Chronic Lithium Treatment Inhibits Amiloride-Sensitive Sodium Transport in the Rat Distal Nephron

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ABSTRACT
Chronic treatment of rats with lithium leads to Na\(^+\) loss and a reduced antinatriuretic response to aldosterone, suggesting that lithium reduces conductive Na\(^+\) transport in the distal nephron. This was investigated in the present study by measuring the renal response to aldosterone infusion followed by amiloride in chronically instrumented conscious rats given lithium for 3 to 4 weeks to achieve plasma Li\(^+\) concentrations of approximately 0.5 mM. A servo-controlled infusion system was used to maintain sodium and water homeostasis, thereby preventing misinterpretation of the findings as a consequence of drug-induced changes in Na\(^+\) balance. In a control group of rats, Na\(^+\) excretion decreased in response to aldosterone (p < .01) and subsequent amiloride administration led to a marked increase in Na\(^+\) excretion (p < .001). In contrast, in the lithium-treated group, there was no significant response to either aldosterone or amiloride. It is concluded that long-term treatment with lithium, even when plasma Li\(^+\) concentrations are below 1 mM, reduces aldosterone-stimulated Na\(^+\) transport through the amiloride-sensitive Na\(^+\) channels in the principal cells of the distal nephron.

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ABBR EVIATIONS: C, renal clearance; FE, fractional excretion; GFR, glomerular filtration rate; ΔFE\(_{Na}\), amiloride-sensitive Na\(^+\) reabsorption; FE\(_{Na-amiloride}\), fractional load of Na\(^+\) to the amiloride-sensitive site; MAP, mean arterial blood pressure; DOCA, deoxycorticosterone acetate.

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wet-mash diet containing 500 mmol of Na⁺ and 200 mmol of K⁺ per kg dry weight for at least 4 weeks before experimentation. Half of the rats in addition were given Li⁺ in the food (100 mmol/kg dry weight) for 3 to 4 weeks immediately before experimentation. All rats were given free access to tap water, and the lithium-treated rats in addition were given free access to 0.46 M NaCl solution to compensate for the Li⁺-induced renal loss of Na⁺ (Jensen et al., 1976).

**Surgical Preparation.** Ten to 20 days before experimentation, the animals were anesthetized with halothane/N₂O. Using aseptic surgical techniques, sterile Tygon catheters were advanced into the abdominal aorta and the inferior caval vein via the femoral vessels, and a sterile chronic suprapubic bladder catheter was implanted into the bladder. All catheters were produced and fixed, with small modifications, as described by Petersen et al. (1991). After instrumenta-
tion, the rats were infused with saline (5 ml), given a long-lasting analgesic (Temgesic; Reckitt & Coleman, Hull, UK 10 µg/animal, s.c.), and allowed to recover from anesthesia. The arterial and venous catheters were sealed with 50% glucose solution containing 500 U of heparin and 10,000 U of strepkinase per ml. After the operation the rats were returned to the animal unit and housed individually. After a recovery period of 5 to 6 days, the rats were acclimatized to restriction by three daily training sessions in the restraining cages. The duration of each daily session was increased stepwise from 1 to 3 h a day.

**Experimental Groups.** Within each group (lithium-treated or control), the animals were allocated randomly to one of three experimental procedures: 1) infusion of vehicle alone throughout the experiment, 2) infusion of aldosterone throughout the experiment, or 3) infusion of aldosterone throughout the experiment, together with amiloride for the final 90 min.

**Clearance Protocol.** Each experiment comprised a 15-min bolus period for [³H]inulin and aldosterone (where applicable), a 120-min equilibration period, three 20-min urine collection periods, an 8-min bolus period for amiloride (where applicable), followed by a 22-min equilibration period and by three 20-min urine collection periods during which amiloride or vehicle was infused.

The experiments were carried out between 8 AM and 1 PM, with the conscious rats immobilized in restraining cages. The rats were connected to infusion pumps via the venous catheter and to a Baxter Uniflow blood pressure transducer via the arterial catheter. Through the pressure transducer a continuous intra-arterial infusion of 150 mM glucose solution containing heparin (100 U/ml) at a rate of 5 µl/min was given to keep the arterial catheter open. In addition, the animals received throughout the experiment an i.v. infusion of 150 mM glucose solution (bolus 0.6 ml, sustained 10 µl/min) containing [³H]inulin (Amersham International, Aylesbury, UK) (bolus 3.6 µCi, sustained 0.068 µCi/min), as well as 150 mM glucose solution at a rate of 30 µl/min to maintain an adequate urine flow necessary for elimina-
tion of bladder-emptying errors. Aldosterone (Sigma; bolus 6 µg, sustained 0.1 µg/min) was included with the [³H]inulin infusion. Amiloride (Merck, Darmstadt, Germany) was administered i.v. as a bolus delivered over 8 min (256 µg) followed by sustained infusion (8 µg/min) in 150 mM glucose solution (5 µl/min).

