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Lithium fluxes in *Paramecium* and their relationship to chemoresponse

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Paramecia respond to environmental stimuli by altering swimming behavior to disperse from or accumulate in the vicinity of the stimulus. We have found, using the T-maze assay, that treatment of paramecia with LiCl in a time- and concentration-dependent manner modifies the normal response to folate, acetate, and lactate from attraction to no response or even repulsion. Responses to NH_4Cl were unaffected and to cAMP were variably affected by LiCl. Cells incubated in the presence of K^+, or both Na^+ and K^+, but not Na^+ alone reliably recovered attraction to folate. Treatment of cells with 4 mM LiCl for 1 h dramatically slowed swimming speed from about 1 mm/s in NaCl or KCl (control) to 0.18 mm/s in LiCl. Li-treated cells subsequently incubated in 4 mM NaCl, KCl or sequentially in KCl and NaCl for a total of 20 min increased their swimming speed to 0.35, 0.45 and 0.67 mm/s, respectively. Paramecia readily took up Li^+ in Na^+- and K^+-free media reaching intracellular concentrations of 5–10 mM in 10 mM extracellular Li^+. Efflux of intracellular Li^+ was stimulated 35% by extracellular 10 mM NaCl and 185% by 10 mM KCl over 10 mM choline chloride. Incubation of cells in 10 mM LiCl for 1 h inhibited the rate of Ca^{2+} efflux by 44% compared to cells in 10 mM NaCl. This may relate to the mechanism by which Li^+ perturbs chemoresponse. A mutant with defects in Ca homeostasis responds normally to NH_4Cl, but not to any of the stimuli that are affected by LiCl.

Introduction

Paramecium tetraurelia is a ciliate that exhibits chemosensory swimming behavior [1]. The detection of specific environmental chemicals, which generally represent food cues, causes the cells to accumulate in areas of higher concentration of the stimuli [2]. The stimulus-response transduction pathway appears to be mediated by external receptors specific for the attractants [3,4], and when the receptors are sufficiently occupied, the plasma membrane hyperpolarizes [5]. Alterations of membrane potential have been shown to modulate directly the pattern of ciliary beating that ultimately controls chemoresponse (i.e. accumulation or dispersal of the cells) [6]. Hence, the measurement of swimming behavior is indicative of membrane potential changes and transduction events that occur at the molecular level. For this reason, *Paramecium* is an attractive system in which to study chemosensory transduction.

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The mechanism of the attractant-stimulated hyperpolarization has been elusive. An extensive study by Preston and Van Houten [5] has ruled out Na^+ and K^+ as the current-carrying ions, and there is only indirect evidence that channel-independent active extrusion of Ca^{2+} may account for the attractant-stimulated hyperpolarization. Electrophysiological evidence for Ca^{2+} efflux is difficult to obtain, given that the active transport of Ca^{2+} is sustainable over a very large electrochemical gradient.

In our efforts to perturb Ca^{2+} metabolism in order to study any subsequent effects on chemoresponse, we found that Li^+ profoundly alters attraction behavior. Li^+ does not appear to affect the turnover of inositol phospholipids or inositol phosphates, thereby affecting chemoresponse. (Indeed, to date, no function has been attributed to phosphoinositide metabolism in *Paramecium* [7]). However, we have found Li^+ to affect Ca^{2+} fluxes. Li^+, therefore, presents a pharmacological tool for the study of chemoresponse.

Materials and Methods

Cell culture. Paramecium tetraurelia, 51-S, sensitive to killer, was grown in culture medium which consisted

of wheat grass extract supplemented with Na_2HPO_4, Tris-HCl, 0.66 g/l proteose peptone and 1 mg/l stigmasterol [8]. Paramecia were added to the culture medium 24 h after it had been inoculated with *Klebsiella pneumoniae* and were cultured until late log phase at 25°C. The cells were harvested by filtering the medium through tissue paper (Kim Wipes, Kimberly–Clark) followed by centrifugation at $350 \times g$ for 1 min in oil-testing centrifuge tubes. The cell pellet was collected with Pasteur pipet and used as indicated in the individual experiments.

