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KINETICS OF LITHIUM EFFLUX THROUGH THE (Na,K)-PUMP OF HUMAN ERYTHROCYTES

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Summary

Evidence from the kinetics of transport supports the hypothesis that cellular Li, Li_c, interacts with the internal aspect of the (Na,K)-pump as a congener of Na. Li_c and Na_c compete for the same sites on the internal aspect of the pump. Li_c promotes Na-activated K influx and Na_c promotes Li-activated K influx. Cellular K inhibits Li-activated K influx, indicating that the interaction of K_c with the internal aspect of the pump is qualitatively different from the interaction with either Na_c or Li_c. The Hill coefficients for Na-promoted and Li-promoted K influx are similar and are both greater than unity, indicating the same number of multiple intracellular sites per pump for the two cations. The stoichiometry of coupling between efflux and K influx is also similar for Na and Li, and is close to 3 Na or 3 Li to 2 K. The Li-activated K influx appears to be independent of the residual Na which remains in cells prepared in Na-free solutions.

Lithium interacts with the external aspect of the (Na,K)-pump of human red cells as a congener of K. The evidence is that Li is transported into red cells by the ouabain-sensitive pump [1], and that Li promotes active Na efflux [2—4]. Furthermore, with low external concentrations of both K and Li, external Li 'coactivates' active K influx [5].

There is recent evidence that, in contrast, intracellular Li can interact with the pump as a congener of Na. In cells with an Na concentration of the order of 1 mmol/l cells, there is an ouabain-sensitive efflux of Li through the (Na,K)-

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Abbreviation: PCMBS, *p*-chloromercuribenzenesulfonate.

pump which is dependent on external K and is coupled to K influx [6]. The Li-activated K influx is unaffected by lowering the orthophosphate concentration in the cells, indicating that the K influx is mediated by the pump in its normal Na-K exchange mode and not in the K-K exchange mode [6]. (K-K exchange is inhibited by incubation with inosine, which reduces orthophosphate concentration [7], whereas K influx by Na-K exchange is not inhibited [6].) Therefore, intracellular Li promotes K influx and seems itself to be transported outward as though it were Na, not K.

Further evidence for the Na-like interaction of Li with the intracellular aspect of the (Na,K)-pump comes from studies on $(Na^+ + K^+)$-ATPase from porcine kidney and red cell membranes [8]. In both preparations, ouabain-inhibitable hydrolysis of ATP was observed in 130 mM LiCl without added Na or K. In a more critical experiment, Li promoted phosphorylation of the ATPase from porcine kidney in the presence of ATP and Mg, an action generally attributed to intracellular Na. But these enzyme preparations do have the disadvantage that concentrations of ligands at the two surfaces cannot be controlled separately. More recently, qualitative evidence for an Na-like action of Li was obtained with inside-out vesicles made from red cell membranes, a preparation which allows conclusions to be drawn about sidedness of action of ligands [9].

We offer here kinetic evidence from intact cells for an Na-like action of Li at the internal aspect of the (Na,K)-pump. The evidence is that intracellular Li and Na interact competitively in activating K influx, and that K inhibits this activation by Li. We also have considered the role of bound Na in intact cells in the action of intracellular Li [8] and the stoichiometry of the pump when exchanging Li for K [6].

Materials and Methods

Blood was drawn from healthy adult donors by venipuncture using heparin as anticoagulant. The erythrocytes were washed by centrifugation at 4°C in isotonic buffered choline chloride (160 mM choline chloride, 10 mM Tris-HCl, pH 7.4).

Alteration of cellular cation concentrations. This was carried out in all experiments by reversibly increasing the cation permeability using the method of Sachs [13]. Cells were incubated for 20 h or more at 4°C in solutions containing 0.1 mM *p*-chloromercuribenzenesulfonate (PCMBS) and the desired concentrations of NaCl, KCl, LiCl, and choline chloride. Normal permeability was restored by incubating the cells for 1 h at 37°C in the presence of 2 mM dithiothreitol.

$[X]_c$ and $[X]_o$ indicate the intracellular and external concentrations of X, respectively; X_c and X_o are used to indicate intracellular and external X without reference to concentration.

