Mouse model of type II Bartter’s syndrome. I. Upregulation of thiazide-sensitive Na-Cl cotransport activity

Alessandra Cantone, Xinbo Yang, Qingshang Yan, Gerhard Giebisch, Steven C. Hebert, and Tong Wang

Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut

Submitted 26 December 2007; accepted in final form 27 March 2008

Cantone A, Yang X, Yan Q, Giebisch G, Hebert SC, Wang T. Mouse model of type II Bartter’s syndrome. I. Upregulation of thiazide-sensitive Na-Cl cotransport activity. Am J Physiol Renal Physiol 294: F1366–F1372, 2008. First published April 2, 2008; doi:10.1152/ajprenal.00608.2007.—ROMK-deficient (Romk−/−) mice exhibit polyuria, natriuresis, and kaliuresis similar to individuals with type II Bartter’s form of hyperprostaglandin E syndrome (HPS; antenatal Bartter’s syndrome). In the present study, we utilized both metabolic and clearance studies to define the contributions of specific distal nephron segments to the renal salt wasting in these mice. The effects of furosemide, hydrochlorothiazide, and benzamil on urinary Na+ and K+ excretion in both wild-type (Romk+/+) and Romk−/− mice were used to assess and compare salt transport by the Na+–K+–2Cl− cotransporter (NKCC2)-expressing thick ascending limb (TAL), the Na+–Cl− cotransporter (NCC)-expressing distal convoluted tubule (DCT1/DCT2), and the epithelial Na+ channel (ENaC)-expressing connecting segment (CNT) and collecting duct (CD), respectively. Whole kidney glomerular filtration rate was reduced by 47% in Romk−/− mice. Furosemide-induced increments in the fractional excretion rate of Na+ and K+ and absolute excretion of Na+ and K+ were significantly blunted in Romk−/− mice, consistent with a major salt transport defect in the TAL. In contrast, hydrochlorothiazide produced an exaggerated natriuresis in Romk−/− mice, indicating upregulation of salt absorption by the DCT. Benzamil resulted in a similar increment in absolute Na+ excretion in both Romk+/+ and Romk−/−, indicating no significant upregulation of Na+ transport by ENaC in ROMK null mice. Moreover, hydrochlorothiazide increased the fractional K+ excretion rate in Romk−/− mice, confirming our recent observation that maxi-K+ channels contribute to distal K+ secretion in the absence of ROMK.

ROMK knockout; furosemide; hydrochlorothiazide; benzamil

THE AVAILABILITY of ROMK null mice (Romk−/−) (15) has made it possible to study electrolyte transport along the nephron in the absence of this small-conductance, 35-pS K+ channel (Kcnj1; Kir1.1) (2, 15, 16). ROMK is highly expressed in the cortical and medullary thick ascending limbs (TAL), connecting tubule segment (CNT), and cortical collecting duct (CCD) in the mammalian kidney, where it serves to recycle K+ across the apical membrane in TAL and secrete K+ in the CNT and CCD (11). We have previously reported the absence of both 35- and 70-pS K+-recycling channels in the TAL of Romk−/− mice, consistent with the severe impairment of salt absorption in the TAL seen in these mice (15, 16).

We have suggested that our inbred Romk−/− mouse provides a good animal model for the study of the pathogenesis and potential therapy of type II Bartter’s syndrome subgroup of hyperprostaglandin E syndrome (HPS) (2, 9, 15). HPS is a severe form of Bartter’s syndrome, a hypokalemic renal salt-wasting disorder characterized by metabolic alkalosis, normotensive hyperaldosteronism, and increased renin and PGE2 production (7, 22). Despite the absence of ROMK, renal K+ wasting and hypokalemia are observed in type II Bartter’s, although they are less severe than found in other HPS genotypes [e.g., with mutations in Na+–K+–2Cl− cotransporter (NKCC)] (20). Our recent study in Romk−/− mice (2) has provided a potential explanation for the continued kaliuresis seen in type II Bartter’s patients. We found that potassium secretion in the late distal convoluted tubule of Romk−/− mice was mediated by maxi-K (BK) channels and suggested that this channel was activated by the high distal flow rates (diuresis) observed in these mice.