T-maze assay. Paramecia were assayed for their behavioral responses to attractants using the modified T-maze assay as described by Van Houten et al. [9]. In these assays, populations of cells were placed in a T-maze (stopcock), which gives the cells equal access to a test and a control solution. Both solutions contained 1 mM $Ca(OH)_2$, 1 mM citric acid and approx. 1.3 mM Tris base to adjust the pH to 7.05 (buffer A). The test solution contained the attractant (e.g. 2 mM disodium folate), and the control solution was balanced for monovalent cations by the addition of the appropriate salt (e.g. 4 mM NaCl). Thus, the test and control solutions differed only by their content of folate, acetate, lactate, or cAMP vs. Cl^- and NH_4^+ vs. Na^+. To run the assay, paramecia were suspended in the control solution and placed into the stopcock of the T-maze. At the start of the assay the stopcock was opened to allow access to the test and control solutions. After 30 min the stopcock was closed, the solutions collected, and the number of cells in each solution was determined. Based on the number of cells found in the test and control solutions we calculated the index of chemokinesis (I_{che}), which is equal to the number of cells in the test solution divided by the number of cells in the test and control solutions. Thus, an I_{che} of 0.5 indicates that the cells are not responsive to the test solution, an $I_{che} > 0.5$ indicates that the cells are attracted to the test solution, and an $I_{che} 0.5$ indicates that the cells are repelled by the test solution. All incubation as well as test and control T-maze solutions consisted of buffer A with the indicated salt (pH 7.05).

Lithium uptake. Harvested paramecia were rinsed in 100 ml of buffer containing 0.25 mM $Ca(OH)_2$, 1 mM citrate and approx. 5.2 mM Tris base to yield a pH of 7.05 (buffer B). The cells were collected by centrifugation and resuspended in an appropriate volume of buffer B and allowed to equilibrate for 30 min. LiCl (50 mM) prepared in buffer B (pH 7.0) was added to the cell suspension to a final concentration of 10 mM and rapidly mixed. At the indicated times, 0.5 ml samples were removed from the suspension and assayed for the amount of Li^+ taken up into the cells.

Intracellular Li^+ (and/or K^+) content was determined using methods modified from Hansma and Kung [10]. Centrifuge tubes made by sealing the ends of Pasteur pipets were filled to within 1 inch of the top with 2% sucrose dissolved in buffer B. The 0.5 ml cell samples were layered over the sucrose solution and centrifuged at 2000 rpm for 1 min in an IEC HN-SII centrifuge. The tips of the tubes containing the rinsed cells were etched with a diamond pencil, broken off and crushed in conical polystyrene centrifuge tubes in 1–2 ml of distilled water. Two drops of glacial acetic acid were added to each tube. They were vortexed and heated to 80–90°C for 10 min. After cooling, the samples were centrifuged at full speed in a clinical centrifuge for 5 min. The supernatant was removed and analyzed for Li^+ or K^+ content using a Corning model 51-Ca flame photometer. Li^+ and K^+ standards were used for calibration with each set of samples.

To measure Li^+ efflux, cells were loaded for 1 h in 10 mM LiCl buffer. The cell suspension was divided into equal portions, centrifuged at $350 \times g$ for 1 min and the supernatants aspirated. The cell pellets were rapidly resuspended in 10 mM choline chloride, 10 mM NaCl or 10 mM KCl (in buffer B). Samples (0.5 ml) were taken at the indicated times and treated as above to analyze for the content of Li^+ remaining in the cells.

$^{45}Ca^{2+}$ efflux. Harvested paramecia were rinsed and resuspended in a small volume of buffer B (5–10 ml). $^{45}CaCl_2$ (2 μCi/ml final concentration) was added to the suspension and the cells were incubated overnight under a humidified atmosphere to prevent evaporation. After the cells no longer incorporated counts (equilibrium was reached after approximately 6 h), the suspension was divided into equal portions and a small volume of concentrated LiCl or NaCl was added to the suspension to yield a final concentration of 10 mM. The cells were incubated for 1 h longer to allow the uptake of Li^+. After this time the suspensions were centrifuged and the supernatant was aspirated. The cell pellets were rapidly resuspended in buffer B (without $^{45}Ca^{2+}$) containing the same salt composition. Samples (0.5 ml) were taken from each suspension at the appropriate times and centrifuged (as above) through 2% sucrose dissolved in buffer B [10]. The tips containing the cells were crushed in scintillation vials containing 1 ml of 2% Triton X-100 to dissolve the cells. Scintillation fluid was added and the content of $^{45}Ca^{2+}$ was analyzed in a Beckman LS 7000 scintillation counter. The data points were found to fit to exponential rate equations using the Medas curve fitting program.

Swimming speed analysis. Time-exposure, dark field photographs were taken to measure swimming speed under various ionic conditions. Paramecia were pelleted and resuspended in solutions of 4 mM NaCl, KCl or LiCl (dissolved in buffer A) for 1 h. Cells that were incubated in LiCl and subsequently allowed to recover were incubated in 4 mM KCl or NaCl or sequentially in each for a total of 20 min as indicated in the legend

to Table III. Aliquots of the cells from each incubation condition were placed onto glass slides and allowed to recover from the mechanical stimulation for 1 min [11]. The slides were illuminated from the side by a high intensity light. The pictures were taken with an exposure time of 5 s using a Polaroid camera equipped with a 50 mm lens. The movements of the cells appear as white streaks and the distances traveled over the 5 s time exposure were measured.

