Evidence for in Vivo Effect of Lithium on p-Aminohippurate Transport in Rat Kidney, Preliminary Study

Kumi SUGIHARA, Akemi TACHIBANA and Munekazu GEMBA*
Department of Pharmacology, Osaka College of Pharmacy, Kawai, Matsubara, Osaka 580, Japan
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Abstract—We examined the effect of lithium on rat renal handling of p-aminohippurate (PAH) and accumulation of organic ions by rat kidney cortical slices. When infused intravenously with lithium at the rate of 0.13 mmoles/kg/min, decreased renal clearance of PAH as well as no significant changes in glomerular filtration rate and plasma PAH level was observed at the first clearance period during lithium infusion. As we expected, tubular secretion of PAH also was decreased significantly by the infusion of lithium. Therefore, it is suggested that the decrease in the clearance of PAH was due to the decrease in the tubular secretion of PAH. After four days of injections with lithium (4 mmoles/kg, i.p., once a day), a significant decrease in PAH accumulation in the slices was detected. No inhibition of tetraethylammonium accumulation was observed. Lithium pretreatment did not alter water content and extracellular space of the slices. The results suggest that lithium selectively inhibits the organic anion transport system in kidney with the in vivo treatment and follows our previous work in which we showed the in vitro effect of lithium on organic anion accumulation in the slices.

Lithium has been used in the treatment of manic-depressive psychosis; however, its effect on kidney function has been recognized (1, 2). The effect of lithium on p-aminohippurate (PAH) transport, one of the functions of proximal tubules in the kidney (3), was shown in our previous report (4), in which lithium decreased the accumulation of PAH, as a prototype for organic anions, in rat kidney cortical slices in vitro, whereas lithium had no effect on that of tetraethylammonium (TEA), as a prototype for organic cations, in the slices. Our previous work has cast doubt on a conclusion in a recent study reporting that the inhibitory effect of lithium on tubular PAH transport may be indirect via raising plasma 2-oxoglutarate, an inhibitor of PAH transport (5). In that study, the concentration of lithium in plasma was not determined concerning evaluation of the pharmacological suitability of the dose of lithium used.

In this paper, we examined the effect of lithium on renal handling of PAH in rats using a renal clearance technique. We also determined the accumulation of organic ions, PAH and TEA, in the slices prepared after the intraperitoneal administration of lithium to rats. In these experiments, we measured the lithium level in plasma. It is the purpose of the present experiment to evaluate whether these in vivo effects of lithium correspond to the previous in vitro data.

Materials and Methods
Renal clearance technique: Renal clearance was examined in male Sprague-Dawley rats, weighing 250–300 g, anesthetized with sodium pentobarbital (30 mg/kg, i.p.) according to the experimental schedule shown in Fig. 1. After the operative procedures, 200 mg of inulin per kg and 20 mg of PAH per kg were intravenously injected via a femoral vein as priming doses. Sustaining solution (0.5 mg of PAH/kg/min–8 mg of inulin/kg/min in 0.9% NaCl) was then
infused with an infusion pump at the rate of 0.085 ml per min per rat. Additional anesthetic was sometimes given intravenously in doses of 2.5 mg/rat/0.5 ml per one application when required during experiments. An equilibration period of about 100 min was permitted before the first clearance period. At each clearance period, urine was collected from a PE-10 catheter secured in the urinary bladder through a suprapubic incision at intervals of 15 min, and the urine volume was determined. Blood was collected at the mid-point of urine collection from a PE-50 catheter placed into the femoral artery. After two control clearance periods, lithium as the chloride salt was infused into the femoral vein with the sustaining solution at a dose of 0.13 mmoles/kg/min for assessing the effect of lithium on renal functions. Urine and plasma were analyzed for the concentration of PAH and inulin by the methods of Bratton and Marshall (6) and Roe et al. (7), respectively. Lithium in plasma was determined with an atomic absorption spectrophotometer (Hitachi 170-10). The glomerular filtration rate (GFR) was determined by measuring the clearance of inulin. Tubular secretion of PAH was determined by subtracting the ultrafiltered fraction of plasma PAH from PAH in urine. The ultrafilterable fraction of plasma PAH was assessed by measuring the unbound (protein-free) concentration of PAH by centrifugation of the plasma using a Centriflo membrane cone (CF-25, Amicon Corp., Lexington, MA). The data of the treatment periods (first and second clearance periods during infusion of lithium—third and fourth clearance periods shown in Fig. 1, respectively) were compared to the value of the control clearance period (the mean calculated from the results of the first and second clearance periods before infusion of lithium).

