Model explaining the relation between distal nephron Li$^+$ reabsorption and urinary Na$^+$ excretion in rats

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Model explaining the relation between distal nephron Li$^+$ reabsorption and urinary Na$^+$ excretion in rats. Am. J. Physiol. 274 (Renal Physiol. 43): F445–F452, 1998.—Li$^+$ may be reabsorbed via an amiloride-sensitive mechanism in the collecting ducts of rats administered a low-Na$^+$ diet. This was investigated by measuring the increase in fractional urinary excretion of Li$^+$ (FELi) in response to amiloride in conscious rats at two different levels of plasma Li$^+$ concentration and after administration of bendroflumethiazide (BFTZ), angiotensin III (ANG III), and aldosterone (Aldo). The results confirmed that amiloride increased (FELi) in rats on a low-Na$^+$ diet (20 ± 1 to 35 ± 1%, means ± SE), whereas no increase was observed in rats on a normal Na$^+$ diet (37 ± 1 to 38 ± 1%). The lithiuretic effect of amiloride was abolished by preadministration of BFTZ (32 ± 1 to 33 ± 2%) to Na$^+$-deprived rats and 2) increased by ANG III (27 ± 3 to 33 ± 2%) and Aldo (25 ± 2 to 37 ± 2%) in Na$^+$-replete rats. Amiloride-induced changes in FELi were independent of plasma Li$^+$ concentration but inversely related to the fractional excretion of Na$^+$ and the amiloride-sensitive excretion of K$^+$. These results are compatible with the hypothesis that a low tubular Na$^+$ concentration reduces end-tubular Na$^+$ reabsorption and results in hyperpolarization of the apical membrane, thus favoring Li$^+$ uptake into the cells.

Renal lithium ion clearance is used as an index of the delivery of tubular fluid from the proximal tubules to the loop of Henle in humans and Na$^+$-replete rats. The technique is based on the assumptions 1) that Li$^+$ in the proximal tubule is reabsorbed to the same extent as Na$^+$ and water so that the concentration of Li$^+$ in the proximal tubular fluid remains constant and 2) that transtubular transport of Li$^+$ is negligible in the postproximal segments of the tubule, i.e., the loop of Henle, the distal convoluted tubules, and the collecting ducts (4, 28). Consistent with these assumptions, it has been found repeatedly that the renal clearance of Li$^+$ in Na$^+$-replete rats is unaffected by administration of amiloride, which inhibits apical membrane Na$^+$ entry into the cells of the collecting duct (2). However, during Na$^+$ depletion, the clearance of Li$^+$ is markedly decreased but can be normalized by amiloride, indicating that, in this situation, a significant proportion of the distal tubular load of Li$^+$ may be reabsorbed in the distal nephron (31).

The reason why amiloride-sensitive transport of Li$^+$ is quantitatively significant in Na$^+$-depleted but not in Na$^+$-replete rats is unknown. The limitation in reabsorption of Li$^+$ during Na$^+$-replete conditions may be either the cellular influx across the apical membrane or extrusion through the basolateral membrane, and, in both cases, the distal tubular load of Na$^+$ may be crucial for the reabsorption of Li$^+$. If Na$^+$ and Li$^+$ compete for the same apical transporter, an increased tubular load of Na$^+$ would be expected to result in a parallel reduction in Li$^+$ transport. Furthermore, Li$^+$ extrusion across the basolateral membrane may require a large concentration gradient between the intracellular fluid and the plasma, as the extrusion does not undergo active transport (9). In this situation, hyperpolarization of the apical membrane, which increases apical inward cation transport, may be crucial for elevating the intracellular Li$^+$ concentration to levels necessary for substantial transepithelial Li$^+$ transport.

In the present study, we have examined, by use of a conscious rat model, the mechanism(s) underlying distal tubular Li$^+$ reabsorption by modulation of the Na$^+$ reabsorption. The influence of amiloride on the fractional extraction of Li$^+$ (FELi), an index of distal tubular Li$^+$ reabsorption, was investigated in rats on normal and low-Na$^+$ intakes, in which distal tubular transport rates of Na$^+$ were manipulated by administration of bendroflumethiazide (BFTZ), angiotensin III (ANG III), and aldosterone (Aldo). The effect of an 80–90% reduction in plasma Li$^+$ concentration was also investigated. The results are compatible with the hypothesis that, during Na$^+$-depleted conditions, the apical membrane in the more distal parts of the amiloride-sensitive region of the collecting duct becomes hyperpolarized due to a low-Na$^+$ influx, and distal Li$^+$ reabsorption can occur.