Measurement of inositol phosphates and inositol phospholipids. Phosphoinositol lipids were labeled to equilibrium by incubating cells with ^{32}P (PBS.11A) or ^{3}H-inositol (Amersham). Aliquots of the labeled cells were stimulated with folate or folate and Li. The reaction was stopped by the addition of chloroform/methanol/HCl (100:200:2) followed by vigorous vortexing. The aqueous and organic phases were separated with the addition of chloroform and water followed by a brief centrifugation. The tritiated inositol phosphates present in the aqueous phase were separated by anion exchange chromatography using Dowex-50 columns (Bio-Rad) [12] and counted in a Beckman LS 7000 scintillation counter. The phospholipids, present in the chloroform phase, were separated on silica gel plates (EM Science) that had been impregnated with 1% potassium oxalate. Chloroform/acetone/methanol/acetic acid/water (40:15:13:12:8) was used as the solvent phase [13]. Radioactive phospholipids (^{32}P labeled) were identified by autoradiography. Phosphatidylinositol, which comigrated with an authentic standard (Sigma), was scraped from the plates and quantitated by liquid scintillation counting.

Results

Effect of Li^+ on chemoresponse

Alterations in *Paramecium* swimming behavior in response to chemical stimuli can be quantitated using the modified T-maze assay [9]. In the T-maze, paramecia are normally attracted to 2 mM disodium folate over 4 mM NaCl control, i.e. $I_{che} > 0.5$ (Table I). However, when cells were pretreated in buffer containing 2–4 mM LiCl as the only monovalent cation, the cells' attraction to folate gradually decreased in a concentration and time-dependent manner to the degree that they actually became repelled, i.e. $I_{che} < 0.5$ (Table I). The effective concentration of Li^+ and the time of exposure necessary to elicit a maximal inhibitory response to disodium folate were quite variable between different populations of cells and appeared to depend on various factors including feeding condition, growth state and the temperature at which the cells were cultured. However, for any given population of cells, prolonged exposure or a high concentration of Li^+ eventually evoked a perturbation of the normal chemoresponse. Throughout the course of investigation 4–10 mM LiCl was used in different experiments depending on the response of the cells to Li^+ at that time. It should be noted that Li^+ was not present in the solutions used in the T-maze assay. Therefore, it appears that Li^+ exerts a protracted effect on chemoresponse, possibly from an intracellular site.

TABLE I

Effect of Li^+ on the chemoresponse of Paramecium to folate

Cells were incubated for the indicated length of time in NaCl or LiCl. The cells were subsequently assayed for their response to 2 mM disodium folate vs. 4 mM NaCl using the T-maze assay. $I_{che} > 0.5$ indicates attraction; $I_{che} < 0.5$ indicates repulsion. The data are means of three tests ± S.D. The absolute response to any given set of conditions is quite variable but similar trends have been obtained in many other experiments.

Incubation	Time (min)	I_{che} ± S.D.
2 mM NaCl (control)	30	0.77 ± 0.06
2 mM LiCl	5	0.66 ± 0.04
2 mM LiCl	15	0.60 ± 0.07 *
2 mM LiCl	30	0.34 ± 0.06 *
4 mM NaCl (control)	30	0.76 ± 0.09
4 mM LiCl	5	0.27 ± 0.04 *
4 mM LiCl	15	0.16 ± 0.06 *
4 mM LiCl	30	0.18 ± 0.07 *

* Values are significantly different than control ($P \leq 0.05$) using the Mann–Whitney *U*-test.

We examined the chemoresponse of *Paramecium* to several other attractants following Li^+ treatment (Table II). The responses to sodium acetate and sodium lactate are clearly affected by Li^+, and similar to disodium folate, responses change from attraction to repulsion. Attraction to Na-cAMP is also diminished by Li^+ incubation, in some experiments repulsion being observed; however, the response is more variable than with folate or acetate (data not shown). On the other hand, attraction to ammonium was not as sensitive to Li^+ treatment. Some slight decrease from control was observed, but a change from attraction to repulsion never occurred, even when the same cells were strongly repelled by folate after LiCl treatment (0.90 ± 0.02 vs. 0.75 ± 0.02 for untreated vs. Li^+-treated cells responding to NH_4Cl compared to 0.88 ± 0.06 vs. 0.54 ± 0.14 for the same population of cells untreated vs. treated with Li^+ responding to disodium folate). These results may indicate that attraction to ammonium is mediated by a distinct transduction mechanism (e.g. alteration of intracellular pH).

The data in Table II compare responses of cells preincubated in LiCl with those with no pretreatment, instead of with cells incubated in comparable amounts of NaCl or KCl. Table III shows that use of cells without preincubation is a valid control.

