Lithium Infusion to Study Sodium Handling in Unanesthetized Hypertensive Rats

Jérôme Biollaz, Bernard Waeber, Jacques Diezi, Michel Burnier, and Hans R. Brunner

SUMMARY To investigate renal tubular handling of sodium in various types of experimental hypertension, sodium, lithium, and insulin clearances were measured simultaneously in unanesthetized rats. Fractional excretion of lithium was used as an index of proximal sodium reabsorption. Eight groups of animals, all of the Wistar-Kyoto strain, were studied. Three were hypertensive: spontaneously hypertensive rats (SHR), rats with two-kidney, one clip renal hypertension, and uninephrectomized rats with deoxycorticosterone-salt hypertension. The five normotensive control groups included animals given normal, low, or high dietary sodium loads and rats with reduced renal mass. Fractional excretion of lithium was not influenced by moderate changes of glomerular filtration rate, but was sharply enhanced by sodium loading. Increased blood pressure was associated with enhanced urinary sodium excretion in uninephrectomized deoxycorticosterone-salt hypertensive and two-kidney, one clip hypertensive rats, as a result of decreased distal tubular reabsorption ("pressure natriuresis"). In contrast, SHR showed reduced sodium excretion and decreased fractional excretion of lithium, which suggests that increased sodium reabsorption in the proximal tubule may contribute significantly to the maintenance of hypertension. (Hypertension 8: 117-121, 1986)

KEY WORDS • lithium • sodium handling • spontaneously hypertensive rats • two-kidney, one clip renal hypertension • deoxycorticosterone-salt hypertension

BASED on the elegant systems analysis presented by Guyton and co-workers,1,2 hypertension can develop and be sustained only if the renal capacity to excrete sodium is impaired. Despite numerous attempts to prove the presence of salt retention in experimental and clinical hypertension, however, no decisive evidence has yet been presented. This may be related to the fact that the amount of excess sodium reabsorbed by the kidney is too small to be detected by balance studies.

Micropuncture studies have been used to better characterize the renal sodium handling in normotensive and hypertensive animals. Unfortunately, this technique requires anesthesia and surgery, both of which are known to alter intravascular volume and renal sodium excretion.3,4 Even if intravascular fluid volume is kept stable during the experiment by infusing plasma protein and fluids,5 barbiturates are known to modify the systemic and renal hemodynamics, renin secretion, sympathetic nerve activity, and the sodium reabsorptive process.6,7 Thus, the effects of anesthesia limit the conclusions that can be drawn from such studies.

In 1969, the lithium ion, which is reabsorbed in the proximal tubule in parallel with sodium, was suggested as a test substance for the determination of proximal sodium reabsorption.8 Although controversies still exist as to whether lithium is reabsorbed in the distal nephron,9 the bulk of experimental and human studies support the view that lithium is a good indicator of the proximal sodium reabsorption, as long as the concentration of this ion is kept in a nontoxic range.10 Furthermore, micropuncture studies failed to demonstrate net transepithelial movements of lithium beyond the loop of Henle.11

Therefore, we decided to use urinary lithium clearance to evaluate renal sodium handling of hypertensive animals. To further validate the method, we initially performed experiments in normotensive rats maintained on different salt intakes or subjected to subtotal nephrectomy. The same investigations were then performed in spontaneously hypertensive rats (SHR), a
model of genetic hypertension,\textsuperscript{12, 13} as well as in rats with two-kidney, one clip renal and mineralocorticoid-salt hypertension, the respective prototypes of renin-dependent and sodium-dependent hypertension.

**Materials and Methods**

Male normotensive Wistar-Kyoto rats (WKY) and SHR were obtained from Madörin AG, Füllinsdorf, Switzerland and were placed on either a regular sodium (RS) diet (2.4 mg of sodium per gram of food) or a low sodium (LS) diet (0.5 mg of sodium per gram of food). Both diets were obtained from U.A.R., Geneva, Switzerland. A high salt (HS) diet was achieved by adding 1\% NaCl as drinking fluid to the RS diet.