Unidirectional K influxes. These were determined as previously described [6,14] using ^{86}Rb as a tracer for K [15,16]. Use of ^{86}Rb as a tracer for measuring K influxes has become common in red cells and other cell types as well. Nevertheless, it seemed worthwhile to confirm the validity of this practice, at least for our purposes. We compared K influxes measured simultaneously by three methods: (i) using ^{86}Rb; (ii) from chemical analysis of the increase in

$[K]_c$; and (iii) from chemical analysis of the decrease in $[K]_o$ (see method below for net fluxes). The cells had been made to contain $[K]_c$ at 1.0 mmol/l; they were placed in a medium containing 5 mM K, resulting in a measurable net K influx. In six experiments the mean ouabain-inhibitable K influxes were (in mmol/l cells per h): 3.98 ± 0.49 (^{86}Rb); 3.96 ± 0.33 (increase in $[K]_c$); 3.80 ± 0.41 (decrease in $[K]_o$) (means ± S.E., $n = 6$). Therefore, under the conditions of these experiments, ^{86}Rb can be used as a tracer for K. These results do not show that ^{86}Rb can be used for detailed kinetic analysis of K influx, for example with varying $[K]_o$, but such studies were not attempted in the present work. Active K influx was operationally defined as that portion of the flux inhibited by ouabain (0.1 mM).

In all aliquots of cells, $[K]_c$ was made less than 1.0 mmol/l cell at the beginning of the measurement of the flux (except when high $[K]_c$ was desired). This was done to minimize both K-K exchange and inhibition by K of Na or Li efflux. Likewise, unless otherwise specified, $[Na]_c$ was less than 1.0 mmol/l cells in all aliquots of cells.

Net fluxes. These were determined for Na, K and Li by chemical analysis of cellular and supernatant concentrations before and after experimental incubations. The procedures prior to and during the incubation were the same as those used for unidirectional influxes, except that the hematocrit during the incubation was between 20 and 25% to facilitate the measurement of increases in the concentrations of Na and Li in the supernatant solution. The incubation was stopped by centrifuging the cells for 10 min at 4° C. The packed cells were washed three times by centrifugation and resuspension in cold isotonic $MgCl_2$, and then they were lysed in distilled water. Li concentrations were measured by atomic absorption spectrophotometry (Perkin-Elmer, model 303); Na and K were measured by emission flame photometry (Instrumentation Laboratories, Model 143). Concentrations were expressed as mmol/l cells. The volumes of cells in the incubation mixtures were obtained, as for the unidirectional fluxes, from the hematocrits and hemoglobin concentrations of the hemolysates. To obtain net efflux from the cells using the concentrations of Na and Li in the supernatant solutions, the latter were multiplied by the ratio of the volume of the supernatant to the volume of the cells, $(1 - H)/H$, where H is the hematocrit of a suspension of cells, expressed as a fraction. Per cent hemolysis during the incubations was always less than 2% and the net fluxes were corrected for the contribution by hemolysis. (Lysis would, of course, appear as an ouabain-insensitive flux and the objects of these experiments were the active fluxes.) In some experiments, unidirectional K influx was measured simultaneously with net fluxes.

Net K influxes were obtained from increases in cellular K; net Na and Li effluxes were obtained from increases in the concentrations in the incubation medium, initially free of both Na and Li. (The reduction in external K concentration provided a less accurate measure of K influx, as did the reduction in cellular Li or Na concentrations for effluxes.)

Interpretation of sigmoid kinetics. When a sigmoid curve relates flux to the concentration of a transported ion, the data can be fitted using one of several similar equations based upon related but distinct assumptions. We have used two approaches. The first is the Hill equation [17], the logarithmic form of

which is:

$$\ln\frac{M}{M_{max}-M} = h \cdot \ln[S] - \ln K_H \tag{1}$$

where M and M_{max} are active flux and maximal active flux, respectively, [S] is the concentration of the transported ion, K_H is a constant comprising the interaction factors between binding sites and also the intrinsic association constants, and h is the Hill coefficient. A plot of the left-hand side of Eqn. 1 against ln[S] gives a straight line with a slope of h.

The Hill coefficient has a complex physical significance, related not only to the number of interacting ligand binding sites per pump moiety, but also to the nature of the interaction between the sites [17,18], and the number of sites generally must be determined by a method independent of kinetics. However, when h is greater than unity, it does show that there are multiple ligand binding sites per pump. If h is the same for two different substrates reacting with the same enzyme (e.g., Na, Li, and the (Na,K)-pump), it is tempting to conclude that there are the same number of binding sites for the two substrates. There could conceivably be different numbers of sites and different cooperativities between them giving fortuitously identical h values, though this possibility seems remote. One assumption implicit in the Hill equation is the simultaneous addition of the transported ions to all of their binding sites on each pump.

Another approach to treating sigmoid kinetics for the (Na,K)-pump has been suggested by Garay and Garrahan [19]. They assumed that the multiple Na-loading sites on each pump have identical affinities for Na. They also assumed no interaction or cooperativity between the sites except that transmembrane translocation and release of Na would occur only when all Na sites on each pump were filled. This treatment does not require simultaneous binding of Na to all of its sites. An expression relating transport to $[Na]_c$ and number of sites was obtained from consideration of probabilities of occupancy of the sites by Na:

$$M = \frac{M_{max}}{(1 + K_s/[S])^n} \tag{2}$$

where K_s is an affinity constant and n is the number of Na-loading sites per pump (thus n has a different significance from h in Eqn. 1).

