Inter-organ Relation between Salivary Gland and Kidney in Lithium Excretion. I. Effects of Continuous Stimulation of Salivation on Salivary, Renal and Systemic Clearances of Lithium in Dog*

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(Received June 27, 1988)

The effects of continuous stimulation of salivation on salivary, renal and systemic clearances of lithium were investigated following bolus intravenous administration of lithium chloride (0.145 meq/kg) in three beagle dogs. The salivation was frequently stimulated with citric acid solution, then parotid saliva and mandibular-sublingual saliva were collected separately by means of permanent fistulae. Although the continuous stimulation of salivation markedly increased the salivary clearance of lithium, no significant change was observed in plasma concentrations or systemic clearance of lithium. This was because the decrement in the renal clearance of lithium canceled out the effect of increased salivary clearance. It is suggested that the reabsorption of lithium in the renal tubule was enhanced under the continuous stimulation of salivation, and this seemed to be caused by loss of water or sodium through the salivary glands.

Keywords — lithium; salivary drug excretion; continuous salivary stimulation; inter-organ relation; salivary clearance; renal clearance; systemic clearance; sodium; potassium; dog

Introduction

Lithium is a drug with a long half-life (about 20 h in man) and is often used in the treatment for manic patients. There are many studies2-7 aimed at therapeutic drug monitoring by measuring lithium concentrations in saliva. However, few studies have been carried out in order to investigate the mechanism or kinetics of the salivary excretion of lithium. In our previous paper,8 it was observed that the total salivary clearance of lithium corresponded to 70% of its systemic clearance, though this salivary clearance was the transient value estimated during a very short period, 2 min, when salivation was stimulated. From this result, there is a possibility that the salivary excretion of lithium may significantly contribute to its elimination under continuous stimulation of salivation. This suggestion has also been made for the salivary excretion of urea and phenobarbital.9

This study was carried out to investigate the effects of continuous stimulation of salivation on salivary, renal and systemic clearances of lithium in unanesthetized dogs. The interrelation between salivary and renal excretion of lithium is discussed in relation to the disposition of water, sodium and potassium.

Materials and Methods

Materials — Lithium chloride was of analytical grade (Wako Pure Chemical Industries Ltd., Osaka, Japan). All other reagents were commercial products of analytical grade.

Animals — Three male beagle dogs (purchased from Fuji Life-science Incorporated, Kitakoma, Japan) weighing 11.0—12.0 kg were used without fasting. All beagle dogs had permanent fistulae10 which were used for collection of parotid saliva and mandibular-sublingual saliva separately. The permanent fistulae were made for one of each pair of these salivary glands.

Drug Administration and Collection of Biological Fluids — Each dog received a bolus injection of 0.145 meq/kg of lithium through the cephalic vein as an aqueous solution of lithium chloride (0.290 meq/ml).

The saliva was collected in test tubes by using the same devices as described in the previous
paper\(^{10}\) through Tygon tubings (i.d. 1/16 inch). The salivation was gustatory stimulated by applying 0.4 ml of 10\% citric acid solution containing 20\% sucrose onto the tongue of each dog. The protocol of saliva and blood collection is shown in Fig. 1 in relation to the stimulation of salivation. After the drug administration, three stimulations at 30 s intervals were repeated every 5 min until the end of the experiment for 390 min (Fig. 1-A). The saliva collection was carried out throughout the whole experiment including 2 min saliva sampling to determine the time course of saliva lithium. The blood sample was taken at the midpoint of the 2 min saliva sampling period. In our previous work\(^{8}\) saliva was taken only during the 2 min period corresponding to blood sampling (Fig. 1-B).

The plasma was obtained by centrifugation of the blood sample at 3000 rpm for 15 min. The urine was collected by means of a urethral catheter (Argyle Rob-Nel Catheter, 10 Fr, Japan Sharwood Ltd., Tokyo, Japan).

An experiment without stimulation of salivation was carried out as the control experiment, using the same dogs. In the control experiment, saliva flowing out spontaneously was pooled for 390 min after administration of lithium.

