Female ROMK null mice manifest more severe Bartter II phenotype on renal function and higher PGE$_2$ production

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Yan Q, Yang X, Cantone A, Giebisch G, Hebert S, Wang T. Female ROMK null mice manifest more severe Bartter II phenotype on renal function and higher PGE$_2$ production. Am J Physiol Regul Integr Comp Physiol 295: R997–R1004, 2008. First published June 25, 2008; doi:10.1152/ajpregu.00051.2007.—ROMK null mice with a high survival rate and varying severity of hydropneohsis provide a good model to study type II Bartter syndrome pathophysiology (26). During the development of such a colony, we found that more male than female null mice survived, 58.7% vs. 33.3%. To investigate the possible mechanism of this difference, we compared the survival rates, renal functions, degree of hydropneohsis, as well as PGE$_2$ and TXB$_2$ production between male and female ROMK wild-type and null mice. We observed that female ROMK Bartter’s mice exhibited lower GFR (0.37 vs. 0.54 ml min$^{-1}$ 100 g BW$^{-1}$, P < 0.05) and higher fractional Na$^+$ excretion (0.66% vs. 0.48%, P < 0.05) than male Bartter’s. No significant differences in acid-base parameters, urinary K$^+$ excretion, and plasma electrolyte concentrations were observed between sexes. In the male strain, we assessed the liquid retention rate in the kidney to evaluate the extent of hydropneohsis and observed that 67% of male and 90% of female ROMK null mice were hydropneohmic mice. Urinary PGE$_2$ excretion was higher in both sexes of ROMK null mice: 1.35 vs. 1.10 ng/24 h in males and 2.90 vs. 0.87 ng/24 h in females. TXB$_2$ excretion was higher in female mice in both wild-type and ROMK null mice. The increments of urinary PGE$_2$ and TXB$_2$ were significantly higher in female null mice than males, 233.33% vs. 22.74% of PGE$_2$ and 85.67% vs. 20.36% of TXB$_2$. These data demonstrate a more severe Bartter phenotype in female ROMK null mice, and higher PGE$_2$ and TXB$_2$ production may be one of the mechanisms of this manifestation.

BARTTER’S SYNDROME BELONGS to a group of rare autosomal-recessive disorders, characterized by salt wasting, polyuria, hypokalemic alkalosis with dehydration, and increased renin and aldosterone levels (2, 39), caused by severe reductions of salt absorption along the thick ascending limb of Henle (TAL) (13). Bartter’s syndrome (28, 36) results from mutations in one of five proteins that are linked to loss of salt transport in the TAL (13). Type II Bartter’s syndrome is caused by mutations of the KCNJ1 gene on chromosome 11q24–25, encoding the inward-rectifier potassium channel ROMK (Kir1.1) (13, 41). One of the important physiological functions of ROMK channel is the generation of luminal positive transepithelial potential, which drives the paracellular reabsorption of NaCl and K$^+$ and also provides the apical pathway for K$^+$ recycling. Such K$^+$ recycling is critical for supporting Na$^+$/K$^+$/2Cl$^-$ cotransport activity (45). ROMK knockout mice exhibit similar spectra as Bartter’s syndrome in patients (24). The significant reduction of Cl$^-$ absorption along the loop of Henle has been demonstrated in ROMK knockout mice (24). We have also demonstrated that ROMK protein expression in TAL and cortical collecting duct (CCD) and apical small-conductance K$^+$ channel activity was absent in apical membranes from either TAL or CCD in knockout mice (26) and that these mice exhibited characteristics of Bartter’s syndrome, including polyuria and Na$^+$ and K$^+$ wasting with compensatory increased water and food intake (26). The first study from ROMK null mice reported by Lorenz and Shull shows that the mortality of ROMK null mice was very high and less than 5% survived to weaning at 21 days; the survival rate did not improve significantly by daily subcutaneous injections of either indomethacin or isotonic saline (24). However, the survival rate significantly increased by selectively crossing surviving ROMK null mutants with heterozygotes from litters, in which there were surviving null mutants (25, 26). Since more male than female null mice survived, 58.7% vs. 33.3%, during the development of a higher survival colony, we further investigated the sex difference in renal functions by both metabolic cage and renal clearance methods. The survival rates and degree of hydropneohsis in male and female ROMK null mice were also compared. We also examined urinary excretion of both PGE$_2$ and TXB$_2$ in wild-type and ROMK null mice and compared the differences between sexes. Our data show that female null mice exhibit a more severe Bartter’s phenotype with a lower GFR, more severe hydropneohsis, higher PGE$_2$, and TXB$_2$ excretion, and earlier death.

