Inter-organ Relation between Salivary Gland and Kidney in Lithium Excretion. II. Salivary, Renal and Systemic Clearances of Lithium under Continuous Stimulation of Salivation in Water Loaded Dogs

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This study was undertaken to investigate the effects of water loading on lithium clearance in dogs under the continuous stimulation of salivation as well as to clarify the mechanism of the inter-organ relation between salivary gland and kidney in lithium excretion. Dogs were given intravenously 0.145 meq/kg of lithium chloride followed by the continuous stimulation of salivation with citric acid solution. Fifty ml of water was loaded orally 7 times at 1-h intervals. Parotid and mandibular-sublingual salivas were collected separately by means of permanent fistulae.

(1) Plasma concentrations of lithium were not significantly different from either those in the experiment with continuous stimulation under no water loading or those in the control experiment without continuous stimulation. (2) Salivary clearance of lithium was markedly increased compared with that in the control experiment. The urinary flow rate, which was decreased under the continuous stimulation of salivation without water loading, was restored by the water loading. The renal clearance of lithium, which was also decreased under the continuous stimulation, remained at the reduced level under the condition of this study. Consequently, the systemic clearance of lithium did not change from that in the experiment under the continuous stimulation without water loading or that in the control experiment. (3) It was suggested that the salivary and renal excretion mechanisms of lithium might be similar to those of potassium, since a similar interrelation between salivary and renal clearances was observed for potassium. (4) It was suggested that the reduction in the renal clearance of lithium under the continuous stimulation of salivation was attributed to the sodium loss caused by the excessive salivation.

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

Introduction

Previously, we investigated the pharmacokinetic characteristics of salivary lithium excretion under the continuous stimulation of salivation in dogs.¹ Under this condition, the systemic clearance of lithium did not change significantly in spite of the marked increase in the salivary clearance. It was due to the reduction in the renal clearance which canceled out the effect of the increased salivary clearance. It is of interest to explore the mechanism of this inter-organ relation between salivary gland and kidney in lithium excretion as well as the excretory mechanisms of lithium through these organs. Under the continuous stimulation of salivation, large amounts of water and sodium were excreted from the body by the excessive salivary secretion.² The interrelation between salivary and renal excretions of lithium seemed to relate to the water or sodium loss due to the excessive salivation. It is known that dehydration and a negative electrolyte balance reduce the rate of lithium elimination in man and rats.³

The present study was carried out to investigate the effects of water loading on salivary, renal and systemic clearances of lithium under the continuous stimulation of salivation in dogs. Sodium and potassium clearances were also determined simultaneously in order to explore the mechanism of the inter-organ relation between salivary gland and kidney in lithium excretion.

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Materials and Methods

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

Animals — Three male beagle dogs (Fuji Life-science Incorporated, Kitakoma, Japan) weighing 11.0—12.0 kg were used without fasting. All beagle dogs had been operated to form permanent fistulae for collecting parotid saliva (Pr) and mandibular-sublingual saliva (MS) separately. The permanent fistulae had been made for one of each pair of these salivary glands.

Drug Administration and Sampling of Plasma, Saliva and Urine — A bolus injection of lithium chloride (0.145 meq/kg) was given to the dogs through the cephalic vein in aqueous solution (0.290 meq/ml).

The saliva and blood were collected in accordance with the protocol in our preceding paper. Salivation was gustatorily stimulated by applying 0.4 ml of 10% citric acid solution containing 20% sucrose onto the tongue of each dog. After administration of lithium chloride, three stimulations at 30 s intervals were repeated every 5 min. The saliva was collected throughout the experiment (for 390 min), including 2-min periods of saliva sampling to determine the time course of salivary lithium concentration.

The blood sample was taken into an ice-chilled heparinized tube at each mid point of the 2-min saliva sampling. The plasma was obtained by centrifugation of the blood sample at 3000 rpm for 15 min. Urine was collected throughout the experiment by means of urethral cathetel (Argyle Rob-Nel Cathetel, 10 Fr, Japan Sharwood Ltd., Tokyo, Japan).

The experiment without the gustatory stimulation of salivation was regarded as the control experiment, where spontaneously secreted saliva was pooled for 390 min after the drug administration.

Water Loading — Distilled water warmed to approximately 35 °C was loaded orally to the dogs using Tygon tubing (i.d. 1/16 inch). Fifty ml of the water was given 7 times at 1-h intervals starting 25 min before the lithium administra-

tion.