Materials and Methods

Animals. Specific pathogen-free female Wistar rats (200–250 g) were obtained from the Department of Experimental Medicine, Panum Institute, University of Copenhagen (Copenhagen, Denmark). They were housed in a temperature (22–24°C) and humidity (40–70%)-controlled room with a 12:12-h light-dark cycle (light on from 6:00 AM to 6:00 PM). The rats were given free access to tap water and pelleted rat diet with a K$^+$ content of 220 mmol/kg and Na$^+$ contents of either 5 mmol/kg (Na5 diet) or 200 mmol/kg (Na200 diet) for at least 8 days before experimentation. Three days before the clearance study, lithium citrate was added to the food (12 and 7 mmol/kg for Na200 and Na5, respectively) to obtain measurable plasma Li$^+$ concentration ([Li$^+$]) without influencing the urinary Li$^+$ excretion. The time course of the Li$^+$ concentration in plasma is shown in Figure 1.
renal function (26). In one group of rats, Li⁺ was withdrawn 1 day before the experiment to reduce the plasma [Li⁺] in this group (Na5-low Li). Animal preparation. Animals were surgically prepared for clearance studies 2 wk before experimentation as described previously (21). In summary, animals were anesthetized with halothane-N₂O, and Tygon catheters were inserted into the abdominal aorta and the inferior caval vein via femoral vessels. A suprapubic bladder catheter was also implanted. After instrumentation, animals were housed individually. During the last week before clearance studies, rats were adapted to the restraining cage used for these experiments by training them for two periods of ~90 min each.

Clearance technique. On the day of the experiment, the rats were transferred to restraining cages, and the catheters were opened and flushed with 150 mM glucose containing 20 U/ml of heparin. Throughout the experiments, the animals received an infusion of this solution at a rate of 0.25 ml/h to keep the arterial catheter open. In addition, each animal received throughout the experiment an infusion of 150 mM glucose (bolus 0.5 ml, sustained 0.5 ml/h) containing [³H]inulin (batch no. 119–121; bolus 5 µCi, sustained 5 µCi/h; Amersham). LiCl (bolus 3.3 µmol, sustained 3.3 µmol/h), and 1.0 ml/h of 150 mM glucose as vehicle for drugs. Each experiment comprised a 15 min inulin-Li⁺ bolus period, a 105-min equilibration period, three 30-min preamiloride periods, and three 30-min periods during which amiloride was added to the ongoing intravenous infusion.

The total rate of infusion of 150 mM glucose was kept constant at 1.75 ml/h in all experiments. In addition, during administration of BFTZ or amiloride, urinary fluid losses exceeding 1.75 ml/h were replaced by infusion of a 150 mmol/l NaCl solution by a computer-driven servo-controlled system. This system, which includes a personal computer, an electronic balance (type MC 1; Sartorius, Goettingen, Germany), and an infusion pump (Perfusor Secura; Braun Melsungen, electronic balance (type MC 1; Sartorius, Goettingen, Germany), monitored urine flow rate gravimetrically at 5-min intervals and adjusted the rate of the pump accordingly (5).

Blood samples of 250 µl were collected from the arterial catheter at the end of the equilibration period, after the preamiloride periods, and at the end of the experiment. All blood samples were replaced immediately with heparinized donor blood. At the end of the experiment, all catheters were sealed with 50% glucose, 500 units of heparin, and 10,000 units of streptokinase per milliliter, and the rats were returned to the animal unit. When different experiments were performed on the same animal, they were separated by at least 1 wk, allowing full recovery between experiments.

Drugs were dissolved in 150 mM glucose and were given as an intravenous priming dose followed by a maintenance infusion at a rate of 0.5 ml/h. The priming doses of BFTZ (83 µg), ANG III (1.5 µg), and amiloride (0.17 mg) were given over a period of 5 min, whereas Aldo (600 pmol) was given over a period of 15 min together with the inulin bolus. The corresponding maintenance infusions provided 250 µg/h, 1.5 µg/h, and 600 µmol/h, respectively.

Analyses. Urine volume was determined gravimetrically. Na⁺ concentration ([Na⁺]), K⁺ concentration ([K⁺]), and [Li⁺] in plasma and urine were determined by atomic absorption spectrophotometry, using a Perkin-Elmer model 2380 atomic absorption spectrophotometer. In the Na5-low Li group, plasma and urine [Li⁺] were determined by flameless atomic absorption spectrophotometry, using a Perkin-Elmer electrothermal atomic absorption spectrophotometer (16, 24). [³H]-inulin in plasma and urine was determined by liquid scintillation counting using a Packard Tri-Carb liquid scintillation analyzer (model 2250CA). The urinary excretion rate of amiloride was determined in eight of the Na5 and eight of the Na200 rats given vehicle infusion. Urinary amiloride concentrations were determined using a high-performance liquid chromatography system with fluorometric analysis. Blood pressure and heart rate were determined continuously using Baxter UniFlow pressure transducers (Bentley Laboratories) connected to Hugo Sachs pressure and heart couplers. Signals were displayed on a Watanabe Instruments WR 3101 Line recorder Mark VII (Watanabe Instruments).

Calculations. Values for renal clearance (C) and fractional excretion (FE) were calculated using standard formulas

\[ C = \frac{U \times V}{P} \]

\[ \text{FE} = 100 \times \frac{C}{GFR} \]

where \( U \) is the concentration in the urine, \( V \) is the urine flow rate, and \( P \) is the concentration in plasma. [³H]Inulin clearance (\( C_{\text{in}} \)) was used as a marker for the glomerular filtration rate (GFR).

The urinary amiloride concentrations were 346 ± 95 (means ± SE) and 170 ± 18 (means ± SE) µmol/l, and the excretion rates were 7.3 ± 0.8 and 6.5 ± 0.8 nmol·min⁻¹·100 g body wt⁻¹ in the Na5-vehicle and the Na200-vehicle groups, respectively. From these values, intratubular amiloride concentrations were estimated to range from 5 to 10 µmol/l in the proximal tubules and higher in the cortical collecting ducts. These concentrations are well below those reported to block the Na⁺-H⁺ exchange mechanism in the proximal tubules (inhibitory constant \( K_i > 50–100 \mu M \)) but above the concentrations required to block movement of Na⁺ through the conductive Na⁺ channel \( K_i < 1 \mu M \); see Refs. 2 and 11).