TABLE II

Effect of Li^+ treatment on the response of Paramecium to various attractants

Cells were treated with 10 mM LiCl for 30 min, or used directly (control) before they were centrifuged and resuspended in the appropriate control buffer (5 mM NaCl) and tested in T-mazes for their response to the indicated attractant. Attraction to folate under identical conditions was used for comparison with each experiment.

Attractant	Control	*n*	Pretreated	*n*
5 mM sodium acetate	0.74 ± 0.07	9	0.29 ± 0.04	8
2.5 mM disodium folate	0.73 ± 0.11	9	0.45 ± 0.22	8
5 mM sodium lactate	0.75 ± 0.14	9	0.50 ± 0.21	9
2.5 mM disodium folate	0.74 ± 0.14	9	0.32 ± 0.20	9
5 mM NH_4Cl	0.82 ± 0.14	12	0.67 ± 0.12	12
2.5 mM disodium folate	0.78 ± 0.12	9	0.43 ± 0.17	9
5 mM potassium acetate	0.73 ± 0.13	21	0.54 ± 0.10	21

TABLE III

The effect of incubation in various salt solutions on the response of Paramecium to the attractant disodium folate in the T-maze

Cells were assayed directly from culture medium or incubated in the indicated salt solution for 30 min before being assayed for response to 2 mM disodium folate vs. 4 mM NaCl.

Treatment	I_{che}	*n*
None	0.87 ± 0.04	6
10 mM NaCl	0.85 ± 0.02	6
10 mM KCl	0.88 ± 0.06	6
10 mM LiCl	0.42 ± 0.07 *	5

* I_{che} is significantly different ($P \leq 0.05$) from the untreated conditions by the Mann–Whitney *U*-test.

To examine recovery from Li^+ treatment, we incubated cells in LiCl to perturb chemoattraction, and subsequently assayed their chemoresponse after allowing the cells time in NaCl or KCl solutions. As shown in Table IV, Li^+-treated cells did not exhibit significant attraction to 2 mM disodium folate even after 1 h for recovery in 4 mM NaCl. However, when Li^+-treated cells recovered in 4 mM KCl before being assayed for chemoresponse, they exhibited a strong attraction to 2 mM disodium folate. A similar approach was used to test recovery of attraction to sodium acetate. Li^+-treated cells allowed to recover in KCl for 1 h were strongly attracted to sodium acetate, while Li^+-treated cells incubated in NaCl did not consistently recover their attraction to sodium acetate, although the response was much more variable (Table V). These results are somewhat unexpected, since Li^+ often acts as an analog of Na^+ and Na^+/Li^+ exchange is the primary pathway for Li^+ efflux in most, if not all, cell types studied [14]. Due to the design of the experiment, the recovery in K^+ was followed by exposure of the cells to Na^+ for 30 min during the T-maze assay. Therefore, it is possible that both K^+ and Na^+ and not K^+ alone is optimal for recovery from Li^+ treatment. The appropriate test of this would be to incubate cells in KCl followed by T-mazes with K^+ salts (KOAc vs. KCl, for example). However, technically this was not

TABLE IV

Reversibility of the effect of Li^+ treatment on chemoresponse to folate

Cells were treated in 4 mM LiCl for 45 min. A fraction of the cells were then assayed directly while others were allowed to recover for 1 h in 4 mM NaCl or 4 mM KCl before being assayed for chemoresponse. The T-maze contained 2 mM disodium folate vs. 4 mM NaCl. $I_{che} \geq 0.5$ indicates attraction to folate; $I_{che} \leq 0.05$ indicates repulsion from folate. The data are the means of *n* determinations ± S.D.

Treatment	Recovery incubation	I_{che}	*n*
4 mM LiCl	none	0.39 ± 0.12	6
4 mM LiCl	4 mM NaCl	0.54 ± 0.11	6^{ns}
4 mM LiCl	none	0.50 ± 0.08	9
4 mM LiCl	4 mM KCl	0.76 ± 0.14	9 *

^{ns} The I_{che} is not significantly different ($P \geq 0.05$) than the LiCl treated cells.

* The I_{che} is significantly different ($P \leq 0.05$) than the LiCl treated cells by the Mann–Whitney *U*-test.

TABLE V

Reversibility of the effect of Li^+ treatment on chemoresponse to acetate

Cells were incubated in 4 mM or 10 mM LiCl for 45 min and then assayed immediately (no recovery incubation) or incubated in NaCl or KCl for 60 min before being assayed for response to 5 mM sodium acetate vs. 5 mM NaCl in the T-maze.