Determination of Salivary Flow Rate and Lithium, Sodium and Potassium Concentrations — Salivary flow rate (μl/min/kg) was estimated from the sample weight assuming the specific gravity of saliva to be 1.00.3

Lithium, sodium and potassium concentrations in the biological samples were determined with a flame photometer (Shimadzu AA-630-12, Shimadzu Seisakusho Co., Ltd., Kyoto, Japan). The dilution of the biological samples was done in the same way as in our preceding paper.2

Calculations — The area under the curve of plasma concentrations for lithium corresponding from time 0 to 390 min (AUCPl,390) was estimated for each dog by the trapezoidal rule. Area under the curve of saliva (Pr and MS) and urine concentrations for lithium (AUCPr,390, AUCMS,390 and AUCUr,390) were calculated from the mean lithium concentration in saliva and urine, respectively, multiplied by 390 min. Saliva/plasma or urine/plasma concentration ratio (S/P or U/P ratio) for lithium was calculated as the ratio of area under the curve of saliva or urine concentrations to that of plasma concentrations for lithium.

Salivary or renal clearance of lithium was calculated by dividing the amount of lithium excreted into saliva or urine within 390 min by AUCPl,390. Systemic clearance was considered to be the sum of the salivary and renal clearances assuming that lithium was not excreted via any other route than kidneys and salivary glands in this experiment, since lithium elimination is known to take place almost exclusively through the kidneys under normal conditions.3

Statistical Analysis — Inter-individual variations of data were analyzed by means of analysis of variance. Differences in the mean values of data were analyzed by the paired t-test when there were significant inter-individual variations, or by Student's t-test when no inter-individual variation was observed. Data were expressed in the mean ± S.D. unless exceptions are shown.
Results and Discussion

Plasma Concentration of Lithium

Plasma and saliva concentrations of lithium are shown in Fig. 1 together with plasma concentrations in the control experiment.\(^2\) No significant difference was observed in the plasma concentrations at all sampling points between the continuous stimulation experiment with water loading and the control experiment. In our preceding paper,\(^2\) it was reported that there was also no difference in the plasma lithium concentrations between the experiment with continuous stimulation of salivation under no water loading and the control experiment. The area under the curve of plasma concentrations for lithium corresponding from time 0 to 390 min (AUC\(_{\text{pl,390}}\)) was shown in Table I together with that of saliva and urine. AUC\(_{\text{pl,390}}\) was not significantly different among these three experimental conditions at p = 0.10 by Student's t-test. The area under the curve corresponding from time 0 to infinity was also calculated by estimating the area from 390 min to infinity using one-exponential extrapolation. No significant dif-

![Fig. 1. Plasma and Saliva Lithium Concentrations under Continuous Stimulation of Salivation with Water Loading in Three Beagle Dogs (LiCl 0.145 meq/kg, i.v.)](image)

- ○, plasma; △, Pr; □, MS; ●, plasma in control experiment.\(^3\) Each point with a vertical bar represents the mean ± S.D. (n = 3).

| TABLE I. | AUC\(_{\text{pl,390}}\), AUC\(_{\text{pr,390}}\), AUC\(_{\text{MS,390}}\) and AUC\(_{\text{Ur,390}}\) for Lithium under Continuous Stimulation of Salivation with Water Loading in Three Beagle Dogs (LiCl 0.145 meq/kg, i.v.) |

<table>
<thead>
<tr>
<th>AUC(_{\text{pl,390}}) (meq·min/l)</th>
<th>Control (^a)</th>
<th>Stimulated (^a)</th>
<th>Stimulated + water loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(_{\text{pl,390}})</td>
<td>91.2 ± 10.3</td>
<td>88.0 ± 4.14</td>
<td>97.3 ± 9.34</td>
</tr>
<tr>
<td>AUC(_{\text{pr,390}})</td>
<td>385 ± 70.7</td>
<td>182 ± 1.67 (^b)</td>
<td>191 ± 13.6 (^b)</td>
</tr>
<tr>
<td>AUC(_{\text{MS,390}})</td>
<td>249 ± 16.8</td>
<td>178 ± 14.3 (^b)</td>
<td>208 ± 10.5 (^c,d)</td>
</tr>
<tr>
<td>AUC(_{\text{Ur,390}})</td>
<td>4950 ± 1920</td>
<td>5250 ± 2730 (^c)</td>
<td>3990 ± 2340 (^c,f)</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. (n = 3). \(^a\) From ref. 2. \(^b\) Significantly different from the control at p < 0.01 by Student's t-test. \(^c\) Significantly different from the control at p < 0.05 by Student's t-test. \(^d\) Different from the stimulated at 0.05 < p < 0.10 by Student's t-test. \(^e\) Different from the control at 0.05 < p < 0.10 by the paired t-test. \(^f\) Different from the stimulated at 0.05 < p < 0.10 by the paired t-test.