METHODS

Animal Care, Reproduction, and Growth

The ROMK wild-type and knockout mouse colonies were generated as described previously (26). All mice were housed at the Yale animal facility with a temperature- and humidity-controlled environment and a 12:12-h light-dark cycle. To produce ROMK null mice, four cross combinations were designed to confirm that the ROMK genotype was controlled by one recessive gene segregation pattern. These four combinations are normal heterozygous (female) × heterozygous (male), heterozygous (female) × homozygous (male), homozygous (female) × heterozygous (male), and homozygous (female) × homozygous (male). The protocol used for genotyping pups was described previously and was followed in current experiments (26). After genotyping, mice were separated into four groups: wild-type male and female groups and knockout male and female groups. The body weight was taken every week from birth to 6 wk and was recorded monthly until the 22nd mo.

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Metabolic Experiments

The metabolic cage experiments were performed by methods described previously in our laboratory (26). Briefly, two mice (6 mo old) were placed in a metabolic cage (Lab Products, Seaford, DE) and trained for 2 days with free drinking water and standard pellet rodent chow. After training, mice that exhibited normal eating and drinking behavior were used for the experiments. Two mice from the same group (same sex, genotype, and age) were housed in a single cage and the 24-h urine volume and food and water intake were measured and recorded for 3 days. Urinary Na⁺ and K⁺ concentrations were measured by a flame photometer (IL 943 Automatic Flame Photometer).

Renal Clearance and Blood Gas Measurements

Renal clearance experiments were carried out by following the methods previously developed in our laboratory (43). The mice were anesthetized by Inactin (100 to 150 mg/kg body wt ip) and placed on a thermostatically controlled surgical table, maintaining body temperature at 37°C. A tracheostomy was performed, the left jugular vein was exposed and was cannulated for intravenous infusion, and a carotid artery catheterized for arterial blood collection. The bladder was catheterized via a suprapubic incision for timed urine collections.

After surgical preparation, 0.05 ml of isotonic saline was given intravenously to replace surgical fluid loss. Subsequently, a priming dose of 10 μCi of [methoxy-3H] inulin (New England Nuclear, Boston, MA) was administered in 0.3-ml isotonic saline, and a maintenance infusion of Ringer solution containing 10 μCi/ml of inulin, at a rate of 0.41 ml/h. An equilibration period of 60 min was followed by 30-min collection periods. Arterial blood samples were taken in the middle of each urine collection period, and urine and plasma Na⁺ and K⁺ concentrations were measured by standard flame photometry. Absolute (E_{Na}, E_{K}) and fractional (FE_{Na}, FE_{K}) excretion rates were calculated by standard methods (42). The blood for pH, PCO₂, and HCO₃⁻ measurements was collected by retroorbital bleeding and measured with a blood-gas analyzer (Blood-gas Corning 850, Corning Medical and Scientific, Corning, NY).

Evaluation of Hydronephrosis

Kidney hydronephrosis was evaluated by the liquid retention rate (LRR) at aged (12–18 mo) wild-type and ROMK null mice. The liquid retention was examined by transecting the kidney in the coronal plane and draining retained liquid in the kidney with Kimwipes paper. Kidneys were weighed before and after cutting and draining, and the ratio of liquid weight and fresh kidney weight was calculated as LRR. Two methods were used to assess hydronephrosis: one is average LRR, in which two kidneys from each mouse were calculated; another is hydronephrosis scales, in which only the kidney with the higher LRR was used. On the basis of maximum LRR of wild-type mice and 75% percentile of LRR in knockout mice, we rated three hydronephrosis scales: normal, slight, and severe hydronephrosis. Because of LRR variation in two kidneys of the same animal, each mouse’s LRR was represented by the higher LRR kidney to evaluate hydronephrosis scales in knockout mice.

Measurement of Urinary PGE₂ and TXB₂

Urine collection. Mice were housed in clear metabolic cages with free access to normal food and water. Urine samples were collected in tubes over 16 h. The collecting tubes were immersed in ice during collection.

