Low-NaCl diet increases H-K-ATPase in intercalated cells from rat cortical collecting duct

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Silver, Randi B., Han Choe, and Gustavo Frindt.
Low-NaCl diet increases H-K-ATPase in intercalated cells from rat cortical collecting duct. Am. J. Physiol. 275 (Renal Physiol. 44): F94–F102, 1998.—Extracellular K+-dependent H+ extrusion after an acute acid load, an index of H/K exchange, was monitored in intercalated cells (ICs) from rat cortical collecting tubule (CCT) using ratiometric fluorescence imaging of the intracellular pH (pHi) indicator, 2,7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF). The hypothesis tested was that 12- to 14-day NaCl deprivation increases H-K-ATPase in rat ICs. The rate of H/K exchange in the low-NaCl ICs was double that of controls. In control ICs, H/K exchange was inhibited by Sch-28080 (10 µM). In the low-NaCl ICs, it was partially blocked by Sch-28080 or ouabain (1 mM). Simultaneous addition of both inhibitors abolished K-dependent pH recovery. The induced H/K exchange observed with NaCl restriction was not due to elevated plasma aldosterone as evidenced by experiments on ICs from rats implanted with osmotic minipumps administering aldosterone such that plasma K levels were comparable to those of NaCl-deficient rats. The results suggest that NaCl deficiency induces two isoforms of H-K-ATPase in ICs and that this effect is not mediated by elevated plasma aldosterone.

MATERIALS AND METHODS

Biological Preparations

Pathogen free Sprague-Dawley rats of either sex (Charles River Laboratories, Kingston, NY) weighing between 100–150 g were used for these experiments. Rats were fed either a normal diet (Purina Formulab 5008, Na content 2.8 g/kg, K content 11 g/kg) or a low-NaCl diet (diet 902902, Na content 3.8 mg/kg, K content 8.6 g/kg; ICN Biochemicals, Cleveland, OH) for 12–14 days. In one series of experiments, osmotic minipumps were implanted subcutaneously (model 2002; Alza, Palo Alto, CA). The pumps contained aldosterone (Sigma Chemical, St. Louis, MO) dissolved in polyethylene glycol 300, which were administered at an infusion rate of 250 µg/kg body wt · day−1 for 12–14 days; a plasma level of 10.2 ± 0.3 ng/ml was achieved. These minipumps were implanted into osmotic minipumps in rats on a low-NaCl diet and maintained on a low-NaCl diet (21).

Tubules were prepared and mounted in the chamber as previously described (27). Briefly, rats were killed by cervical dislocation, the kidneys were removed, and CCTs were dissected free and opened to form a flat epithelium. Sometimes two tubules were used from each animal.

Solutions were gravity fed into a manually operated six-port Hamilton valve. The solution leaving the valve went directly into a miniature water-jacketed glass coil (Radnotti Glass Technology, Monrovia, CA) for regulating the solution temperature. The warmed solution entered the experimental chamber, which was mounted on the stage of an inverted epifluorescence microscope (Nikon Diaphot). The temperature of the superfusate in the chamber was maintained at 37°C.
Tubules were superfused with HEPES-buffered solutions as described in Table 1. Bafilomycin A1 (LC Labs, New Bedford, MA; and Alexis, San Diego, CA) was added to potassium Ringer solutions (solutions a and b) from a 10 mM stock (3 parts ethanol, 1 part dimethylformamide) for a final concentration of 10 nM. Individual vials (50 µg) of the hydroxymethyl derivative of 2,7-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF-AM; Molecular Probes) were stored dry at 0°C and reconstituted in DMSO (at a concentration of 10 mM) for each experiment. The final loading concentration of dye was 5 µM in sodium Ringer solution. Sch-28080, a gift from Dr. A. Barnett at Schering Plough, was stored dry at 0°C and reconstituted in DMSO (at a concentration of 10 mM) for each experiment. The final loading concentration of dye was 5 µM in sodium Ringer solution. N-acetyl-D-glucosamine (NMDG) powder in the K- and Na-free calibration solutions. Phosphoric acid 2.5 2.5 2.5 CaCl₂ 2.0 2.0 2.0 2.0 2.0 2.0 pH 7.4 7.4 7.4 7.4 7.8 6.5