Treatment	Recovery incubation	I_{che}	*n*
45 min 4 mM LiCl	none	0.42 ± 0.12	3
60 min 4 mM NaCl	none	0.24 ± 0.24	3
60 min 4 mM KCl	none	0.78 ± 0.03 *	3
45 min 10 mM LiCl	none	0.11 ± 0.01	3
None	60 min 10 mM NaCl	0.32 ± 0.02 *	3
None	60 min 10 mM KCl	0.71 ± 0.08 *	3

* I_{che} is significantly different ($P \leq 0.05$) than the no recovery incubation conditions by the Mann–Whitney *U*-test.

possible because, as shown in Table II, Li^+-treated cells were already recovering in the T-mazes with KOAc. This, combined with the less robust chemoresponse in K^+ salts, made an adequate test difficult. However, other indications (below) point to a combination of K^+ and Na^+ as optimal for recovery from LiCl.

Swimming speed analysis

Direct observation of *Paramecium* swimming behavior using time-exposure dark field photography reveals that Li^+ exposure in Na^+- and K^+-free buffers dramatically alters motility. Cells incubated in 4 mM LiCl for 1 h exhibited a much slower forward swimming speed (0.18 ± 0.09 mm/s) compared to cells similarly incubated in NaCl or KCl (1.02 ± 0.31 and 0.95 ± 0.30 mm/s, respectively) (Table VI).

Since the observations made with the T-maze assay suggested that K^+ may be more effective than Na^+ in reversing the effect of Li^+ on chemoattraction, we examined the swimming behavior of Li^+-treated cells after subsequent exposure to Na^+ and K^+ (Table VI). Paramecia were treated in 4 mM LiCl for 1 h followed by a recovery period of 20 min in 4 mM NaCl or 4 mM KCl or allowed to recover 10 min each in KCl and subsequently NaCl. The 20 min time period was chosen since Na^+ or K^+-stimulated Li^+ efflux has plateaued within this time (see below). The cells in KCl had a significantly greater swimming speed (ANOV, $P \leq 0.05$) than the cells in NaCl solution (0.47 ± 0.16 vs. 0.35 ± 0.22 mm/s). Li^+-treated cells sequentially incubated in KCl and NaCl for a total of 20 min had a significantly greater swimming speed (0.67 ± 0.27 mm/s) than cells recovering in either Na^+ or K^+ alone ($P \leq 0.05$).

TABLE VI

Swimming speed of Paramecium after various ionic treatments

Cells were incubated in the various ionic buffers as indicated below. After the last incubation a small aliquot of the cell suspension (0.15 ml) was placed onto a glass slide and allowed to equilibrate for 1 min before a dark field photograph was taken as described in Materials and Methods. The swimming speed represents the mean distance traveled by the cells (n) divided by the time of exposure (5 s) ± S.D. Recovery conditions were incubation in 4 mM NaCl or KCl for 20 min or KCl followed by NaCl for 10 min each. All LiCl treated cell conditions were significantly slower than cells treated in KCl or NaCl ($P \leq 0.05$). The cells allowed to recover in NaCl, KCl or KCl and NaCl were significantly faster ($P \leq 0.05$) than cells treated with LiCl alone. Significance was determined by ANOV.

Treatment conditions	Recovery incubation	Swimming speed (mm/s)	n
4 mM KCl, 1 h	none	0.95 ± 0.30	35
4 mM NaCl, 1 h	none	1.02 ± 0.31	36
4 mM LiCl, 1 h	none	0.18 ± 0.09	34
4 mM LiCl, 1 h	NaCl	0.35 ± 0.22	58
4 mM LiCl, 1 h	KCl	0.47 ± 0.16	63
4 mM LiCl, 1 h	KCl, NaCl	0.67 ± 0.27	37

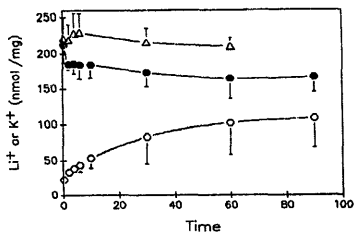

Fig. 1. Li^+ uptake and K^+ efflux in *Paramecium* in NaCl or LiCl. Concentrated LiCl or NaCl was added to equal cell suspensions in standard buffers to yield a 10 mM final concentration. Samples were collected and processed as described in Materials and Methods and analyzed for their content of Li^+ and/or K^+ by flame photometer. △, K^+ content of cells in 10 mM NaCl; ●, K^+ content of cells in 10 mM LiCl; ○, Li^+ uptake by cells in 10 mM LiCl. Data are the means ± S.E. of three experiments conducted in duplicate.

Li^+ fluxes

We have studied Li^+ fluxes in *Paramecium* in an attempt to correlate Li^+ movements into and out of the cells with the behavioral observations. We also examined the K^+ content of the cells treated with Li^+, since it has become apparent in the behavioral studies that K^+ metabolism may somehow be linked to Li^+ fluxes.