The same expression can be derived more rigorously using rapid equilibrium kinetics derived for enzymes, assuming a random multireactant system and assuming no release of product (transmembrane translocation) unless all substrate sites are filled (therefore a sequential enzyme). For a terreactant system (three substrate sites; the conclusion of Garay and Garrahan [19] about Na sites on the pumps) with all of the substrates identical to one another, the velocity equation is [17]:

$$M = \frac{M_{max} \cdot [S]^3/\alpha\beta\gamma K_A K_B K_C}{1 + \frac{[S]}{K_A} + \frac{[S]}{K_B} + \frac{[S]}{K_C} + \frac{[S]^2}{\gamma K_A K_B} + \frac{[S]^2}{\beta K_A K_C} + \frac{[S]^2}{\alpha K_B K_C} + \frac{[S]^3}{\alpha\beta\gamma K_A K_B K_C}} \tag{3}$$

where α, β and γ are coefficients for the interactions between the sites and K_A, K_B and K_C are equivalent affinity constants. In the special case of Garay and

Garrahan, the interaction coefficients are all unity, the affinity constants, K_s, are all the same, and Eqn. 3 reduces to Eqn. 2 with $n = 3$. Multiplying the nth root of the reciprocal of Eqn. 2 by S gives:

$$\frac{[S]}{M^{1/n}} = \frac{K_s}{M_{max}^{1/n}} + \frac{[S]}{M_{max}^{1/n}} \quad (4)$$

If the number of sites for substrates per pump is 3, a plot of $[S]/M^{1/3}$ against [S] will yield a straight line with slope of $M_{max}^{1/3}$ and intercept on the abscissa of K_s [19].

Effect of residual bound Na_c on Li-activated K influx. Cells were prepared with different amounts of residual Na_c (apparently bound) by exposing them to two successive PCMBS solutions as follows: in the first incubation, half the cells were suspended in 150 mM choline chloride, half in 140 mM choline chloride + 10 mM KCl. The presence of KCl in the PCMBS solution led to a slight increase in the amount of Na_c retained by the cells. * For the second PCMBS incubation, each aliquot of cells was divided in half, one incubated in 150 mM choline chloride, the other in 50 mM LiCl + 100 mM choline chloride. Each incubation was for 24 h with three changes of the solutions. Cation permeability was restored as usual in solutions containing dithiothreitol; the K influx and cellular cation concentrations were measured as described above.

Chemicals. Choline chloride was obtained from Nutrition and Chemical Division of Syntex Agri-Business, Inc. (Springfield, MO, U.S.A.), and was used without further purification; fresh stock solutions were made the day before use. *p*-Chloromercuribenzenesulfonate (PCMBS) and dithiothreitol were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). ^{86}Rb and ^{22}Na were obtained as chlorides in aqueous solution from New England Nuclear Corp. (Boston, MA, U.S.A.).

Results

Interaction between Na_c, and Li_c, and K_c in activating the (Na,K)-pump

To test the hypothesis that Li_c acts as a congener of Na_c, we determined the kinetics of the interactions between Li and Na and between Li and K in activating K influx. If Li is acting as a congener of Na, there should be a competitive interaction between Li and Na, and mutual stimulation of K influx (if there are multiple Na-loading sites per pump). When K-K exchange is suppressed, K_c should inhibit activation of K influx by Li (the interaction may or may not be simple competition).

Accordingly, K influx was measured in three kinds of experiments: (i) $[Li]_c$ varied with fixed $[Na]_c$; (ii) $[Na]_c$ varied with fixed $[Li]_c$; (iii) $[Li]_c$ varied with fixed $[K]_c$. The concentrations of the fixed or invariant species were set near the $K_{1/2}$ (concentration at half-saturation): approx. 5 mmol/l for Na; approx. 50 mmol/l for Li [6].

Activation of K influx by Li_c: effect of Na_c

Fig. 1 shows a double-reciprocal plot of active K influx as a function of

* We cannot account for this effect of K. In any case, the effect was never large (see Table II).

$[Li]_c$ in the presence and absence of a fixed $[Na]_c$. In cells that were nominally Na-free (less than 1.0 mmol/l), the curvilinear relationship between the reciprocals of K influx and Li_c (sigmoid in a direct plot) shows that Li interacts with more than one site on each pump to activate K influx. With $[Na]_c$ fixed at 4.5 mmol/l, Li-promoted K influx is further stimulated, though now the data fit a straight line. Extrapolations to M_{max} of the two curves are nearly the same, indicating that Na and Li interact competitively when promoting K influx. The linear relationship between K influx and $[Li]_c$ in the reciprocal plot with fixed $[Na]_c$ suggests that Li interacts with only one site per pump when Na occupies one (or more) sites per pump.

