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Li^+ stimulation of ouabain-sensitive respiration and (Na^+-K^+)-ATPase of kidney cortex of ground squirrels

There is a striking and well-known correspondence between the ability of K^+ and Na^+ to stimulate membrane "transport" ATPase[1,2] and the ability of those same ions to stimulate their own transport[1,2]. Inevitably, this close correspondence has been interpreted to mean not only that transport and ATPase are coupled activities, but also that the ions which stimulate the ATPase are the same ones that are being transported (for example see refs. 3 and 4). Although other ions such as Rb^+, Cs^+, NH_4^+, and Li^+ can in many cases substitute for K^+ in stimulating ATPase, few attempts have been made to compare the kinetics for activation of the enzyme by these ions with that of their own transport. Li^+ is of particular interest because in human erythrocytes it can substitute either for internal Na^+ or external K^+ in stimulating the enzyme[5]. In lactose-treated erythrocytes, however, there is only a slight active uptake of Li^+ and no active extrusion[6]. (Only net movements of Li^+ can be studied since the radioactive isotopes of Li^+ are all very short-lived.)

In the course of searching for a suitable inert substitute for Na^+ in incubation media for kidney slices from ground squirrels (see ref. 7) we found respiration of slices was inhibited by ouabain in media with Li^+ as the main cation just as it is in media with Na^+ as the principal cation. Kidney slices seemed a very useful preparation for comparing the effects of Li^+ on ouabain-sensitive ATPase and cation transport, since in this tissue (in contrast to erythrocytes) the net movement of ions are rapid, the tissue is rich in (Na^+-K^+)-ATPase and the rate of respiration of the intact cell is thought to reflect the (Na^+-K^+)-ATPase activity.

Slices of kidney cortex from ground squirrel were made freehand as described elsewhere[8,9]. K^+ was leached from slices in glucose-free media with nitrogen as the gas phase and either 140 mM NaCl or 140 mM LiCl as the principal constituent. Subsequent incubation was in similar media with glucose and oxygen. Uptake of oxygen by slices was measured in Warburg manometers. Ion contents of slices following incubation were determined on acid digests of the dried tissue by flame photometry. (Na^+-K^+)-ATPase activity was determined in the nuclear mitochondrial fraction of kidney cortex of ground squirrels as described elsewhere[10,11].

When slices were leached in LiCl so that they became loaded with Li^+ and depleted of K^+ and were then incubated in Li^+ medium; the Q_{O_2} was 14.7 μl O_2/mg dry wt. per h compared with 16.6 in Na^+-loaded slices, a difference which was not statistically significant (Table I). Ouabain inhibited respiration to the same extent in slices incubated in Li^+ medium as in the slices incubated in Na^+ medium.

When slices are incubated in Na^+-rich medium which initially contains no K^+, respiration is lower than in media with 4–6 mM K^+ (ref. 7, Table I). When kidney cortex slices from ground squirrel were incubated in K^+-free medium with 4–6 mM Li^+, however, respiration was not significantly less than that in control media with K^+ (Table I). Again, this stimulation by Li^+ was inhibited by ouabain.

These effects of Li^+ on respiration of kidney may be peculiar to ground squirrels, since they have not been observed in rabbit[12].

In general, the effects of Na^+ and K^+ on transport are 2-fold. Obviously, each

TABLE I

EFFECT OF Li^+ ON RESPIRATION OF SLICES OF KIDNEY CORTEX OF GROUND SQUIRRELS

Mean ± S.E. is indicated.

Conditions of incubation	(*N*)	Q_{O_2} (*µl O_2/mg dry wt. per h*)
A. Li^+ substituting for Na^+		
(All slices incubated with 4–6 mM K^+ in medium)		
Slices leached in Na^+, incubated in 140 mM Na^+	(16)	16.6 ± 0.5
+ 25–125 µM ouabain	(16)	10.4 ± 0.4
Slices leached in Li^+, incubated in 140 mM Li^+	(16)	14.7 ± 0.8
+ 25–125 µM ouabain	(16)	10.7 ± 0.5
B. Li^+ substituting for K^+		
(All slices leached in 140 mM Na^+, incubated in 140 mM Na^+)		
With no added K^+	(16)	12.7 ± 0.5
With 4–6 mM K^+ in medium	(16)	15.4 ± 0.6
+ 25–125 µM ouabain	(11)	9.0 ± 0.5
With 4–6 mM Li^+ in medium	(16)	14.7 ± 0.3
+ 25–125 µM ouabain	(12)	7.9 ± 0.7

ion is necessary for its own transport, and in addition each ion stimulates transport in the opposite direction of the other ion. Thus, in kidney slices, as in other tissues, extrusion of Na^+ is necessary for K^+ uptake[3,9], and removal of K^+ from the medium reduces the extrusion of Na^+ (ref. 8). In the present study when Li^+ was substituted for Na^+ during incubation, there was no net uptake of K^+. When Li^+ was substituted for K^+ in a Na^+ medium, the extrusion of Na^+ was quite variable but was generally less than half that in media with K^+ and in some cases was negligible.

Changes in tissue Li^+ are more difficult to determine owing to the lower sensitivity of the flame photometer to Li^+. To date the results indicate that there is no net extrusion of Li^+ from slices loaded with Li^+ and incubated in a Li^+ medium. There may be slight uptake of Li^+ when Li^+ is substituted for external K^+, but if so it is only a small fraction of the uptake of K^+ that occurs *in vitro* under comparable conditions of 100 to 200 µequiv/g dry wt. (ref. 9).

