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CELLULAR UPTAKE OF LITHIUM VIA AMINO ACID TRANSPORT SYSTEM A

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Summary

We now add to the agencies by which cells take up lithium the process of cotransport with neutral amino acids via System A. In the Ehrlich cell various natural and synthetic amino acids, depending on their structure, can cause substantial accelerations of Li^+ uptake over a considerable range of levels of Na^+, Li^+ and H^+. Half the maximal augmentation of uptake, namely 1.2 mequiv. Li/kg cell water per 15 min, was obtained for 5.4 mM alanine in a double-reciprocal plot. Alanine also stimulated the exodus of Li^+ from the Ehrlich cell. The human red blood cell, lacking System A as it does, becomes an imperfect model for studying cellular uptake of Li^+. Until the Li^+ dependence of amino acid uptake in the reticulocyte is known, reticulocytosis can be suspected of contributing to the interpersonal variations seen in Li^+-for-Na^+ exchange.

Because lithium is extensively used in treating manic depressive illness, the mode of its uptake by cells receives considerable interest, especially the possibility of interpersonal differences. The human red blood cell has served as a model cell for such studies. A mode of uptake [1—3] and exodus [2,3] sensitive to ouabain is attributed to the Na^+/K^+ pump. One ouabain-insensitive mode of migration [2,3] which shows interpersonal differences [4] is attributed to an exchange diffusion with Na^+; another ouabain-insensitive component is sensitive to inhibitors of anion movement and is considered to be a leak [2,3].

It occurred to us that this choice for the mature red blood cell as the model cell might cause another mode of Li^+ migration to be neglected, namely

Abbreviation: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-ethane sulfonic acid.

co-transport with neutral amino acids via transport System A [5,6] for which either Na^+ or Li^+ may serve as cosubstrate, the amino acid structure determining which alkali ion is preferred and to what extent [7]. The mature red blood cell of man and some other mammalian species lacks SystemA and other alkali-ion-dependent transport systems for amino acids.

Our present results show for the Ehrlich ascites tumor cell that the presence of System A allows uptake and exodus of Li^+ by exchange for Na^+ along with any of a number of neutral amino acids. Since System A is ubiquitously present in cells, this contribution to Li^+ uptake needs to be taken into account.

Ehrlich ascites tumor cells, propagated and handled as described elsewhere [8—11] were incubated in a modified Ringer medium, usually containing 2 mM Li^+, 10 mM Na^+, 130 mM choline and 1 mM ouabain, buffered with *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid, (HEPES), at pH 7.0 except where indicated otherwise. In manipulating the levels of Na^+, Li^+, and choline their total was kept unchanged. The uptake of Li^+, usually during 15 min at 37°C, was terminated by dilution with ice-cold medium, followed by brief centrifugation. Extracts of the cells were analyzed for Li^+ by flame photometry. When a neutral amino acid was added, the choline chloride content was lowered correspondingly to maintain an unchanged osmotic pressure. To study effects on Li^+ exodus, the cells were first incubated 30 min in 5 mM Li^+, at which time the apparent concentration of Li^+ in the cell water was 5.2 mM.

The effects of 20 mM levels of each of four System A substrates on the uptake of Li^+ by the Ehrlich cells during 15 and 45 min are shown in Fig. 1. Serine and homoserine were about as effective as alanine and *S*-methylcysteine, whereas glycine, threonine, and cycloleucine (1-aminocyclopentanecarboxylic acid) were about as effective as methionine and proline. Small

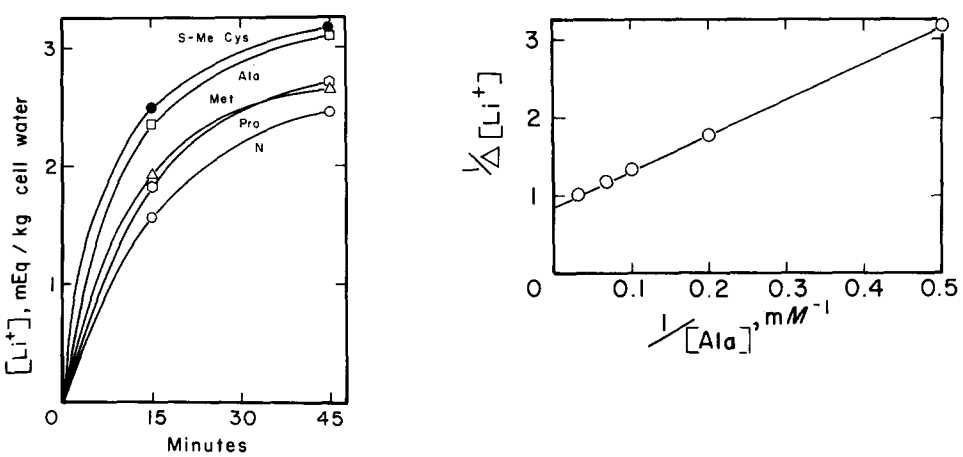

Fig. 1. Effect of each of four L-amino acids at 20 mM on Li^+ uptake by the Ehrlich cell, $[Li^+]$ = 2 mM. S-Me Cys, *S*-methylcysteine; Ala, alanine; Met, methionine; Pro, proline; N, no amino acid.