Assuming that amiloride completely blocked the conductive Na⁺ channels in the collecting ducts and that Na⁺ is not reabsorbed by other mechanisms in this segment or further downstream (22), the reabsorption (R) of Na⁺ expressed as a fraction (f) of the load delivered to the reabsorbive site can be calculated as

\[ R_{\text{Na-amil}} = \left( V_2 \times U_{\text{Na2}} - V_1 \times U_{\text{Na1}} \right) / V_2 \times U_{\text{Na2}} \]

where \( V_1 \times U_{\text{Na1}} \) and \( V_2 \times U_{\text{Na2}} \) represent the absolute excretion of Na⁺ before and after administration of amiloride, respectively. To account for changes in GFR and plasma [Na⁺] between the two observation periods, these variables should be factored, resulting in the following equation expressing the relative (r) amiloride-sensitive Na⁺ reabsorption

\[ R_{\text{Na-amil}} = \left( V_2 \times U_{\text{Na2}} / P_{\text{Na2}} / GFR_2 \right) - \left( V_1 \times U_{\text{Na1}} / P_{\text{Na1}} / GFR_1 \right) \times \left( (V_2 \times U_{\text{Na2}} / P_{\text{Na2}} / GFR_2) / (V_1 \times U_{\text{Na1}} / P_{\text{Na1}} / GFR_1) \right) \]

which is equal to the equation

\[ R_{\text{Na-amil}} = (P_{\text{Na2}} - P_{\text{Na1}}) / P_{\text{Na2}} \]

Similarly, the relative amiloride-sensitive Li⁺ reabsorption can be expressed as

\[ R_{\text{Li-amil}} = (P_{\text{Li2}} - P_{\text{Li1}}) / P_{\text{Li2}} \]

The expressions \( R_{\text{Na-amil}} \) and \( R_{\text{Li-amil}} \) are similar, as no differences between GFR and \( P_{\text{Na}} \) are seen before or after administration of amiloride. In contrast, as \( P_{\text{Li}} \) showed time-dependent changes in some series, a meaningful estimate of the relative reabsorption of Li⁺ in the amiloride-sensitive segment can only be accomplished through determination of \( R_{\text{Li-amil}} \). Furthermore, Li⁺ and Na⁺ transport in the amiloride-
sensitive segment can be directly compared using the expressions $r_{RLi-Amil}$ and $r_{RRNa-Amil}$. Because the Na+ load on which $r_{RRNa-Amil}$ is based (FE $Na^+$) may be underestimated because of Na+ reabsorption in the distal nephron by mechanisms not being amiloride sensitive, the true $r_{RRNa-Amil}$ may be less than the calculated value. However, this inaccuracy is not important for the data presentation and interpretation performed.

Data presentation and statistics. Renal clearance variables are presented as mean values of the last two preamiloride periods and the last two periods during amiloride infusion. Effects of amiloride were evaluated statistically by comparing the two average values using Student’s paired t-test. Differences were considered statistically significant at the 0.05 level. Values presented are means ± SE.

RESULTS

Heart rate, mean arterial blood pressure, GFR, and plasma electrolytes before and during amiloride are given in Table 1. Between series, the blood pressure values were similar except for the series given ANG III. Administration of ANG III increased blood pressure by ~15%. In most experiments, GFR was not influenced by the dietary Na+ intake and the various pharmacological treatments, but, in the ANG III series, it was reduced by ~20% compared with the other appropriate control series. Plasma [Li+] averaged 125–170 μmol/l, except in the Na5-low Li group in which plasma [Li+] was 10–20% of this level. There were no significant differences in plasma [Na+] or [K+] among the groups.

The renal handling of Na+ and Li+ is reported in terms of fractional excretion before and during amiloride administration (Table 2). The difference between the FE $Li^+$ before and during amiloride administration (delta FE $Li^+$) may be taken as an index of the $Li^+$ reabsorption in the distal tubular segments, predominantly in the collecting ducts (Table 3). With regard to the general pattern of changes in FE $Li^+$, it is noticeable that 1) conditions during which distal tubular reabsorption of $Na^+$ is intensified, resulting in low fractional excretion rates of $Na^+$, were associated with amiloride-reversible reductions in the FE $Li^+$; and 2) FE $Li^+$ after amiloride always was close to 35% irrespective of large changes in Na+ intake or plasma [Li+] or of administration of Aldo, ANG III, or thiazide.