Nevertheless, the high survival rate of our inbred Romk−/− mice suggests that specific genes have been selected which could have mitigated fluid and electrolyte losses. Consequently, the selected adaptive changes in the Romk−/− mouse could have altered the mouse phenotype compared with that observed in HPS. Diuretic pharmacotyping has been used to separate the various phenotypes of hypokalemic renal salt-wasting disorders (22) and can be used to compare responses in Romk−/− mice and type II HPS. The differential sensitivities to diuretics has established that the defect in HPS was localized to the TAL (25). Thus individuals with HPS exhibit impaired diuretic and natriuretic responses to furosemide, while the effect of this diuretic in Gitelman’s disorder is normal (22). In contrast, individuals with Gitelman’s disorder exhibit a markedly reduced response to thiazide diuretics that inhibit the thiazide-sensitive NaCl cotransporter localized in the distal convoluted tubule (DCT), while the response to this diuretic is exaggerated in HPS.

In the present paper (part one of a series of two), we address several questions. First, is furosemide-sensitive Na+–K+–2Cl− cotransport activity dependent on the expression of ROMK? Second, is thiazide-sensitive Na+–Cl− cotransporter activity modulated by increased delivery of salt and water in ROMK null mice? Third, is ENaC activity modulated by enhanced NaCl delivery in the late nephron in ROMK null mice? Finally, is the ROMK-dependent K+ secretion reduced in ROMK null mice? The effects of furosemide, hydrochlorothiazide, and benzamil were examined by two types of experiments: metabolic studies in conscious animals with urine collections before and after injection of diuretics; and renal clearance studies in anesthetized animals. We show that Romk−/− mice exhibit a reduced glomerular filtration rate (GFR) and a diuretic pharmacotyping profile similar to that of type II HPS. In the companion study (27a), we show the adaptive changes in...
expression of renal sodium and water transporters that accompany these diuretic responses.

**MATERIALS AND METHODS**

**Mice.** The original Romk<sup>−/−</sup> breeder pairs were obtained from G. E. Shull (15). The Romk<sup>−/−</sup> mice used in the present studies were obtained by crossing heterozygous males and females for several generations (17). Survival of Romk<sup>−/−</sup> mice was >50% compared with the low survival rate of the original ROMK null (Kcnj1 null) mice, and the Romk<sup>−/−</sup> mice exhibited a markedly reduced incidence of hydronephrosis. Romk<sup>+/+</sup> littermates are genetically identical to Romk<sup>−/−</sup> mice except for deletion of Kcnj1. All mice were given tap water ad libitum and maintained on standard rodent chow (1.2% K).

Genotypes of heterozygous mice, weighing 30–40 g, to investigate the expression of renal sodium and water transporters that accompany these diuretic responses.

**AJP-Renal Physiol**  
**VOL 294  JUNE 2008  www.ajprenal.org**

After a tracheotomy, the left carotid artery and left jugular vein were cannulated with polyethylene tubing (PE-10). The arterial catheter was then connected to a pressure transducer to monitor blood pressure and to take blood samples, while the venous catheter was connected to a syringe pump for saline infusion. After surgical fluid loss was replaced with isotonic saline, the mice were given a priming dose of 10 μCi of [methoxy-3H]julin, followed by a maintenance infusion in isotonic saline containing 10 μCi/h at a rate of 0.4 ml/h throughout the experiment. The bladder was cannulated with a PE-50 tube for urine collection.

After a 60-min balance period, 30-min urine samples were collected. Blood samples (30 μl) were taken after each clearance period. After two control periods, furosemide (5 mg/kg), hydrochlorothiazide (30 mg/kg), or benzamil (0.8 mg/kg) was given intravenously as a bolus injection. Furosemide, hydrochlorothiazide, and benzamil were purchased from Sigma (St. Louis, MO).

In the control group, a similar amount of vehicle was administered. After the intravenous bolus, four additional 30-min urine and blood collections were made. The hematocrit did not change during the experiment. Urine and plasma Na<sup>+</sup> and K<sup>+</sup> concentrations were measured by flame photometry (type 480 Flame Photometer, Corning Medical and Scientific, Corning, NY). Blood pressure, urine volume, GFR, E<sub>Na</sub>, E<sub>K</sub>, F<sub>Na</sub>, F<sub>K</sub>, and plasma Na<sup>+</sup> and K<sup>+</sup> concentrations were calculated by standard methods.