Slice technique: Lithium as the chloride salt at doses of 2 and 4 mmoles/kg/day was given intraperitoneally once a day for four days to the rats. Control animals received only vehicles. Twenty-four hours posterior to the final administration of lithium, the kidneys were removed and decapsulated for slice preparation with a razor blade. Blood was collected, and then the plasma was obtained by centrifugation. Plasma samples were analyzed for lithium with an atomic absorption spectrophotometer. The experimental procedures of the slice technique were essentially the same as those already described (8). To be brief, approximately 140 mg of the slices were placed in flasks con-
taining 10 ml of an incubation medium with the following composition in mM: NaCl, 134; KCl, 5.9; CaCl₂, 1.5; MgCl₂, 1.2; glucose, 11.5; Hepes (N-2-hydroxyethylpiperazine-N' -2-ethanesulfonic acid) buffer (pH 7.4), 5.8; and PAH, 0.074. It further contained 1% inulin which was added to estimate the extracellular space of the slices. For the experiment of TEA accumulation in the slices, the medium contained 0.1 mM TEA plus 5 nCi/ml ¹⁴C-TEA (4.4 mCi/mnmole, New England Nuclear) and 100 nCi/ml methoxy-³H-inulin (384 mCi/g, New England Nuclear) instead of PAH and inulin. After incubation for 30 min at 37°C with a gas phase of 100% oxygen, the samples of the slices and media were used for the spectrophotometric analyses of PAH and inulin. Radioactivities of ³H and ¹⁴C in the slices and media were measured with scintillator as described previously (9).

The slice-to-medium concentration ratios (S/M) for PAH and TEA were calculated by dividing the amount of PAH or TEA in one ml of intracellular water in the slices by the corresponding amount in one ml of the medium. This method probably represents the active transport of PAH and TEA in the slices.

Statistics: Data are reported as means ±S.E. Statistical analysis was performed by analysis of variance. Significant difference at the P<0.05 level was determined among multiple comparisons with Dunnett’s test (10).

Results

Clearance studies: To check the influence of anesthesia and surgical injuries on the renal functions, the animals were treated in the same way as shown in Fig. 1 except that PAH and inulin were infused without concurrent infusion of lithium through all the clearance periods. A satisfactory function in this preparation was evidenced by normal values of the parameters tested, which were generally similar to those early reported for rats (11), and the respective parameter was reasonably stable in four clearance periods (data not shown).

Lithium, when infused intravenously in concentrations that did not change significantly glomerular filtration rate (GFR) and plasma concentration of PAH (Pₚₐ₉) during the first lithium-period, caused the decreased clearance of PAH (Cₚₐ₉), with the increase in urine flow which was probably observed as a result of the effect of the cation on vasopressin action (12) (Table 1). More profound reduction in renal clearance of PAH as well as significant changes in GFR and plasma concentration of PAH was observed during the second period of lithium infusion. The concentration of lithium in plasma during the infusion was somewhat higher in the second period (15.06±1.4 mEq/l) than in the first period (10.70±0.5 mEq/l).

Because of the reduction in renal clearance of PAH by lithium, we sought to determine whether lithium affected tubular secretion of PAH. Figure 2 illustrates that the tubular secretion of PAH showed a significant difference between the control period and the first period of lithium infusion and that the infusion resulted in a more pronounced decrease in tubular secretion of PAH during

| Table 1. Effect of lithium on parameters of renal functions in rats infused with lithium at the rate of 0.9 mg per kg per min |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Clearance       | Urine flow      | GFR             | Pₚ₉             | Cₚ₉             |
| periods         | (ml/g·min)      | (ml/g·min)      | (µg/ml)         | (ml/g·min)      |
| Control         | 25.0±3.1        | 1.24±0.06       | 13.0±1.5        | 3.68±0.6        |
| Lithium infusion|                 |                 |                 |                 |
| First period    | 45.5±4.5**      | 1.18±0.06       | 15.2±1.5        | 2.30±0.3**      |
| Second period   | 35.5±3.2*       | 0.96±0.06**     | 19.5±2.0*       | 1.79±0.2*       |

Each control value was obtained by calculating the mean of two consecutive clearance periods before infusion of lithium (I and II indicated in Fig. 1). First and second periods during infusion of lithium correspond with period III and IV shown in Fig. 1, respectively. *Kidney weight. The values are the means±S.E. of ten experiments. **P<0.05, ***P<0.01, when compared to the respective control.
the second period, with which lithium concentration in plasma tended to rise.