The following procedures were performed in normotensive rats under ether anesthesia. The two-kidney, one clip model of renal hypertension was prepared by placing a U-shaped silver clip (0.2 mm internal diameter) on the left renal artery; the animals were then maintained on the RS diet for 4 weeks. The remnant kidney model was prepared by a right nephrectomy, followed 2 weeks later by the excision of both poles of the remaining kidney. Three days later the rats were placed on a HS diet for 1 additional week. The deoxycorticosterone (DOC)-salt hypertensive rats were either uninephrectomized (DOC 1) or sham-operated (DOC 2) and then injected weekly subcutaneously with desoxycorticosterone pivalate (Percorten pivalate, 30 mg/kg body weight) for 3 weeks and kept on the HS diet. In addition, normotensive animals were maintained for 1 week on either a LS, RS, or HS diet. The SHR received the RS diet throughout the study. The characteristics of the eight different study groups are outlined in Table 1. In this table, the body weight refers to the day of the experiment.

The dome of the urinary bladder was removed in all animals under light ether anesthesia 24 hours before the experiments. On the day of the study, all the animals had free access to food and water until 3 hours before the experiments. To measure glomerular filtration rate (GFR) as the urinary clearance of inulin, the unanesthetized rats kept in lucite restraining cages were infused (0.1 ml/min) through a tail vein with 5\% glucose containing a suitable amount of inulin (Laevosan, Linz, Austria) to maintain a plasma concentration of approximately 1 mg/ml. Lithium chloride was added to the infusion to obtain stable plasma concentrations of approximately 0.6 mmol/L. Venous blood samples (200 \mu l) were obtained from a hindpaw and collected in tubes coated with ammonium heparinate. A 1-hour equilibration period preceded two 30-minute clearance periods. Urine was collected directly from the urethral orifice into plastic tubes.

Immediately after completion of the renal function studies, rats were anesthetized with ether and the right external iliac artery was cannulated with a PE-50 catheter containing a heparinized 0.9\% NaCl solution. They were allowed a 3-hour recovery period before their blood pressure was measured with a transducer (Statham, Hato Rey, Puerto Rico) connected to an electrogalvanometer (Phillips 2000, Eindhoven, Netherlands) and recorded on a light-sensitive oscillograph (Mannarp 150, Electronic Institute Ltd., London, England). One milliliter of blood was then drawn in less than 20 seconds through the arterial catheter for determination of plasma renin activity. Blood and urine samples were analyzed for sodium, potassium, and lithium content by direct flame photometry and urine samples were analyzed for sodium, potassium, and lithium content by direct flame photometry and urine samples were analyzed for sodium, potassium, and lithium content by direct flame photometry and urine samples were analyzed for sodium, potassium, and lithium content by direct flame photometry and urine samples were analyzed for sodium, potassium, and lithium content by direct flame photometry and urine samples were analyzed for sodium, potassium, and lithium content by direct flame photometry.

Plasma renin activity was measured by radioimmunoassay.\textsuperscript{14} The average of the two clearance periods was used for analysis. Numerical results are given as means ± SEM. The normality of the distribution was tested, and a logarithmic transformation made when necessary. The significance of differences between means was evaluated with a one-way analysis of variance followed by the Student’s t test. Regression analysis was performed using the least-squares method, and the slopes of the regression lines were compared using a common slope regression test based on Student’s t test.\textsuperscript{16}

The characteristics of the study groups are summarized in Table 2 (see pages 120, 121). The GFR varied from 2 to 7 ml/kg/min and was lowest after subtotal nephrectomy (remnant kidney group) and unilateral nephrectomy (DOC 1 group). In the normotensive animals, both absolute and fractional (FE\textsubscript{Na}) sodium excretion was related to the sodium content of the diet. Both absolute and fractional sodium excretion were markedly increased in rats with renal (+214\% and +180\% respectively) or DOC 1 hypertension (+407\% and +441\% respectively). Plasma lithium concentration decreased with increasing sodium loads except in rats with markedly decreased GFR (DOC 1 and remnant kidney groups). Plasma lithium concentration ranged from 0.52 to 0.85 mmol/L, well below toxic levels that could have directly influenced renal sodium handling.