FE $Li^+$ was markedly reduced by a low-Na+ diet (Na5-vehicle group), and this reduction was completely reversed by amiloride. The difference amounted to 16% of the filtered load equal to ~40% of the estimated distal delivery of Li+ (Table 3). A practically identical reversal of FE $Li^+$ was observed after amiloride in the Na5-low Li group in which plasma [Li+] was reduced by 80–90%. Here, amiloride increased FE $Li^+$ with 14% of the filtered load, indicating that the amiloride-reversible reduction in FE $Li^+$ induced by the low-Na+ diet was independent of [Li+] in plasma. Administration of BFTZ completely reversed the decrease of FE $Li^+$ otherwise observed in rats on a low-Na+ diet (Na5-BFTZ group vs. the Na5-vehicle and Na200-vehicle groups), and subsequent administration of amiloride did not affect FE $Li^+$ of the Na5-BFTZ group at all. Similar to the effects of dietary Na+ restriction, pretreatment with Aldo led to a reduction of FE $Li^+$, which also was fully reversed by subsequent administration of amiloride. A less pronounced effect was seen after the administration of ANG III. All conditions during which fractional Na+ excretion was reduced (low-Na+ intake, Aldo or ANG III pretreatment) were associated with a decrease in FE $Li^+$, which was reversible by amiloride, whereas conditions during which fractional excretion of Na+ was normal or high (normal Na+ diet, thiazide + normal Na+ diet or thiazide + low-Na+ diet) revealed FE $Li^+$ values close to 35% and completely amiloride insensitive.

The amiloride-sensitive reabsorption of Na+ and Li+ expressed as a fraction of the distal tubular load ($r_{RLi-Amil}$ and $r_{RRNa-Amil}$, respectively) showed almost the same pattern as $delta FE Na^+$ and $delta FE Li^+$ (Table 3). In general, $r_{RRNa-Amil}$ was ~95% in the groups with low-Na+ excretion (Na5-vehicle, Na5-low Li, and Na200-Aldo), suggesting an almost complete distal tubular Na+ reabsorption. In the groups with a high Na+ excretion (Na200-vehicle, Na200-BFTZ, and Na5-BFTZ), $r_{RRNa-Amil}$ ranged from 35 to 80% and was thereby somewhat lower compared with rats exhibiting Na+ retention. $r_{RLi-Amil}$ was ~35% in groups with a low-Na+ excretion, but, in contrast to $r_{RRNa-Amil}$, it completely vanished in groups with a high Na+ excretion. These data emphasize that $r_{RRNa-Amil}$ and $r_{RLi-Amil}$ did not change proportion-

Table 1. Heart rate, mean arterial blood pressure, GFR, and blood parameters

<table>
<thead>
<tr>
<th>Period</th>
<th>Na200-Vehicle</th>
<th>Na200-BFTZ</th>
<th>Na5-Vehicle</th>
<th>Na5-Low Li</th>
<th>Na5-BFTZ</th>
<th>Na200-Aldo</th>
<th>Na200-ANG III</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>Control</td>
<td>391 ± 7</td>
<td>369 ± 34</td>
<td>396 ± 13</td>
<td>396 ± 14</td>
<td>425 ± 15</td>
<td>391 ± 13</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>Control</td>
<td>374 ± 9</td>
<td>393 ± 22</td>
<td>391 ± 12</td>
<td>379 ± 16</td>
<td>423 ± 11</td>
<td>391 ± 12</td>
</tr>
<tr>
<td>GFR, µl·min⁻¹·100 g body wt⁻¹</td>
<td>Control</td>
<td>1,028 ± 52</td>
<td>963 ± 26</td>
<td>999 ± 44</td>
<td>1,021 ± 72</td>
<td>933 ± 25</td>
<td>986 ± 60</td>
</tr>
<tr>
<td>P&lt;sub&gt;L&lt;/sub&gt;, µmol/l</td>
<td>Control</td>
<td>1,068 ± 54</td>
<td>1,002 ± 34</td>
<td>1,060 ± 43</td>
<td>1,025 ± 52</td>
<td>951 ± 42</td>
<td>1,022 ± 61</td>
</tr>
<tr>
<td>P&lt;sub&gt;Na&lt;/sub&gt;, µmol/l</td>
<td>Control</td>
<td>144 ± 2</td>
<td>141 ± 1</td>
<td>143 ± 1</td>
<td>141 ± 1</td>
<td>141 ± 1</td>
<td>141 ± 1</td>
</tr>
<tr>
<td>P&lt;sub&gt;K&lt;/sub&gt;, µmol/l</td>
<td>Control</td>
<td>4.6 ± 0.2</td>
<td>4.2 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>4.9 ± 0.2</td>
<td>4.2 ± 0.1</td>
<td>4.5 ± 0.2</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, no. of rats. MAP, mean arterial pressure; GFR, glomerular filtration rate; $P_{<L}$, $P_{Na}$, and $P_{K}$, plasma concentrations of $Li^+$, $Na^+$, and $K^+$, respectively; BFTZ, bendroflumethiazide; Aldo, aldosterone; ANG III, angiotensin III; and Na200, 200 mmol/kg Na; Na5, 5 mmol/kg Na.
Table 2. Renal parameters before and during blockade of distal tubular reabsorption of Li\(^+\) with amiloride

