

SODIUM UPTAKE AND MEMBRANE EXCITATION IN *PARAMECIUM*

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ABSTRACT

Although the phenotypes of many membrane-excitation mutants of *Paramecium* are best expressed in Na⁺-containing solutions, little is known about the role of Na⁺ in membrane excitation in *Paramecium*. By measuring ²²Na fluxes, we have shown that: (a) The total cellular Na⁺ content is equivalent to a cytoplasmic concentration of 3–4 mM, if the Na⁺ concentration is uniform throughout the cell. (b) The kinetics of Na⁺ uptake can be divided into a saturable Na⁺ uptake with an apparent $K_m = 0.15$ mM and a nonsaturable Na⁺ uptake seen at higher Na⁺ concentrations up to 20 mM. (c) The rate of Na⁺ uptake in high Na⁺ solutions is correlated with the duration of backward swimming and membrane excitation in wild type *Paramecium* and the mutants fast-2 and paranoiac. (d) Na⁺ uptake is inhibited at 4°C. From these results, we postulate that Na⁺ uptake is faster when the membrane is depolarized than when it is at the resting potential level.

KEY WORDS *Paramecium* · excitable membranes · chemotaxis · sodium fluxes · behavioral genetics of microorganisms

Paramecium tetraurelia is a good system in which to investigate the biochemical mechanism of membrane excitation, because it is a well-studied unicellular organism that can be readily cultured and genetically manipulated. Furthermore, membrane excitation can be easily observed in *Paramecium* by its effect on the swimming behavior of the cell. It is known that a Ca⁺⁺ action potential causes the cilia to beat backward, which in turn causes the paramecium to swim backward (6). To understand this process on a molecular level, Kung and co-workers have been carrying out a “genetic dissection” of behavior and membrane excitation in *Paramecium*. They have isolated mutants at

over 18 gene loci that show abnormal behavior and membrane electrophysiology in response to chemical stimulation with various cations (15). The most successful early methods for isolating mutants relied on abnormal responses to Na⁺-containing solutions, and many of the mutants show clear-cut behavioral abnormalities only in Na⁺ (13, 14, 27). This is surprising, since the electrophysiology of *Paramecium* is based upon inward Ca⁺⁺ and outward K⁺ currents, and Na⁺ has not been shown to play a major role in membrane excitation in *Paramecium*. On the other hand, it is reasonable that Na⁺ would be important to *Paramecium*, which lives in fresh and brackish water where Na⁺ is a major cation.

Another way of investigating the membrane physiology of Na⁺ in *Paramecium* is by measuring ²²Na fluxes. In this paper, we describe the kinetics

of ^{22}Na uptake and provide evidence that membrane depolarization causes an increased uptake of Na^+ .

MATERIALS AND METHODS

Supplies

Cerophyl (powdered, dehydrated rye leaves) was obtained from Cerophyl Laboratories, Inc., Kansas City, Mo. Tris, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES), and murexide (ammonium purpurate) were obtained from Sigma Chemical Co., St. Louis, Mo. Sodium-22 was obtained from ICN K & K Laboratories Inc., Plainview, N. Y., and New England Nuclear Boston, Mass.

Cell Stocks

All strains were *Paramecium tetraurelia* (previously named *Paramecium aurelia* syngen 4). The wild type *Paramecium* was stock 51s (non-kappa bearing). The two paranoiac mutants were: "PaC," strain d4-150, genotype *PaC PaC*, and "fna^P," strain d4-149, genotype *fna^P fna^P*. The fast-2 mutant was strain d4-91, genotype *fna fna*. All mutants were derived from 51s. The two paranoiac mutations are unlinked. The fast-2 and paranoiac mutations *fna* and *fna^P* map at the same genic locus (30). The mutants were supplied by Professor Ching Kung (University of Wisconsin, Madison).