Fig. 2. Concentration dependence of stimulation of Li^+ uptake during 15 min by L-alanine.

and equivocal effects were seen with 2-(methylamino)-isobutyric acid and 4-aminobutyric acid. Examination of Fig. 3 in ref. 7 will show that the intensity of these effects are positively correlated with the Li^+ uptake produced by high levels of each of these α-amino acids in that study, and negatively correlated with the concentration of Li^+ required to cause a half-maximal uptake of the amino acid in the absence of added Na^+.

Fig. 2 shows that the rate of uptake of Li^+ produced by various alanine levels corresponds to the Michaelis-Menten equation, a half-maximal stimulation occurring at 5.4 mM alanine, and 1.2 mequiv. Li/kg cell water per 15 min being the maximal uptake obtained with alanine.

The increasing rate of Li^+ uptake produced by 20 mM serine, as a function of the external Li^+ concentration at $[Na^+]$ = 10 mM was determined (Fig. 3) and Fig. 4 shows the effect on the stimulation at 2 mM Li^+ produced by varying $[Na^+]$ from 0 to 100 mM, with choline replacement.

The effect of serine was obtained from pH 6.6 to 7.8 (Fig. 5); the lower, difference curve corresponds approximately to the pH profile obtained for the Na^+-dependent System A uptake.

External alanine at 20 mM stimulated the loss of lithium previously introduced into Ehrlich cells (Table I), provided that Na^+ was included in the medium.

The various aspects shown here for amino acid effects on Li^+ migration correspond to the predictions for exchange of Li^+ for Na^+ by cotransport by way of System A. These aspects include a dependence of the intensity of the effect on amino acid structure. Considering that SystemA has been found to make a major contribution to neutral amino acid uptake in virtually all cells of the animal organism tested except red blood cells, it became undesirable to place total confidence on the red blood cell as a model for the study of cellular uptake of Li^+ in patients. Because the presence of a

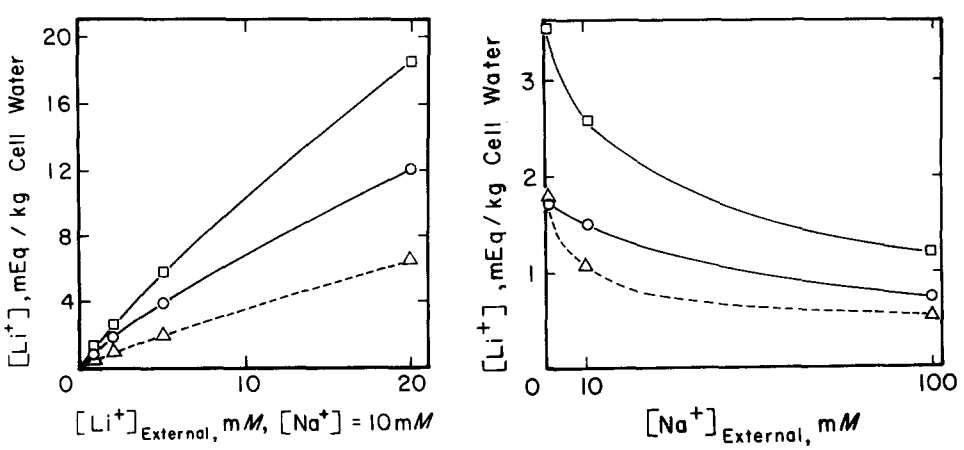

Fig. 3. Effect of Li^+ concentration on stimulation of Li^+ uptake during 15 min by 20 mM serine, $[Na^+]$ = 10 mM. □——□, 20 mM serine; ○——○, no amino acid; △- - - - -△, difference.

Fig. 4. Effect of Na^+ concentration on stimulation of Li^+ uptake during 15 min by 20 mM serine at 2 mM Li^+. Symbols as in Fig. 3.

TABLE I

Li^+ RETAINED IN EHRLICH CELLS AFTER 5 MIN IN OUABAIN-CONTAINING, pH 7 HEPES BUFFER, WITH AND WITHOUT ALANINE PRESENT

Prior loading 30 min in 5 mM Li^+ had brought the cells to 5.2 mequiv./kg cell water.

Medium	Residual Li^+ (mequiv./kg cell water)	Extra Li^+ retained
$[Na^+]$ = 100 mM		
No alanine	1.60	+50%
20 mM alanine	1.07	
$[Na^+]$ = 0 (choline)		
No alanine	1.14	+ 5%
20 mM alanine	1.08	

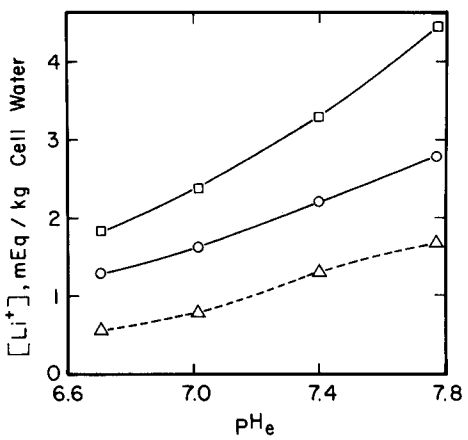

Fig. 5. Effect of pH on stimulation of Li^+ uptake during 15 min by 20 mM serine. Symbols as in Fig. 3.

system transporting lithium with amino acids has not yet been formally excluded for the reticulocyte [12], as it has in the pigeon erythrocyte [13], reticulocytosis remains a possible factor in accounting for interpersonal differences in lithium uptake by red cell populations. The Na^+-dependent Li^+ countertransport varied by 5-fold among 39 apparently healthy male volunteers [4].

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