<table>
<thead>
<tr>
<th>Period</th>
<th>Na200-Vehicle</th>
<th>Na200-BFTZ</th>
<th>Na5-Vehicle</th>
<th>Na5-Low Li</th>
<th>Na5-BFTZ</th>
<th>Na200-Aldo</th>
<th>Na200-ANG III</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FE Li, % Control</td>
<td>37 ± 1</td>
<td>33 ± 2</td>
<td>20 ± 1</td>
<td>24 ± 1</td>
<td>32 ± 1</td>
<td>25 ± 2</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>Amiloride</td>
<td>38 ± 1</td>
<td>32 ± 2</td>
<td>35 ± 1*</td>
<td>38 ± 4*</td>
<td>33 ± 2</td>
<td>37 ± 2*</td>
<td>33 ± 2*</td>
</tr>
<tr>
<td>FENa, % Control</td>
<td>0.53 ± 0.11</td>
<td>4.62 ± 0.54</td>
<td>0.05 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>2.61 ± 0.54</td>
<td>0.11 ± 0.03</td>
<td>0.38 ± 0.06</td>
</tr>
<tr>
<td>Amiloride</td>
<td>2.61 ± 0.29*</td>
<td>7.28 ± 0.93*</td>
<td>1.72 ± 0.35*</td>
<td>2.18 ± 0.37*</td>
<td>7.33 ± 0.92*</td>
<td>1.93 ± 0.27*</td>
<td>1.57 ± 0.20*</td>
</tr>
<tr>
<td>FE K, % Control</td>
<td>17.6 ± 2.5</td>
<td>31.6 ± 3.9</td>
<td>11.4 ± 2.0</td>
<td>16.9 ± 3.0</td>
<td>42.9 ± 3.1</td>
<td>15.2 ± 1.8</td>
<td>15.0 ± 2.3</td>
</tr>
<tr>
<td>Amiloride</td>
<td>1.3 ± 0.4*</td>
<td>7.6 ± 1.2*</td>
<td>1.4 ± 0.6*</td>
<td>3.0 ± 1.4*</td>
<td>6.1 ± 1.4*</td>
<td>2.4 ± 0.9*</td>
<td>2.0 ± 0.52*</td>
</tr>
<tr>
<td>FE V, % Control</td>
<td>1.90 ± 0.19</td>
<td>6.58 ± 0.61</td>
<td>1.34 ± 0.20</td>
<td>1.74 ± 0.20</td>
<td>4.97 ± 0.82</td>
<td>1.60 ± 0.16</td>
<td>1.88 ± 0.20</td>
</tr>
<tr>
<td>Amiloride</td>
<td>3.78 ± 0.36*</td>
<td>8.56 ± 0.93*</td>
<td>2.74 ± 0.50*</td>
<td>3.79 ± 0.48*</td>
<td>8.83 ± 0.98*</td>
<td>3.04 ± 0.35*</td>
<td>2.89 ± 0.17*</td>
</tr>
<tr>
<td>U Na(_200)/U Li</td>
<td>16 ± 3</td>
<td>149 ± 14</td>
<td>3 ± 1</td>
<td>13 ± 3</td>
<td>76 ± 21</td>
<td>4 ± 1</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Amiloride</td>
<td>86 ± 10*</td>
<td>265 ± 28</td>
<td>55 ± 11*</td>
<td>568 ± 133*</td>
<td>209 ± 33*</td>
<td>44 ± 5*</td>
<td>37 ± 3*</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, no. of rats. FE Li, fractional Li\(^+\) excretion; FENa, fractional Na\(^+\) excretion; FE K, fractional K\(^+\) excretion; FE V, fractional water excretion; U Na\(_200\)/U Li, ratio between concentration of Na\(^+\) and Li\(^+\) in urine. Control versus amiloride: *P < 0.001, †P < 0.01, and ‡P < 0.05.

Effect of BFTZ on Li\(^+\) reabsorption in the distal tubule. Amiloride blocks conductive Na\(^+\) channels in the collecting ducts and has often been used as a tool to recognize distal nephron Li\(^+\) reabsorption (13, 27, 31). Micropuncture studies have shown that, in the doses employed in these studies, as well as in the present study, absolute and fractional proximal reabsorption of Li\(^+\), Na\(^+\), and water is not influenced, nor is the reabsorption of Na\(^+\) and Na\(^+\) in the loop of Henle. The increase of fractional urinary Li\(^+\) excretion observed in Na\(^+\)-depleted rats is due to inhibition of Li\(^+\) reabsorption in the collecting ducts (7, 29, 32). An increased urine flow rate during amiloride administration, as observed in the present study, has consistently been found and is due to decreased water reabsorption in the collecting ducts (7, 29).

Effect of BFTZ on Li\(^+\) reabsorption in distal nephron. The results of the present study confirm previous observations that dietary Na\(^+\) depletion in rats leads to reabsorption of Li\(^+\) via an amiloride-sensitive transport mechanism (27, 31). This amiloride-sensitive reabsorption of Li\(^+\) is located between the last accessible part of the distal nephron and the end of the collecting ducts (32). In the present study, we show, for the first time, that Li\(^+\) transport at these distal nephron sites is abolished by administration of the thiazide diuretic.