**Statistics.** All the data are expressed as means ± SE. Statistical evaluation was performed using Student’s t-test. *P* < 0.05 was considered statistically significant.

**RESULTS**

**Effects of diuretics on renal metabolic parameters in Romk<sup>+/+</sup> and Romk<sup>−/−</sup> mice.** Control data in wild-type (Romk<sup>+/+</sup>) and ROMK null (Romk<sup>−/−</sup>) mice, shown in Fig. 1 and Table 1, are similar to those reported previously by us (1). Body weight, arterial blood pressure, and plasma Na<sup>+</sup> and K<sup>+</sup> concentrations were measured from 6-h collections from individual cages and calculated to means ± SE.

![Fig. 1. Baseline renal metabolic studies in Romk<sup>+/+</sup> (wild-type; filled bars) and Romk<sup>−/−</sup> (ROMK null; open bars) mice. Water intake (A), urine volume (B), and absolute urinary Na<sup>+</sup> (E<sub>Na</sub>; C) and K<sup>+</sup> (E<sub>K</sub>; D) excretion were measured from 6-h collections from individual cages and calculated to means ± SE.](http://ajprenal.physiology.org/DownloadedFrom/)
Table 1. Basal conditions of wild-type and ROMK knockout mice used in renal clearance studies

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>BW, g</th>
<th>BP, mmHg</th>
<th>PNa, meq/l</th>
<th>PK, meq/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Romk⁺/+</td>
<td>20</td>
<td>36.0±4.1</td>
<td>100.9±13.8</td>
<td>149.2±6.1</td>
<td>4.1±0.4</td>
</tr>
<tr>
<td>Romk⁻/⁻</td>
<td>17</td>
<td>35.5±4.1</td>
<td>87.2±14.8</td>
<td>155.1±8.3</td>
<td>3.8±0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE, n, No. of animals; BW, body weight; BP, mean blood pressure; PNa, plasma sodium; PK, plasma potassium.

was 131.8 ± 7.6 vs. 45.18 ± 6.8 μeq·6 h⁻¹·100 g body wt⁻¹; water intake, 3.05 ± 0.37 vs. 1.08 ± 0.1 ml/6 h (P < 0.01, n = 18).

Figure 2 summarizes the effects of diuretics on water and electrolyte excretion for metabolic studies in Romk⁺⁺/⁺⁺ and Romk⁻/⁻ mice. Both furosemide and hydrochlorothiazide produced significant diuresis, natriuresis, and kaliuresis in Romk⁺⁺/⁺⁺ mice. Furosemide and hydrochlorothiazide significantly increased 6-h urine volume by 2.33-fold and 1.5-fold, respectively (Fig. 2A). Furosemide increased ENa by 0.92-fold and EK by 0.67-fold (Fig. 2, A and C). Hydrochlorothiazide increased ENa by 1.05-fold and EK by 0.32-fold (Fig. 2, B and C). In Romk⁻/⁻ mice with high basal UV rates, UV was unaltered by diuretics (Fig. 2A). Furosemide had no significant effect on ENa in Romk⁻/⁻ mice (Fig. 2B). However, the ENa values in the control and furosemide-treated Romk⁻/⁻ mice were similar to that in Romk⁺⁺/⁺⁺ mice given furosemide (111.9 vs. 101.2 μeq·6 h⁻¹·100 g body wt⁻¹). As expected, ENa in Romk⁻/⁻ mice is indistinguishable in Romk⁺⁺/⁺⁺ mice on furosemide and in Romk⁻/⁻ mice as both inhibit NaCl reabsorption in the TAL. Similarly, furosemide had no significant effect on EK in Romk⁻/⁻ mice (108.7 ± 12.2 vs. 82.9 ± 0.7 μeq·6 h⁻¹·100 g body wt⁻¹, P > 0.05, Fig. 2C). In sharp contrast, hydrochlorothiazide produced a more profound natriuretic response in Romk⁻/⁻ mice compared with wild-type littermates; ENa increased 2.1-fold in Romk⁻/⁻ mice, from 101.2 ± 11.6 to 316.1 ± 66.7 μeq·6 h⁻¹·100 g body wt⁻¹ (Fig. 2B). The latter is consistent with an upregulation of Na⁺ transport in the DCT, a response supported by the hypertrophy and hyperplasia of the DCT shown in the companion paper (27a). Despite the natriuretic effect of hydrochlorothiazide in Romk⁻/⁻ mice, this diuretic did not produce a significant increment in kaliuresis (Fig. 2C).