PGE₂ measurement. PGE₂ concentration was determined by using a PGE₂ enzyme immunoassay (EIA) kit-monoconal (catalog No. 514010, Cayman Chemical, Ann Arbor, MI). The assay was performed according to the manufacturer’s instructions. Briefly, after diluting samples to the optional concentration, 50 μl of each sample along with a serial dilution of PGE₂ standard samples were mixed with appropriate amounts of acetylcholinesterase-labeled tracer and PGE₂ antiserum and incubated at 4°C for 18 h. After the wells were emptied and rinsed with wash buffer, 200 μl of Eillman’s reagent containing substrate for acetylcholinesterase was added. The enzyme reaction was carried out on a slow shaker at room temperature for 1–2 h. The plates were read with Microplate Reader (Benchmark Microplate Reader, Bio-Rad, Hercules, CA) at 405 nm. The results were analyzed by Cayman Chemical’s computer spreadsheet.

Thromboxane B₂ measurement. Thromboxane B₂ (TXB₂) concentration was determined by using a TXB₂ EIA Kit (catalog no. 519031, Cayman Chemical, Ann Arbor, MI). The assay was very similar to the PGE₂ measurement. Briefly, 50 μl of each diluted sample (1:10) along with a serial dilution of TXB₂ standard samples were added to the plate well, followed by 50 μl TXB₂ AChE tracer and TXB₂ antiserum, and the plate was incubated at room temperature for 18 h. After the wells were emptied and rinsed with wash buffer 5 times, 200 μl of Eillman’s reagent containing substrate for acetylcholinesterase was added to each well and developed on a slow shaker at room temperature for 1 h. Methods to read the plate and calculate the result were same as above in PGE₂ measurement.

Statistical analysis. Data are presented as the means ± SE. Statistical significance was assessed by using paired and unpaired t-test depending on whether paired or unpaired experiments were conducted. Pearson’s correlation analysis was used for testing correlations between PGE₂, TXB₂ excretion, and LRR.

RESULTS

Sex ratio and survival rate in male and female ROMK Barter’s mice. The sex ratio of newborn pups and their survival rates were examined in male and female ROMK knockout mice. The results are summarized in Fig. 1. The study included 138 male and 141 female newborns. The ratio of male and female is close to 1:1, indicating no differences of reproductive and embryonic development between sexes in ROMK null mice. Because most of nonsurviving ROMK knockout mice died between birth and 15 days (26), and death was rare after weaning for up to 6 mo, the survival rates were compared at the weaning stage of development (3 wk old). The average survival rates were calculated from animals produced from different cross combinations, +/−X+/−, +/−X−/−, −/−X+/− and −/−X−/−. As shown in Fig. 1, the male and female survival rates were similar.
knockout pups had significantly higher survival rates than female pups. The rates are 58.7% in males and 33.3% in females, respectively.

Growth trends in male and female ROMK Bartter’s mice. The growth trends were evaluated by measuring age-dependent changes of body weight in ROMK wild-type and null male and female mice. Body weights at birth, and their weekly changes before weaning in all types of mice are summarized in Fig. 2, showing significantly lower weights from both sexes of ROMK null mice at almost all age periods. The lower body weight in ROMK null mice is consistent with slower development and growth in children with Bartter’s syndrome (30, 36).

Figure 3 summarizes the body weight changes recorded for a time period of 2 yr in all types of mice. On the basis of the general changes of body weight, mouse growth can roughly be divided into three periods; namely, the developmental, growth continuation, and stabilization of body weight periods. Figure 3 shows that the body weight quickly increased in the first 6 mo and then slowed from 7 to 14 mo; after 15 mo, the body weight remained relatively constant. These three periods of body weight changes are clearly demonstrated in both male and female wild-type mice, although the overall body weights are significantly lower in female than male mice (Fig. 3). In ROMK knockout mice, mean body weights are lower in both male and female mice, with no significant differences in the rate of increase in body weight in the developmental period. However, after 7 mo, the body weight becomes constant without significant change, and the continuation growth period is absent in the ROMK null mice. The difference between male and female null mice also appeared after 14 mo. In that period, the body weight was constant in males but significantly reduced in female null mice, suggesting the possibility of progressive renal functional impairment in female mice. Indeed, aged females exhibited higher death rates than males (17.5% vs. 10.53%) when evaluated for the period of 15 to 26 mo.