![Table 1. Characteristics of the Study Groups](https://example.com/table1.png)

**Table 1. Characteristics of the Study Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Sodium diet</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n = 5)</td>
<td>Normal</td>
<td>279 ± 4</td>
</tr>
<tr>
<td>SHR (n = 6)</td>
<td>Normal</td>
<td>321 ± 4</td>
</tr>
<tr>
<td>2K1C (n = 8)</td>
<td>Normal</td>
<td>209 ± 6</td>
</tr>
<tr>
<td>WKY (n = 6)</td>
<td>High</td>
<td>308 ± 9</td>
</tr>
<tr>
<td>DOC 1 (n = 5)</td>
<td>High</td>
<td>274 ± 8</td>
</tr>
<tr>
<td>DOC 2 (n = 5)</td>
<td>High</td>
<td>272 ± 7</td>
</tr>
<tr>
<td>Remnant kidney (n = 9)</td>
<td>High</td>
<td>273 ± 8</td>
</tr>
<tr>
<td>WKY (n = 6)</td>
<td>Low</td>
<td>320 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

2K1C = two-kidney, one clip renal hypertensive rats; DOC 1 = deoxycorticosterone-salt hypertensive uninephrectomized rats; DOC 2 = rats receiving DOC and salt, with both kidneys intact.
Blood pressure in DOC 2 rats was not significantly higher than in remnant kidney rats and rats on the LS, RS, or HS diets. All these groups were later considered to be normotensive.

Changes in GFR did not influence fractional lithium reabsorption except in the remnant kidney group, which showed a 60 to 70% decrease in renal function. The effects of various sodium loads on fractional urinary lithium excretion (FE\textsubscript{L\textsubscript{t}}) and plasma lithium concentration in normotensive rats with intact renal function are depicted below in Figure 1. It is apparent that the amount of filtered lithium escaping reabsorption is directly related to the amount of sodium in the diet.

The relationships between FE\textsubscript{Na} and FE\textsubscript{L\textsubscript{t}} in normotensive and hypertensive rats are shown in Figure 2. A close linear correlation was present in both groups (\( y = 20.40x + 12.09, r = 0.95 \); \( y = 5.22x + 15.13, r = 0.83 \) respectively). The slope of the two regression lines differed markedly (\( p < 0.001 \)), however, so that for a given increase in FE\textsubscript{Na}, a larger increase in FE\textsubscript{L\textsubscript{t}} was observed in hypertensive than in normotensive rats.

Discussion

The present study attempted to assess possible alterations in tubular handling of sodium in various types of experimental hypertension. To differentiate between proximal and distal nephron function in unanesthetized rats, we elected to use urinary clearance of lithium as an index of proximal sodium reabsorption. Clearly, such a procedure is based on the assumption that no net transport of lithium occurs beyond the proximal tubules. This issue has been discussed extensively recently, and several lines of evidence suggest that urinary clearance of lithium can indeed be used for such a purpose. The evidence includes, most notably, the fact that no net transport of lithium could be measured by micropuncture techniques along distal tubules and that lithium clearance is closely correlated with changes in proximal sodium reabsorption in conditions known to entail alterations in fluid delivery out of the proximal tubules.

Our studies indirectly confirm that lithium excretion correlates positively with changes in proximal sodium reabsorption, since variations in extracellular volume resulting from different oral sodium loads entailed expected changes in FE\textsubscript{Na}. For example, the largest FE\textsubscript{Na} in our series was measured in rats with remnant kidneys; a marked inhibition of proximal sodium reabsorption has been shown by micropuncture techniques to occur in animals with reduced renal mass. Conversely, "distal" sodium reabsorption (i.e., FE\textsubscript{L\textsubscript{t}} - FE\textsubscript{Na}), notably in groups on the RS, HS, and LS diets and in the remnant kidney group, was directly correlated to end-proximal sodium delivery (i.e., FE\textsubscript{L\textsubscript{t}}), which indicates enhanced distal sodium reabsorption at increased rates of delivery to the distal tubules. This

![Figure 1. Effect of sodium diet on fractional excretion of lithium (FE\textsubscript{L\textsubscript{t}}) and on plasma lithium concentration in normotensive WKY with normal renal function. LS = low sodium; RS = regular sodium; HS = high sodium; DOC 2 = deoxycorticosterone-salt hypertensive rat with both kidneys intact.](image)

