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THE DIFFERENTIAL EFFECT OF Li^+ ON MICROSOMAL ATPase IN CORTEX, MEDULLA AND PAPILLA OF THE RAT KIDNEY

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SUMMARY

1. The effect of Li^+ on microsomal ATPase was studied in the cortex, medulla and papilla of the rat kidney.

2. At Li^+ concentrations, *in vitro*, of 5–50 mM no significant effect on Mg^{2+}-ATPase activity in any part of the kidney was observed. At concentrations of 100–150 mM a slight inhbition of the activity of this enzyme was found.

3. At *in vitro* Li^+ concentrations of 5–50 mM $(Na^+ + K^+)$-dependent ATPase activity was significantly increased in the kidney medulla. In the cortex and papilla an increased activity of $(Na^+ + K^+)$-dependent ATPase was found only at 10 mM LiCl. At a concentration of 150 mM Li^+ inhibited $(Na^+ + K^+)$-dependent activity in the medulla and cortex.

4. *In vivo* administration of LiCl at a dose of 2 mequiv/kg daily for 14 days caused a significant increase in $(Na^+ + K^+)$-dependent ATPase in the medulla and papilla but not in the cortex of the kidney.

5. The significance of these findings with respect to the clinical effect of Li^+ is discussed.

6. The experiments reported demonstrate a selective effect of Li^+ on $(Na^+ + K^+)$-dependent ATPase in the kidney medulla as compared to this enzyme in the kidney cortex.

INTRODUCTION

Lithium salts have been introduced successfully in the treatment of manic depressive disease[1]. With the increased clinical use of Li^+ side effects are continuously recognized and reported[2]. Among the "side effects", there is also a diabetes insipidus-like syndrome expressed by increased intake of water and excretion of large volumes of urine[3,4]. Since the doses of Li^+ used clinically result in plasma levels of less than 2 mequiv/l one cannot ascribe the effect of Li^+ to phenomena observed *in vitro* where various parameters of cell function were studied when Na^+ was completely replaced by Li^+ (over 100 mM)[5,6]. Administration of Li^+ to animals at doses similar to those used clinically is also followed by a diabetes insipidus-like state[3,7].

In addition to increased urine volume, Li^+ also augments Na^+ loss, at least in the initial phase of treatment[8]. Therefore, Li^+ seems to affect also Na^+ reabsorption

in the kidney in addition to the decrease in concentrating ability of the kidney. It has been reported that Li^+ can replace, *in vitro*, either Na^+ or K^+ in the activation of $(Na^+ + K^+)$-dependent microsomal ATPase[9,10] (ATP phosphohydrolase, EC 3.6.1.3). However, replacement of Na^+ or K^+ cannot be the mechanism of Li^+ action *in vivo* since the tissue concentrations of Li^+ achieved at therapeutic doses are negligible as compared to the concentrations of Na^+ and K^+[11].

We, therefore, considered of interest to study the effect of various concentrations of lithium, *in vitro*, on the activity of microsomal ATPase from the kidney. Furthermore, since Na^+ transport in the kidney cortex probably serves a different function (sodium balance[12]) while Na^+ transport in the medulla and papilla serve mainly the function of urine concentration[13], it seemed of interest to compare the effect of Li^+ on microsomal ATPase in the different regions of the kidney.

Finally, the effect of *in vivo* administration of Li^+ on microsomal ATPase activity in the different regions of the kidney was also studied.

MATERIALS AND METHODS

Male rats of the Hebrew University strain were used throughout the experiments. Weight 250–300 g. The rats were sacrificed by dislocation of the neck and the kidneys were removed immediately, weighed and kept on ice. The preparation was then carried on as previously described[14]. Each kidney was cut sagittally; the papilla (the pale portion projecting into the pelvis) was cut and separated; then the medulla (dark red) containing the thick ascending loops of Henle, was separated from the cortex and finally the outer part of the cortex was cut. The intermediate zone, including the inner portion of the cortex and outer portion of the medulla, where the exact limit is not very clear, was discarded. The three regions of the kidney were homogenized in 10 vol. of cold 0.25 M sucrose containing 2 mM EDTA, 25 mM Tris buffer (pH 7.2) and 0.07% deoxycholate. The homogenates were centrifuged at $1000 \times g$ for 10 min and the supernatant was separated and spun at $10000 \times g$ for 10 min. The supernatant was aspirated and centrifuged at $100000 \times g$ for 30 min. All centrifugations were carried out at 4 °C. The final sediment was resuspended in 0.25 M sucrose and served as the enzyme preparation.