Activation of K influx by Na_c: effect of Li_c

Fig. 2 shows a double-reciprocal plot of the active K influx from an experiment complementary to the one in Fig. 1: the cells containing varying concentrations of Na without Li and with $[Li]_c$ fixed at 40 mmol/l. The results are similar to those obtained with varying Li. With Na alone the relationship between K influx and $[Na]_c$ is curvilinear, consistent with other reports that

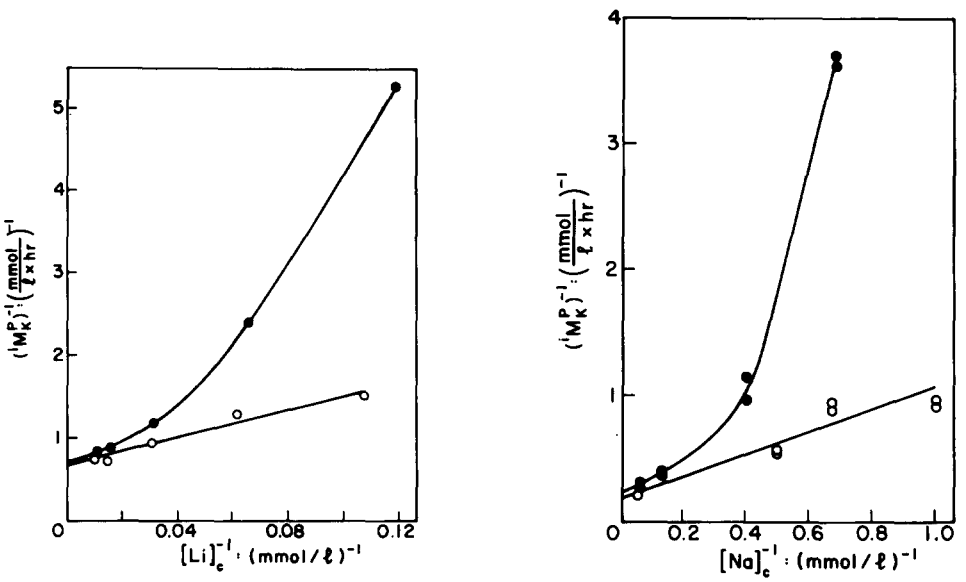

Fig. 1. Double-reciprocal plots of active K influx against $[Li]_c$ in cells nominally Na-free or with constant $[Na]_c$. Cellular cation concentrations were varied to give the desired $[Li]_c$ and $[Na]_c$, with the balance made up by choline. (•) Na-free cells ($[Na]_c$ = 1.0 mmol/l cells); (○) $[Na]_c$ fixed at 4.5 mmol/l cells (mean, n = 10). Fluxes were measured in an Na-free medium containing choline chloride (157 mM), KCl (3 mM), glucose (5 mM), and Tris-HCl (10 mM) at pH 7.4. $^iM_K^P$, active K influx. Points represent means of two determinations from one experiment. The lines were fitted by eye. The maximal velocity of influx (M_{max}) for Li-activated K influx was about 1.5 mmol/l per h, both in the presence and absence of Na_c. Similar results were obtained in four other experiments.

Fig. 2. Double-reciprocal plots of active K influx with varying $[Na]_c$ in the presence or absence of a fixed $[Li]_c$. The experimental procedure was the same as for Fig. 1. (•) Li-free cells; (○) fixed $[Li]_c$ at 40 mmol/l cells (mean, n = 10). Points represent single determinations from one experiment. M_{max} for Na-promoted K influx was about 5.7 mmol/l per h, both in the presence and absence of Li_c. Similar results were obtained in three other experiments.

Na interacts with more than one site on the pump to promote K influx [13, 19,20]. With fixed $[Li]_c$, K influx was further activated at each $[Na]_c$, although M_{max} was unchanged, and the relationship became linear. This is further evidence for the competitive nature of the interaction between Li and Na at multiple sites on each pump in activating K influx. At low concentrations of either Na or Li, the addition of the other ion stimulates the pump by occupying one (or more) of the multiple sites required for activation of the pump.

Hill coefficients for Na_c and Li_c

The results above show that Li, like Na, activates K influx at multiple binding sites on each pump and that Li and Na are competing at the same sites. It seemed worthwhile to compare the Hill coefficients for activation of the pump by Na in Li-free cells and by Li in (nominally) Na-free cells. Fig. 3 shows such K influxes plotted according to Eqn. 1. The slopes, h, are about the same for Li_c and Na_c (1.6 and 1.5, respectively). Though these numbers do not give the number of sites per pump, their similarity suggests that the number of sites is the same for Li and Na. Furthermore, if bound Na were participating in the activation of the pump by Li, h for Li would be unlikely to be as large as h for Na.