Culturing and Harvesting Cells

Cells were grown in flasks of autoclaved Cerophyl medium bacterized with *Enterobacter aerogenes* (10, 29). "Improved culture medium" (10) was used for the early experiments (Figs. 1-3). This medium has 10 mM Na^+ and 6 mM K^+ , as measured by flame photometry. A culture medium of lower Na^+ content (0.5 mM Na^+ and 6 mM K^+) was developed for later experiments to minimize Na^+ contamination of the cells. This medium contained the extract of 5 g of Cerophyl per liter, with 1 mM citric acid, 1 mM Tris-HCl, 4 mM Tris, 0.5 mM CaCl_2 , 0.5 mM NaCl.

Cell cultures were filtered through gauze and centrifuged for 2 min at 330 g in 100-ml pear-shaped bottles. The cell pellets were removed and washed by recentrifugation in either buffer A (0.1 mM CaCl_2 , 0.02 mM EDTA, 0.7 mM Tris, 2 mM HEPES, pH 7) or buffer B (0.33 mM calcium citrate, 0.33 mM citric acid, 1 mM Tris, 1 mM HEPES, pH 7.2) (see figure captions). Buffer B has ~ 0.3 mM free Ca^{++} , as measured by murexide dye-binding (3, 24). Buffer A was developed during the course of the experiments to have a more precise free Ca^{++} concentration (0.08 mM).

Measuring ^{22}Na Uptake

Aliquots of cells in buffer were pipetted into plastic vials. $^{23}\text{NaCl}$ and $^{22}\text{NaCl}$ were added, either simultane-

ously or sequentially, to the desired final concentrations. To measure ^{22}Na uptake, duplicate 0.5-ml samples of the cell suspension were layered over 1 ml of wash solution in centrifuge tubes made by melting the tips of 6-inch Pasteur pipettes (10, 11). The tubes were centrifuged for 20 or 30 s at 600 g in an International Centrifuge, Model K (International Equipment Co., Damon/IEC Div., Needham Heights, Mass.) After centrifugation, the tips of the centrifuge tubes, containing the cell pellets, were cut off. The radioactivity of the cell pellets was measured in a Nuclear Chicago well-type gamma counter (Nuclear-Chicago, Des Plaines, Ill.). Protein determinations (17) and/or cell counts were made on aliquots of the cell suspension.

The wash solution through which cells were centrifuged was buffer with 1% sucrose and 10 mM NaCl added. This washing procedure removed at least 97% of the extracellular ^{22}Na , as determined from the radioactivity of the wash solution directly above the cell pellet.

Measuring Total Cellular Na^+

Wild type paramecia were concentrated 10- to 20-fold and incubated overnight in culture medium containing 0.5 mM $^{23}\text{NaCl}$ and $^{22}\text{NaCl}$ to allow equilibration of ^{22}Na with the cells. Overnight incubation was sufficient for total equilibration of ^{22}Na , since the ^{22}Na content of cells incubated for 7 h was 70-99% of the ^{22}Na content at 20 h, and the ^{22}Na content did not increase between 20 and 30 h. The total cellular ^{22}Na was measured as described above by centrifuging aliquots of cells.

The mean cell volume was $\sim 0.2 \mu\text{l}/10^3$ cells, as determined both by calculating the volume of a cylindrical cell, $40 \mu\text{m} \times 140 \mu\text{m}$, and by directly measuring the volume of packed cells in the tip of a calibrated "Pasteur-pipet" centrifuge tube.

Measuring Swimming Behavior

Cells in culture fluid were suspended in several volumes of buffer for at least 15 min before beginning behavioral observations. Single cells were then transferred into one of the Na^+ -containing solutions under a dissecting microscope, and the swimming behavior was timed immediately with a stopwatch. The swimming behavior of the paranoiac mutants was best quantified as the duration of the initial period of backward swimming. The swimming behavior of the fast-2 mutant was quantified as the number of swimming reversals or "backward jerks" in the first 30 s after transfer. The swimming behavior of wild type was measured in one or the other of these two ways, depending on the mutant to which it was being compared. In each experiment, swimming behavior was measured on groups of five cells in each Na^+ solution. The data are presented as means for at least 20 cells.