Table 1. Composition of solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>NaCl</th>
<th>Na₂HPO₄</th>
<th>KCl</th>
<th>MgCl₂</th>
<th>Glucose</th>
<th>NH₄Cl</th>
<th>NMDG-Cl</th>
<th>Phosphoric acid</th>
<th>CaCl₂</th>
<th>pH</th>
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</thead>
<tbody>
<tr>
<td>Ringer (1)</td>
<td>135.0</td>
<td>2.5</td>
<td>5.0</td>
<td>1.0</td>
<td>2.0</td>
<td>1.0</td>
<td>130.0</td>
<td>2.5</td>
<td>2.0</td>
<td>7.4</td>
</tr>
<tr>
<td>NH₄Cl-Cl (2)</td>
<td>10.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
<td>2.0</td>
<td>130.0</td>
<td>2.5</td>
<td>2.0</td>
<td>7.4</td>
</tr>
<tr>
<td>0 Na (3)</td>
<td>10.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
<td>2.0</td>
<td>130.0</td>
<td>2.5</td>
<td>2.0</td>
<td>7.4</td>
</tr>
<tr>
<td>K Ringer (4)</td>
<td>130.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>135.0</td>
<td>25.0</td>
<td>25.0</td>
<td>7.8</td>
</tr>
<tr>
<td>Calibration A</td>
<td>130.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>135.0</td>
<td>25.0</td>
<td>25.0</td>
<td>7.8</td>
</tr>
<tr>
<td>Calibration B</td>
<td>130.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>135.0</td>
<td>25.0</td>
<td>25.0</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Values are in mM, except for pH. To titrate the solutions to the appropriate pH, NaOH was used in the Na-containing Ringer solution, N-methyl-D-glucamine (NMDG) powder in the K- and Na-free solutions, and KOH in the Na-free calibration solutions.
H-K-ATPase function was assayed as the rate of K-dependent intracellular alkalinization in response to an acute acid load of 10 mM NH₄⁺. As part of this protocol and in all of the experiments presented in this study, bafilomycin A₁ (100 nM), a vacuolar H-ATPase inhibitor (2), was added to all solutions from the NH₄Cl acid pulse solution (solution 2) through the end of the experimental protocol at a concentration used by others to inhibit H-ATPase activity in rat late distal tubule (17, 18, 34).

K-Dependent Intracellular Alkalinization in Control ICs

An example of the response to the acid pulse protocol by three ICs from a single tubule from a control rat is shown in Fig. 2. Each representative point is the mean ± SE of the three ICs simultaneously studied from this tubule. The pHi value shown on the ordinate was determined from the 490/440 fluorescence ratio and the in situ calibration performed on this tubule. The solution changes are depicted at the top of Fig. 2. As shown, upon removal of NH₄⁺, Na⁺, and K⁺ (solution 3), the pHi fell more than 1 pH unit from the initial pHi in all of the cells monitored. As expected, no pH recovery was observed, because of the continued presence of bafilomycin A₁ in the Na- and K-free superfusing solution. Readdition of 5 mM K to the superfusate resulted in a small and partial K-dependent pH recovery at a mean rate of 0.045 ± 0.01 pH units/min. The K-dependent pH recovery rate from all of the ICs studied in control tubules averaged 0.07 ± 0.01 pH units/min, which is similar to rates reported in earlier and similar studies on control rabbit ICs (5, 26, 27).