The lines in Fig. 3 were from two separate experiments. M_{max} in the experiment on Li cells was uncharacteristically low, but not uniquely so. Comparison of the intercepts on the ordinates in Figs. 1 and 2 shows that the values for M_{max} varied several-fold between experiments. The explanation lies with the long incubations in the cold in solutions of PCMBS. It is not surprising that this treatment led to differences in M_{max}; the differences are not critical to the conclusions drawn.

Li_c as an activator of a sequential terreactant system

Fig. 4 shows the activation of K influx by Li_c plotted according to Eqn. 4, but setting the exponent at 1/1, 1/2. and 1/3, i.e., assuming 1, 2 or 3 sites for Li on each pump. The lowest value of n which yields a straight line when [S] is plotted against $[S]/M^{1/n}$ gives the number of sites if the assumptions of Garay and Garrahan [19] hold. Fig. 4 is consistent with two or three sites for Li_c. Though the line for $n = 3$ appears slightly better than for $n = 2$, this was not a consistent finding. The same results were found for Na_c, that is a slightly better fit for $n = 3$ than $n = 2$ (results not shown), consistent with the earlier results of Garay and Garrahan, though these authors reported a clearer difference between the curves for $n = 2$ and 3.

The values obtained for the exponents, h (Fig. 3) and n (Fig. 4), were not the same, nor should they be since they have different physical significances.

Activation of the pump by Li_c: effect of K_c

The nature of the relationship between Li_c and K_c when interacting with the internal aspect of the pump was determined by observing the effect of a fixed $[K]_c$ on the Li activation of K influx. Fig. 5 shows a double-reciprocal plot of active K influx as a function of $[Li]_c$ in either the presence or absence of fixed $[K]_c$ (27 mmol/l cells). All aliquots of cells had been preincubated in isotonic choline chloride containing 10 mM inosine for 1 h at 37°C before the flux mea-

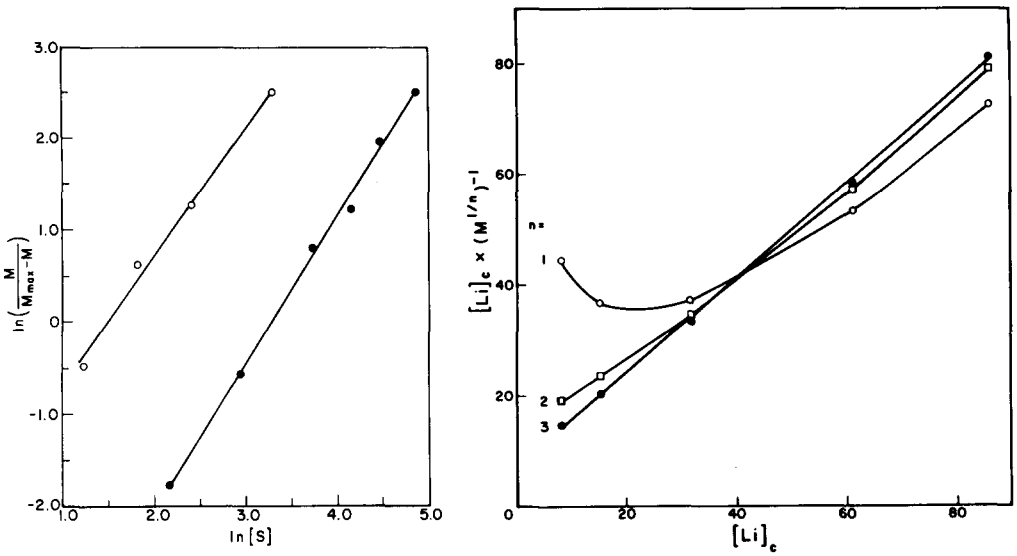

Fig. 3. Hill plots of Na-activated and Li-activated K influx. The experimental procedure was the same as for Fig. 1. (○) cells with varying $[Na]_c$ (Li-free); (●) varying $[Li]_c$ (nominally Na-free). Points represent single determinations from one experiment. Values of M_{max} used to calculate

$$\ln \frac{M}{M_{max} - M}$$

(see Eqn. 1) were (in mmol/l per h) 3.6 for Na and 1.4 for Li. K_H was (in mmol/l cells) 4.8 for Na_c and 27.6 for Li_c. The number of similar experiments was three for Li cells and two for Na cells.