Acute effects of diuretics on GFR and electrolyte excretion in Romk⁺⁺/⁺⁺ and Romk⁻/⁻ mice. We used clearance studies to compare the acute effects of furosemide and hydrochlorothiazide on renal function and fractional electrolyte excretion in Romk⁻/⁻ and Romk⁺⁺/⁺⁺ mice. GFR in the Romk⁻/⁻ mice was about fourfold higher than in the original ROMK null mice (15) but still remained ~48% lower than in wild-type mice (Fig. 3A and Table 2). Figure 3 summarizes the results of baseline clearance experiment data; UV, GFR, FE Na, and FE K are compared. GFR in ROMK null mice was 48% lower, similar to data previously reported by Lorenz et al. (15). Inspection of Fig. 3 shows that UV was higher, FE Na doubled, and FE K was also significantly increased. These phenotypes are similar to the electrolyte imbalance of Bartter’s syndrome. In contrast to the metabolic studies, furosemide and hydrochlorothiazide acutely increased UV in Romk⁻/⁻ mice as well as in Romk⁺⁺/⁺⁺ mice (Table 2). However, the diuretic effect of furosemide was greatly blunted. The difference between this series and that in Fig. 2 is that the animals were anesthetized and infused and diuretics were applied by intravenous bolus. The absolute amount of diuretic effect produced by hydrochlorothiazide was markedly enhanced (4.85 vs. 1.21 ml/min), but due to higher UV in the basal condition the increase from the control period was about same (1.4- vs. 1.2-fold in Romk⁻/⁻ compared with Romk⁺⁺/⁺⁺ mice). Benzamil increased UV by 62% in Romk⁺⁺/⁺⁺ (P < 0.05) and by 57% in Romk⁻/⁻ mice, but the change did not reach statistical significance. In Romk⁺⁺/⁺⁺ mice, both furosemide and

Fig. 2. Effects of diuretics on renal metabolic parameters in Romk⁺⁺/⁺⁺ (wild-type; filled bars) and Romk⁻/⁻ (ROMK null; open bars) mice. Urine volume (A), ENa (B), and EK (C) were measured from 6-h collections from individual mice in metabolic cages before (Control) and after administration of furosemide (Furo) or hydrochlorothiazide (HCTZ). Furosemide or hydrochlorothiazide was given by intraperitoneal injection at concentrations of 5 and 30 mg/kg, respectively. *P < 0.05 compared with control period. #P < 0.05 compared with the Romk⁻/⁻ mice.
hydrochlorothiazide acutely reduced GFR by ~50%, while benzamil had no significant effect (Table 2). In contrast, none of the diuretics reduced GFR below the control values in Romk⁻/⁻ mice. This lack of reduction in GFR by furosemide and hydrochlorothiazide also indicated impaired tubuloglomerular feedback in Romk⁻/⁻ mice.

All three diuretics produced acute increases in both ENa and FEK in Romk⁺/+ and Romk⁻/⁻ mice (Table 2). The acute increment in FEK resulting from furosemide administration was markedly blunted in Romk⁻/⁻ compared with Romk⁺/+ mice (Fig. 4A), but nevertheless was twofold greater than in the absence of diuretics in these ROMK null mice. In sharp contrast, the natriuretic effect of hydrochlorothiazide was greatly enhanced in Romk⁻/⁻ mice, a finding similar to that in the metabolic studies. Benzamil, the diuretic inhibitor of ENaC, produced a similar increment in FEK in wild-type and ROMK null mice. Table 2 summarizes the effects of three diuretics on renal clearance of Na⁺ and K⁺.

Values are means ± SE. n, No. of animals; UV, urine volume; GFR, glomerular filtration rate; ENa, absolute sodium and potassium excretion, respectively; FENa, and FEK, fractional excretion of sodium and potassium, respectively; Furo, furosemide; HCTZ, hydrochlorothiazide. Significant difference from same group of animals in control periods: *P < 0.05, †P < 0.001, ‡P < 0.0001.