It should be noted that the cells used for ^{22}Na uptake studies were harvested by centrifugation, while the cells used for swimming behavior were not. The effect of this

variable has not been quantified; but the swimming behavior of cells in ^{22}Na uptake studies was qualitatively the same as the measured behavior, and the differences in behavior between mutants and wild type were obvious. The interpretation of data is based on the changes in swimming behavior and ^{22}Na uptake and not the absolute amounts of behavior and uptake.

Statistical Analyses

Na-uptake data are presented as means \pm standard deviations. Student's *t* test (5) was used to determine the statistical significance of differences between means.

The distribution of data on swimming behavior is not Gaussian, since most groups contained some cells that did not swim backward at all. Therefore only the means are given, and the Mann-Whitney U test (5) was used to determine the significance, at the 5% confidence limit, of differences between groups.

RESULTS

Total Cellular Na

The total cellular Na content of wild type paramecia in culture medium containing 0.5 mM Na^+ was 46 ± 7 nmol Na/mg protein ($n = 6$), or ~ 0.7 nmol Na/ 10^3 cells. The cells were somewhat starved after being incubated overnight in a concentrated suspension but were otherwise healthy; there was no cell death in these suspensions for at least 2 d. The Na content per cell decreased significantly as the cells starved, however, and the variability in Na content was large.

If Na^+ is distributed uniformly throughout the cell, the Na content corresponds to a cytoplasmic concentration of 3–4 mM. This assumption is reasonable, since we have found previously that the total cellular K^+ concentration of ~ 20 mM (10, 11) is comparable to the cytoplasmic concentration estimated from electrophysiological data.¹

Na Uptake

The time-course of ^{22}Na uptake can be described in three stages (Figs. 1 and 2A): (a) A rapid Na uptake before the first data point can be taken, followed by (b) a linear increase in ^{22}Na from 15 s to 2 or 3 min, followed by (c) a slower increase in ^{22}Na at later times. When cells were chilled at 4°C for 20 min or more in solutions of 0.1–20 mM Na^+ , the linear portion (b) of Na uptake decreased to 50% or less of the rate at room temperature. The intercept at zero time was

¹ Y. Naitoh, University of Tsukuba, Ibaraki, Japan, personal communication.

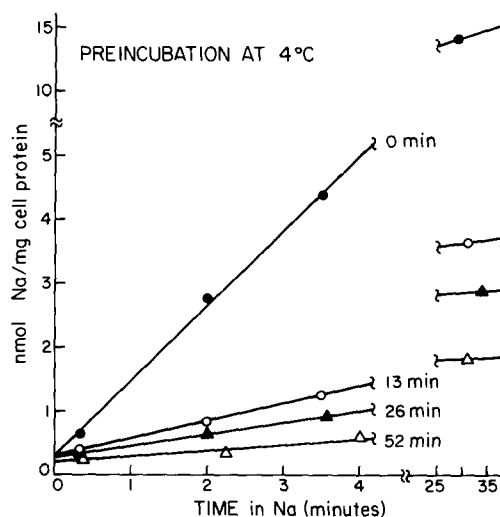


FIGURE 1 Time-course of uptake of ^{22}Na by *Paramecium* preincubated for different lengths of time at 4°C in buffer B. Concentration of $^{22}\text{NaCl}$ was 0.1 mM.