In 10 mM LiCl buffer, and in the absence of other monovalent cations, paramecia readily took up Li^+ (Fig. 1). Association of Li^+ with cells at short time periods, before the first sample was removed at 15 s, was very rapid and this apparent uptake probably includes binding to external sites on the cells. (The assay measures only incorporated Li^+ that is not readily washed off by centrifugation, i.e. both externally bound and internal Li^+.) After the rapid initial phase (0–2 min) the cells exhibited a slightly slower uptake (2–8 min) followed by much slower but sustained uptake for the rest of the study (10–60 min). The uptake curve does not conform to simple kinetic equations so that a meaningful single rate can not be calculated. Nevertheless, the results clearly show that paramecia take up substantial quantities of Li^+ over a time scale similar to that observed for Li^+ inhibition of chemoattraction. Using the conversion factors for volume and protein of paramecia reported by Hansma and Kung [10], we calculate that cells incubated in 10 mM Li^+ for 1 h, on the average, took up enough Li^+ to yield an intracellular concentration of 5–10 mM. Measurements of K^+ content in the same Li^+-treated cells and in NaCl-treated control cells reveals that Li^+ treatment caused a net loss of (up to 50% of) cellular K^+ whereas there was no net K^+ loss from Na^+-treated cells (Fig. 1).

To measure Li^+ efflux, cells were loaded in 10 mM LiCl for 1 h and then rapidly resuspended in Li^+-free medium containing 10 mM choline chloride, 10 mM NaCl or 10 mM KCl. The effluxes of Li^+ over time, under all three conditions (Fig. 2), very closely fit single exponential rate equations determined with the Medas curve fitting program. Based on the first minute of efflux we calculate that Na^+ slightly stimulated the rate of Li^+ efflux over choline from 5.3 nmol/mg per min to 7.2 nmol/mg per min, while K^+ doubled the rate of Li^+ efflux over Na^+ with a rate of 15.1 nmol/mg per min (mean of three experiments). Hence, the somewhat puzzling fact that K^+ was more effective than Na^+ in reversing the effect of Li^+ on chemoresponse and motility may be explained by the greater efficacy with which K^+, as compared to Na^+, stimulates the loss of Li^+ from the cells.

Ca^{2+} efflux

We examined the efflux of Ca^{2+} from cells that were incubated in 10 mM NaCl or 10 mM LiCl for 1 h prior to the start of the assay. After the 1 h incubation the cells were resuspended in a similar medium without $^{45}Ca^{2+}$ and the content of $^{45}Ca^{2+}$ remaining in the cells over time was determined (Fig. 3). The efflux of Ca^{2+} closely conforms to a single exponential rate as determined by the Medas curve fitting program. Based on the first minute, the Li^+-treated cells had a rate of Ca^{2+} efflux that was 56% of the Na^+-treated control cells.

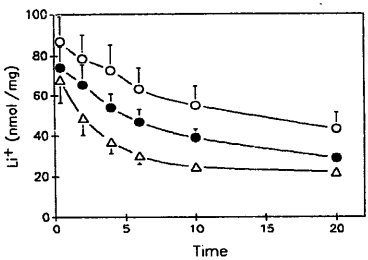

Fig. 2. Li^+ efflux from *Paramecium* in 10 mM choline chloride, 10 mM NaCl or 10 mM KCl. Cells were incubated in 10 mM LiCl for 1 h to allow the uptake of Li^+. The cell suspension was divided into three equal fractions and centrifuged. The cell pellets were rapidly resuspended in medium containing 10 mM choline chloride, 10 mM NaCl or 10 mM KCl. Samples were taken from each suspension as described in Materials and Methods and the content of Li^+ in the cells was determined by flame photometry. ○, Li^+ efflux in choline chloride; ●, Li^+ efflux in NaCl; △, Li^+ efflux in KCl. Data are the means ± S.E. of three experiments conducted in duplicate. S.E. is smaller than the data point symbol if not shown.

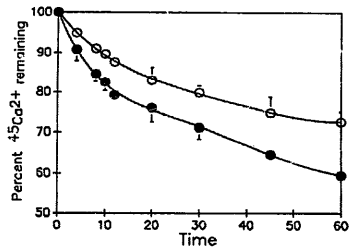

Fig. 3. $^{45}Ca^{2+}$ efflux from *Paramecium* in 10 mM NaCl or 10 mM LiCl buffers. Cells were labelled to equilibrium with $^{45}Ca^{2+}$ as described in Materials and Methods. One hour before Ca^{2+} measurements were initiated, concentrated NaCl or LiCl was added to equal portions of the cell suspension to yield a final concentration of 10 mM. After the incubation, the cells were centrifuged and the cell pellets were rapidly resuspended in the same medium without $^{45}Ca^{2+}$. Samples were taken as described in Materials and Methods and the amount of $^{45}Ca^{2+}$ remaining in the cells over time was determined. ●, $^{45}Ca^{2+}$ efflux in 10 mM NaCl; ○, $^{45}Ca^{2+}$ efflux in 10 mM LiCl. Data are the means ± S.E. of four or five experiments conducted in duplicate.