**Studies with slices:** The rats were pretreated with lithium for 4 days (2 and 4 mmoles/day/kg, i.p., once a day). When the double dose of lithium (4 mmoles/day/kg, i.p.) was used, there was a significant increase in urine volume in rats (data not shown), and the dose approximately doubled the concentration of lithium in plasma (0.63±0.2 mEq/l).

The effect of lithium pretreatment on organic ion accumulation in kidney cortical slices is illustrated in Fig. 3. The slices from rats treated with lithium (4 mmoles/kg, i.p., 4 days, once a day) achieved a lower S/M ratio of PAH than the slices of saline-injected rats. However, no difference between TEA accumulation by the slices from lithium-treated rats and that from control rats was detected.

Lithium injection for 4 days at a dose (4 mmoles/kg, i.p., once a day) which caused the decrease in PAH accumulation had no effect on total tissue water or extracellular water in the slices after incubation (data not shown). Therefore, the decreased PAH accumulation induced by lithium pretreatment is not likely to be due to the effect of the cation on the water distribution in the slices.

**Discussion**

Previous studies showed that addition of lithium to an incubation medium resulted in inhibition of PAH accumulation in rat kidney cortical slices, but had no detectable effect on TEA accumulation in vitro (4). Evidence for the in vivo effect of lithium on PAH transport in kidney was obtained in the present study using the renal clearance technique and the kidney cortical slice technique. The suitability of the slice technique for tubular transport studies has been well established (13). In these experiments, the in vivo effect of lithium on tubular secretion of PAH and PAH accumulation was observed in the intact animals infused with lithium and in the slices from rats administered lithium for 4 days, respectively (Figs. 2 and 3). Therefore, the present results confirmed the previous finding that lithium interfered selectively with PAH transport and expanded this aspect into the observation of the in vivo action of the cation.

In the present clearance studies, lithium infusion decreased not only the renal clearance of PAH but also decreased tubular secretion of the anion (Table 1, Fig. 3). It is suggested from these results that the decrease in renal clearance of PAH is due not to the
tendency for GFR to decrease, but due to the decrease in tubular secretion of PAH. We think that the results obtained with the slice technique probably exclude hemodynamic variables as factors contributing to the decreased tubular secretion of PAH in lithium-infused rats. Recently, it has been reported that lithium may inhibit PAH transport indirectly by raising plasma 2-oxoglutarate which could compete for the PAH transport mechanism (5). However, it would be expected from our previous (4) and present results carried out with the slice technique in the absence of 2-oxoglutarate in the incubation medium that the inhibitory effect of lithium on the PAH transport mechanism was not secondary to a competition of the two substances for a common transport mechanism.

The concentration of lithium in plasma found to cause significant inhibition of tubular secretion of PAH was considerably higher than that found pharmacologically (therapeutic plasma level of lithium <1.5 mM). However, in the case of slice studies with repeatedly lithium-pretreated rats, we found a significant effect on PAH accumulation at the lithium concentration of 0.63 mEq/l in plasma, which ranged within the therapeutic plasma level (>0.5 mM). This raises the possibility that in chronic administration, lithium may be contributing to lowered urinary PAH excretion by inhibiting tubular secretion of PAH.

Of interest is the selective inhibitory effect with lithium on PAH transport since lithium inhibited PAH accumulation without reducing TEA accumulation. It is well-known that numerous organic anions are actively secreted by renal proximal tubules through a transport system common to that for PAH (14). The present results are of general interest because these organic anions include not only several drugs but also endogenous anions such as prostaglandins and cyclic nucleotides (15). Lithium administered as a drug may affect the physiological or pharmacological effect of these organic anions by inhibiting tubular secretion although we have no data for these cases.

Finally, our studies raise the question of how lithium affects the transport system for organic anions in kidney cortical cells. However, the mechanism by which lithium inhibits PAH transport remains unknown from the present results, and it is an aim of further experiments to elucidate that.

In summary, the present results demonstrated that the renal transport of PAH in lithium-treated rats was significantly inhibited as compared to that of PAH in the control and then lithium exerted its characteristic effect on proximal tubular function. This paper followed the previous report (4) in which we showed that lithium inhibited selectively PAH accumulation in rat kidney cortical slices in vitro.

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