Table 3. Renal responses to administration of amiloride

<table>
<thead>
<tr>
<th>Period</th>
<th>Na200-Vehicle</th>
<th>Na200-BFTZ</th>
<th>Na5-Vehicle</th>
<th>Na5-Low Li</th>
<th>Na5-BFTZ</th>
<th>Na200-Aldo</th>
<th>Na200-ANG III</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td></td>
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</tr>
<tr>
<td>Delta FE Li, %</td>
<td>0.2 ± 1.2</td>
<td>-1.2 ± 2.5</td>
<td>15.7 ± 1.7*</td>
<td>14.3 ± 4.5*</td>
<td>0.2 ± 1.9</td>
<td>12.4 ± 1.3*</td>
<td>5.8 ± 1.7*</td>
</tr>
<tr>
<td>Delta FENa, %</td>
<td>2.08 ± 0.25*</td>
<td>2.66 ± 0.48*</td>
<td>1.67 ± 0.35*</td>
<td>2.13 ± 0.37*</td>
<td>4.66 ± 0.44*</td>
<td>1.82 ± 0.26*</td>
<td>1.20 ± 0.18*</td>
</tr>
<tr>
<td>Delta FE K, %</td>
<td>16.3 ± 2.5*</td>
<td>24.0 ± 4.0*</td>
<td>10.0 ± 1.5*</td>
<td>13.9 ± 3.2*</td>
<td>38.0 ± 2.2*</td>
<td>12.8 ± 2.2*</td>
<td>13.0 ± 2.0*</td>
</tr>
<tr>
<td>rRLi-Amiloride, %</td>
<td>0.3 ± 3</td>
<td>-7.9 ± 14</td>
<td>44 ± 4*</td>
<td>34 ± 8†</td>
<td>-1 ± 6</td>
<td>34 ± 3*</td>
<td>20 ± 6†</td>
</tr>
<tr>
<td>rRNAmiloride, %</td>
<td>80 ± 3*</td>
<td>35 ± 4*</td>
<td>96 ± 1*</td>
<td>98 ± 1*</td>
<td>66 ± 5*</td>
<td>94 ± 1*</td>
<td>72 ± 6*</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, no. of rats. rRLi-Amiloride, relative Li\(^+\) reabsorption in the amiloride-sensitive segment; rRNAmiloride, relative Na\(^+\) reabsorption in the amiloride-sensitive segment. Significantly different from zero: *P < 0.001, †P < 0.01, and ‡P < 0.05.
BFTZ. Thiazide diuretics inhibit Na\(^{+}\)-Cl\(^{-}\) cotransport in the distal convoluted tubules, just proximal to the amiloride-sensitive region (1, 6). BFTZ does not interfere with the tubuloglomerular feedback because of its lacking inhibition of the carbonic anhydrase in the proximal tubules (3) and does not inhibit Li\(^{+}\) reabsorption in Na\(^{+}\)-repleted rats (12). Furthermore, BFTZ does not impair the amiloride-sensitive Na\(^{+}\) transport as seen from the similar natriuretic responses to amiloride in the Na\(^{+}\)-Vehicle and the Na\(^{+}\)-BFTZ series (Table 2).

The ability of BFTZ to abolish amiloride-sensitive Li\(^{+}\) reabsorption suggests that Li\(^{+}\) transport at distal nephron sites is inhibited by increased Na\(^{+}\) delivery to the amiloride-sensitive mechanism. Indeed, this would also account for previous findings that the diuretics acetazolamide and furosemide, which act in the proximal tubule and in the thick ascending limb of Henle's loop, respectively, also suppress amiloride-sensitive Li\(^{+}\) reabsorption (13, 30, 32). The alternative explanation that Li\(^{+}\) reabsorption in the distal nephron could be mediated by a thiazide-sensitive mechanism seems unlikely, since BFTZ and amiloride did not display an additive lithiuretic effect, in contrast to what was observed for Na\(^{+}\).

Mechanism of Li\(^{+}\) transport in distal nephron. The mechanism of Li\(^{+}\) reabsorption in distal nephron segments is not yet known with certainty, but, since it is blocked by amiloride, Li\(^{+}\) movement via the amiloride-sensitive Na\(^{+}\) channel is presumably involved (2). This channel is located in the apical cell membrane of collecting duct principal cells, and it displays a slightly higher permeability for Li\(^{+}\) than for Na\(^{+}\) (20). The number of open Na\(^{+}\) channels is increased by Na\(^{+}\) depletion (18) or chronic Aldo administration (19), but, even in Na\(^{+}\)-replete animals, a significant number of channels are active, as also suggested by the increase of FE\(_{\text{Na}}\) in response to amiloride observed in previous studies and in the present study. It is therefore surprising that Li\(^{+}\) is only reabsorbed in the distal nephron in Na\(^{+}\)-depleted rats and not in Na\(^{+}\)-replete rats.

Because the number of apical Na\(^{+}\) channels is limited and determines the amiloride-sensitive Na\(^{+}\) reabsorption, it is obvious that Na\(^{+}\) and Li\(^{+}\) compete for cellular influx across the apical membrane. This has led to speculations of the importance of the [Na\(^{+}\)]/[Li\(^{+}\)] ratio for the distal tubular Li\(^{+}\) reabsorption (25, 30, 32). Thus it has been hypothesized that an increase in the [Na\(^{+}\)]/[Li\(^{+}\)] ratio in distal tubular fluid arriving to the amiloride-sensitive channels may account for suppression of Li\(^{+}\) ions transport. However, whereas the distal tubular [Na\(^{+}\)]/[Li\(^{+}\)] ratio undoubtedly influences the number of Li\(^{+}\) being transported by the channels, this does not necessarily imply that the amiloride-induced increase of FE\(_{\text{Li}}\) is influenced. Thus a decrease of plasma [Li\(^{+}\)] leads to a proportional decrease of tubular fluid [Li\(^{+}\)] and a proportional decrease of the number of Li\(^{+}\) being reabsorbed, but the fraction of Li\(^{+}\) being
reabsorbed is supposedly not dependent on the plasma [Li\(^+\)]. The reduction of FE\(_{Li}\) in response to dietary Na\(^+\) restriction is accordingly not changed when plasma Li\(^+\) is lowered, despite the fact that the tubular [Na\(^+\)]/[Li\(^+\)] ratio is increased. In agreement with this, it was found that, although the urinary [Na\(^+\)]/[Li\(^+\)] ratio in the Na5-low Li series was less favorable for distal tubular Li\(^+\) transport than in the Na5-vehicle series (13 ± 3 vs. 3 ± 1, respectively), the increase in FE\(_{Li}\) during amiloride infusion (delta FE\(_{Li}\)) was similar in the two series (14 vs. 15%, respectively). Furthermore, the urinary [Na\(^+\)]/[Li\(^+\)] ratio in the Na5-low Li series was similar to that of the Na200-vehicle series (13 ± 3 vs. 16 ± 3, respectively) in which delta FE\(_{Li}\) was zero. The data thus show that differences in the distal tubular [Na\(^+\)]/[Li\(^+\)] ratio cannot explain the increase in amiloride-inhibitable Li\(^+\) reabsorption observed in rats transferred from a high to a low dietary Na\(^+\) intake.