To establish whether the effects of Li^+ on respiration could be interpreted as reflecting an influence of (Na^+-K^+)-ATPase, the enzymatic activity was determined directly. The results shown in Fig. 1a indicated that with 20 mM K^+ a concentration of 50 mM Li^+ produced about 31% of the total stimulation achieved with 100 mM Na^+ and 20 mM K^+ (*i.e.* above a baseline with only 5 mM Mg^{2+} and no Na^+ or K^+ present). Activation caused by 50 mM Li^+ was statistically significant. When 100 mM Na^+ and no K^+ were present, 30 mM Li^+ produced 55% of maximal stimulation (Fig. 1b). With 20 mM K^+ and 100 mM Li^+ or with 100 mM Na^+ and 50 mM Li^+, ouabain reduced the activity to a level not significantly different from baseline.

Since Li^+ could substitute to some extent for either Na^+ or K^+ it was of interest to see whether ATPase activity could be stimulated by Li^+ alone. In Fig. 1c it can be seen that no concentration of Li^+ between 0 and 125 mM caused a significant rise in ATPase activity in the absence of both Na^+ and K^+. Addition of ouabain to the medium with 120 mM Li^+ did not further decrease activity.

The results show that, as in human erythrocytes, Li^+ can substitute for Na^+ or K^+ in stimulating the ouabain-sensitive (Na^+-K^+)-ATPase activity and the ouabain-sensitive part of respiration of cells of kidney cortex of ground squirrels. Net transport of Li^+, either in or out, is probably negligible. Coupled transport of the counter ion is either abolished (K^+ uptake in presence of Li^+) or reduced to a greater extent than stimulation of respiration and ATPase (Na^+ extrusion with low concentrations of Li^+ in the medium). While there may be several explanations of these results, they nevertheless raise the possibility that stimulation of transport related ATPase can be uncoupled from actual translocation of ions.

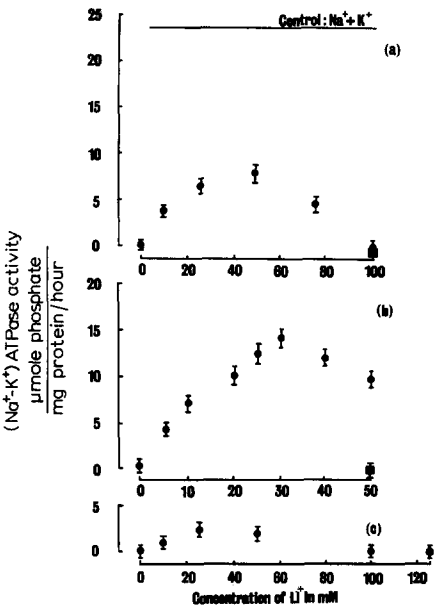

Fig. 1. Stimulation of membrane ATPase activity by Li^+. All experiments were performed at 37°, pH 8 for 3 min with 5 mM ATP, 5 mM Mg^{2+} and varying concentrations of cations. ●, values with varying Li^+; ■, values with 125 μM ouabain added to samples with Li^+; horizontal line, ATPase activity in presence of 20 mM K^+, 100 mM Na^+; baseline, ATPase in medium with Mg^{2+} alone and no alkali metals. Number of cases is 8 in all instances; means ± S.E. are indicated. (a) Li^+ substituting for Na^+. Media contained 20 mM K^+. (b) Li^+ substituting for K^+. Media contained 100 mM Na^+. (c) Li^+ substituting for both Na^+ and K^+.

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Subcellular distribution of thiamine pyrophosphokinase activity in rat liver and erythrocytes

The transport of thiamine across the cell surface membrane is of basic importance for the study of thiamine metabolism in mammalian tissues. Until now only little is known about the mechanism of uptake of thiamine into the cell.

Since thiamine pyrophosphokinase was found exclusively in the membrane fraction (spheroplasts) of *Escherichia coli*, the participation of this enzyme in the transport of thiamine across the membrane barrier has been postulated[1].

The aim of the present study was to check whether this hypothesis was applicable to mammalian cells. Therefore the distribution of thiamine pyrophosphokinase in the subcellular fractions of rat liver and rat erythrocytes was investigated. The results provide evidence that thiamine pyrophosphokinase is localized in the soluble phase of both types of cells. A preliminary report on the fractionation of erythrocytes has been published[2].

Male albino Sprague-Dawley rats of 220–280 g weight, raised on a commercial stock diet, were decapitated after they had been starved for 12 h.

Nucleotides and phosphorylated sugars were obtained from C. F. Boehringer & Soehne, 68 Mannheim, Germany. Bovine serum albumin was a product of the Behringwerke, 355 Marburg, Germany and Dextran 150 of Pharmacia, Uppsala, Sweden. Thiamine(thiazole-2-^{14}C)hydrochloride was purchased from the Radiochemical Centre, Amersham, England.

Rat liver cell fractionation was carried out according to the method of DE DUVE *et al.*[3]. For the fractionation of erythrocytes, blood was collected in heparinized glass vessels. The white blood cells were removed almost completely with the "buffy layer". The erythrocytes were washed twice with 0.15 M NaCl and hemolysed by addition of 4 vol. of distilled water. The suspension was frozen and thawed twice. Hemolysate supernatant was obtained by centrifugation of the hemolysate at 105000 × *g* for 1 h. The erythrocyte membrane fraction (ghosts) was prepared according to the method of REGA *et al.*[4]. Leucocytes were obtained by the procedure of HOLLDORF *et al.*[5].