Fig. 4. Active K influx in cells with varying $[Li]_c$ plotted according to Eqn. 4, where $[Li]_c/M^{1/n}$ vs. $[Li]_c$ should give a straight line when n is the number of intracellular sites per pump activated by Li. M, active K influx. The results were plotted with n set at 1, 2, or 3. Similar results were obtained in two other experiments.

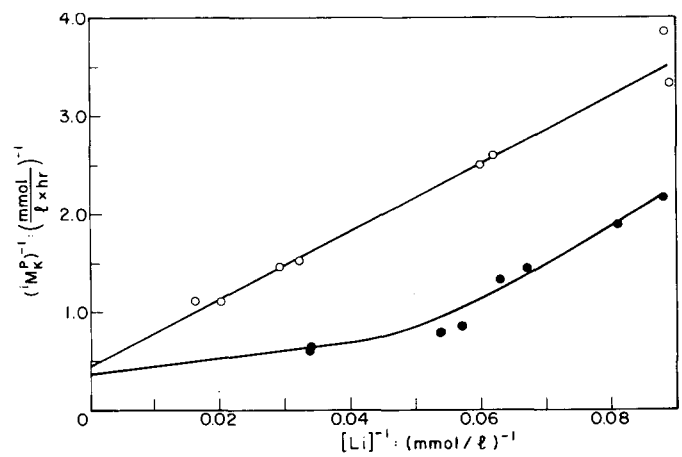

Fig. 5. Double-reciprocal plots of active K influx with varying $[Li]_c$ in the presence or absence of fixed $[K]_c$. (●) K-free cells; (○) $[K]_c$ = 27 mmol/l. The experimental procedure was the same as for Fig. 1. The cells had been preincubated for 1 h at 37°C in medium containing inosine (10 mM) in order to deplete cellular orthophosphate and thereby reduce K-K exchange. M_{max} of Li-activated K influx was about 2.7 mmol/l per h in Li cells and 2.0 in (Li + K) cells. The points are single determinations from one experiment. Similar results were obtained in two other experiments.

surement. This procedure minimized K-K exchange by depleting the cellular concentration of orthophosphate, which is required for K-K exchange [7]. For the cells with varied $[Li]_c$ and neither K_c nor Na_c, there was a curvilinear relationship as in Fig. 1. With fixed $[K]_c$ the Li-promoted K influx was inhibited, and the relationship between the reciprocals of the flux and $[Li]_c$ became linear. The interaction between K_c and Na_c was reported to be competitive [19,20]. Fig. 5 seemed to indicate a competitive interaction between Li_c and K_c, but the results are not sufficiently extensive to be conclusive. Nevertheless, the results are clear in showing that K inhibits the activation by Li of K influx, in contrast to Na, which promotes it (Fig. 1).

Stoichiometry of coupling: Li : K and Na : K

In our previous work, we suggested that the coupling ratio of the pump when exchanging Li for K was 1 : 1 [6], in contrast to the ratio of 3 Na : 2 K in the normal mode of the pump's operation in human red cells. However, the similarity of the Hill coefficients for Na and Li (Fig. 3) is inconsistent with different coupling ratios for Li and K as compared to Na and K. Therefore, we re-examined the Li : K coupling ratio by making simultaneous measurements of net fluxes of K and either Na or Li. To improve the accuracy, the experiments were run at high hematocrits (20% or more) and net effluxes of Na and Li were obtained from the increases in extracellular [Na] and [Li] rather than from the changes in intracellular concentrations, a less accurate measure of net efflux.

Table I shows the results of seven experiments. The coupling ratio of 1.46 observed for Na-K exchange is tolerably close to the published value of 1.5 [10—12]. With the $[Na]_c$ of the Li cells at 1 mmol/l, a small but measurable Na efflux was observed (equal to 9% of the Li efflux). This must be added to the

TABLE I

STOICHIOMETRY OF COUPLING FOR Na:K AND Li:K DETERMINED BY NET FLUXES OF K, Na AND Li

Concentrations were measured before and after incubation for 1 h in a medium given for Fig. 1, but with 5 mM $[K]_o$. Net K influx (^iM_K) was obtained from increases in $[K]_c$. Net effluxes of Na ($^oM_{Na}$) and Li ($^oM_{Li}$) were obtained from increases in the $[Na]_o$ and $[Li]_o$ of the incubation medium, initially free of both Na and Li. M^P represents active fluxes. The fluxes were determined in quintuplicate in seven separate experiments; values are means ± S.E. (n = 7). The hematocrits were approx. 20%. Due to the presence of a small but measurable net Na efflux in the Li cells, the Na and Li effluxes were combined in calculating the coupling ratio in these cells. The coupling ratios are given as the means of the ratios from seven experiments, not the ratio of the means of the fluxes. Both ratios (Na cells and Li cells) are significantly different from 1.0 ($P < 0.05$).