These results do, however, not answer the question whether the lack of distal tubular Li\(^+\) reabsorption seen in rats on a normal Na\(^+\) diet can be explained by competition alone or if a difference in basolateral membrane transport between Na\(^+\) and Li\(^+\) contributes as well. The affinity and maximal rate of Li\(^+\) transport via the apical membrane is slightly higher than that of Na\(^+\) (20). In consequence, if competition was the only reason for the lack of Li\(^+\) reabsorption, r\(_{Li-Amil}\) would be expected to be similar to r\(_{Na-Amil}\) in Na\(^+\)-replete rats and vary proportionally. Nevertheless, it was found that the r\(_{Li-Amil}\) was less than r\(_{Na-Amil}\) in Na\(^+\)-replete rats (0 ± 3 vs. 80 ± 3, respectively), and overall r\(_{Na-Amil}\) and r\(_{Li-Amil}\) did not vary proportionally. Although the true r\(_{Na-Amil}\) may be slightly smaller than indicated above, because of a non-amiloride-sensitive Na\(^+\) transport, an r\(_{Li-Amil}\) of around zero is in any case markedly lower than the r\(_{Na-Amil}\) values reported. This suggests that the lack of distal tubular Li\(^+\) reabsorption during Na\(^+\)-replete conditions cannot be explained by simple competition of transport across the apical membrane between Na\(^+\) and Li\(^+\) alone.

An alternative model. Because modulation of Li\(^+\) transport across the distal tubule via the amiloride-sensitive mechanism cannot be explained by changes in the relative concentration of Na\(^+\) and Li\(^+\) in distal tubular fluid or by competition alone between Na\(^+\) and Li\(^+\) for entrance through the channels of the apical membrane, an alternative model is warranted. Of importance for the transcellular transport of Li\(^+\) across the distal tubular epithelium is the Li\(^+\) pathway across the basolateral membrane. The efflux of Li\(^+\) at the antiluminal side of the cell does not occur via the Na\(^+\)-K\(^+\)-ATPase (9) and may rely on either the Na\(^+\)-Li\(^+\) antiporter previously described in other “tight” epithelia (14) or even simple diffusion, through as yet undefined channels driven by the prevailing electrochemical gradient. This difference in basolateral transport capacity for Na\(^+\) over Li\(^+\) may explain why the amiloride-sensitive Li\(^+\) reabsorption is quantitatively insignificant during Na\(^+\)-replete conditions. Considering that Li\(^+\) may rely on passive extrusion mechanisms at the basolateral membrane, accumulation inside the cell will occur and thereby diminish the driving force for influx via the luminal membrane Na\(^+\) channels. If the extrusion is entirely passive without being coupled to inward transport of Na\(^+\), the intracellular [Li\(^+\)]/[Li\(^+\)] above which basolateral Li\(^+\) transport occurs cannot be estimated. Given a potential difference across the basolateral membrane of 83 mV (23) and a plasma [Li\(^+\)] of ~0.15 mM, the Nernst equation indicates that [Li\(^+\)] must exceed 3.3 mM before efflux across the basolateral membrane is initiated. The [Li\(^+\)] in late distal tubular fluid is about two times that of plasma (4), i.e., 0.30 mM. Thus, using 3.3 mM as an estimate of [Li\(^+\)], it follows that the potential difference across the apical membrane (PD\(_{a}\)) must be higher than 64 mV (lumen positive) before Li\(^+\) influx across the apical membrane occurs. However, this equilibrium potential for Li\(^+\) may not be strictly correct, since basolateral Li\(^+\) extrusion is presumably coupled to Na\(^+\) influx (14), resulting in a lower intracellular-to-plasma Li\(^+\) concentration ratio than that obtained by passive distribution. Ratios between one and four have been reported in the literature from nonepithelial tissues (9), but, as the transport of Li\(^+\) into the distal tubulus cell is facilitated by the presence of a conductive Na\(^+\) channel, a higher [Li\(^+\)] in tubular cells must be expected compared with the nonepithelial tissues. Based on an intracellular/plasma [Li\(^+\)] ratio of ~10, the Nernst equation indicates, with the same set of assumptions as above, that the PD\(_{a}\) in distal tubule cells must exceed 43 mV (lumen positive) before Li\(^+\) influx across the apical membrane occurs.