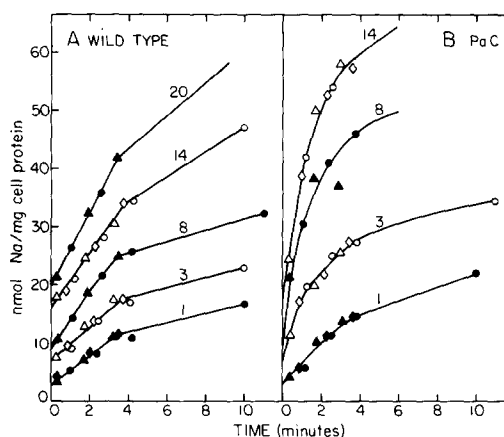


FIGURE 2 Time-course of uptake of ^{22}Na by *Paramecium* incubated in buffer B containing 1, 3, 8, 14, or 20 mM NaCl . Circles, triangles, and diamonds represent three identical samples at each Na concentration in which the first and subsequent time points were staggered, to have enough early time points for determining whether the uptake between 15 s and 3 min was linear.

approximately the same for 4°C and room-temperature incubations, however (Fig. 1). This suggests that the early Na uptake (a) is a fast process, perhaps surface binding of Na, that can be separated from the linear increase in Na (b) during the next 2–3 min.

Naitoh and Yasumasu (22) also have evidence that Na^+ and other cations bind rapidly to the surface of *Paramecium*. This is reasonable, since

the anionic surface charges on a membrane are partially neutralized by either binding or loose association of the cations in the bulk solution (18). Goodford (8) has also suggested that Na^+ and Ca^{++} might bind rapidly to smooth muscle cells, on the basis of tracer flux data.

The linear rates of Na uptake (*b*) were plotted as a function of the extracellular Na concentration (Fig. 3). The rate of Na uptake increases with increasing concentrations of extracellular Na up to 20 mM. There is a rapid increase in the rate of Na uptake between 0 and 1 mM extracellular Na ("low-Na solutions"), followed by a slower increase between 1 and 20 mM extracellular Na ("high-Na solutions"). The Lineweaver-Burke plot of Na uptake in low-Na solutions is linear (Fig. 4), suggesting that there may be a saturable component for the uptake of Na at low concentrations. The kinetics of this system depend on the extracellular Ca^{++} concentration. In a solution of 0.1 mM Ca^{++} (Fig. 4), the apparent K_m is ~ 0.15 mM and the apparent V_{max} is ~ 4 nmol Na/mg protein per min.

Na uptake in high-Na solutions is probably the sum of several processes. Wild type paramecia

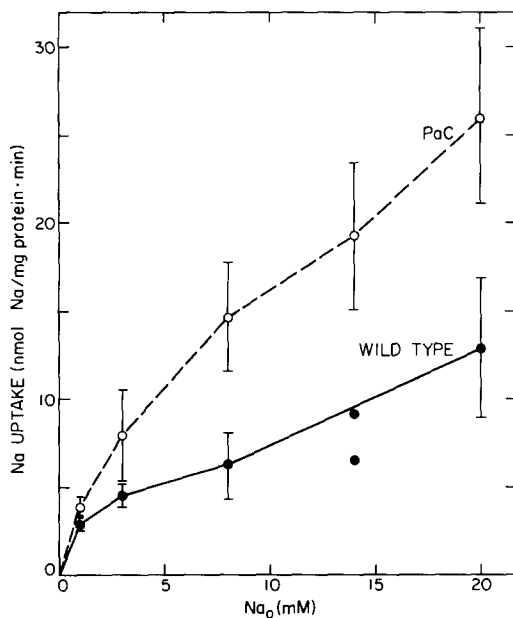


FIGURE 3 Rate of ^{22}Na uptake vs. extracellular Na^+ concentration for wild type *Paramecium* and paranoiac mutant PaC. Rates of ^{22}Na uptake were determined from the ^{22}Na content of cells 20 s and 1.5 min after adding ^{22}Na . Data are means \pm SD; $n = 4$ or 5. Cells were incubated in buffer B.