Throughout the experiment, water and Na⁺ balance was main-
tained by a computer-driven servo-control system taking into ac-
count all the extra fluid given by the various pumps (Spannow et al., 1997). From the bladder catheter, urine passed a Na⁺-sensitive electrode that performed one measurement of urinary Na⁺ per sec-
ond (Novabiochem, Waltham, MA). Urine was collected in vials ar-
ranged in an autosampler placed on an electronic balance. The au-
tosampler was operated by a photocell, which allowed change of the
vial without touching the balance. Data on urine production (weight on scale) and urinary Na⁺ were sampled continuously on an IBM-
compatible computer, which, in turn, controlled the infusion rates of two independent infusion pumps that delivered 150 mM glucose solution and 300 mM NaCl solution, respectively. Urinary output of Na⁺ and fluid were integrated over 5 min, thus causing a 5-min delay in changes of Na⁺ and glucose infusion rates.

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.

**Maintenance of Na⁺ Electrode.** The Na⁺ electrode was cali-
ibrated with standard solutions containing 10, 50, and 100 mM NaCl solution in 5 mM KCl solution. After each experiment, the electrode was conditioned with “Na/pH solution” (Novabiochem) and then per-
fused with 10 mM NaCl solution in 5 mM KCl solution until the next experiment while the Na⁺ concentration was measured continuously and data were collected. In this way, the stability and readings of the electrode could be checked by one glimpse at the computer screen before the start of the experiment and, if necessary, the calibration adjusted. After the experiment, the computer-calculated Na⁺ excre-
tion was compared with the Na⁺ excretion based on measurements of urinary Na⁺ by flame-emission photometry. In every case, there was a good agreement between the two values.

**Analysis.** Urine volume was determined gravimetrically. Concentra-
tions of Na⁺ and potassium in plasma and urine and Li⁺ in plasma were determined by flame-emission photometry. [³H]inulin concentrations in plasma and urine were determined by liquid scintillation counting on a Packard Tri-Carb liquid scintillation analy-
zer. Fifteen microliters of the sample and 285 µl of water were mixed with 2.5 ml of Ultima Gold (Packard Instruments, Meriden, CT).

**Calculations.** Renal clearances (C) and fractional excretions (FE) were calculated by the standard formulas:

\[ C = U \times V / P; \quad FE = C / GFR \]

where \( U \) is the urine concentration, \( V \) is the urine flow rate, \( P \) is the plasma concentration, and \( GFR \) is the glomerular filtration rate as measured by [³H]inulin clearance.

Calculations of the fractional delivery of Na⁺ to the amiloride-
sensitive segment have made the assumptions that amiloride blocked completely the conductive Na⁺ channels in the principal cells and that Na⁺ was not reabsorbed by any other mechanisms in the collecting duct in quantitatively significant amounts. Thus, the fractional delivery of Na⁺ to the amiloride-sensitive segment can be estimated as the FE\(_{Na-pre-amil}\) during administration of amiloride (\( FE_{Na-pre-amil} \)) and amiloride-sensitive Na⁺ reabsorption can be calcu-
ated as the difference between the values for FE\(_{Na-pre-amil}\) and postamiloride administration (ΔFE\(_{Na} \)). It is possible that FE\(_{Na-pre-amil}\) is underestimated using this method, because there may be some Na⁺ reabsorption in the collecting duct via amiloride-insensitive mecha-
nisms. Furthermore, calculated values for ΔFE\(_{Na} \) will be influenced by any changes in the fractional delivery of Na⁺ to the amiloride-
sensitive segment between pre- and postamiloride measurements. However, it is assumed that any such inaccuracies would apply to both groups of rats; thus, they should not prevent between-group comparisons being made.