Phosphoinositide metabolism

The potential role of phosphoinositide metabolism in the signal transduction pathway of *Paramecium* chemoreception was assessed by looking for attractant-induced changes in the levels of inositol phospholipids or their metabolites. The phosphoinositide pools were readily labeled with ^{32}P and ^{3}H-inositol. However, under no conditions (i.e. incubation with 2.5 mM disodium folate or disodium folate and 4 mM LiCl) could we detect the stimulated formation of inositol phosphates, or changes in the pools of phosphatidylinositol or phosphatidic acid (data not shown).

Also suggesting that a transduction pathway independent of inositol lipid hydrolysis is involved, we found that incubation of cells in 1 μM TPA for up to 17 h had no affect on chemoattraction to folate (data not shown).

Discussion

The primary conclusion of this report is that *Paramecium* readily takes up Li^+ and that intracellular Li^+ directly alters chemoreception and motility in the cells. This conclusion is supported by the observation that the time course of Li^+ uptake is similar to the time course for the loss of chemoresponse upon incubation with Li^+ and that the ionic conditions which stimulate the efflux of cellular Li^+ also tend to reverse the effect of Li^+ treatment on chemoresponse and motility. The mechanism of Li^+ transport into and out of *Paramecium* is not the focus of this paper, but in

the endeavor to understand the behavioral aspects of Li^+ treatment on chemoresponse we discovered that K^+ is 2-fold more effective than Na^+ at stimulating Li^+ efflux from Li^+-loaded cells (Fig. 2), and that Li^+ displaces intracellular K^+ (Fig. 1). These observations are quite curious in light of the fact that Li^+ usually functions as an analog of Na^+ and that in most systems studied, if not all, Li^+ efflux is primarily carried by a Na^+/Li^+ exchange mechanism [14]. In human erythrocytes, 75% of Li^+ efflux occurs via Na^+/Li^+ counter exchange, and replacement of external Na^+ with choline or K^+ inhibits the rate of Li^+ efflux by 75% [15]. In our system, the rate of Li^+ efflux is reduced by only 26% when choline is substituted for Na^+, and in K^+ medium, the rate of Li^+ efflux is actually double compared to the Li^+ efflux of cells in Na^+. These data strongly suggest that Na^+/Li^+ exchange is not a primary mechanism of Li^+ efflux in *Paramecium*.

A logical candidate for the Li^+ carrier in *Paramecium* is a Na^+/K^+ pump. In erythrocytes, under non-physiological conditions, i.e. no extracellular K^+ and low intracellular Na^+, it has been demonstrated that the Na^+/K^+ pump will transport Li^+ in place of either Na^+ or K^+ [16]. Under normal physiological conditions for the erythrocyte, however, no Li^+ is transported by the Na^+/K^+ pump due to the comparatively low affinity of Li^+ for the Na^+ and K^+ sites [18,19]. *Paramecium* lives in a freshwater environment of low ionic concentration and the intracellular concentrations of Na^+ and K^+ are estimated to be only 3–4 mM and approximately 15–20 mM, respectively [15,16]. Therefore, if we place *Paramecium* in buffer with Li^+ as the only monovalent, inorganic cation and assume that Li^+ largely displaces intracellular Na^+, as well as some K^+, we have basically created the 'non-physiological' conditions used to stimulate Li^+ efflux by the Na^+/K^+ pump in erythrocytes. A major departure from the erythrocyte model, however, is that the K^+-stimulated Li^+ efflux is not sensitive to 0.2 mM ouabain (data not shown). It should be noted that a ouabain-sensitive Na^+/K^+ pump has yet to be unequivocally identified in *Paramecium* and the mechanisms of active transport of Na^+ and K^+ are virtually unknown [20]. In this light, Li^+ may be useful as a tool in further studies of ion regulation in *Paramecium*.

Our laboratory is interested in elucidating the mechanism of signal transduction in the chemoresponse of *Paramecium*. We originally examined the effect of Li^+ on chemoattraction in hope of ascertaining whether phosphoinositide metabolism may be part of the signal transduction pathway. The initial results showed that Li^+ treatment did, indeed, perturb normal chemoreception (as we predicted if phosphoinositide turnover were involved), and it appeared that inhibition of inositol phosphate recycling might be the locus of Li^+ interference in chemoresponse. Moreover, Li^+ inhibition of Ca^{2+} efflux (Fig. 3) also tended to support a role for phosphoinositide metabolism in the release of intracellular Ca^{2+} and its subsequent efflux. Nevertheless, direct biochemical analysis of phosphoinositide metabolism revealed no evidence of attractant stimulated changes in the levels of the inositol phospholipids or the soluble inositol phosphates (data not shown). Similarly a combination of Li^+ and attractant had no effect on inositol phospholipid pools; and incubation of *Paramecium* for up to 17 h in 1 μM TPA had no effect on chemoattraction (unpublished results). Therefore, our conclusion is that the inhibitory effect of Li^+ upon chemoresponse must involve a mechanism(s) distinct from Li^+ perturbation of phosphoinositide metabolism, since there is no direct evidence that phosphoinositide metabolism is involved in *Paramecium* chemoreception.

