramecium and the K-resistant mutant d4-521. Data points are the means of duplicate determinations; three experiments are shown. The mean Ba content of cells at zero time (100%) was 0.62 nmol Ba/10^3 cells for wild type and 0.40 for mutant d4-521

Ba uptake per second of backward swimming is much smaller in the resistant mutant d4-521 than in wild type (Table 1). The intermediate mutant d4-524 has an intermediate Ba uptake per second backward swimming during the first and third time intervals and an uptake similar to wild type during the second time interval (Table 1).

From one time interval to the next, there is often a large decrease in backward swimming, Ba uptake or Ba uptake per sec backward swimming. There are many variables that were not measured in these experiments, such as the ratio of Ba to Ca currents and the duration of each period of backward swimming. Therefore, the decreases in Ba uptake per sec backward swimming in later time intervals might or might not reflect actual changes in the mean Ba + Ca current per sec of membrane depolarization. We did not measure ^{45}Ca influx to answer these questions because Browning and Nelson (1976) have shown that there is a rapid efflux of ^{45}Ca from *Paramecium* at room temperature, which masks the ^{45}Ca influx during membrane excitation. They were able to detect an excitation-dependent influx of ^{45}Ca in *Paramecium* at 0 °C, where Ca efflux was slow, but the cell condition was poor at this temperature.

Ba Efflux Is Normal in K-Resistant Mutant d4-521

The efflux of ^{133}Ba was measured to test the possibility that the most resistant mutant actually has a faster Ba efflux than wild type instead of a slower Ba influx as indicated in Table 1. The results (Fig. 4) show that there is no difference in Ba efflux between wild type and the most resistant mutant d4-521. The time course of ^{133}Ba efflux is similar to that reported by Ling and Kung (1980). The mean Ba efflux from Ba-loaded wild type cells is 0.07 nmol Ba/10^3 cells in the first 6 min, while the mean Ba uptake during the first 6 min is 0.74 nmol Ba/10^3 cells; therefore, the Ba efflux during uptake experiments should be less than 10% of the influx.

Discussion

Paramecium has a Ca action potential; therefore it has a voltage-dependent Ca channel that carries the inward current during the action potential. Dunlap (1977) and Ogura and Takahashi (1976) have shown that these Ca channels are located on the part of the surface membrane of *Paramecium* that covers the cilia. Thus, the Ca entering during an action potential enters directly into the cilia, where it serves at least two functions. First, as the intraciliary Ca rises to about 10^{-6} M, the cilia begin to beat backward, and the cell swims backward (Naitoh & Kaneko, 1973). Second, as the intraciliary Ca continues to increase, the Ca channels inactivate or close (Brehm & Eckert, 1978). This inactivation of the Ca channels probably requires a higher concentration of intraciliary Ca than ciliary reversal, since the Ca influx during an action potential is sufficient to raise the intraciliary concentration much higher than 10^{-6} M. When the Ca channels inactivate, the cilia beat forward again, presumably because the intraciliary Ca is rapidly pumped out (Browning & Nelson, 1976; Eckert & Brehm, 1979). Thus it appears that a small influx of Ca will cause ciliary reversal, and a larger influx will cause Ca-channel inactivation.

Ba^{++}, like Ca^{++}, can enter the Ca^{++} channels of *Paramecium* and stimulate backward swimming (Naitoh & Kaneko, 1973). Unlike Ca^{++}, Ba^{++} causes little inactivation of Ca^{++} channels (Eckert & Brehm, 1979).

Evidence for a Ca Channel Defect

The main evidence for a Ca-channel defect in the K-resistant mutants is that d4-521 and perhaps d4-524