Assay of ATPase was performed as previously described[15]. The incubation mixture consisted of 100 mM NaCl, 10 mM KCl, 4 mM $MgCl_2$, 4 mM ATP, 33 mM Tris buffer (pH 7.5) and 0.05–0.2 ml of the enzyme preparation. Total volume of incubation was 3.0 ml.

Each preparation was incubated with and without the addition of ouabain at a final concentration of $1 \cdot 10^{-3}$ M. Th edifference between the two is $(Na^+ + K^+)$-dependent ATPase while the activity in the presence of ouabain is Mg^{2+}-ATPase.

Incubation was carried out at 37 °C for 5–20 min, depending on the activity in the various regions of the kidney. Inorganic phosphate liberated was assayed according to the method of Baginski and Zak[16]. Protein was assayed according to the method of Lowry *et al.*[17].

Li^+ was added to the incubation mixture as LiCl in final concentration of 5–150 mM.

In vivo administration of Li^+ was carried out at a dose of 2 mequiv/kg, divided into two daily doses, injected intraperitoneally at 8 a.m. and at 4 p.m. The administra-

tion of LiCl was continued for 14 days. 18 h after the last injection, the rats were sacrificed, as described above, and the microsomal ATPase from the different regions of the kidney was prepared. The assay of ATPase activity was carried out in the absence of Li^+.

RESULTS

Fig. 1 shows the effect of addition of LiCl on microsomal ATPase activity in the cortex, medulla and papilla of the rat kidney. Mg^{2+}-ATPase activity was unaffected by Li^+ at concentrations of 5–50 mM. At 100 and 150 mM LiCl the Mg^{2+}-ATPase activity was significantly inhibited in all parts of the kidney (the inhibition ranging from 20–35% in the different parts of the kidney).

$(Na^+ + K^+)$-dependent ATPase was affected by Li^+ differently according to the region of the kidney. At Li^+ concentrations of 5–50 mM, $(Na^+ + K^+)$-dependent ATPase was significantly enhanced in the papilla and cortex only at

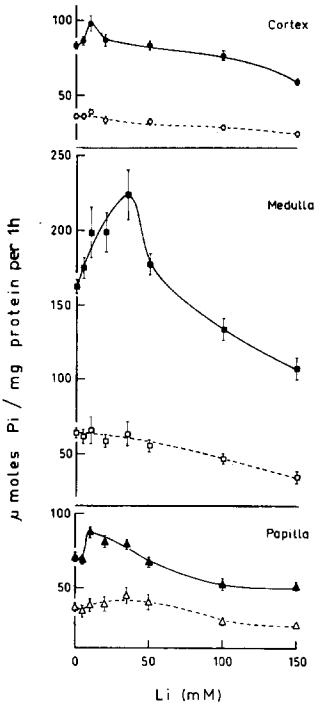

Fig. 1. Effect of Li^+ on microsomal ATPase activity in different parts of the rat kidney. Broken lines, Mg^{2+}-ATPase activity; continuous lines, total ATPase activity; the difference between the broken and continuous lines, $(Na^+ + K^+)$-dependent ATPase. Upper panel, activity in the cortex ($n = 16$); middle panel, activity in the medulla ($n = 12$); lower panel, activity in the papilla ($n = 10$). Vertical bars denote S.E. Mg^{2+}-ATPase: decreased activity at 100 and 150 mM LiCl compared to controls (no LiCl), significant at $P < 0.01$ in the cortex, medulla and papilla. $(Na^+ + K^+)$-dependent ATPase: in the cortex, increase at 10 mM LiCl ($P < 0.05$) and decrease at 150 mM LiCl ($P < 0.01$); in the medulla, increase at 10 mM LiCl ($P < 0.02$), at 20 and 35 mM LiCl ($P < 0.01$) and at 50 mM LiCl ($P < 0.05$), decreased activity at 150 mM LiCl ($P < 0.01$); in the papilla, increased activity at 10 mM LiCl ($P < 0.01$).