	Initial cellular concentrations (mmol/l cells)			
	[K]	[Na]	[Li]	[choline]
Na cells	4.4	19.9	—	~85
Li cells	4.0	1.0	25.3	~85

	Net active fluxes (mmol/l cells per h)			
	$^iM_K^P$	$^oM_{Na}^P$	$^oM_{Li}^P$	Coupling ratios
Na cells	3.96 ± 0.26	5.55 ± 0.56	—	Na:K 1.46 ± 0.12
Li cells	0.73 ± 0.12	0.08 ± 0.02	0.89 ± 0.18	(Li + Na):K 1.50 ± 0.22

TABLE II

EFFECT OF RESIDUAL BOUND Na ON K INFLUX ACTIVATED BY Li_c

Results are means from six identical experiments, each performed in duplicate. Two aliquots of cells, A and B, were prepared with two levels of residual $[Na]_c$, that in A higher than in B, as described in Materials and Methods. Each aliquot was then divided into two, one of which was made to contain a high $[Li]_c$, approx. 55 mmol/l. Shown below are the means of $[Na]_c$ for aliquots A and B and the mean of the ratios (±S.E., n = 6). (B/A is the mean of the ratios and not the ratio of the means). Also shown are active K influxes, ${}^{i}M_K^P$, for A and B and the mean of their ratios (±S.E., n = 6).

Cells	$[Na]_c$ (mmol/l cells)			${}^{i}M_K^P$ (mmol/l cells per h)		
	A	*B*	*B/A*	*A*	*B*	*B/A*
Li	0.79	0.45	0.74 ± 0.11	0.84	0.93	1.03 ± 0.12
Li-free	0.56	0.41	0.73 ± 0.03	0.08	0.05	1.31 ± 0.70

net Li efflux in determining the coupling ratio. A mean ratio of 1.50 is obtained, which is indistinguishable from 1.46 found for Na : K. Both ratios were significantly greater than unity.

Effect of residual bound Na_c on Li-activated K influx

When cells are prepared in PCMBS solutions with no added NaCl, Na_c is routinely 0.5—1.0 mmol/l cells. This residual Na, apparently bound, does not activate K influx [6]. Nevertheless, it was suggested that it might be required for activation of K influx by Li_c [8]. In an attempt to test this suggestion, cells were prepared with two different levels of residual Na. The cells were also made to contain Li, and the active K influx was measured. Li-free cells were prepared at the same time with two levels of residual Na_c. Table II shows the results of six such experiments. The two levels of Na_c in the Li-cells were 0.79 mmol/l cells (aliquot A) and 0.45 mmol/l (aliquot B). The mean of the ratios (not the ratio of the means) of Na_c, B/A, was 0.74. The rates of active K influx were about the same in aliquots A and B (mean of ratios, B/A, = 1.03). In the Li-free cells with about the same levels of Na_c there was negligible active K influx. From the results in Table II, it appears that the residual bound Na has no effect on the activation of K influx by Li_c, though the range of concentrations of bound Na we were able to achieve was not great.

Discussion

This study was undertaken to test the hypothesis that Li interacts with the internal aspect of the (Na,K)-pump as congener of Na. The results support the hypothesis in the following ways:

(i) Na and Li compete for the same sites on the intracellular aspect of the pump, and each species of ion stimulates K influx promoted by low concentrations of the other cation.

(ii) The Hill coefficients for Na-promoted and Li-promoted K influx are both greater than unity, and are about the same (approx. 1.5), indicating multiple sites and the same number of sites per pump for Na and Li. Plotting the kinetic data according to Eqn. 4 led to the same conclusion, that Li interacts with the Na-loading sites, and with all of them.

(iii) The stoichiometry of coupling between K influx and both Na efflux and Li (+Na) efflux is similar, and close to the established ratio of 3 Na : 2 K.

(iv) Cellular K inhibits the Li-activated K influx while cellular Na promotes it, indicating that the interactions of Na_c and K_c with the Li-activation sites are qualitatively different.

(v) Finally, although measurable residual Na remains in the cells, it appears to play no role in the activation of the pump by cellular Li.

Lithium is an effective therapeutic agent in the treatment of some patients with affective disorders (see Refs. 21 and 22 for references), and there have been attempts to implicate differences in cation transport in red cells between responders to Li therapy and non-responders [23—26]. The transport system involved is an ouabain-insensitive Na-Li exchange, apparently independent of the (Na,K)-pump [27,28], though a slightly lower activity of the (Na,K)-pump has been reported for patients with affective disorders than for controls [29]. Interestingly, Li therapy seems to result in stimulation (18%) of both the (Na,K)-pump and ($Na^+ + K^+$)-ATPase of red cells [30], but this result is difficult to interpret.

