Effect of Lithium on Organic Ion Transport in Rat Kidney Cortical Slices

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Lithium is presently well-known as a pharmacological agent for use in the treatment of manic-depressive mental disorders. However, the renal effects of lithium compounds have been reviewed as side effects (1, 2). Previous studies reported the lithium-induced histological changes in proximal tubules as well as in the distal and collecting tubules in the kidney (3, 4). A recent report has indicated that lithium-induced damage was morphologically evident in connecting, collecting and distal tubules, but not observed in the proximal ones (5). Though further studies on the exact anatomic sites of lithium-induced renal damage are necessary, it would be interesting to obtain information about the biochemical effects of lithium salts on the physiological process in proximal tubules. It has already been reported that an administration of lithium to rats resulted in reduced function of the proximal tubules by affecting the transport of electrolytes (6). The secretion of p-aminohippurate (PAH) and tetraethylammonium (TEA) as representatives of organic acids and bases, respectively, is also one of important functions of proximal tubules in the kidney (7). It is probable that in vitro concentrative uptake of organic ions by renal slices reflects, at least qualitatively, in vivo tubular secretion (8, 9). This study was to investigate the effect of lithium on organic ion transport using kidney cortical slices.

Kidney cortical slices were prepared from male Sprague-Dawley rats as described previously (10), and the slice technique used in this study has also been described previously (11). To be brief, the slices were placed in an ice-cold medium containing 137 mM NaCl, 5.9 mM KCl, 1.5 mM CaCl₂, 1.2 mM MgCl₂, 11.5 mM glucose and 5.8 mM Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer, pH 7.4. Thereafter, the slices were incubated for 30 min at 37°C with a gas phase of 100% oxygen in 10 ml of incubation medium; its composition was identical to the medium described above, except that it further contained 0.074 mM PAH and 1% inulin, which was added to estimate the extracellular space of the slices. After incubation, the slices were homogenized with 10% trichloroacetic acid and then centrifuged. A sample of the medium was deproteinized by adding trichloroacetic acid. Supernatants obtained by centrifugation of the extracts of the slices and media samples were used for the spectrophotometric analyses of PAH and inulin. For the experiment of TEA accumulation in the slices, the incubation medium contained 0.1 mM TEA, 5 nCi/ml ¹⁴C-TEA (4.4 mCi/m mole, New England Nuclear) and 100 nCi/ml methoxy-³H-inulin (384 mCi/g, New England Nuclear) instead of PAH and inulin. Radioactivities of ³H and ¹⁴C in the slices digested in NaOH and media were measured after being mixed with scintillator as described previously (12). The accumulation of PAH and TEA was calculated as the ratio of the concentrations of these compounds in the intracellular fluid to those in the medium (S/M ratio). Statistical analyses were performed by Student’s t-test.

Table 1 shows the effects of lithium salts on PAH accumulation in the slices. At a concentration of 0.3 mM, Li₂CO₃ significantly inhibited the accumulation of PAH by 12.3%. There was no significant difference among the S/M ratios of PAH accumulation.

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decreased by \( \text{Li}_2\text{CO}_3 \) (1 mM), \( \text{LiCl} \) (2 mM) and \( \text{LiNO}_3 \) (2 mM). These data suggest that with respect to the anion form of the lithium salts, the relative inhibitory potency of lithium salts was almost in the same order as that for PAH accumulation when the same equivalents of lithium ions (2 mEq/L) were present in the medium.

As shown in Table 2, though TEA was avidly taken up by the slices, LiCl up to 5 mM had no effect on the accumulation of TEA in the slices.

Previous investigations speculated that lithium increased renal excretion of dicarboxylic acids by reducing renal tubular reabsorption (13, 14). Recently, Wright et al. demonstrated that lithium acted as a potent inhibitor of the Na\(^+\)-dependent uptake of a dicarboxylic acid, succinate, into renal luminal membrane vesicles, but did not inhibit Na\(^+\)-coupled glucose and amino acid transport into the vesicles (15). The present study indicated that lithium was a potent inhibitor of PAH transport in rat kidney cortical slices. PAH accumulation by slices is Na\(^+\)-dependent (16, 17), and the accumulation in slices reflects preponderantly the process localized at the antiluminal membranes (18, 19). Our study offered another demonstration for the effect of lithium on the Na\(^+\)-dependent membrane transport system in the kidney.

In contrast to the effect of lithium on PAH accumulation in the slices, TEA accumulation was unaffected by lithium, thus confirming the selective action of lithium on organic anion transport processes. The mechanism by which lithium inhibits PAH accumulation, however, remains to be elucidated. It could be speculated that lithium affects the pharmacokinetics of organic anionic drugs in rats by reducing their renal tubular secretion. This assumption is only suggestive based on the present in vitro results, but can be investigated further by in vivo experiments. Further study is in progress along this line.

### Table 1. Effects of lithium salts on PAH accumulation by kidney cortical slices

| Additions   | N | PAH accumulation (S/M) |
|-------------|---|------------------------|
| None        | 7 | 12.32±0.32             |
| \( \text{Li}_2\text{CO}_3 \) 0.3 mM | 5 | 10.81±0.38*            |
| \( \text{Li}_2\text{CO}_3 \) 1.0 mM | 5 | 7.64±0.57**            |
| LiCl        | 5 | 7.10±0.65**            |
| \( \text{LiNO}_3 \) 2.0 mM | 5 | 6.81±0.33**            |

The slices were incubated at 37°C for 30 min in the medium containing the indicated concentration of lithium salt. The pH value of the medium containing 1.0 mM \( \text{Li}_2\text{CO}_3 \) was higher than that of the "None" control medium (7.66 vs 7.40). There was no significant difference in PAH accumulation between both pH values of the media in the absence of \( \text{Li}_2\text{CO}_3 \). N: The number of experiments. S/M: Slice-to-medium concentration ratio of p-aminophenylphosphate (PAH). Data are expressed as the mean±S.E. *: Significantly different from the "None" control at \( P<0.02 \) and \( P<0.001 \), respectively.

### Table 2. Effect of LiCl on TEA accumulation by kidney cortical slices

| Additions | TEA accumulation (S/M) |
|-----------|------------------------|
| None      | 24.04±1.70             |
| LiCl 1.0 mM | 23.43±1.78     N.S. |
| LiCl 5.0 mM | 20.75±0.74     N.S. |

The slices were incubated at 37°C for 30 min in the medium containing the indicated concentration of LiCl. S/M: Slice-to-medium concentration ratio of tetraethylammonium (TEA). Data are expressed as the mean±S.E. of six experiments. N.S.: Not significant.
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