Comparison of 24-h urine output, Na\(^+\) and K\(^+\) excretion. Urine output, total Na\(^+\) and K\(^+\) excretion, and water and food intake were examined in conscious mice by metabolic cage experiments. Data are summarized in Table 1 and show significantly higher urine volume and Na\(^+\) and K\(^+\) excretion in both sexes of ROMK null mice, consistent with salt and water wasting seen in Bartter’s patients and previously reported in ROMK null mice (24, 26). Food and water intakes in ROMK null mice were also significantly higher than those in wild-type mice of both sexes. Such an increase of water and food intake is expected and compensates for the salt and water wasting in ROMK Bartter’s mice. There were no significant differences in urine output or absolute Na\(^+\) and K\(^+\) excretion between sexes either in wild-type mice or in ROMK null mice. However, food and water intake were significantly higher in female than male ROMK null mice, indicating more water wasting may occur in female null mice.

Comparison of GFR, Urine Volume, Na\(^+\), and K\(^+\) Excretion

Urine volume, GFR, and fractional Na\(^+\) and K\(^+\) excretion were examined by renal clearance methods in all groups of anesthetized mice. The results are summarized in Table 2. Urine volumes were much higher in both sexes in ROMK-null mice, consistent with the polyuria in Bartter’s syndrome. In addition to the lower GFR in ROMK-null mice compared with wild-type mice of both sexes, the female ROMK-null mice had even lower GFR than males. This result demonstrates more severely impaired renal functions in female ROMK-null mice.

No differences in fractional Na\(^+\) and K\(^+\) excretions were observed between sexes in wild-type mice (Table 2). FE\(_{\text{Na}}\) was significantly elevated in knockout mice of both sexes, consistent with sodium wasting in Bartter’s syndrome. FE\(_{\text{Na}}\) was higher in females than male knockout mice, indicating more severe sodium wasting in female null mice. FE\(_{\text{K}}\) is also higher in knockout mice in both sexes. However, there was no statistically significant sex difference in FE\(_{\text{K}}\) in female null mice. Plasma electrolyte and acid-base parameter measurements were summarized in Table 3 and showed no significant difference in Na\(^+\), K\(^+\), and Cl\(^-\) concentrations among all groups except for male knockout mice, which was statistically lower than male wild-type. In addition, blood pH and HCO\(_3^-\) had no significant difference between genotypes in each sex. The blood pH and HCO\(_3^-\) levels are slightly lower than those measured from arterial blood (44), since the mixed blood might
Comparison of food and water intake, urine output, sodium and potassium excretion between male and female ROMK wild-type and null mice

Comparison of LRR to Evaluate Hydronephrosis

Because no methods were available to determine the presence of, or to quantify the severity of, hydronephrosis in mice, we examined whether the amount of liquid retention in the kidney would provide a reasonable index to hydronephrosis. This seemed reasonable since hydronephrosis would reduce renal mass and increase kidney fluid retention in a dilated, hydronephrotic collecting system. Thus, a higher LRR than normal would be considered hydronephrosis. Figure 4 showed the LRR in male and female wild-type and ROMK-null mice kidney. There was no difference between sexes in wild-type mice. In ROMK-null mice, the average LRR was higher in female than male: 17.75 ± 2.46% (n = 36, from 18 mice) in male and 21.44 ± 2.31% (n = 40, from 20 mice) in female. Because of the large variation of LRR from each kidney, this difference was not statistically significant. Further, we determined the normal range of LRR values and the 75% percentile LRR in wild-type mice. The LRRs were low and ranged between 2.6 and 8.9%, and the 75% percentile LRR was 5.2% from 24 kidneys in adult wild-type mice aged 12–18 mo. Neither LRR ranges nor the 75% percentile LRRs showed difference between the sexes or between two kidneys from individual wild-type mice. Thus, we used these normal values in wild-type mice to indicate the absence of hydronephrosis.

In 76 kidneys from ROMK-null mice aged 12–18 mo, LRR range was larger (1.4–65%) and the 75% percentile LRR was higher (25.8%) than in wild-type mice. On the basis of the findings that LRRs are less than but close to 10% in all wild-type mice and the 75% percentile LRR in ROMK-null mouse kidneys is about 25%, we used 10 and 25% as thresholds to rate normal (LRR ≤10%), slight hydronephrosis (10% < LRR ≤25%), and severe hydronephrosis (LRR > 25%) mice.