Slopes of the K-dependent (and Na-dependent) intracellular alkalinization rates were calculated from the point where recovery had actually started to the leveling off point, as illustrated in Fig. 2. The time lag sometimes observed between solution changes and the cellular response reflects the time it may take for the solution to exit the Hamilton valve, become warm, and then replace the preexisting solution in the chamber. The solution marker on the trace reflects the moment right after the valve is manually turned to the appropriate solution. A summary of the responses of all of the ICs from control tubules is shown in Table 3. Under control conditions, H/K exchange raised pHi by 0.2 pH units to a mean pHi of 6.72 ± 0.04.

Introducing Na back to the superfusate resulted in additional recovery at a rate of 0.54 ± 0.12 pH units/min. This Na-dependent recovery reflects basolateral Na/H exchange similar to that described in rabbit CCT (27, 36).

Response to Sch-28080

To test whether the K-dependent intracellular alkalinization observed in control tubules was due to an H-K-ATPase, the same protocol was carried out in the presence of the gastric H-K-ATPase inhibitor Sch-28080 at a concentration known to completely inhibit the gastric isoform (10 µM) (33). Figure 3 is an experi-
mental trace of the means ± SE from three ICs in the same control tubule exposed to the NH₄Cl protocol. As shown in Fig. 3, there was virtually no K-dependent pHᵢ recovery in the presence of the imidazopyridine inhibitor, as indicated by the low rate of pHᵢ recovery 0.01 ± 0.01 pH units/min. As shown in Table 3, the mean overall rate of the Sch-28080-insensitive K-dependent pHᵢ recovery in all of the ICs from control tubules was 0.03 ± 0.01 pH units/min, with the Sch-28080-sensitive component being -0.04 pH units/min. Sch-28080 did not appear to affect the rate of Na/H exchange. As shown in Fig. 3, readmittance of Na to the superfusate resulted in a rapid intracellular alkalinization response back to the initial pHᵢ at a rate of 0.85 ± 0.10 pH units/min.

To test whether the Sch-28080-insensitive component of the K-dependent pHᵢ recovery rate could be due to a ouabain-sensitive isoform of H-K-ATPase, the acid pulse protocol described above was performed in the presence of 1 mM ouabain. The Kᵢ for ouabain inhibition of the α-subunit of the putative colonic H-K-ATPase expressed in oocytes in the presence of 5 mM extracellular K was close to 1 mM (6).

Figure 4 compares the K-dependent pHᵢ recovery rates in the absence and presence of ouabain and/or Sch-28080. Only the addition of Sch-28080 alone or with ouabain significantly inhibited the K-dependent pHᵢ recovery rate (P < 0.01). Ouabain alone did not have any affect on the K-dependent pHᵢ recovery rate. Also, the rate of K-dependent pHᵢ recovery was the same in the presence of Sch-28080 and Sch-28080 and ouabain combined (see Table 3). Taken together, these data demonstrate that a Sch-28080-sensitive H-K-ATPase predominates as the primary H/K exchanger in ICs under control conditions. A summary of all of these results is shown in Table 3.

**Effect of Low-NaCl Diet**

Having demonstrated H/K exchange in individual ICs from control tubules, we next tested the hypothesis that NaCl restriction induces H/K exchange in ICs of the CCT. Experiments similar to those described above were performed on tubules dissected from rats maintained on a NaCl-restricted diet.

Figure 5 is representative record from one experiment of four ICs identified in a tubule from a rat maintained on a low-NaCl diet. Bafilomycin A₁ (100 nM) was present from the acid pulse through the end of the protocol. As shown in Fig. 5, upon removal of NH₄⁺, Na⁺, and K⁺, the pHᵢ fell from an initial mean pHᵢ of ~7.5 to 6.7. No pHᵢ recovery was observed in the absence of extracellular Na and K. With readmittion of K to the superfusate, partial pHᵢ recovery occurred, returning the pHᵢ to a mean of 6.85. For the four ICs in this tubule, the K-dependent rate of alkalinization averaged 0.11 ± 0.04 pH units/min. As shown in Table 3, for all of the ICs studied under low-NaCl conditions, the mean K-dependent pHᵢ recovery rate was roughly double that observed under control conditions [0.03 ± 0.02 pH units/min for low NaCl (n = 35 cells)] vs. 0.07 ± 0.01 pH units/min for control (n = 38 cells).