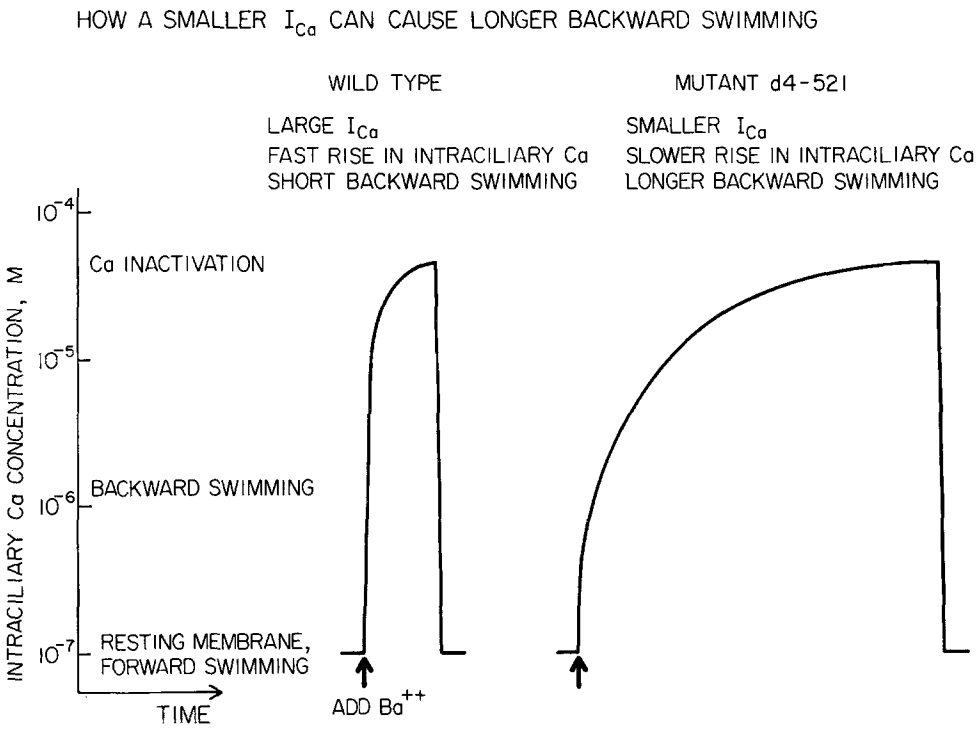

Fig. 5. Explanation of how a smaller Ca current could cause longer backward swimming in Paramecium. The intraciliary Ca concentration for Ca inactivation is an estimate from Eckert and Brehm (1979); the intraciliary Ca concentrations for backward swimming and forward swimming are estimates from Naitoh and Kaneko (1973). *Left:* Wild type shows brief episodes of backward swimming lasting a few seconds each in Ba-buffer and a faster Ba^{++} uptake. Perhaps the Ca-uptake is also faster in Ba-buffer and therefore the intraciliary Ca concentration rises quickly to the concentration required for inactivation or closing of Ca channels. *Right:* Mutant d4-521 swims backward continuously for a minute or more in Ba-buffer and has a slower Ba^{++} uptake. Perhaps its Ca uptake is also slower and therefore the intraciliary Ca concentration rises slowly to the concentration required for inactivation of Ca channels. We postulate that when the Ca channels have been inactivated the intraciliary Ca falls quickly to the concentration in forward-swimming cells and the membrane potential returns quickly to the resting level. More data is needed on the actual kinetics for Ca efflux and the changes in membrane potential, intraciliary Ca concentration, and swimming direction of *Paramecium* in these high concentrations of Ba^{++}

have a much lower Ba influx per second backward swimming than wild type (Table 1) and that Ba goes through the Ca channel (Ling & Kung, 1980). The K-resistant mutants also show other differences from wild type in their responses to Ba: (1) d4-521 has a lower Ba content than wild type after 15 min or more in Ba-buffer (Fig. 3). (2) d4-521 "overreacts" to Ba by swimming backward longer in Ba than wild type (Table 1; C.L. Shusterman & C. Kung, *personal communication*). (3) d4-521 and d4-524 survive longer in Ba than wild type. We have also shown that Ba efflux is normal in d4-521 and that K turnover in both K-resistant mutants is not significantly different from wild type for all parameters measured. All of this data is consistent with the hypothesis of a Ca-channel defect in the K-resistant mutants.

Another strong piece of evidence in favor of a Ca-channel defect comes from the genetic analyses of Shusterman (*personal communication*), who has shown that the double mutant of d4-521 and d4-524 has the "pawn" phenotype. Pawns are mutants that cannot swim backward, because they do not have functional Ca channels (Kung & Eckert, 1972). It is reasonable that two mutations affecting the Ca channel might combine to give a mutant with no Ca-channel function.