| TABLE II. | Salivary Flow Rate and Saliva/Plasma Concentration Ratio for Lithium under Continuous Stimulation of Salivation with or without Water Loading in Three Beagle Dogs (LiCl 0.145 meq/kg, i.v.) |

<table>
<thead>
<tr>
<th>Salivary flow rate (μl/min/kg)</th>
<th>Control (^a)</th>
<th>Stimulated (^a)</th>
<th>Stimulated + water loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary rate (μl/min/kg) Pr</td>
<td>0.987 ± 0.315</td>
<td>35.6 ± 8.84 (^b)</td>
<td>41.7 ± 12.9 (^b)</td>
</tr>
<tr>
<td>MS</td>
<td>18.4 ± 10.9</td>
<td>58.3 ± 5.84 (^b)</td>
<td>68.3 ± 4.59 (^b,c)</td>
</tr>
<tr>
<td>Saliva/plasma concentration ratio (^d)</td>
<td>Pr</td>
<td>4.21 ± 0.732</td>
<td>2.07 ± 0.0844 (^b)</td>
</tr>
<tr>
<td>MS</td>
<td>2.75 ± 0.473</td>
<td>2.03 ± 0.241 (^e)</td>
<td>2.15 ± 0.221</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. (n = 3). \(^a\) From ref. 2. \(^b\) Significantly different from the control at p < 0.01 by Student's t-test. \(^c\) Different from the stimulated at 0.05 < p < 0.10 by Student's t-test. \(^d\) Ratio of area under the curves of saliva to plasma concentrations for lithium. \(^e\) Different from the control at 0.05 < p < 0.10 by Student's t-test.
ference was observed in the area corresponding from time 0 to infinity among three experimental conditions (control: 267 ± 133, stimulated: 242 ± 124, stimulated + water loading: 243 ± 63.8 meq min/l, mean ± S.D., n = 3) at p = 0.10 by the paired t-test.

**Lithium Clearance by Salivary Gland and Kidney**

Table II shows the salivary flow rate and the saliva/plasma concentration ratio (S/P ratio) of lithium. While the S/P ratio tended to be lower in the continuous stimulation experiment than the control experiment, a remarkable increase was observed in the salivary flow rate for both Pr and MS. The S/P ratio of lithium possibly changed in relation to the salivary flow rate. Burgen\(^{6}\) has reported that at lower salivary flow rates, the S/P ratio of lithium was higher (2 to 3 times the plasma level) in Pr of anesthetized dogs. We have also observed a negative correlation between the S/P ratio and salivary flow rate for both Pr and MS in unanesthetized dogs.\(^{7}\)

In our preceding paper,\(^{2}\) a decreasing tendency in the renal clearance of lithium was observed under the continuous stimulation of salivation. It was supposed that the decrease in the renal clearance might be due to water loss by the excessive salivation, since urinary flow rate also showed a lower mean value. Hence, we expected that the water loading could overcome the decrease in the renal clearance. In this study, the urinary flow rate recovered to the level in the control experiment by the water loading as shown in Table III. However, the urine/plasma concentration ratio of lithium was decreased under this experimental condition (Table III), and the mean value of the renal clearance remained lower than that in the control experiment as shown in Fig. 2, where CLS and CLR represent the salivary clearance for the sum of Pr and MS and the renal clearance, respectively.

The extremely high lithium concentration in urine (40–60 times the plasma concentration, Table III) was due to the difference in the fraction of reabsorbed lithium and water to glomerular filtration.\(^{3}\) Under normal condition, 70–80% of lithium filtered by glomeruli is reabsorbed in renal tubule,\(^{3,8}\) while more than 99% of water is reabsorbed.

Figure 2 also shows the considerable increase in the salivary clearance of lithium by the continuous stimulation of salivation. This was due to the remarkable increase in the salivary flow rate (Table II), because the salivary clearance of lithium was in proportion to the salivary flow rate in dogs as we reported previously.\(^{7}\)

Our preceding study\(^{2}\) showed that the systemic clearance of lithium was not increased by the continuous stimulation since the reduction in the renal clearance canceled out the increase in the salivary clearance. The water loading did not affect this inter-organ relation. The salivary and renal clearances remained at the increased and decreased level, respectively, as shown in Fig. 2. Hence, the systemic clearance, which was shown as the sum of CLS and CLR in Fig. 2, did not differ from that in the control experiment (control: 527 ± 270, stimulated: 575 ± 243, stimulated + water loading: 554 ± 115 µl/min/kg, mean ± S.D., n = 3, not significantly different at p = 0.10 by the paired t-test). This result was contrary to our expectation that the decrease in the renal clearance of lithium would be attributed to the water loss by the ex-
Salivary and Renal Excretion of Lithium

Fig. 2. Lithium Clearances under Continuous Stimulation of Salivation with or without Water Loading in Three Beagle Dogs (LiCl 0.145 meq/kg, i.v.)