Table 1. Comparison of food and water intake, urine output, sodium and potassium excretion between male and female ROMK wild-type and null mice

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>BW, g</th>
<th>Food intake, g/24 h</th>
<th>Water intake, ml/24 h</th>
<th>UV, ml/24 h</th>
<th>ENa, μEq/24 h</th>
<th>EK, μEq</th>
<th>ENa (% of BW)</th>
<th>EK (% of BW)</th>
<th>MBP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>20</td>
<td>27.60 ± 0.65</td>
<td>0.82 ± 0.07</td>
<td>1.05 ± 0.12</td>
<td>0.36 ± 0.05</td>
<td>33.7 ± 3.85</td>
<td>82.8 ± 8.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KO</td>
<td>26</td>
<td>25.96 ± 0.72</td>
<td>1.18 ± 0.08*</td>
<td>3.36 ± 0.22*</td>
<td>2.21 ± 0.13*</td>
<td>57.1 ± 4.65*</td>
<td>146.1 ± 11.23*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>10</td>
<td>22.60 ± 1.17†</td>
<td>1.53 ± 0.16†</td>
<td>4.43 ± 0.37†</td>
<td>2.34 ± 0.16*</td>
<td>58.8 ± 6.91*</td>
<td>146.7 ± 14.40*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N, number of animals; BW, body weight; UV, Urine volume; ENa and EK, Absolute Na+ and K+ excretion. *Significant difference between wild-type and knockout mice in same sex. †Significant difference between sexes in same genotype.

be obtained by retro-orbital bleeding in this study. As we reported previously (26), the hematocrit was higher in ROMK-null mice, suggesting that extracellular fluid is reduced in null mice.

Comparison of LRR to Evaluate Hydronephrosis

Fig. 5 showed the percentages of knockout mice with normal, slight, and severe hydronephrosis in both sexes. The results indicated that 33, 27, and 40% males and 10, 40, and 50% females exhibited normal, slight, and severe hydronephrosis, respectively. 33% of males, but only 10% of females were without hydronephrosis, and 67% of male and 90% female were hydronephrotic, indicating that female ROMK mice had more significant hydronephrosis than males.

Comparison of PGE2 and TXB2 Production

The urinary PGE2 amount was compared in wild-type and ROMK-null mice in both sexes, as shown in Fig. 6A. In wild-type mice, the PGE2 production was slightly lower in female than male, 0.87 ± 0.06 (n = 6) vs. 1.10 ± 0.1 ng/24 h (n = 12), P = 0.1. In contrast, female null mice had significantly higher PGE2 excretion (2.90 ± 0.21 vs. 1.35 ± 0.19 ng/24 h, n = 13, 25, respectively, P = 0.0043) compared with wild-type, consistent with high prostaglandin excretion in the urine in Bartter’s syndrome (7, 9). The increments of PGE2 excretion in ROMK-null mice over wild-type mice were 233.33% in female and only 22.73% in male. Fig. 6B shows the urinary TXB2 excretion in wild-type and ROMK-null mice in both sexes. The female wild-type mice exhibited higher urinary TXB2 production than males, 3.21 ± 0.31 vs. 1.67 ± 0.21 ng/24 h (n = 8 for both sexes, P = 0.0011). The TXB2 excretion increased by 20.36% from 1.67 ± 0.21 to 2.01 ± 0.31 ng/24 h (n = 8, 20, respectively, P = 0.41) in male ROMK-null mice. Meanwhile, the TXB2 increased by 85.67% from 3.21 ± 0.31 to 5.96 ± 0.62 ng/24 h in female ROMK-null mice (n = 8, 14, P < 0.0045). The difference in TXB2 excretion between male and female ROMK-null mice was extremely significant.

To investigate whether severe hydronephrosis is correlated to increased PGE2 or TXB2 production, we compared the LRR, PGE2, and TXB2 concentrations between male and female ROMK-null mice (Fig. 7). The correlation analysis was performed, and the significance of the curves was calculated by
In contrast, PGE\(_2\) excretions were higher in female ROMK-null mice (Fig. 7A). The slope of the first-order regression curve was \(r = -0.01984\), and the correlation coefficient (Pearson’s \(r\)) was \(-0.22381\), \(P = 0.43\). In contrast, PGE\(_2\) excretions were higher in female ROMK-null mice as the LRR was elevated. The slope was 0.1036; Pearson’s \(r\) was 0.9147, \(P = 0.0039\). Fig. 7B displays the relationship of hydronephrosis and urinary TXB\(_2\) excretion in male and female ROMK-null mice. Urinary TXB\(_2\) excretion was much higher at almost all points in female than male null mice. TXB\(_2\) excretions failed to exhibit correlation with LRR in male mice; however, LRR and TXB\(_2\) excretion seemed to be negatively correlated in female null mice. The slope was \(-0.081\), Pearson’s \(r\) was \(-0.7966\) and \(P = 0.032\) (Fig. 7B).