As shown in Fig. 5, introducing Na back to the superfusate resulted in additional recovery for these four ICs at a mean rate of 0.15 ± 0.03 pH units/min.

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### Table 2. Blood and urine pH and electrolyte values

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>pH (mm)</th>
<th>HCO₃⁻ (meq/l)</th>
<th>Na⁺ (meq/l)</th>
<th>K⁺ (meq/l)</th>
<th>Cl⁻ (meq/l)</th>
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</thead>
<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>6</td>
<td>7.51 ± 0.03*</td>
<td>24.0 ± 1.3</td>
<td>141 ± 1</td>
<td>5.5 ± 0.1</td>
<td>99 ± 1*</td>
</tr>
<tr>
<td>Low-NaCl rats</td>
<td>6</td>
<td>7.50 ± 0.03*</td>
<td>23.4 ± 1.6</td>
<td>134 ± 3</td>
<td>6.0 ± 0.2t</td>
<td>100 ± 1*</td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>3</td>
<td>6.6 ± 0.2</td>
<td>13 ± 2</td>
<td>20.4 ± 3.5</td>
<td>3.6 ± 0.2</td>
<td>19.0 ± 2.9</td>
</tr>
<tr>
<td>Low-NaCl rats</td>
<td>4</td>
<td>6.6 ± 0.2*</td>
<td>15 ± 2</td>
<td>0.13 ± 0.02</td>
<td>2.6 ± 0.3</td>
<td>1.9 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Blood samples were obtained either by heart puncture or ocular bleeding. Urine was collected from individual rats placed in metabolic chambers and collected under mineral oil over a 24-h period; *n = 3 rats; †n = 5 rats.

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**Fig. 2. Effect of 5 mM extracellular K on intracellular pH (pHᵢ) recovery after acute exposure to an NH₄Cl acid pulse in intercalated cells (ICs) from a control rat. Y-axis, pHᵢ as determined from the intracellular calibration of the dye in this tubule. This trace represents the mean ± SE response of the three ICs in this tubule. Bafilomycin A₁ (100 nM) was present in the superfusate from the NH₄Cl until the end of the protocol. Tubule was initially superfused with sodium Ringer solution (NaR) and then changed to 10 mM NH₄Cl. Acute exposure to NH₄Cl resulted in acidification after its removal. Upon removal of the NH₄⁺, Na⁺, and K⁺, the pHᵢ fell from an initial value of 7.75 to 6.60. Recovery was absent in K-free and Na-free (0 K, 0 Na) solution. Readdition of 5 mM K resulted in partial recovery of pHᵢ to 6.75 at a mean rate of 0.045 pH units/min. The slope or rate of the K-dependent pHᵢ recovery was calculated at the beginning of recovery process as illustrated in the trace. Readdition of Na to the superfusate resulted in further recovery of pHᵢ, to 7.30 at a rate of 0.54 pH units/min.**

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**Fig. 4. Comparison of the K-dependent pHᵢ recovery rates in the absence and presence of ouabain and/or Sch-28080.**
LOW-NaCl DIET INDUCES H-K-ATPASE IN INTERCALATED CELLS

Table 3. Mean IC pHi response to an acute acid load before and after readdition of K