**Measurement of Salivary Flow Rate**

The salivary flow rate (ml/min/kg) was estimated from the sample weight and averaged for the 390 min; the specific gravity of saliva was assumed to be 1.00.\(^{11}\)

**Determination of Lithium, Sodium and Potassium**

Lithium and potassium concentrations in the biological fluids were determined with a flame photometer (Shimadzu AA-630-12, Shimadzu Seisakusho Co., Ltd., Kyoto, Japan) after appropriate dilution with a concentrated solution of sodium (100 meq/l). Sodium concentrations were determined after dilution with a concentrated solution of potassium (100 meq/l). These dilution procedures were done to compensate for changes in the flame emission owing to the existence of other ions.

**Estimation of Clearances**

Salivary or renal clearance of lithium was calculated from the following equation,

\[
\frac{\text{excreted amount of lithium in saliva or urine within 390 min}}{\text{AUC}_{390}} = \text{salivary or renal clearance}
\]

where \(\text{AUC}_{390}\) is the area under the plasma concentration–time curve for lithium within 390 min following the administration, estimated for each dog by applying the trapezoidal rule. The salivary clearance from Eq. 1 actually accounts for a part of the systemic clearance. On the other hand, the salivary clearance which we reported previously\(^{8}\) was a transient value corresponding only to the 2 min period with stimulated salivation, and it would make little contribution to the lithium elimination.

In this experiment, the systemic clearance was considered to be the sum of salivary and renal clearances, assuming that lithium was not excreted via any other route than the kidneys and salivary glands, because lithium elimination is thought to take place almost wholly through the kidneys under normal conditions.\(^{12,13}\)

**Statistical Analysis**

Differences among data were analyzed by means of the paired \(t\)-test.

**Results and Discussion**

**Plasma Lithium Concentration**

Figure 2 shows the plasma and saliva lithium
concentration–time curves under the condition of continuous stimulation of salivation together with the plasma concentrations in the control experiment. We had expected that the stimulation of salivation would promote the excretion of lithium into saliva and result in a more rapid decline in the plasma concentrations of the drug. However, little difference was observed between the plasma concentrations under stimulation of salivation and those in the control experiment, as shown in Fig. 2. No significant difference was observed between the areas under the curves of plasma concentrations for lithium under these two experimental conditions, as shown in Table III. Then, salivary and renal clearances of lithium were examined.

Salivary Clearance

Table I shows that the saliva/plasma concentration ratio for lithium in the continuous stimulation experiment was smaller than that in the control experiment. However, salivary flow was markedly increased by applying the citric acid solution in the continuous stimulation experiment. Consequently, both salivary excretion and salivary clearance of lithium were increased for parotid saliva (Pr), mandibular-sublingual saliva (MS) and the sum of them (Pr+MS), as shown in Table I.

The mean value for the salivary clearance (CL Pr+MS, 0.192 ml/min/kg) obtained under the continuous stimulation of salivation was about two-thirds of the value we observed previously at (0.271 ml/min/kg, the estimate for Pr+MS). This may be because these values corresponded to different sampling periods. Our previous value corresponded to the 2 min period with three stimulations of salivation (see Fig. 1) and salivary flow rate, the major determining factor of salivary clearance of lithium, was kept very high during the 2 min period. On the other hand, the salivary clearance obtained in this study was estimated for the whole time of the experiment. The salivary flow rate became lower as a result of being averaged for 390 min than that during the 2 min period.

Since 11.6% of the dose was excreted into

| Table I. Saliva/Plasma Concentration Ratio, Salivary Excretion and Salivary Clearance of Lithium under Continuous Stimulation of Salivation in Three Beagle Dogs (LiCl, 0.145 meq/kg, i.v.) |
|--------------------------------------------------|--|--|
| | Control | Stimulated |
| Saliva/plasma concentration ratio \( a \) | Pr | 4.21 \( \pm \) 0.732 | 2.07 \( \pm \) 0.0844 \( b \) |
| | MS | 2.75 \( \pm \) 0.473 | 2.03 \( \pm \) 0.241 \( c \) |
| Salivary excretion of lithium \( b \) (% of dose) | Pr | 0.252 \( \pm \) 0.0320 | 4.48 \( \pm \) 1.14 \( b \) |
| | MS | 3.21 \( \pm \) 1.92 | 7.17 \( \pm \) 1.23 \( c \) |
| | Pr+MS | 3.46 \( \pm \) 1.95 | 11.6 \( \pm \) 0.0577 \( b \) |
| Salivary clearance of lithium (ml/min/kg) | CL Pr | 0.00404 \( \pm \) 0.000832 | 0.0733 \( \pm \) 0.0163 \( b \) |
| | CL MS | 0.0510 \( \pm \) 0.0307 | 0.119 \( \pm \) 0.0260 \( b \) |
| | CL Pr+MS | 0.0550 \( \pm \) 0.0313 | 0.192 \( \pm \) 0.0107 \( b \) |