**Data Presentation and Statistics.** All values are presented as means ± S.E.M. The values for renal clearance variables are derived from the averages of the three preamiloride periods and of the three periods during amiloride infusion. Overall statistical comparisons were performed by one-way ANOVA (between groups), one-way ANOVA for repeated measures (within group), or two-way ANOVA for repeated measures for two-way classified data (group and time). Individual comparisons within or between groups were performed by subsequent use of Student’s paired or unpaired t test. Differences were considered statistically significant at the 0.05 level.

**Results**

Renal Na⁺ excretion was measured continuously during the experiments by the Na⁺ electrode (Fig. 1). In the control rats, Na⁺ excretion decreased significantly in the time inter-
val 0 to 195 min in each of the two subgroups given aldoste-
lytes before and during amiloride infusion are given in Table 1. The blood pressure was significantly higher in the lithium-treated group than in the control group. There were no significant differences in plasma Na\(^+\) or K\(^+\) between the two groups. Administration of amiloride influenced neither MAP nor the plasma electrolyte concentrations.

Renal clearance data are shown in Table 2. In the rats given vehicle only, GFR was significantly higher in the lithium-treated rats than in the control animals. C\(_{Na}\) and F\(_{ENa}\) also tended to be higher in the Li\(^+\)-treated rats, although statistical significance was not quite reached. In the control animals, there was a tendency to a reduction in C\(_{Na}\) and F\(_{ENa}\) in response to aldosterone (compared with vehicle) and a significant increase in C\(_{Na}\) and F\(_{ENa}\) in response to amiloride. In the lithium-treated group, aldosterone had no significant effect on C\(_{Na}\) and F\(_{ENa}\); furthermore, although there was some increase of C\(_{Na}\) and F\(_{ENa}\) in response to amiloride, the increase was less pronounced than that observed in the control group, and in any case some increase (although statistically insignificant) was also observed in the two time-control lithium-treated subgroups not given amiloride. Moreover, in lithium-treated rats, neither C\(_{Na}\) nor F\(_{ENa}\) was significantly higher in the aldosterone-amiloride subgroup during period 4 + 5 + 6 than in the aldosterone alone subgroup during the corresponding period.

After blockade of Na\(^+\) reabsorption in the collecting ducts by amiloride, F\(_{ENa}\) was very similar in the control group and the lithium-treated group (3.45 \pm 0.74\% versus 3.46 \pm 0.33\%), suggesting that the load of Na\(^+\) to the amiloridesensitive site was similar in the two groups. In contrast, in the same time period there was a marked difference in F\(_{ENa}\) between the two groups given aldosterone alone (0.72 \pm 0.32\% versus 2.54 \pm 0.55\%, \(p < .05\)), reflecting a difference in Na\(^+\) reabsorption through the amiloride-sensitive channels. Fractional potassium excretion was not significantly different between the two groups before administration of amiloride and decreased to similar values after the administration of amiloride.

Further information about the amiloride-sensitive Na\(^+\) reabsorption appears from inspection of results from individual rats (Fig. 2). In control rats, the amiloride-sensitive Na\(^+\) reabsorption (\(\Delta F_{ENa}\)) rose linearly with increasing load of Na\(^+\) to the amiloride-sensitive site (F\(_{ENa-amil}\)), at least when the latter did not exceed 5\%. In one extreme data set (not shown), F\(_{ENa-amil}\) and \(\Delta F_{ENa}\) were 9.2 and 6.2\%, respectively, suggesting that when the delivered load is very high the capacity for Na\(^+\) reabsorption may be exceeded. In marked contrast to the control group, \(\Delta F_{ENa}\) in the Li\(^+\)-