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of Tubes</th>
<th>No. of Cells</th>
<th>Initial</th>
<th>Acid Load (0 K, 0 Na)</th>
<th>Recovery (+ K)</th>
<th>ΔpHi/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7 (6)</td>
<td>38</td>
<td>7.34 ± 0.04</td>
<td>6.53 ± 0.02</td>
<td>6.72 ± 0.04</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Control + Sch</td>
<td>7 (5)</td>
<td>26</td>
<td>7.36 ± 0.08</td>
<td>6.59 ± 0.03</td>
<td>6.66 ± 0.05</td>
<td>0.03 ± 0.01*</td>
</tr>
<tr>
<td>Control + ouabain</td>
<td>4 (3)</td>
<td>16</td>
<td>7.96 ± 0.05</td>
<td>6.71 ± 0.05</td>
<td>6.87 ± 0.06</td>
<td>0.05 ± 0.01*</td>
</tr>
<tr>
<td>Control + Sch + ouabain</td>
<td>5 (3)</td>
<td>28</td>
<td>7.63 ± 0.07</td>
<td>6.55 ± 0.03</td>
<td>6.65 ± 0.03</td>
<td>0.03 ± 0.01*</td>
</tr>
<tr>
<td>Low Na</td>
<td>5 (4)</td>
<td>35</td>
<td>7.38 ± 0.04</td>
<td>6.57 ± 0.03</td>
<td>6.79 ± 0.04</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>Low Na + Sch</td>
<td>5 (5)</td>
<td>26</td>
<td>7.24 ± 0.05</td>
<td>6.55 ± 0.06</td>
<td>6.69 ± 0.06</td>
<td>0.05 ± 0.02†</td>
</tr>
<tr>
<td>Low Na + ouabain</td>
<td>3 (2)</td>
<td>19</td>
<td>7.36 ± 0.06</td>
<td>6.74 ± 0.01</td>
<td>7.00 ± 0.04</td>
<td>0.06 ± 0.01*</td>
</tr>
<tr>
<td>Low Na + Sch + ouabain</td>
<td>4 (3)</td>
<td>22</td>
<td>7.37 ± 0.06</td>
<td>6.40 ± 0.03</td>
<td>6.42 ± 0.02</td>
<td>0.01 ± 0.01‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; IC, intercalated cell; pHi, intracellular pH. Baflomycin A1 was present in all of the experiments and, where indicated, 10 µM Sch-28080 (Sch) and/or 1 mM ouabain, all of which were added at the time of the NH4Cl prepulse and maintained through the end of the protocol. The number of rats used for each study is in parentheses after the number of tubules. ΔpHi/min, rate of K-dependent pHi recovery. *P < 0.01, †P < 0.001, and ‡P < 0.0001. Comparisons of the K-dependent pHi recovery rates were made between control ICs with and without Sch-28080 and or ouabain and low-NaCl ICs with and without the inhibitors.

Response to Sch-28080 and Ouabain in NaCl-Deficient Rats

To test whether the increased rate of K-dependent pHi recovery observed in the low-NaCl ICs was due to stimulation of the Sch-28080-sensitive H/K exchanger, pHi recovery was monitored in the presence of 10 µM Sch-28080. A representative result from one experiment on a tubule is shown in Fig. 6. As shown, readdition of 5 mM K in the presence of Sch-28080 partially inhibited K-dependent pHi recovery with pHi going from 6.6 to 6.75. The rate of K-dependent intracellular alkalinization in the presence of Sch-28080 for the two ICs in this tubule was 0.07 pH units/min. In all of the ICs studied in the presence of Sch-28080 from low-NaCl rats, the average rate of K-dependent pHi recovery was 0.05 ± 0.02 pH units/min. The rate of this recovery is less than one-half of that observed in ICs from NaCl-deficient rats without inhibitor (0.13 ± 0.02 pH units/min) (Table 3). These data suggested that an additional Sch-28080-insensitive component was contributing to the rate of K-dependent intracellular alkalinization in the ICs from low-NaCl rats.

To examine the possibility that NaCl restriction induces a ouabain-sensitive isoform of H-K-ATPase in CCT, we compared rates of K-dependent intracellular alkalinization in the absence and presence of ouabain and Sch-28080.