Table 2. Effects of furosemide, hydrochlorothiazide, and benzamil on urine volume, GFR, E_Na, E_K, F_E_Na, and F_E_K

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>UV, ml/min</th>
<th>GFR, ml/min</th>
<th>ENa, μeq·min⁻¹·100 g BW⁻¹</th>
<th>Ek, μeq·min⁻¹·100 g BW⁻¹</th>
<th>FENa, %</th>
<th>FEK, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Romk⁺/+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>1.18 ± 0.13</td>
<td>0.92 ± 0.06</td>
<td>0.39 ± 0.06</td>
<td>0.52 ± 0.04</td>
<td>0.35 ± 0.019</td>
<td>17.45 ± 1.90</td>
</tr>
<tr>
<td>Furo</td>
<td>9</td>
<td>12.1 ± 1.4 ‡</td>
<td>0.56 ± 0.04‡</td>
<td>3.86 ± 0.55‡</td>
<td>0.95 ± 0.09‡</td>
<td>3.99 ± 0.44‡</td>
<td>49.75 ± 3.40‡</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>1.09 ± 0.16</td>
<td>0.95 ± 0.11</td>
<td>0.45 ± 0.1</td>
<td>0.76 ± 0.11</td>
<td>0.37 ± 0.05</td>
<td>23.48 ± 1.95</td>
</tr>
<tr>
<td>HCTZ</td>
<td>5</td>
<td>2.39 ± 0.33†</td>
<td>0.41 ± 0.05†</td>
<td>1.39 ± 0.2†</td>
<td>0.99 ± 0.15†</td>
<td>2.05 ± 0.23†</td>
<td>68.18 ± 6.80‡</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0.80 ± 0.10</td>
<td>0.84 ± 0.09</td>
<td>0.29 ± 0.03</td>
<td>0.53 ± 0.08</td>
<td>0.27 ± 0.06</td>
<td>17.3 ± 3.50</td>
</tr>
<tr>
<td>Benzmila</td>
<td>6</td>
<td>1.30 ± 0.10‡</td>
<td>0.77 ± 0.07</td>
<td>1.04 ± 0.13‡</td>
<td>0.10 ± 0.02‡</td>
<td>1.12 ± 0.22‡</td>
<td>3.24 ± 0.50‡</td>
</tr>
<tr>
<td>Romk⁻/⁻</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>3.25 ± 0.56</td>
<td>0.43 ± 0.06</td>
<td>0.38 ± 0.08</td>
<td>0.78 ± 0.08</td>
<td>0.71 ± 0.07</td>
<td>51.49 ± 8.00</td>
</tr>
<tr>
<td>Furo</td>
<td>5</td>
<td>8.53 ± 1.81*</td>
<td>0.31 ± 0.04</td>
<td>1.13 ± 0.27*</td>
<td>1.14 ± 0.20</td>
<td>2.24 ± 0.36†</td>
<td>105.8 ± 8.17†</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>3.46 ± 0.79</td>
<td>0.49 ± 0.08</td>
<td>0.39 ± 0.07</td>
<td>0.83 ± 0.11</td>
<td>0.76 ± 0.15</td>
<td>49.23 ± 5.17</td>
</tr>
<tr>
<td>HCTZ</td>
<td>7</td>
<td>8.31 ± 1.41*</td>
<td>0.39 ± 0.04</td>
<td>2.82 ± 0.49‡</td>
<td>1.09 ± 0.10</td>
<td>6.93 ± 0.83‡</td>
<td>85.16 ± 5.98‡</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>2.23 ± 0.40</td>
<td>0.51 ± 0.06</td>
<td>0.47 ± 0.05</td>
<td>0.57 ± 0.07</td>
<td>0.60 ± 0.07</td>
<td>30.6 ± 4.00</td>
</tr>
</tbody>
</table>
| Benzmila | 5   | 3.50 ± 0.40 | 0.49 ± 0.05 | 1.38 ± 0.2‡               | 0.31 ± 0.03‡             | 2.42 ± 0.44† | 20.5 ± 3.00*  

Fig. 3. Baseline renal clearance studies in Romk⁺/+ (wild-type; filled bars) and Romk⁻/⁻ (ROMK null; open bars) mice. Glomerular filtration rate (GFR; A), urine volume (B), and fractional Na⁺ (FENa; C) and K⁺ (FEK; D) excretion are shown.
to DCT and CCD, factors known to stimulate K\(^+\) secretion (12). The reduced kaliuresis observed with benrazil is consistent with the known reduction in K\(^+\) secretory driving force following inhibition of ENaC in distal nephron segments (18, 19). Despite the absence of ROMK in distal nephron segments, furosemide and hydrochlorothiazide produced significant, albeit blunted, acute kaliuresis in Romk\(^{-/-}\) mice. As expected, benzamil reduced FEK in Romk\(^{+/+}\) mice but also significantly blunted kaliuresis in Romk\(^{-/-}\) mice.