<table>
<thead>
<tr>
<th>MAP (mm Hg)</th>
<th>Control, (n = 11)</th>
<th>Lithium Treated, (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(_{Na}) (mmol/liter)</td>
<td>Preamiloride 103 \pm 2</td>
<td>113 \pm 3*</td>
</tr>
<tr>
<td>Amiloride</td>
<td>105 \pm 3</td>
<td>115 \pm 1*</td>
</tr>
<tr>
<td>Amiloride +</td>
<td>138 \pm 3</td>
<td>141 \pm 2</td>
</tr>
<tr>
<td>P(_{K}) (mmol/liter)</td>
<td>Preamiloride 3.9 \pm 0.1</td>
<td>3.8 \pm 0.2</td>
</tr>
<tr>
<td>Amiloride</td>
<td>4.0 \pm 0.1</td>
<td>3.8 \pm 0.1</td>
</tr>
<tr>
<td>P(_{Li}) (mmol/liter)</td>
<td>Preamiloride 0.52 \pm 0.07</td>
<td>0.43 \pm 0.07</td>
</tr>
<tr>
<td>Amiloride</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(P_{Na}, P_{K}, P_{Li}\) plasma concentration of Na\(^+\), K\(^+\), and Li\(^+\), respectively. Control versus lithium treated, \(^* p < .05\).
Amiloride blocks conductive apical Na⁺ channels in principal cells in the collecting duct and is used as a tool to recognize Na⁺ reabsorption through these channels (Benos, 1982). The dose employed in the present study leads to intratubular amiloride concentrations well below those reported to block the Na⁺-H⁺ exchange mechanism in the proximal tubules but above the concentrations required to block movement of Na⁺ through the conductive Na⁺ channel (Shalni et al., 1998). In line with this, micropuncture studies have demonstrated that Na⁺ reabsorption in the proximal tubule, the loop of Henle, or the distal tubule is not influenced and that the increased fractional urinary Na⁺ excretion is entirely a result of inhibition of Na⁺ reabsorption in the collecting duct (Fransen et al., 1992; Shirley et al., 1992; Walter et al., 1995). An increased urine flow rate during amiloride administration, as observed in the present study, has been found consistently and is due to decreased water reabsorption in the collecting ducts (Shirley et al., 1992).

As explained in Materials and Methods, 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 (Shirley et al., 1992), the load of Na⁺ delivered to the amiloride-sensitive site can be estimated as the urinary excretion of Na⁺ after administration of amiloride (FENa-amil). That FENa-amil in control and Li⁺-treated rats was almost identical suggests that the load of Na⁺ to the amiloride-sensitive site was similar in the two groups and that the marked difference in Na⁺ excretion in the absence of amiloride was a result of a difference in Na⁺ reabsorption through the amiloride-sensitive channels in the distal nephron. Furthermore, in control rats, amiloride-sensitive Na⁺ reabsorption was found to increase with increasing Na⁺ load, as shown by the close correlation between Δ FENa and FENa-amil (Fig. 2). In contrast, in Li⁺-treated rats, Δ FENa did not rise with increasing Na⁺ load. Thus, these findings reaffirm that chronic Li⁺ treatment is associated with a reduction in amiloride-sensitive Na⁺ reabsorption. Although micropuncture studies in anesthetized animals have indicated that high doses of Li⁺ can inhibit Na⁺ reabsorption in additional nephron segments including proximal and distal convoluted tubules (Martinez-Maldonado et al., 1975; Hecht et al., 1978), the inhibitory site of action is likely to depend critically on the plasma Li⁺ concentration (very