If d4-521 and perhaps d4-524 have Ca channels that carry lower-than-normal currents, can one explain the other aspects of their phenotype? Yes, the other Ba-related defects can all be explained fairly well, at least in simple terms. Perhaps d4-521 swims backward longer than wild type in Ba because its inward Ba+Ca current is large enough to cause ciliary reversal but not large enough to cause Ca-channel inactivation (Fig. 5). Perhaps the mutants survive longer in Ba than wild type because the intracellular Ba does not rise to toxic levels as quickly in the mutants.

The K-related aspects of the mutants' phenotypes can also be explained by the hypothesized Ca-channel defect. Basically, the mutants don't die and don't "adapt" to high K as readily as wild type. High K causes prolonged membrane excitation and backward swimming in Paramecium (Naitoh, 1968; Kung, 1971). Perhaps wild type *Paramecium* dies not of "K-overdose" but of "Ca-overdose" in high K because of the Ca influx during the prolonged membrane excitation. The mutants, whose Ca channels may carry smaller currents (Table 1), might not accumulate Ca as quickly; thus, the mutants would not die. This hypothesis remains untested.

Hildebrand and Dryl (1976) have proposed that *Paramecium* "adapts" to K because Ca entering through the Ca channels inactivates them by binding at intraciliary sites. Their model would suggest that the K-resistant mutants do not "adapt" normally because the Ca influx through the Ca channels is too small. Therefore, one can explain all aspects of the phenotype of the K-resistant mutants by hypothesizing that their Ca channels are defective and carry smaller-than-normal currents.

Evidence Against a Ca Channel Defect

There is one piece of data that is hard to reconcile with the hypothesis that the Ca channels in the K-resistant mutants carry smaller-than-normal currents. If Ba- and K-resistance are actually due to a slower Ba- and Ca-accumulation during membrane excitation, then all mutants with the pawn phenotype should be both Ba- and K-resistant, since they show little or no Ca-channel function. In fact, the pawn B mutant is both Ba- and K-resistant, as predicted; but the pawn A mutant is resistant only to Ba (C.L. Shusterman, *personal communication;* H.G. Hansma, *unpublished results*). The pawn A mutant has a leaky phenotype, i.e., some membrane excitation. It is not clear whether this discrepancy reflects a failure of the hypothesis or a lack of understanding of the pawn A mutation.

Another problem with the hypothesized Ca-channel defect is that it assumes that the measured Ba fluxes are also a reflection of differences in Ca currents between wild type and mutants. However, no one has measured the ratio of Ca to Ba currents in *Paramecium*. Perhaps the K-resistant mutants have a defect in the Ba-selectivity of the Ca channel, but their Ca currents are approximately normal. Although this is possible, it seems less probable.

Volgate-clamp experiments show that the inward currents in wild type *Paramecium* decay in a few milliseconds to a low level, although the membrane is still depolarized and the ciliary beat is still reversed (Eckert & Brehm, 1979). Therefore, the Ba fluxes measured here would represent mainly the small persistent currents across the depolarized membrane. The Ba fluxes per second backward swimming given in Table 1 correspond to currents of about 0.1 to 0.8 nA/cell, which is reasonable. The peak inward currents in *Paramecium tetraurelia* are 5–6 nA, but these large currents last only about 3 msec (Oertel, Schein & Kung, 1977; Satow & Kung, 1979).

There is one interesting detail about "adaptation" that cannot be explained by these data. When wild type cells "adapt" to high K, they lose their avoiding response to Ba but retain their avoiding response to Na (Schusterman et al., 1978., H.G. Hansma, *unpublished results*). The phenotypes of two classes of mutants also show a difference between Na and Ba. The K-resistant mutant d4-521 "overreacts" to Ba but reacts normally to Na. The "paranoiac" mutants have the opposite phenotype; they "overreact" to Na but react fairly normally to Ba (Kung et al., 1975). These data all suggest that Na and Ba cause membrane excitation in very different ways.

In their preliminary characterization of the K-resistant mutants, Shusterman et al. (1978) suggest that these mutants might have defects in either their Ca channels, their K-channels, or their regulation of K permeability. These measurements of ion fluxes have provided strong evidence for the first possibility.