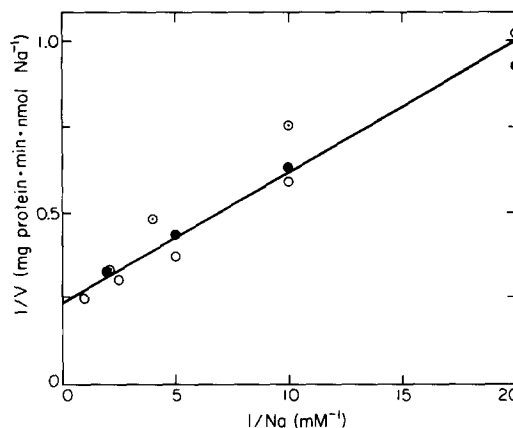


FIGURE 4 Kinetics of ^{22}Na uptake by wild type *Paramecium* in low- Na^+ solutions. Double-reciprocal plot of the rate of ^{22}Na uptake vs. extracellular Na^+ concentration from 0.05 to 1 mM. Data are from three experiments with cells in buffer A.

show an increasing rate of swimming reversals and action potentials as the Na concentration rises, and our evidence suggests that the uptake of Na is higher when the membrane is excited. Thus, in high-Na solutions, the Na uptake is probably the sum of the saturable (low-Na) component plus diffusion across the resting membrane plus diffusion across the excited membrane.

A Correlation between Membrane Excitation and Na Uptake

When cells are incubated in high-Na solutions, there is a strong correlation between membrane excitation and Na uptake. This correlation is shown below in several kinds of experiments, in which membrane excitation is measured as backward swimming or swimming reversals.

Wild type *Paramecium* and three mutants were used in these studies. Two paranoiac mutants, PaC and *fna^p*, "over-react" to high-Na solutions by swimming backward for as much as a minute or more, which corresponds to a long plateau of membrane depolarization (15). Wild type, in contrast, reacts to Na^+ by jerking back and forth repeatedly; the backward jerks and the corresponding membrane depolarizations usually last less than a second. The fast-2 mutant "under-reacts" to Na^+ by swimming fast forward initially, and its membrane hyperpolarizes (13, 15, 28). Different scales were used to quantify these different behaviors (see Materials and Methods).

The first correlation between Na uptake and

membrane excitation is that the paranoiac mutant PaC has a much faster Na uptake than wild type in solutions of 3–20 mM Na. Since the earliest time points for PaC are not linear (Fig. 2B), the data in Fig. 3 are not true rates; nonetheless, there is a clear difference between PaC and wild type. We have previously shown (11) that PaC swims backward in 8 mM Na for approximately the first 35 s and in 20 mM Na for ~1 min. Wild type, in contrast, swims backward for <5 s after transfer to high-Na⁺ solutions ([11] and Table I).

The second correlation is that there is a decrease in both ²²Na uptake and backward swimming when paranoiac mutants are preincubated in nonradioactive NaCl before the addition of ²²Na (Fig. 5). The two paranoiacs, *fna*^P and PaC, have