Figure 4 compares the mean K-dependent pHi recovery rates for all of the low-NaCl ICs studied in the absence and presence of Sch-28080 and/or ouabain. The K-dependent rate of intracellular alkalinization is greatest in the low-NaCl ICs (roughly double that measured in control ICs; Fig. 4 and Table 3). Addition of 1 mM ouabain to the superfuse, a concentration that did not have any effect on the K-dependent pHi recovery rate in control ICs (Fig. 4), significantly inhibited the K-dependent pHi recovery rate from 0.13 ± 0.02 to 0.06 ± 0.01 pH units/min (P < 0.01). This inhibition of the K-dependent pHi recovery rate was similar to that observed in the presence of Sch-28080 (0.05 ± 0.02 pH units/min), which was also significantly lower than back to the initial pHi. The mean Na-dependent pHi recovery rate for 35 ICs studied with low NaCl was 0.28 ± 0.04 pH units/min.

Fig. 3. Effect of Sch-28080 on K-dependent pHi recovery from an acute acid load in ICs from a control rat tubule. Y-axis, pHi, as determined from intracellular calibration of the dye in these cells. This trace represents the mean ± SE response of the three ICs in this tubule. Solution changes are the same as Fig. 2. Baflomycin A1 (100 nM) and Sch-28080 (10 µM) were present in the superfuse from NH4Cl until the end of the protocol. Addition of blocker prevented the K-dependent H efflux observed in Fig. 2 but did not affect Na-dependent recovery.

Fig. 4. Comparison of K-dependent ΔpHi/dt in absence and presence of Sch-28080 and ouabain in control ICs. K-dependent ΔpHi/dt (pH units/min) rates are compared in ICs from control rats with and without Sch-28080 (Sch; 10 µM) and ouabain (OAU; 1 mM) added to the superfuse in all of the ICs studied from control rats. **P < 0.01. Values are means ± SE. All experiments were carried out in the presence of baflomycin A1 (100 nM).
control rates (P < 0.001). Adding both Sch-28080 and ouabain together to the superfusate totally inhibited the rate of K-dependent pH\textsubscript{i} recovery (0.01 ± 0.01 pH\textsubscript{i} units/min), suggesting that both isoforms of H-K-ATPase contribute to K-dependent pH\textsubscript{i} recovery in ICs from NaCl-restricted rats.

**Effect of Aldosterone**

Because chronically maintaining rats on a NaCl-deficient diet results in elevated endogenous plasma aldosterone levels, we speculated that aldosterone may be the mediator responsible for inducing the H-K-ATPase exchange rate in ICs from low-NaCl rats. To test this idea, a group of rats was implanted with osmotic minipumps, which continuously administered aldosterone for 12–14 days at levels comparable to that measured in our previous study on low-NaCl rats (21). In our present study, the amount of aldosterone delivered by the pumps was 250 µg·kg body wt\textsuperscript{-1}·day\textsuperscript{-1} for 12–14 days. This was the same amount of aldosterone put in the pumps used in our previous study (21), which produced plasma aldosterone levels of 750 ng/dl in identical rats. Figure 8 compares the K-dependent pH\textsubscript{i} recovery rates in the ICs from the minipump rats with control ICs and ICs from rats maintained on the low-NaCl diet. The rate of K-dependent pH\textsubscript{i} recovery measured in ICs from the rats infused with aldosterone was comparable to that measured in control rats (0.06 ± 0.01 vs. 0.07 ± 0.01 pH\textsubscript{i} units/min). These results demonstrate that aldosterone alone is not responsible for the enhanced rate of K-dependent intracellular alkalinization observed in ICs from low-NaCl animals. Some other as yet unidentified mechanism is responsible for this induced change.
DISCUSSION