I thank Cheryl Shusterman, Ching Kung, and Kit Yin Ling for giving me the mutants and their unpublished data, Paul Brehm for sharing with me his knowledge of *Paramecium* electrophysiology, and George Taborsky for sharing his lab with me. This work was supported by NSF grant PCM77-02672.

References

Brehm, P., Eckert, R. 1978. An electrophysiological study of the regulation of ciliary beating frequency in *Paramecium. J. Physiol. (London)* **283**:557

Browning, J. 1976. Studies on the excitable membrane of *Paramecium aurelia*. Ph.D. Thesis. University of Wisconsin, Madison

Browning, J.L., Nelson, D.L. 1976. Biochemical studies of the excitable membrane of *Paramecium aurelia. Biochim. Biophys. Acta* **448**:338

Campbell, R.C. 1974. Statistics for Biologists. Cambridge University Press, London

Dunlap, K. 1977. Localization of calcium channels in *Paramecium caudatum. J. Physiol. (London)* **271**:119

Eckert, R. 1972. Bioelectric control of cilia. *Science* **176**:473

Eckert, R., Brehm, P. 1979. Ionic mechanisms of excitation in *Paramecium. Annu. Rev. Biophys. Bioeng.* **8**:353

Hansma, H.G. 1979. Sodium uptake and membrane excitation in *Paramecium. J. Cell Biol.* **81**:374–381.

Hansma, H.G. 1981. Turnover of monovalent cations in *Paramecium. J. Exp. Biol. (in press)*

Hansma, H.G., Kung, C. 1976. Defective ion regulation in a class of membrane-excitation mutants in *Paramecium. Biochim. Biophys. Acta* **436**:128

Hildebrand, E., Dryl, S. 1976. Significance of Ca^{2+} and K^+ ions for the excitation of the protozoan membrane. *Bioelectrochem. Bioenerg.* **3**:543

Kung, C. 1971. Genic mutants with altered system of excitation in *Paramecium aurelia*. *Z. Vergl. Physiol.* **71**:142

Kung, C., Chang, S.-Y., Satow, Y., Van Houten, J., Hansma, H. 1975. Genetic dissection of behavior in *Paramecium*. *Science* **188**:898

Kung, C., Eckert, R. 1972. Genetic modification of electric properties in an excitable membrane. *Proc. Natl. Acad. Sci. USA* **69**:93

Ling, K.-Y., Kung, C. 1980. Ba^{++} influx measures the duration of membrane excitation in *Paramecium*. *J. Exp. Biol.* **84**:73

Naitoh, Y. 1968. Ionic control of the reversal response of cilia in *Paramecium caudatum*. *J. Gen. Physiol.* **51**:85

Naitoh, Y., Kaneko, H. 1973. Control of ciliary activities by adenosine-triphosphate and divalent cations in Triton-extracted models of *Paramecium caudatum*. *J. Exp. Biol.* **58**:657

Nelson, D.L.,, Kung, C. 1978. Behavior of *Paramecium*: Chemical physiological and genetic studies. *In*: Receptors and Recognition, Series B. G.L. Hazelbauer, editor. Vol. 5, 76–100. Chapman and Hall

Oertel, D., Schein, S., Kung, C. 1977. Separation of membrane currents using a *Paramecium* mutant. *Nature (London)* **264**:120

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

Satow, Y., Hansma, H.G., Kung, C. 1975. The effect of sodium on "Paranoiac" – A membrane mutant of *Paramecium*. *Comp. Biochem. Physiol.* **54A**: 323

Satow, Y., Kung, C. 1979. Voltage sensitive Ca-channels and the transient inward current in *Paramecium tetraurelia*. *J. Exp. Biol.* **78**:149

Shusterman, C.L., Thiede, E.W., Kung, C. 1978. K^+-resistant mutants and "adaptation" in *Paramecium*. *Proc. Natl. Acad. Sci. USA* **75**:5645

Sonneborn, T.M. 1950. Methods in the general biology and genetics of *Paramecium aurelia*. *J. Exp. Zool.* **113**:57

Received 9 September 1980; revised 31 October 1980