Thus, the question remains open: what is the biochemical basis for the observed effect of Li^+ on chemoattraction? Currently we have two hypotheses which we consider the most plausible:

(1) There is electrophysiological evidence to suggest that modulation of Ca^{2+} efflux may be an essential component of the chemoresponse mechanism (see Ref. 5), and as we have shown here, measurements of Ca^{2+} efflux in wild-type cells suggest that intracellular Li^+ inhibits the rate of Ca^{2+} efflux (Fig. 3). Also in support of a role for Ca^{2+} homeostasis in chemoresponse, behavioral data (T-maze assays) gathered on a putative Ca^{2+} homeostasis mutant, K-*shy* [21], reveal these cells are unable to accumulate in folate and acetate but show normal accumulation in NH_4Cl [22]. Curiously, LiCl affects responses of normal cells to folate, acetate and lactate but has little effect on attraction to NH_4Cl (Table II) [22]. Although this evidence is correlative, it suggests that Ca^{2+} metabolism may be a common factor which, if perturbed in any manner, will disrupt normal chemoresponse. We are presently studying the mechanism of Li^+ inhibition of Ca^{2+} efflux in greater detail and have found that Li^+ may inhibit a Ca^{2+}-pumping ATPase by displacing intracellular K^+ which is needed for optimal activation of the pump [23].

(2) Reports indicate that low millimolar concentrations of Li^+ will immobilize demembranated flagella from sea urchin sperm and the flagella will resume their normal beat when Li^+ is removed [24]. Li^+ appears to interact with a regulatory factor which controls microtubule switching and ultimately controls flagellar oscillation [25]. Presently we have no experimental evidence that Li^+ directly affects the ciliary beat of *Paramecium*, but the remarkable structural homology between cilia and flagella from diverse species, and the fact that Li^+ uptake dramatically decreases the motility of *Paramecium* in a reversible manner (as in sperm flagella), prompts speculation that Li^+ may be affecting ciliary function. This hypothesis

loses credibility, however, when considering that cells remain almost normally responsive to NH_4Cl despite Li^+ treatment. If a general effect on motility and not on chemosensory transduction were at work, the response to NH_4Cl might not be spared the Li^+ effect. However, it is conceivable that LiCl acts exclusively at the level of the axoneme to affect motility and not in upstream steps of the signal transduction pathway that are initiated by stimuli such as acetate and folate. Possibly the NH_4Cl signal transduction pathway couples differently to the axoneme compared to the other pathway and NH_4Cl-induced behavior is left intact while the acetate- or folate-pathway is affected at the level of the axoneme. Until we know more about the two pathways, we cannot make this distinction.

The variable response of *Paramecium* to different attractants following Li^+ incubation may indicate that a complex interplay of Li^+ effects are involved (i.e. inhibition of Ca^{2+} efflux and ciliary motility) in changing chemoattraction to repulsion. In theory, the organic attractants folate, acetate and lactate are perceived via external receptors that, when occupied, hyperpolarize the cell, possibly by activating a Ca^{2+} pump. On the other hand, the attractant ammonium is thought to influence cellular behavior through a direct effect on internal pH [26,27], bypassing the receptor-mediated transduction pathway. The responses of the cells to ammonium were the least influenced by Li^+ treatment. For the receptor mediated attractants that may require Ca^{2+} efflux, the tendency to become repellents may result from the combined effects of Li^+ on ciliary motility and Ca^{2+} efflux.

In mammalian cells, Li^+ has been found to interfere with other cell functions, which, if present in *Paramecium*, could conceivably be part of the chemoreception signal transduction pathway. The two most likely possibilities from other cell types are inhibition by Li^+ of G-protein-mediated, receptor-response coupling [28] and inhibition by Li^+ of adenylate cyclase activity [29]. In the case of these two cellular functions, no ligand-stimulated G-protein turnover or ligand-stimulated adenylate cyclase activity has been reported to date in *Paramecium*. In agreement with this, we have been unable to find any attractant stimulated changes in cAMP levels [30]. Therefore, the effects of Li^+ on Ca^{2+} homeostasis and/or ciliary activity remain the most promising possibilities in the search for the mechanism of Li^+ perturbation of chemoresponse.

Acknowledgments

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