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### Table 2

Renal clearance data (mean ± S.E.M.)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Period</th>
<th>GFR</th>
<th>CNa</th>
<th>FENa</th>
<th>FEK</th>
<th>FEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>Vehicle</td>
<td>1 + 2 + 3</td>
<td>855 ± 44</td>
<td>14.6 ± 5.2</td>
<td>1.76 ± 0.69</td>
<td>13.9 ± 2.6</td>
<td>3.06 ± 0.80</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>4 + 5 + 6</td>
<td>965 ± 54</td>
<td>15.3 ± 6.5</td>
<td>1.53 ± 0.59</td>
<td>15.1 ± 3.0</td>
<td>3.78 ± 1.19</td>
</tr>
<tr>
<td>Control (n = 6)</td>
<td>Aldo</td>
<td>1 + 2 + 3</td>
<td>1005 ± 67</td>
<td>7.0 ± 3.4</td>
<td>0.75 ± 0.41</td>
<td>19.2 ± 1.6</td>
<td>2.22 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>Aldo</td>
<td>4 + 5 + 6</td>
<td>902 ± 72</td>
<td>5.8 ± 2.0</td>
<td>0.72 ± 0.32</td>
<td>17.0 ± 1.7</td>
<td>1.72 ± 0.37</td>
</tr>
<tr>
<td>Control (n = 11)</td>
<td>Aldo + Amil</td>
<td>1 + 2 + 3</td>
<td>961 ± 61</td>
<td>5.9 ± 2.2</td>
<td>0.60 ± 0.24</td>
<td>17.6 ± 2.8</td>
<td>2.65 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>Aldo + Amil</td>
<td>4 + 5 + 6</td>
<td>997 ± 39</td>
<td>34.8 ± 7.2</td>
<td>3.45 ± 0.74</td>
<td>1.9 ± 0.4</td>
<td>5.14 ± 0.88</td>
</tr>
<tr>
<td>Lithium (n = 7)</td>
<td>Vehicle</td>
<td>1 + 2 + 3</td>
<td>1093 ± 51</td>
<td>28.7 ± 5.6</td>
<td>2.53 ± 0.41</td>
<td>11.8 ± 2.2</td>
<td>7.96 ± 1.37</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>4 + 5 + 6</td>
<td>1213 ± 61</td>
<td>36.7 ± 8.9</td>
<td>2.89 ± 0.61</td>
<td>8.9 ± 2.0</td>
<td>8.55 ± 1.89</td>
</tr>
<tr>
<td>Lithium (n = 5)</td>
<td>Aldo</td>
<td>1 + 2 + 3</td>
<td>1135 ± 39</td>
<td>23.0 ± 4.2</td>
<td>2.03 ± 0.39</td>
<td>12.1 ± 2.4</td>
<td>7.15 ± 1.05</td>
</tr>
<tr>
<td></td>
<td>Aldo</td>
<td>4 + 5 + 6</td>
<td>1192 ± 44</td>
<td>30.2 ± 6.6</td>
<td>2.54 ± 0.55</td>
<td>10.4 ± 2.2</td>
<td>8.47 ± 1.76</td>
</tr>
<tr>
<td>Lithium (n = 8)</td>
<td>Aldo</td>
<td>1 + 2 + 3</td>
<td>1028 ± 51</td>
<td>21.0 ± 3.4</td>
<td>2.00 ± 0.28</td>
<td>12.7 ± 1.6</td>
<td>9.91 ± 1.07</td>
</tr>
<tr>
<td></td>
<td>Aldo + Amil</td>
<td>4 + 5 + 6</td>
<td>1063 ± 66</td>
<td>37.2 ± 4.7</td>
<td>3.46 ± 0.33</td>
<td>2.5 ± 0.4</td>
<td>10.82 ± 0.87</td>
</tr>
</tbody>
</table>

Δ CNa, sodium clearance; Δ FENa, fractional Na⁺ excretion; Δ FEK, fractional K⁺ excretion; Δ FEV, fractional water excretion; Aldo, aldosterone infusion; Amil, amiloride infusion. Period 1 + 2 + 3 versus period 4 + 5 + 6, ^p < 0.05, ^p < 0.01, ^p < 0.001; Aldo versus Aldo + Amil during period 4 + 5 + 6, ^p < 0.05, ^p < 0.01, /p < 0.001. Control-vehicle versus lithium-vehicle during period 1 + 2 + 3 or period 4 + 5 + 6, respectively, ^p < 0.05, ^p < 0.01.
Lithium Inhibits Amiloride-Sensitive Sodium Transport

1999

high in the studies cited) and on the duration of treatment (Thomsen, 1973). It is clear from the present investigation in conscious animals that the natriuretic effect of moderate plasma Li+ concentrations (~0.5 mM) is entirely due to inhibition of Na+ reabsorption at the amiloride-sensitive site.

Previous studies showing that Li+ inhibits Na+ transport through the amiloride-sensitive Na+ channel in toad urinary bladder (Singer et al., 1972; Herrera et al., 1985) and turtle bladder (Arruda et al., 1980; Bank et al., 1982) are consistent with the findings of the present investigation. Further evidence for inhibition of the amiloride-sensitive Na+ channel comes from studies showing a blunted renal response to DOCA or aldosterone in Li+-treated animals (Rodomski et al., 1950; Schou, 1958; Baer et al., 1973; Iaina et al., 1982). However, those studies were carried out in animals with intact adrenal glands and, therefore, do not exclude the possibility that the blunted response could be a result of an increased level of endogenous aldosterone secretion secondary to lithium-induced sodium losses; high circulating levels of endogenous aldosterone would be expected to prevent any further effect of exogenous aldosterone. Thomsen et al. (1976) attempted to overcome this problem by using adrenalectomized rats. They used the voluntary intake of hypertonic NaCl solution as an index of renal Na+ losses and found that the reduction in salination consumption, which occurs in re-

Lithium Inhibits Amiloride-Sensitive Sodium Transport

amiloride-induced sodium losses; high circulating levels

References


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