The BCECF-loaded, split tubule preparation was used in combination with dual-excitation digital imaging to study H/K exchange in ICs in response to an imposed acidosis. ICs were visually identified under epillumination by their much brighter appearance compared with the neighboring principal cells. Our results indicate that maintaining rats on a NaCl-restricted diet induces a ouabain-sensitive H/K exchanger in the ICs that is not observed under control conditions. With chronic NaCl deficiency, both the Sch-28080- and the ouabain-sensitive H/K exchangers contribute equally to the K-dependent pH recovery rate after an acute acid pulse. The net effect is an increased rate of K-dependent pH recovery from an acid load in the low-NaCl ICs compared with control ICs. In both control and low-NaCl ICs, the K-dependent intracellular alkalinization in response to the acid load resulted in only a partial recovery of the pHj (Figs. 2 and 5). The magnitude of this pHj recovery was not different in the low-NaCl ICs compared with controls (0.22 ± 0.02 for low-NaCl ICs vs. 0.19 ± 0.03 pH units for control ICs). This type of response has also been observed in ICs from rabbits under control and chronically acidotic conditions (26, 27). In ICs from newborn rabbits, however, the K-dependent pHj recovery was more complete, returning the pHj back to the basal value (5). This difference in response between the ICs from newborns and adults indicates that the H-K-ATPase is capable of functioning at pHj > 7.0 but there is some underlying factor in mature animals under the conditions of these studies that limits the function of the H-K-ATPase.

In all of the experiments, readdition of Na to the superfusate results in additional pHj recovery; however, the rates are different between the controls and low-NaCl ICs. The ICs from control rats had much higher rates than ICs from low-NaCl rats and aldosterone minipump rats [0.44 ± 0.04 (n = 38 ICs) for control, vs. 0.28 ± 0.04 pH units/min (n = 35 ICs) for low NaCl, vs. 0.31 ± 0.04 pH units/min (n = 32 ICs) for aldosterone minipump]. This difference is also evident by comparing the Na-dependent slope in a control tubule (Fig. 2) with that measured in ICs from a low-NaCl rat tubule (Fig. 5). We did not explore the reasons for these differences. They may be related to the higher plasma aldosterone levels in the low-NaCl and aldosterone minipump rats compared with controls.

The isoform or variant of the ouabain-sensitive H-K-ATPase, induced in the ICs with NaCl restriction, remains to be determined. Although the pharmacological profile of this exchanger has not yet been fully characterized, 1 mM ouabain appeared to inhibit H/K exchange. We do not know whether this dose of ouabain was maximal. It also remains to be determined whether this H-K-ATPase is inhibited by a higher concentration of Sch-28080 than that used in this study (10 μM). Based on enzymatic assays, it was reported recently that another H-K-ATPase isof orm may be expressed in rat CCT with K depletion (3). The enzymatic activity for this H-K-ATPase was inhibited with 1 mM ouabain and 100 μM Sch-28080.

Our finding suggesting that two isoforms of H-K-ATPase function in ICs of NaCl-deprived rats is consistent with mRNA expression studies. The mRNA for the α-subunit of gastric H-K-ATPase has been detected in CCT from normal rats (1). Similarly, mRNA for the α-subunit of the putative distal colonic H-K-ATPase has also been detected in this nephron segment from control rats (31). By means of a modified immunoprecipitation technique, protein levels associated with the α-subunit of colonic H-K-ATPase and gastric H-K-ATPase have been measured in kidneys from control rats (15). Furthermore, this study reported the finding that a K-deficient diet increased the abundance of protein for the colonic isoform of H-K-ATPase, whereas that of the gastric isoform remained at control levels (15). Differential expression of H-K-ATPase isoforms was also observed at the mRNA level in kidney cortex from NaCl-deprived rats (35). With Northern hybridization techniques, a fourfold increase in the mRNA level for the α-subunit of colonic H-K-ATPase was found with NaCl restriction, but the level of message for the α-subunit of the gastric isoform remained at control levels (35). This is similar to the results of Sangan et al. (23), who also saw an increase in the mRNA level for colonic H-K-ATPase in renal cortex with NaCl depletion.