10 mM, whereas in the medulla the enzyme activity was enhanced at all Li^+ concentrations up to and including 50 mM. The maximal activation of the enzyme in the medulla was over 60% and even at 50 mM LiCl there was still 24% enhancement as compared to no significant change in the cortex and inhibition of 21% in the papilla at this concentration of Li^+. At a concentration of 150 mM LiCl the ($Na^+ + K^+$)-dependent ATPase from both cortex and medulla were inhibited, however the inhibition was larger in the medulla (36%) than in the cortex (26%). No significant inhibition in the enzyme from the papilla was observed at the higher Li^+ concentrations.

Chronic Li^+ administration to rats caused increased urine volume and an increased Na^+ excretion as shown in Table I. The increased Na^+ excretion could be due to a reduced rate of Na^+ reabsorption along the nephron.

TABLE I

EFFECT OF Li^+ ON URINE VOLUME, OSMOLALITY AND SODIUM EXCRETION IN THE RAT

Results are given as mean ±S.E. *n*, number of animals. Data are for the fourteenth day of Li^+ treatment, *i.e.* one day preceding sacrifice of the animals. Li^+ was given intraperitoneally, 2 mequiv/kg per day, divided into two doses.

	Urine volume (*ml/kg × 24 h*)	*Urine osmolality* (*mosmoles/kg water*)	*Sodium excretion* (*mequiv/kg × 24 h*)
Control ($n=8$)	24.7 ± 6.0	1745 ± 189	4.29 ± 0.76
Li^+-treated ($n=8$)	139.7 ± 22.3	828 ± 89	9.03 ± 1.15
	$t=4.986$	$t=4.928$	$t=3.544$
	$P<0.001$	$P<0.001$	$P<0.005$

The effect of chronic Li^+ treatment on microsomal ATPase activity in the different parts of the kidney is shown in Fig. 2. The activity *in vitro* of both Mg^{2+}-ATPase and ($Na^+ + K^+$)-dependent ATPase in the kidney cortex was unchanged by Li^+ administration *in vivo*.

On the other hand, in the medulla and papilla microsomal ($Na^+ + K^+$)-dependent ATPase was significantly elevated (by 80% and 60%, respectively) while Mg^{2+}-ATPase was somewhat increased only in the kidney papilla after chronic Li^+ treatment. Li^+ treatment *in vivo*, therefore, increased selectively ($Na^+ + K^+$)-dependent ATPase in the kidney medulla and papilla but not in the cortex.

It is also noteworthy that the weight of the kidneys from the Li^+-treated rats was significantly higher than that of the control animals (1248±20, $n=28$, compared to 1171±21 mg/kidney, $n=28$, $t=2.625$, $P<0.01$).

DISCUSSION

The experiments reported here demonstrate that Li^+ can enhance ($Na^+ + K^+$)-dependent ATPase of the kidney medulla *in vitro*. Previously it has been reported

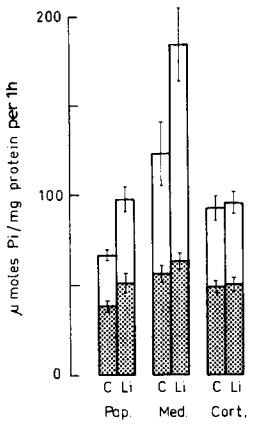

Fig. 2. Effect of Li^+ treatment *in vivo* on microsomal ATPase activity in different parts of the rat kidney. Rats were given 2 mequiv/kg daily of LiCl intraperitoneally for 14 days. The microsomes of the different parts of the kidney were then assayed for ATPase activity *in vitro* in the absence of Li^+. Dotted columns, Mg^{2+}-ATPase; white columns, $(Na^+ + K^+)$-dependent ATPase. Vertical bars, S.E. C, control; Li, Li^+-treated rats. Pap., papilla ($n = 10$); Med., medulla ($n = 16$); Cort., cortex ($n = 16$). No significant change in Mg^{2+}-ATPase in any part of the kidney. $(Na^+ + K^+)$-dependent ATPase, increased in papilla and medulla ($P < 0.05$).

that Li^+ can replace either Na^+ or K^+ in activating microsomal ATPase although the maximal activity obtained was less than that with Na^+ and K^+[9,10]. Our experiments demonstrate that Li^+ concentrations of 5–50 mM, when added to the incubation medium which already contains Na^+ and K^+ at the optimal concentrations further increase $(Na^+ + K^+)$-dependent ATPase in the medulla. Therefore, neither substitution for Na^+ nor for K^+ plays any role under these conditions. Furthermore, the simple addition of electrolyte cannot explain the increased activity of the enzyme since addition of excess Na^+ does not enhance the medullary ATPase activity in the rat kidney (unpublished observations).