**DISCUSSION**

Diuretic pharmacotyping was used to assess the functional characteristics and selected adaptive responses of distal nephron segments in our high-surviving Romk\(^{-/-}\) mice. The diuretic and natriuretic responses in Romk\(^{-/-}\) mice consisted of a reduced effect of furosemide, an exaggerated response to hydrochlorothiazide, and an unaltered response to benzamil. Thus the diuretic responses observed in Romk\(^{-/-}\) mice were similar to that in type II HPS patients (22). The primary underlying assumptions used to interpret our results are that 1) each diuretic specifically inhibits a single transport mechanism (furosemide inhibition of NKCC2; hydrochlorothiazide inhibition of NCC; benzamil inhibition of ENaC); 2) at the diuretic doses used, each diuretic has a major effect on its targeted transport mechanism; 3) inhibition of any single transport mechanism can have both upstream (e.g., effects on tubuloglomerular feedback) and downstream (altered delivery of salt and water) effects on other salt and water transport pathways. Where relevant, we discuss some of these limitations in interpretation of our findings in the sections below.

GFR in Romk\(^{-/-}\) mice was ~48% of that observed in wild-type mice. This reduction in GFR is unlikely to be due to enhanced tubuloglomerular feedback since this was severely impaired in the original Romk\(^{-/-}\) mice (15). Given that single-nephron GFR was unaltered in Romk\(^{-/-}\) mice (15), the reduction in whole kidney GFR could be the result of a reduction in functional glomerular number. Indeed, we found that the total number of glomeruli was reduced by 48% in Romk\(^{-/-}\) mice compared with the Romk\(^{+/+}\) (28).

The response to the loop diuretic furosemide was markedly attenuated, consistent with a key role for ROMK-dependent apical K\(^+\) recycling in salt reabsorption by the TAL (5, 8, 10). This finding underscores a functional defect of the TAL as the basic mechanism for renal NaCl wasting in the ROMK-deficient subgroup of HPS (15). In the 6-h metabolic study, the effect of furosemide on UV and ENaC was completely absent in Romk\(^{-/-}\) mice, while in the clearance study an acute, albeit markedly blunted, diuretic effect of furosemide was observed. The former may have been due to incomplete compensation of the rapid diuretic-induced renal losses of Na\(^+\) and water. In contrast in the clearance study, fluid and electrolyte losses were minimized by intravenous replacement. Similarly attenuated, but not absent, acute diuretic and natriuretic responses to furosemide have been observed in children with HPS (20). At least two possibilities may explain the remaining acute diuretic effect of furosemide in Romk\(^{-/-}\) mice and in the clinical syndrome. First, a significant portion of fluid and Na\(^+\) absorption in the TAL may be independent of K\(^+\) recycling in ROMK null animals. This possibility is supported by the free-flow micropuncture data in the original analysis of the Romk\(^{-/-}\) mouse that showed the persistence of a fraction of sodium reabsorption along the loop of Henle (15). To potentially account for this remaining salt absorption, we (26) and others (1) had previously observed a furosemide-sensitive but K\(^+\)-independent mechanism for NaCl absorption by the mouse TAL. More recently, this K\(^+\)-independent mode of salt absorption has been linked to a splice variant of the NKCC2 (21). Second, furosemide and torsemide, but not bumetanide, have been shown to reduce absorption by the NaCl cotransport mechanism in the rat early DCT using the in vivo microperfusion technique (27) and to reduce Na\(^+\) entry into DCT cells by electron microprobe analysis (3). Third, some proximal micropuncture studies have demonstrated a reduction in proximal tubule fluid transport after intravenous furosemide, but
this has not been a consistent finding (23). The proximal tubule effect has been suggested to result from furosemide-mediated inhibition of carbonic anhydrase. Our study does not permit distinguishing among these possibilities.