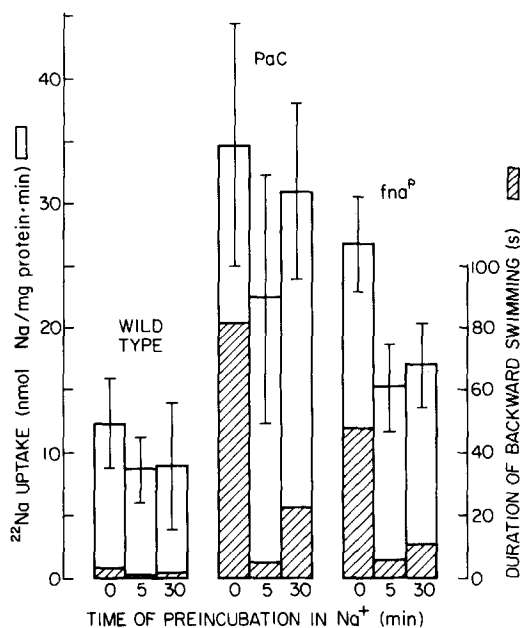


FIGURE 5 *Paramecium* preincubated in nonradioactive NaCl show slower ²²Na uptake and less backward swimming. Cells were washed in buffer A and transferred to either buffer A (0 min preincubation in Na⁺) or buffer A containing 10 mM NaCl (10 mM NaCl solution). Backward swimming was measured on cells transferred from the preincubation solution to 10 mM NaCl solution. ²²Na uptake was measured as in Fig. 2 after adding either trace amounts of ²²Na (5 and 30 min preincubation in Na⁺) or 10 mM ²²NaCl. Significant differences in Na uptake were found for PaC between 0 and 5 min preincubation ($P < 0.05$) and for *fna*^P between 0 min and both 5 min and 30 min ($P < 0.01$). Significant differences in backward swimming were found for PaC between 0 and 5 min, 0 and 30 min, 5 and 30 min, and for *fna*^P between 0 and 5 min, 0 and 30 min.

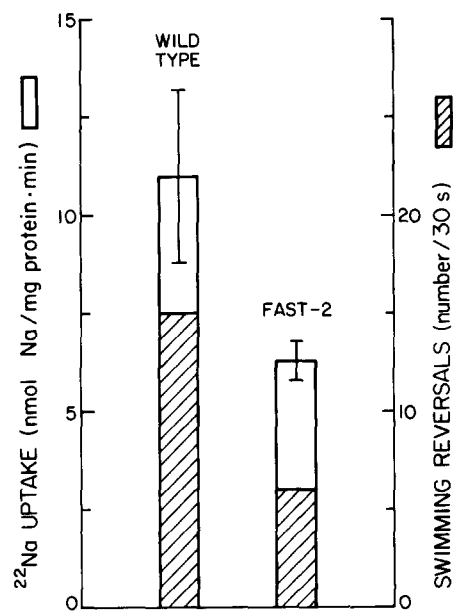


FIGURE 6 Wild type *Paramecium* shows faster Na⁺ uptake and more swimming reversals than fast-2 mutant. Cells were suspended in buffer A. Swimming reversals were measured in buffer A containing 10 mM NaCl. ²²Na uptake was measured as in Fig. 2 after adding 10 mM ²²Na to the cells. Differences between wild type and fast-2 were significant for both Na uptake ($P < 0.01$) and number of swimming reversals.

different, unlinked mutations but similar phenotypes (1, 15, 30). The cause of this “adaptation” to Na⁺ is not known. Wild type shows a similar, but not significant, effect. The third correlation is that both Na⁺ uptake and the frequency of swimming reversals of wild type are significantly greater than those of the fast-2 mutant in 10 mM Na solution (Fig. 6).

Effect of Cations on Na⁺ Uptake and Swimming Behavior

K⁺, Li⁺, and Ca⁺⁺ were tested for their effect on Na uptake and swimming behavior in 10 mM Na solutions (Table I). Na uptake was stimulated by cation addition in only one situation: Wild type cells transferred to Na solution containing 20 mM KCl showed prolonged backward swimming and approximately a two-fold increase in the rate of Na uptake (Table I). This is another example of a correlation between Na uptake and membrane excitation. High K⁺ has previously been shown to cause backward swimming in wild type *Paramecium* (13, 19). In the two paranoiac mutants, KCl