Each value represents the mean \( \pm \) S.D. \( (n = 3) \). \( a \) Ratio of areas under the curves of saliva to plasma concentrations for lithium. \( b \) Significantly different from the control at \( p < 0.05 \). \( c \) Different from the control at \( 0.05 < p < 0.10 \). \( d \) Amount of lithium excreted within 390 min following the administration.
saliva within 390 min under the continuous stimulation of salivation (Table I), it is evident that salivary excretion of lithium made a considerable contribution to its elimination from the body. Nevertheless, the plasma concentration-time curve was not significantly different from that of the control experiment (Fig. 2). It was suggested that lithium excretion via the other route might be changed. Thus, renal clearance was examined, since the major route of excretion of lithium is through the kidneys.\textsuperscript{12,13}

**Renal and Systemic Clearances**

Urinary excretion and renal clearance of lithium under continuous stimulation of salivation are shown in Table II. Although there were relatively great inter-individual variations, both of these values were lower than those in the control. Figure 3 shows the relationship between renal clearance and salivary clearance of lithium for individual dogs. The renal clearance of lithium showed a decreasing tendency with increase in the salivary clearance. Consequently, the decrease in the renal clearance canceled out the effect of the increased salivary clearance, and the systemic clearance of lithium, which was considered to be equivalent to the sum of renal and salivary clearances, was not increased significantly as shown in Table III and Fig. 4. This is probably the reason why no difference was ob-

Table II. Urinary Excretion and Renal Clearance of Lithium under Continuous Stimulation of Salivation in Three Beagle Dogs (LiCl, 0.145 meq/kg, i.v.)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Stimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary excretion of lithium ( % ) (of dose)</td>
<td>28.5 ± 15.6</td>
<td>23.0 ± 14.1 ( b )</td>
</tr>
<tr>
<td>Renal clearance of lithium (ml/min/kg)</td>
<td>0.472 ± 0.279</td>
<td>0.383 ± 0.236 ( c )</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. \( n = 3 \). \( a \) Amount of lithium excreted within 390 min following the administration. \( b \) Significantly different from the control at \( p < 0.05 \). \( c \) Different from the control at 0.05 < \( p < 0.10 \).

Table III. Area under the Curves of Plasma Concentrations within 390 min \( (AUC_{390}) \) and Systemic Clearance (Sum of Salivary and Renal Clearances) of Lithium under Continuous Stimulation of Salivation in Three Beagle Dogs (LiCl, 0.145 meq/kg, i.v.)

<table>
<thead>
<tr>
<th>Dogs</th>
<th>( AUC_{390} ) (meq/l.min)</th>
<th>Systemic clearance (ml/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 1</td>
<td>82.4</td>
<td>0.694</td>
</tr>
<tr>
<td>No. 2</td>
<td>88.7</td>
<td>0.671</td>
</tr>
<tr>
<td>No. 3</td>
<td>102.6</td>
<td>0.216</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>91.2 ± 10.3</td>
<td>0.527 ± 0.270</td>
</tr>
<tr>
<td>Stimulated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 1</td>
<td>83.5</td>
<td>0.681</td>
</tr>
<tr>
<td>No. 2</td>
<td>89.0</td>
<td>0.748</td>
</tr>
<tr>
<td>No. 3</td>
<td>91.6</td>
<td>0.297</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>88.0 ± 4.1</td>
<td>0.575 ± 0.243</td>
</tr>
</tbody>
</table>

Fig. 3. Relationship between Renal Clearance and Salivary Clearance \( (CL_{Fr+MS}) \) of Lithium When Salivation Was Continuously Stimulated (Open Symbols) or Not (Closed Symbols) in Three Beagle Dogs