![Figure 2. Correlation between fractional excretion of sodium (FE\textsubscript{Na}) and lithium (FE\textsubscript{L\textsubscript{t}}). A. Normotensive WKY (FE\textsubscript{L\textsubscript{t}} = 20.40 FE\textsubscript{Na} + 12.09; \( r = 0.95, p < 0.001 \)). B. Hypertensive rats (FE\textsubscript{L\textsubscript{t}} = 5.22 FE\textsubscript{Na} + 15.13; \( r = 0.83, p < 0.001 \)). RMNT K = remnant kidney; DOC I = deoxycorticosterone-salt unipheres tomized rats; GII = two-kidney, one clip renal hypertensive rats. See Figure 1 for key to other abbreviations.](image)
observation is consonant with micropuncture studies indicating that, although net sodium reabsorption along distal tubules is enhanced during saline loading, it is insufficient to prevent marked natriuresis. The weight of evidence, therefore, favors the hypothesis that simultaneous measurements of lithium and sodium clearances allow the delineation of proximal and distal tubular handling of sodium.

Since our experiments included animals with reduced glomerular filtration rates, it appeared important to investigate the relationship between GFR and lithium clearances in the range of the GFR measured in this study. Fractional lithium reabsorption remained nearly constant at all levels of GFR in all groups except the remnant kidney group, which had the lowest GFR and, as stated previously, in which a strong inhibition of proximal tubular sodium reabsorption occurs. Thus, we conclude from these as well as from previous results that moderate changes of GFR do not preclude the use of lithium excretion rate measurements as indicators of proximal tubular sodium reabsorption.

The lithium ion has been shown to interfere with the Na⁺-H⁺ antiport system, as studied in brush border membrane vesicles obtained from rabbit renal cortex. At high doses, lithium has been reported to inhibit proximal and distal sodium reabsorption in rats. Thus, to control and cancel out any possible effects of lithium ions on sodium reabsorption, we used a protocol in which lithium was infused to all experimental groups and plasma concentrations were maintained within a narrow range (0.52–0.65 mmol in 7 groups, 0.85 mmol in 1 group), at levels that are clinically nontoxic.

When the fractional excretions of sodium and lithium were compared, tubular sodium handling clearly appeared to be altered in the three groups of hypertensive rats (renal DOC 1, and SHR). The relative contribution of sodium escaping proximal reabsorption (as evaluated by FE\(_{\text{Na}}\)) to final sodium excretion was decreased in renal hypertensive and DOC 1 rats (see Figure 2), while overall natriuresis was enhanced in these two groups (see Table 2). This observation suggests that the blood pressure increase in both models promotes sodium excretion by inhibiting distal reabsorption and thereby confirms the findings of Kunau and Lameire, who located the pressure natriuresis phenomenon in the distal nephron. In contrast, SHR, in which a reduced overall natriuresis occurred, did not show a pressure natriuresis, a finding that is consonant with previously published work.

The decrease in FE\(_{\text{Na}}\) of SHR, compared with that in normotensive controls on a similar diet, suggests that proximal sodium reabsorption was enhanced in these animals. This enhancement may reflect a primary, intrinsic alteration of proximal reabsorption or may result from relative sodium depletion (e.g., due to pressure diuresis). Since, as mentioned previously, there was no evidence for the occurrence of pressure natriuresis in these animals, the enhancement of proximal tubular sodium reabsorption in this group was most likely related to primary, intrinsic characteristics of the kidneys of SHR.

Of interest is the recent finding that FE\(_{\text{Na}}\) is decreased in another genetic model of hypertension, Dahl salt-sensitive rats. This suggests that a greater proximal tubule reabsorption of sodium may participate in the development of hypertension in this model, too. Interestingly, this natriuretic handicap was revealed only after sodium loading, a prerequisite to increasing the blood pressure of these animals.

In conclusion, our results obtained in unanesthetized rats by using lithium as a marker of the renal proximal tubular handling of sodium suggest that reabsorption of sodium in the proximal tubule is enhanced in rats with genetic hypertension.

### Acknowledgments

The authors thank Ms. Yolande Parisod for technical assistance and Ms. Anne-Françoise Staudé and Anne Campiche for secretarial help.