The experiments presented in this study also demonstrate that aldosterone is not responsible for the enhanced H-K-ATPase activity in ICs from NaCl-depleted rats (Fig. 8). This finding is in agreement with those of Elam-Ong (9), who demonstrated that Sch-28080-sensitive H-K-ATPase activity in the CCT was not altered with increased plasma aldosterone. More recently, it was demonstrated that the level of mRNA expression of the putative colonic K-ATPase was not altered in rat kidney with elevated plasma aldosterone (14). These results in kidney are different from the findings in distal colon, where it has been shown that aldosterone administration via osmotic minipump produced enhanced electroneutral K+ reabsorption (32) believed to be mediated via a K-ATPase (14).

We are left to consider the physiological significance of increased H-K-ATPase function under conditions of NaCl restriction. Under normal conditions in control rats, the reabsorption of Na+ is minimal in the collecting duct as a result of a dearth of Na channels in the principal cells; however, under conditions of NaCl restriction with the resultant elevated plasma aldosterone levels, there is an abundance of apical Na channels in the principal cells (12, 22). The higher density of Na channels as well as increased channel activity under these conditions leads to increased reabsorption of Na+ from the tubular fluid as demonstrated in isolated microperfused rabbit and rat CCTs (24, 30). It is believed that the increase in the principal cell apical membrane Na+ permeability results in a more favorable electrical driving force for secretion of K+ and...
leads to a coupling between Na\(^+\) reabsorption and K\(^+\) secretion (22, 28). NaCl restriction is also known to increase Cl\(^-\) reabsorption in the collecting duct independent of plasma Cl\(^-\) concentration, filtered Cl\(^-\) load, or Cl\(^-\) delivery to the collecting duct. (10).

We speculate that the enhanced H/K exchange observed with NaCl restriction may serve to reabsorb the K\(^+\) secreted in the neighboring principal cell. This would necessitate that H-K-ATPase be located in apical membrane of the ICs. We and others have previously demonstrated apical localization of H-K-ATPase in \(\beta\)-type ICs of rabbit CCT (26, 38). Although we were not able to differentiate between the IC subtypes in rat CCT, ultrastructural analysis of kidney from control rat has shown a predominance of \(\beta\)-type ICs in the CCT (19). In our model of the \(\beta\)-type IC, the apical membrane contains H-K-ATPase and the Cl\(^-\)/HCO\(_3\) exchanger. Under conditions of NaCl restriction, enhanced reabsorption of Na\(^+\) via the principal cells leads to increased excretion of K\(^+\) into the tubular lumen. This secreted K\(^+\) could then be reabsorbed via the H-K-ATPase in the neighboring ICs, concomitant with exchange of luminal Cl\(^-\) for HCO\(_3\). The net effect of these coordinated processes is reabsorption of Na\(^+\), K\(^+\) and Cl\(^-\) and the secretion of H\(^+\) and HCO\(_3\).

In conclusion, we have demonstrated increased H-K-ATPase function in ICs of rat CCT with chronic NaCl deficiency. Based on inhibition by Sch-28080 and ouabain, we conclude that this functional H/K exchange may be due to two distinct isoforms. The signal responsible for the increased H/K exchange appears not to be related to ad-d-base state, plasma K\(^+\) balance, or plasma aldosterone levels. In NaCl depletion, the increased H/K exchange in addition to increased Na\(^+\) reabsorption and K\(^+\) secretion yields net Na\(^+\) for H\(^+\) exchange. This in parallel with apical Cl\(^-\)/HCO\(_3\) exchange in the \(\beta\)-type ICs, may serve to promote net NaCl reabsorption by the CCT rather than H\(^+\) for K\(^+\) exchange.

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