Dog number: 1, ○ ●; 2, △ ▲; 3, □ ■.
Salivary Excretion of Lithium in Dog

Lithium clearance (ml/min/kg)

Control

Stimulated

Fig. 4. Effects of Continuous Stimulation of Salivation on Lithium Clearance in Three Beagle Dogs (LiCl, 0.145 meq/kg, i.v.)

a) Renal clearance. b) Salivary clearance (CL_s = CL_{Pr+MS}). Whole column with a bar represents the mean ± S.D. (n=3) of systemic clearance (CL_s + CL_R).

observed in the plasma lithium concentration–time curves (Fig. 2).

Relation between Salivary and Renal Excretion of Lithium

Under continuous stimulation of salivation, the salivary clearance of lithium was increased, but the renal clearance showed a decreasing tendency. This is a very interesting phenomenon from the viewpoint of "homeostasis" in lithium excretion.

It is known that renal excretion of lithium depends on the glomerular filtration and the tubular reabsorption. Our preliminary experiment without lithium administration showed that the continuous stimulation of salivation did not alter creatinine clearance in the same beagle dogs (stimulated: 2.53 ± 0.492, control: 2.41 ± 0.582 ml/min/kg, n = 3). Therefore, the enhanced reabsorption of lithium may be responsible for the decreasing tendency in the renal clearance of lithium under continuous stimulation of salivation.

Under these experimental conditions, water loss through the salivary glands by the continuous stimulation of salivation was approximately 4% of the body weight of the dogs. It is expected that the renal function to maintain body water or the reabsorption of water may be activated through water loss to the extent seen under these experimental conditions. Figure 5 shows the relationship between urinary and salivary flow rates. When salivation was stimulated, the urinary flow rate showed a tendency to decrease for each dog, supporting the enhanced reabsorption of water in the kidneys. This function to maintain body water might lead to the enhanced reabsorption of lithium and consequently the decreasing tendency in the renal clearance. No significant change was observed in the ratios of areas under the curves of urine to plasma concentrations for lithium (stimulated: 60.0 ± 31.1, control: 55.5 ± 23.9, n = 3). Therefore, it was considered that the change in the urinary flow rate would be related directly to the change in the renal excretion of lithium. It is also known that dehydration reduces the rate of lithium elimination, and this is consistent with our findings.

Water reabsorption in the renal tubule is related closely to the reabsorption of sodium or potassium. The renal clearances of sodium and potassium in this study are presented in Table IV together with the salivary and systemic clearances. Plasma concentrations of sodium and potassium were not changed significantly by the continuous stimulation of salivation (sodium, stimulated: 157.9 ± 6.55, control: 156.0 ± 2.78 meq/l, potassium, stimulated: 4.53 ± 0.398, control: 4.78 ± 0.154 meq/l, n = 3).

As shown in Table IV, the mean values of the renal clearances for both sodium and potassium
were smaller under continuous stimulation of salivation than in the control, though the difference was not statistically significant at the level of $p = 0.05$. These data also seemed to support the assumption that the reabsorption of water and lithium was enhanced under continuous stimulation of salivation. Table IV also shows that the systemic clearance of sodium was markedly increased by the continuous stimulation of salivation, but this was not the case with potassium. The increase in the systemic clearance of sodium was mainly due to the effect of the increased salivary clearance. It is also possible that the inter-organ relation in lithium excretion is related to the increase in the salivary or systemic clearance of sodium.

In the investigation of lithium clearance under continuous stimulation of salivation in dogs, the effect of a significant increase in salivary clearance of lithium was canceled out by the decrement in renal clearance. Consequently, the systemic clearance of lithium was not increased significantly. These findings suggest that an inter-organ relation in lithium excretion exists between kidney and salivary gland. This inter-organ relation seemed to be related to the loss of water or sodium through the salivary glands. We are investigating the effect of water loading on lithium clearance under continuous stimulation of salivation in order to clarify the inter-organ relation between salivary gland and kidney in lithium excretion and to examine whether the systemic clearance of lithium can be increased.

### Acknowledgment
The authors wish to thank Mr. Hiroaki Nishii and Miss Takako Ebara for their technical assistance in this work. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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