### Table 2. Clearances, Blood Pressure, and Plasma Renin Activity in the Eight Groups of Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WKY-RS</th>
<th>SHR</th>
<th>2K1C</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(_\text{Na}) mmol/l</td>
<td>145.3±1.0</td>
<td>143.2±0.8</td>
<td>138.6±1.6*</td>
</tr>
<tr>
<td>P(_\text{Li}) mmol/l</td>
<td>0.60±0.02</td>
<td>0.58±0.01</td>
<td>0.85±0.02*</td>
</tr>
<tr>
<td>C(_\text{in}) (ml/kg/min)</td>
<td>7.0±0.2</td>
<td>5.1±0.4*</td>
<td>4.3±0.4*</td>
</tr>
<tr>
<td>U(_\text{Na})V (μmol/kg/min)</td>
<td>2.8±0.6</td>
<td>1.2±0.2</td>
<td>6.0±1.5†</td>
</tr>
<tr>
<td>U(_\text{Li})V (μmol/kg/min)</td>
<td>0.79±0.04</td>
<td>0.41±0.05*†</td>
<td>0.77±0.06</td>
</tr>
<tr>
<td>FE(_\text{Na}) (%)</td>
<td>0.28±0.06</td>
<td>0.17±0.02</td>
<td>1.14±0.35†</td>
</tr>
<tr>
<td>FE(_\text{Li}) (%)</td>
<td>18.9±1.0</td>
<td>13.7±0.8*</td>
<td>21.8±1.6</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>115±2.6</td>
<td>173±4.2*</td>
<td>203±7.6*</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>7.20±2.5</td>
<td>3.12±0.4</td>
<td>24.0±11.8</td>
</tr>
</tbody>
</table>

Values are means ± SEM. RS = regular sodium; HS = high sodium; LS = low sodium; P\(_\text{Na}\) = plasma sodium concentration; P\(_\text{Li}\) = plasma lithium concentration; C\(_\text{in}\) = inulin clearance; U\(_\text{Na}\)V = urinary sodium excretion; U\(_\text{Li}\)V = urinary lithium excretion; FE\(_\text{Na}\) = fractional excretion of sodium; FE\(_\text{Li}\) = fractional excretion of lithium; MBP = mean blood pressure; PRA = plasma renin activity. See Table 1 for key to other abbreviations.

\(^* p < 0.001, † p < 0.05, ‡ p < 0.01, \text{compared with values for WKY-RS}; § p < 0.05, || p < 0.001, \| p < 0.01, \text{compared with values for WKY-HS}.\)
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<table>
<thead>
<tr>
<th>WKYS-HS</th>
<th>DOC 2</th>
<th>DOC 1</th>
<th>Remnant kidney</th>
<th>WKYS-LS</th>
</tr>
</thead>
<tbody>
<tr>
<td>147.0±1.2</td>
<td>141.1±1.2†‡</td>
<td>144.0±1.6</td>
<td>146.0±1.2</td>
<td>145.2±0.9</td>
</tr>
<tr>
<td>0.56±0.02</td>
<td>0.52±0.02†</td>
<td>0.64±0.03§</td>
<td>0.65±0.03§</td>
<td>0.59±0.03</td>
</tr>
<tr>
<td>5.8±0.2*</td>
<td>4.9±0.3*</td>
<td>2.5±0.2*‖</td>
<td>2.0±0.1*‖</td>
<td>6.0±0.3*</td>
</tr>
<tr>
<td>4.0±0.7</td>
<td>5.8±1.1</td>
<td>7.2±1.5‡§</td>
<td>4.4±0.6</td>
<td>0.33±0.1‡§</td>
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<tr>
<td>0.68±0.07</td>
<td>0.70±0.07</td>
<td>0.44±0.05*§</td>
<td>0.57±0.03</td>
<td>0.40±0.08*§</td>
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<tr>
<td>0.46±0.07</td>
<td>0.83±0.14</td>
<td>2.03±0.45*‡</td>
<td>1.50±0.16*‡</td>
<td>0.04±0.004*‡</td>
</tr>
<tr>
<td>20±1.3</td>
<td>27.1±1.0*§</td>
<td>27.5±2.3*§</td>
<td>43.8±2.4*§</td>
<td>11.1±1.8*§</td>
</tr>
<tr>
<td>125±3.9</td>
<td>131±5.2</td>
<td>183±8.2*‡</td>
<td>130±2.5</td>
<td>123±3.1</td>
</tr>
<tr>
<td>4.8±1.0</td>
<td>0.10±0.01</td>
<td>0.14±0.02</td>
<td>1.10±0.1</td>
<td>12.7±2.9</td>
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