These reasonable estimates may explain why amiloride-sensitive Li\(^+\) reabsorption is insignificant in Na\(^+\)-replete rats (4). PD\(_{a}\) in Na\(^+\)-replete rats is ~50 mV (lumen positive; see Ref. 23) and therefore close to the equilibrium potential for Li\(^+\). This PD\(_{a}\) may therefore not be sufficient to drive a quantitatively significant Li\(^+\) uptake. In addition, this model may explain why Li\(^+\) is reabsorbed in this segment during Na\(^+\) restriction. In this condition, not only is the number of active Na\(^+\) channels increased, but also the Na\(^+\) delivery to the later part of the amiloride-sensitive region is markedly decreased, as indicated by the low urinary Na\(^+\) excretion. This reduces the competition from Na\(^+\), and, furthermore, due to a decrease in Na\(^+\) influx through the apical ion channels, the apical membrane potential becomes relatively hyperpolarized, favoring intracellular uptake of Li\(^+\).

In line with this, Aldo treatment led in the current study to a reduction of Na\(^+\) delivery to the later part of the amiloride-sensitive region, as indicated by a lowering of FE\(_{Na}\), and induced a quantitatively significant amiloride-sensitive Li\(^+\) reabsorption (Table 3). In contrast, Horisberger and Diezli (10) failed to see any reduction in FE\(_{Li}\) in their study on the effect of Aldo in adrenalectomized rats, but this was presumably because FE\(_{Na}\) was not reduced sufficiently. In that study, FE\(_{Na}\) during Aldo administration was 0.5%, which is similar to what was observed in our Na200 control group, in which no distal nephron reabsorption of Li\(^+\) was detectable either.
ANG III was administered as another means of increasing distal tubular Na$^+$ reabsorption (8) and was chosen in favor of ANG II because its effect on mean arterial pressure is significantly less pronounced (8). A modest increase in mean arterial pressure and a 20% reduction in GFR during ANG III infusion documented its expected pharmacological actions. Surprisingly, ANG III did not increase rR$_{Na}$/Am because in the present study; however, it did reduce FE$_{Na}$ below control levels and thereby contributed with a further data point in the relationship between delta FE$_{Na}$ control and delta FE$_{Li}$. The Na$^+$-retaining effect elicited by ANG III was not as marked as for Aldo and, in line with this, the influence on distal tubular Li$^+$ reabsorption was also weaker (Table 3). However, when evaluating the ANG III results in conjunction with the other data obtained, these results complement the continuity in the relation between FE$_{Na}$ control and delta FE$_{Li}$ (Fig. 1B) from a Na$^+$-retaining state to Na$^+$-replete conditions.

Further support for the hypothesis that PD$_a$ influences the amiloride-sensitive distal tubular Li$^+$ reabsorption is derived from the results obtained with BFTZ. It has been established that, as Na$^+$ delivery to the distal nephron rises, the Na$^+$ influx via Na$^+$ channels increases, and the luminal membrane depolarizes (17). Therefore, as Na$^+$ delivery increases, Li$^+$ influx via Na$^+$ channels should fall, which is consistent with the effect of BFTZ observed in the present study. The depolarizing effect of increased Na$^+$ delivery and reabsorption is also reflected by an increase in K$^+$ secretion, since a decrease of PD$_a$ increases the driving force for K$^+$ efflux. Thus, if changes in PD$_a$ are important, amiloride-sensitive Li$^+$ and K$^+$ transport should change in opposite directions as well. Figure 2 shows that this was indeed the case, since a close inverse relationship between amiloride-sensitive Li$^+$ and K$^+$ transport persists.

In conclusion, Na$^+$ is reabsorbed more readily than Li$^+$ at all levels of urinary Na$^+$ excretion. Measurable distal tubular Li$^+$ reabsorption occurs only when the urinary Na$^+$ excretion and hence the [Na$^+$] in the distal tubular fluid are low. Our observations are consistent with the hypothesis that, because Li$^+$ is not extruded from the cell with the Na$^+$-K$^+$-ATPase, it is accumulated intracellularly, and its influx through the apical membrane is dependent of the prevailing PD$_a$. Under Na$^+$-replete conditions, Na$^+$ is reabsorbed in advance of Li$^+$ because it is actively extruded across the basolateral membrane, and the reabsorption of Na$^+$ depolarizes the apical membrane, thereby preventing Li$^+$ from being reabsorbed. Under Na$^+$-depleted conditions, the apical membrane becomes hyperpolarized due to a low-Na$^+$ influx, and distal Li$^+$ reabsorption can occur.

We thank Dr. P. Christensen for performing some of the Li$^+$ analysis by flameless atomic absorption spectrometry and Ellen Philipson and Lene Gertman for excellent technical assistance. Bendroflumethiazide was donated by Leo Pharmaceuticals (Ballerup, Denmark), and amiloride hydrochloride was donated by GEA A/S. Urinary amiloride concentrations were generously analyzed by GEAAS.

This study was supported by the Danish Research Council for Health Sciences, The Novo Nordisk Foundation, The Direktør Ib Henriekens Fond, and The United Kingdom National Kidney Research Fund.

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Received 20 December 1996; accepted in final form 15 October 1997.

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