Each column with a bar represents the mean ± S.D., n = 3, of CL_R or CL_S. a) From our preceding paper. b) Different from the control at 0.05 < p < 0.10 by the paired t-test. c) Significantly different from the control at p < 0.01 by Student's t-test.

excessive salivation and could be restored by the water loading.

Relationship between Lithium Excretion and Potassium or Sodium Excretion

Potassium and sodium excretion into saliva and urine were also examined to find a clue to the inter-organ relation between salivary gland and kidney in lithium excretion.

Plasma concentrations of potassium were 4.78 ± 0.154 meq/l in control experiment, 4.53 ± 0.398 in stimulated and 4.38 ± 0.161 in stimulated + water loading, mean ± S.D., n = 3. Those of sodium were 156.0 ± 2.78 meq/l in control, 157.9 ± 6.55 in stimulated and 155.0 ± 3.20 in stimulated + water loading, mean ± S.D., n = 3.

However, a remarkable increase in the salivary clearance of potassium was observed under the continuous stimulation of salivation with or without water loading, and the mean value of the renal potassium clearance was considerably lower than that in the control experiment as shown in Fig. 3. Therefore, the sum of salivary and renal clearances of potassium was not increased significantly under the continuous stimulation of salivation with or without water loading (control: 608 ± 263, stimulated: 678 ± 159, stimulated + water loading: 646 ± 120 μl/min/kg, mean ± S.D., n = 3) at p = 0.10 by Student's t-test. This means that a similar inter-organ relation between salivary gland and kidney as seen in lithium excretion also exists for

Fig. 3. Potassium Clearances under Continuous Stimulation of Salivation with or without Water Loading in Three Beagle Dogs (LiCl 0.145 meq/kg, i.v.)

Each column with a bar represents the mean ± S.D., n = 3, of CL_R or CL_S. a) From our preceding paper. b) Significantly different from the control at p < 0.001 by Student's t-test. c) Significantly different from the stimulated at p < 0.05 by Student's t-test.

Fig. 4. Sodium Clearances under Continuous Stimulation of Salivation with or without Water Loading in Three Beagle Dogs (LiCl 0.145 meq/kg, i.v.)

Each column with a bar represents the mean ± S.D., n = 3, of CL_R or CL_S. a) From our preceding paper. b) Significantly different from the control at p < 0.01 by Student's t-test. c) Significantly different from the control at p < 0.001 by Student's t-test.
potassium excretion. It seems that the salivary and renal excretion mechanisms of lithium might have some similarity to those of potassium. In rats, it was previously reported that potassium excretion was increased by lithium administration and that renal excretion of lithium was diminished by potassium depletion. On the other hand, no significant correlation was observed between the fraction of the filtered lithium which was reabsorbed by the renal tubule and the similar fraction for potassium in dogs. Further investigations will be needed to elucidate the relationship between the disposition of lithium and potassium in the kidney.

In the present study, the continuous stimulation of salivation with or without water loading resulted in the significantly higher salivary clearance and, consequently, the higher sodium excretion from the body was expected, compared with the control experiment, as shown in Fig. 4. Both lithium and sodium have been known to be reabsorbed in the proximal tubule, but lithium is not reabsorbed in the distal tubule. Under the condition of low sodium intake, however, it was reported that the renal excretion of lithium was decreased because of the activated reabsorption in the distal tubule. The loss of sodium due to the increase in the sodium excretion through the salivary glands might activate the reabsorption of lithium in the renal distal tubule in this study.

In conclusion, this study was undertaken to investigate the effects of water loading on salivary, renal and systemic clearances of lithium under the continuous stimulation of salivation in dogs. Even when water was loaded orally, the increased salivary clearance and the decreased renal clearance of lithium were observed, as seen in the experiment without water loading. It was suggested that a sodium loss, not water loss, induced by the excessive salivation would be responsible for the reduction in the renal clearance of lithium under the continuous stimulation of salivation. It should also be noted that a similar inter-organ relation between salivary gland and kidney was found for both lithium and potassium excretions. In our laboratory, further experiments are being carried out under the condition of continuous stimulation of salivation with sodium loading.

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References