The natriuretic responses to hydrochlorothiazide were significantly enhanced in Romk\(^{-/-}\) mice. Our results show that hydrochlorothiazide increased ENa by 2.1- and 1.05-fold in metabolic studies, increased ENa by 6.23- and 2.08-fold, and increased FE\(\text{Na}\) by 6.17- and 1.68-fold, from renal clearance studies, in Romk\(^{-/-}\) and Romk\(^{+/+}\) mice, respectively (Fig. 4). This exchanged transport activity along the distal convoluted tubule (DCT) is associated with hyperplasia and hypertrophy of this nephron segment [see Ref. 27a]. A similar DCT hypertrophy has been observed in the pseudohypaldosteronism type II (PHA II) mice with WNK4 kinase mutations (14) and in rats following chronic administration of furosemide (4). Adaptation of the DCT with loop diuretics has been attributed in part to increased salt delivery out of the loop of Henle with subsequent absorption by the DCT (13) as well as to activation of the renin-angiotensin system (4). In PHA II mice, DCT hypertrophy has been attributed directly to enhanced Na-Cl cotransport activity (14). However, in type II Bartter’s mice, the adaptive response of the DCT, even in the presence of the lowered GFR, is insufficient to completely reabsorb salt losses from the TAL dysfunction.

Despite hypovolemia and increased renal renin expression and aldosterone levels in Romk\(^{-/-}\) mice (15, 17; see also Ref. 27a), the effect of benzamil on Na\(^+\) excretion was not enhanced. Maintenance of the driving force for Na\(^+\) entry in principal cells depends on membrane polarization by K\(^+\) secretion, and therefore it is likely that the driving force for Na\(^+\) entry was reduced in Romk\(^{-/-}\) mice. Other factors may have contributed to the blunted response to aldosterone. For example, the activity of the kallikrein-kinin system and PGE\(_2\) production (and urinary excretion) is elevated in HPS, and cyclooxygenase (COX) inhibitors reduce both renal PGE\(_2\) and kallikrein excretion as well as NaCl wasting in HPS. While chronic furosemide treatment is associated with CCD hypertrophy, chronic piretanide administration is not; the latter has been attributed to the lack of increased bradykinin elevation with the latter loop diuretic. In addition, both natriuretic and antinatriuretic effects of PGE\(_2\) have been observed in various segments of the collecting duct, complicating the ability to predict specific effects of the elevated PGE\(_2\) in HPS. However, the PGE\(_2\), EP1, EP3, and EP4 receptor responses have been suggested to contribute to the natriuretic effects of PGE\(_2\) in the chronic furosemide model of HPS. Analysis of the expression, distribution, and function of EP receptors and the kallikrein-kinin system in Romk\(^{-/-}\) mice may be informative in assessing their potential role in the lack of a hypertrophic response in collecting ducts in this animal model of HPS. Finally, benzamil could affect a number of other proteins (e.g., TRPP3, Na\(^+\)/Ca\(^{2+}\) exchanger) expressed in the kidney that may have contributed to the unaltered responses to this diuretic in Romk\(^{-/-}\) mice (6, 24).

The acute kaliuresis in Romk\(^{-/-}\) mice after hydrochlorothiazide, while attenuated compared with wild-type mice, persisted in Romk\(^{-/-}\) mice. The blunting of kaliuresis in Romk\(^{-/-}\) mice is consistent with the role of ROMK in K\(^+\) secretion in distal nephron segments. Furthermore, the ENaC blocker benzamil reduced FEK in Romk\(^{-/-}\) mice, suggesting that the mechanism for distal K\(^+\) secretion in Romk\(^{-/-}\) mice is an electrogenic process, i.e., mediated by Na\(^+\) channels. Consistent with this notion, we have recently shown in stationary microperfusion studies that K\(^+\) secretion continues in the late distal convoluted tubule of Romk\(^{-/-}\) mice by iberiotoxin-sensitive BK potassium channels. Since BK channels can be activated by a flow-sensing mechanism in distal epithelial cells, the increased distal delivery of fluid and sodium out of the hypertrophied DCT with hydrochlorothiazide administration is likely to account for the enhanced kaliuresis in Romk\(^{-/-}\) mice with this diuretic.

REFERENCES


AJP-Renal Physiol • VOL 294 • JUNE 2008 • www.ajprenal.org