At higher Li^+ concentrations (100 and 150 mM) microsomal ATPase activity was inhibited, especially the $(Na^+ + K^+)$-dependent ATPase. Inhibition of this enzymatic activity in the kidney medulla and papilla was previously observed in the guinea pig kidney with high concentrations of Na^+ and urea[14]. The inhibition may, therefore, be due to a high ionic or high osmotic concentration.

If Li^+ reaches *in vivo* concentrations in the kidney within the range which causes activation of $(Na^+ + K^+)$-dependent ATPase it is difficult to explain the natriuretic effect of Li^+ on this basis. On the other hand increased Na^+ absorption in the medulla (thick ascending limb of the loop of Henle) would increase the free water load at distal portions of the nephron and thus decrease the concentrating ability, which is already reduced by Li^+, due to inhibition of antidiuretic hormone by Li^+[18]. But this could still not provide an explanation for the increased Na^+ excretion induced by Li^+.

An alternative possibility is that Li^+ produces "uncoupling" of the Na^+ pump and the ATPase activity of the enzyme related to the pump, *i.e.* Li^+ could activate the ATPase while the energy derived from splitting of the ATP was not used for Na^+ transport but was wasted. Such "uncoupling" could be envisaged, *e.g.*, by con-

formational changes in the ATPase caused by Li^+. This possibility is now being further explored. Some support for this view can be found in the observations of Willis and Fang[10]. These authors reported that Li^+ stimulated the respiration of slices of cortex from the squirrel kidney but the uptake of K^+ and extrusion of Na^+ by these slices was inhibited in the presence of Li^+, thus showing a dissociation between energy production (by respiration) and energy utilisation for K^+ accumulation and Na^+ extrusion.

Another interesting facet of the experiments reported here is the selectivity or differentiation in the microsomal ATPase in the various regions of the kidney. We have previously reported that microsomal ATPase activity in the kidney medulla was correlated with the concentrating ability when compared in different species[13]. Administration of saline or water-deprivation decreased $(Na^+ + K^+)$-dependent ATPase selectively in the cortex[19]. In the experiments reported now Li^+ administered *in vivo* caused a selective increase of $(Na^+ + K^+)$-dependent ATPase in the medulla and papilla, but not in the kidney cortex.

The differentiation between microsomal ATPase in various parts of the kidney is also evident under *in vitro* conditions. Thus, we have previously reported that Na^+ and urea selectively inhibited $(Na^+ + K^+)$-dependent ATPase in the guinea-pig medulla and papilla[14]. In the present report a selective effect of Li^+ on rat medullary ATPase is shown. These observations may suggest that there are differences in this enzyme obtained from the various parts of the kidney. Such differences may be related to physiological functions of different parts of the nephron.

The increase of $(Na^+ + K^+)$-dependent ATPase in the kidney medulla and papilla after chronic Li^+ treatment *in vivo* may be the reason for the fact that the natriuresis induced by Li^+ is only temporary[8]. The increase did not reflect activation of the enzyme by Li^+ since it was found *in vitro* with an incubation medium containing only Na^+ and K^+ but no Li^+, and would thus suggest an increased amount of $(Na^+ + K^+)$-dependent ATPase in the microsomal fraction of the kidneys of the Li^+-treated animals. The increase of $(Na^+ + K^+)$-dependent ATPase may result in better Na^+ conservation, and, because it is restricted to the medulla and papilla only, may result in production of more "free water" thus decreasing the urine concentration, apart from any effect on antidiuretic hormone activity. This may be the reason for the lack of responsiveness to antidiuretic hormone of rats chronically treated with Li^+[20].

The increase of $(Na^+ + K^+)$-dependent ATPase in the microsomal fraction of the kidney medulla induced by Li^+ could have been mediated by aldosterone. Thus, we have recently demonstrated that Li^+ administration increased plasma renin level[21] and Murphy *et al.*[8] have found increased aldosterone levels in patients treated with Li^+. Release of renin, due to natriuresis induced by Li^+, may be the initial factor which, then, stimulates aldosterone secretion and the latter diminishes subsequently the natriuretic effect of Li^+ through induction of synthesis of $(Na^+ + K^+)$-dependent ATPase in the kidney medulla.

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