TABLE I
Effect of Cations on Na⁺ Uptake and Backward Swimming

Cell type (phenotype)	Addition to 10 mM Na solution	Na ⁺ uptake*	Backward swimming‡
		nmol Na/mg protein·min	s
Wild type§	None (control)	12.3 ± 3.7	3
	Ca ⁺⁺ 1 mM	6.3 ± 2.3	1
	10 mM	5.2 ± 1.8	2
	K ⁺ 5 mM	13.3 ± 7.6	15
	20 mM	21.8 ± 7.8	100
	Li ⁺ 5 mM	4.2 ± 1.9	3
PaC (paranoiac)	None (control)	34.7 ± 9.7	85
	Ca ⁺⁺ 1 mM	17.5 ± 7.1	60
	10 mM	7.6 ± 3.0	7
	K ⁺ 5 mM	25.0 ± 8.1	69
	20 mM	20.0 ± 4.9	77
	Li ⁺ 5 mM	25.0 ± 3.5	92
fna ^P (paranoiac)¶	None (control)	26.8 ± 3.9	60
	Ca ⁺⁺ 1 mM	15.8 ± 1.6	62
	10 mM	9.7 ± 2.3	37
	K ⁺ 5 mM	18.8 ± 2.8	66
	20 mM	14.4 ± 3.0	61
	Li ⁺ 5 mM	19.8 ± 4.8	62
	20 mM	15.7 ± 2.8	110

* ²³Na uptake was measured as in Fig. 2 after adding 10 mM NaCl and the specified concentrations of CaCl₂, KCl, or LiCl to cells in buffer A.

‡ Backward swimming was measured on cells transferred from buffer A to buffer A containing 10 mM NaCl and the specified concentrations of CaCl₂, KCl, or LiCl.

§ Significant differences in Na uptake were found between control and the following: 1 Ca ($P < 0.02$), 10 Ca, 20 K, 5 Li, 20 Li (all $P < 0.01$). $n = 4-7$. Significant differences in backward swimming were found between control and 20 K, between 5 K and 20 K.

|| Significant differences in Na uptake were found between control and the following: 1 Ca, 10 Ca, 20 K, 20 Li (all $P < 0.01$); between 5 Li and 20 Li ($P < 0.01$); between 1 Ca and 10 Ca ($P < 0.05$). $n = 5-10$. Significant differences in backward swimming were found between control and the following: 1 Ca, 10 Ca, 20 Li; and between 1 Ca and 10 Ca.

¶ Significant differences in Na uptake were found between control and 5 Li ($P < 0.02$), between control and all other solutions ($P < 0.01$); between 1 Ca and 10 Ca ($P < 0.02$). $n = 3-7$. Significant differences in backward swimming were found between control and 10 Ca, control and 20 Li, 1 Ca and 10 Ca, 5 Li and 20 Li.

inhibited Na uptake and did not change the duration of backward swimming significantly.

10 mM CaCl₂ inhibited both Na uptake and backward swimming in the two paranoiac mutants (Table I). This again shows a correlation between membrane excitation and Na uptake. Ca⁺⁺ also inhibited Na uptake in wild type; the number of swimming reversals was not measured. Naitoh (19, 20) has shown that Ca⁺⁺ inhibits backward swimming of wild type *Paramecium* in solutions containing Ca⁺⁺ and either K⁺, Na⁺, Li⁺, or Rb⁺.

20 mM LiCl inhibited Na uptake but stimulated backward swimming in all three cell types. Since

Li⁺ is an analogue of Na⁺, it might be competing with Na⁺ for the same binding or uptake sites. Therefore, it is possible that Li⁺ uptake as well as Na⁺ uptake is greater during membrane excitation and backward swimming.

DISCUSSION

The main conclusion of this work is that Na uptake in *Paramecium* is faster when the cells are swimming backward than when they are swimming forward. Since swimming direction is correlated with membrane potential, it follows that Na uptake is faster when the membrane is in an

excited state than when it is at the resting potential. The evidence for this conclusion is as follows: (a) Paranoiac mutants show much longer backward swimming and much faster Na uptake than wild type in high-Na solutions. (b) Both the duration of backward swimming and the rate of ^{22}Na uptake decrease when the paranoiacs have been preincubated in nonradioactive NaCl. (c) The fast-2 mutant has fewer swimming reversals and slower Na uptake in Na solutions than wild type. (d) Both Na uptake and backward swimming are increased in wild type by adding 20 mM K^+ to the Na solution. (e) Both Na uptake and backward swimming are decreased in paranoiacs by adding 10 mM Ca^{++} to the Na solution.