Given the therapeutic serum concentration of Li (less than 1.2 mM; 1.5 mM is often toxic), the concentrations of Li in red cells of patients (generally less than 1.0 mmol/l), and the low affinity of the pump for Li either inside or outside the cells compared to the affinities of the normal substrates, it is unlikely that the (Na,K)-pump plays more than a minor role in the distribution of Li between plasma and red cells in vivo.

In contrast, active efflux of Li has been claimed from human red cells with both internal and external cation concentrations approximating those in vivo [31]. However, these authors were not able to exclude the ouabain-insensitive Na-Li exchange as the mechanism responsible for their Li efflux. An activation energy of 3 kJ/mol and differing rate constants for influx and efflux of Li were invoked as the evidence for active transport. But the exchange reaction can be expected to have a high activation energy [32] and the Na-Li exchange mechanism has transmembrane asymmetries in its affinities for Na and Li [33]. Moreover, in resealed red cell ghosts, containing no ATP, countertransport of Li against its concentration gradient was demonstrated, driven by an Na concentration gradient [28]. Finally, an Li gradient can be developed with either orientation across the ghost membrane by appropriate manipulation of the Na gradient.

An efflux of Li against its electrochemical potential gradient has been observed in several other cell types, including frog muscle [34], cat spinal neurons [35], frog skin [36], and snail neurons [37], all with nearly physiological intracellular concentrations of Na and K. These fluxes of Li are probably not mediated by the (Na,K)-pump, but rather by an exchange or countertransport system as in red cells [38].

Sigmoid kinetics generally signify positive interaction between ligand binding sites. In deriving Eqn. 2 from Eqn. 3, it was assumed that there is no interaction between Na-loading sites, i.e., that the interaction coefficients, α, β and γ, are all unity. With this assumption, sigmoid kinetics would not be obtained unless it is also assumed that the enzyme ((Na,K)-pump) does not function (no Na transported) unless all substrate sites (Na-loading sites) are filled. Such an

assumption is not generally warranted for enzymes, but is justifiable for the (Na,K)-pump of red cells. The evidence is that the stoichiometry of the pump is fixed at 3 Na : 2 K, even under widely varying conditions, particularly with either low $[K]_o$ or $[Na]_c$ (Refs. 10—12; and Dunham, P.B., unpublished results).

The interaction of Li with the (Na,K)-pump as a congener of both Na and K has important implications for the mechanism, as yet unknown, of selective binding of alkali metal ions to the two surfaces of the pump. At the outer surface of the pump of human red cells the sequence of affinities is Rb > K > Cs > Li as determined from the activation of Na efflux [2] or the coactivation of K influx [5]. This sequence corresponds to series III of Eisenman (with Na omitted) (Refs. 39, 40; see also Ref. 41).

The data available for the sequence of affinities at the intracellular aspect of the pump are less explicit; Cavieres [42], using the results of Maizels [3], obtained the following affinity sequences for Na-loading sites of human red cells: Na > K, Rb > Cs > Li, which is compatible with series VII of Eisenman if K > Rb [40].

These considerations suggest a difference in the nature of the cation binding sites at the two surfaces of the pump. But there is another important consideration, and that is the consequence of the binding of the ions. As determined by the relative effectiveness in inhibiting Na efflux, K_c has a much higher affinity than Li_c for the Na-loading sites. However, the inhibition by K is 'dead end'; efflux of K through the (Na,K)-pump apparently can take place only by K-K exchange (with a $K_{1/2}$ approx. 9 mM; Ref. 43) which is a reversal of the K entry step, and does not represent K substituting for Na in the normal forward exchange process. The pump can also mediate K efflux by a reversal of the entire process [7], but the apparent affinity for K in activating reversal is very low ($K_{1/2}$ approx. 300 mM; Ref. 44). So K inhibits Na efflux with relatively high affinity and acts competitively, i.e., interacts directly with the Na-loading sites, but inhibition of Na efflux is the only consequence of the binding of K to those sites; K is not itself then translocated outward. Li, on the other hand, binds with relatively low affinity to the Na-loading sites, as judged by inhibition of Na efflux [3] and activation of K influx (Ref. 6 and present work). Nevertheless, consequent to the (low affinity) binding of Li to the Na-loading sites (where Li interacts competitively with Na), Li is itself transported outward by the pump working in its normal forward exchange mode. There is no clear explanation for these phenomena indicated by comparing the binding of K and Li to intracellular loading sites: K binds with high affinity but is not translocated; Li binds with low affinity but is translocated. But perhaps caution should be used in attempting to draw conclusions about mechanisms of selectivity from affinity sequences for just one property of a portion of a mechanism as complex as the (Na,K)-pump.

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