These new results on the initial ^{22}Na uptake by paranoiacs support and extend our earlier discovery that paranoiacs have a higher ^{22}Na content than wild type after 30-min incubations in high concentrations of $^{22}\text{NaCl}$ and a much lower total cellular K content (11, 26). Satow et al. (26) have shown that the depolarized membrane has a higher conductance than the resting membrane for the paranoiac mutant PaA in Na solutions (PaA is a third paranoiac mutant whose mutation is in a gene linked to the PaC gene but not the fna^{P} gene). This high conductance is probably due to higher conductances to Ca^{++} , Na^+ , and K^+ in the excited membrane. If the cytoplasmic concentration of Na^+ is $\sim 3\text{--}4$ mM as our data indicate, then the electrochemical gradient for Na^+ for *Paramecium* in 10 mM Na solutions would favor Na influx at membrane potentials more negative than +23 mV.

Are the electrophysiological data for wild type *Paramecium* consistent with our conclusion that Na uptake is faster during membrane excitation? When action potentials are evoked in *Paramecium caudatum* either electrically or by mechanically stimulating the anterior of the cell, the spike overshoot is independent of the extracellular Na concentration (1, 7, 21). This indicates that there is no increase in Na current at the peak of the action potential that can be measured by electrophysiological techniques. The peak inward current in *Paramecium tetraurelia* under voltage clamp is 5 nA/cell, and the minimum detectable current is ~ 0.5 nA (23). In contrast, a minute-long Na current of 0.5 nA/cell corresponds to an Na flux of 20 nmol Na^+ /mg protein per min. By comparing this value with the data in Table I, one can see that the Na uptake during prolonged backward swimming is well within the

noise level of electrophysiological measurements. Therefore, a voltage-dependent Na influx in *Paramecium* is not inconsistent with the electrophysiological data.

This calculation shows that the use of tracer fluxes can be a sensitive way to measure small ionic currents in *Paramecium*. Browning and Nelson (3) and Browning et al. (4) have also measured Ca currents during membrane excitation in wild type *Paramecium* and many behavioral mutants using ^{45}Ca at 0°C . This paper presents the first evidence that there is an Na^+ current across the excited membrane in wild type *Paramecium*.

If there is a voltage-dependent Na current in *Paramecium*, then the simplest explanation is that Na enters through one of the two voltage-dependent channels that have been found in *Paramecium*: the Ca channel or the K channel. It seems more likely that Na^+ would enter through the Ca channel, for the following reasons: First, the K channel in nerve is 100 times less permeable to Na^+ than K^+ (12). Second, the Na channel in the squid giant axon appears to be permeable also to Ca^{++} (9). Third, the Ca channel of *Paramecium* is affected by Na^+ , since Na^+ slows the inactivation of the inward Ca current during voltage clamp.²

It is intriguing to speculate that one or more of the paranoiac mutations might affect the Ca channel such that Na^+ further slows its inactivation, since the paranoiac mutants show very long depolarizations only in Na^+ or Li^+ solutions. Brehm and Eckert (2) have shown recently that the Ca channel in *Paramecium caudatum* is inactivated by Ca^{++} entry into the cell. They also showed that Ba^{++} and Sr^{++} both slow the inactivation of the Ca channel, apparently by reducing the Ca^{++} entry into the cell. In the paranoiac mutants of *Paramecium tetraurelia*, the duration of backward swimming increases with increasing Na concentrations (11). This is all consistent with the model that a paranoiac mutation might alter the relative specificity of the Ca channel for Na^+ and Ca^{++} . The pawn mutants have previously been identified as Ca^{++} -channel mutants, since they have no action potentials (15, 16, 25). Pawn mutations seem to prevent the Ca^{++} channel from opening normally; the paranoiac mutations might prevent the Ca^{++} channel from clos-

² Y. Satow and C. Kung, University of Wisconsin, Madison Wis., personal communication.

ing normally in Na⁺ solutions. More research is needed to test this exciting hypothesis.

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