### DISCUSSION

Mice lacking ROMK exhibit a phenotype similar to Bartter’s syndrome, including salt and water wasting, and high PGE\(_2\) excretion (9, 33). This study shows that female ROMK Bartter’s mice exhibit a greater reduction in GFR, a greater magnitude of hydronephrosis, higher urinary PGE\(_2\) and TXB\(_2\) excretion and reduced growth and survival rates. Although the mechanism of these sex differences is not yet clear, one possible contributing factor is the elevated PGE\(_2\) production in female ROMK-null mice. PGE\(_2\) has been found to inhibit distal nphron NaCl absorption (15, 16), and the inhibition of PGs production by indomethacin and cyclooxygenase-2 (COX-2) inhibitors has been used as the major method to treat Bartter’s patients (11, 31). Such treatment not only prevented salt wasting and dehydration, but also slowed the process of progressive renal failure, especially in Bartter’s children (40).

Three lines of evidence indicate that female ROMK-null mice exhibit more severe and progressive impairment of renal function than males. First, female mice have lower rates of GFR and elevated fractional sodium excretion; second, females had more severe hydronephrosis; and third, females had a greater reduction of body weight and a lower survival rate. It is likely that the sex differences in magnitude of body weight reduction and earlier death in female ROMK-null adults was due to the greater impairment of renal function with hydronephrosis.

The suggestive evidence supports the theory that increased PGE\(_2\) production in female ROMK-null mice may be one of the important contributors to the severity of the Bartter’s phenotype in this sex. COX-2 is a major enzyme in the kidney for PGE\(_2\) synthesis, and its expression increases in high-renin states (12). Higher COX-2 expression has also been detected in Bartter’s patients (21, 31). The role of PGE\(_2\) in Bartter’s syndrome is supported by the observations that inhibition of PG generation with indomethacin and COX-2 inhibitors reduces the salt-wasting dehydration and hypercalciuria in children with hyperprostaglandin E syndrome (6, 18). Previous studies show that the nonselective COX inhibitor indomethacin causes sodium retention and a decline in GFR, but the selective inhibition of COX-2 does not, suggesting that the depression of GFR by indomethacin is due to inhibition of COX-1 (4). The effect of PGE\(_2\) on Na\(^+\) transport in the kidney has been extensively studied, and it has been demonstrated that PGE\(_2\) inhibits net Na\(^+\) absorption in the CCD and inner medullary collecting duct (IMCD) (3) and reduces apical Na\(^+\) channel activity in rabbit cortical collecting tubules (23). PGE\(_2\) also reduces Cl\(^-\) absorption in the loop of Henle (29), inhibits Na\(^+\)/K\(^+\)/2Cl\(^-\) cotransport activity in medullary thick ascend-

### Table 3. Plasma electrolyte and acid-base parameters in male and female ROMK null and wild-type mice

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>PNa, mM</th>
<th>PK, mM</th>
<th>PCI, mM</th>
<th>pH</th>
<th>PHCO(_3), mM</th>
<th>HCT, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>M</td>
<td>151.8±2.5</td>
<td>4.32±0.20</td>
<td>112.8±2.0</td>
<td>7.32±0.03</td>
<td>21.9±0.8</td>
<td>47.8±0.6</td>
</tr>
<tr>
<td>F</td>
<td>8</td>
<td>149.7±1.4</td>
<td>4.37±0.26</td>
<td>112.4±1.0</td>
<td>7.25±0.01</td>
<td>20.9±0.8</td>
<td>44.6±1.34</td>
</tr>
<tr>
<td>KO</td>
<td>M</td>
<td>155.5±1.1</td>
<td>3.68±0.16(^*)</td>
<td>113.8±2.4</td>
<td>7.27±0.02</td>
<td>22.9±0.7</td>
<td>49.3±1.8</td>
</tr>
<tr>
<td>F</td>
<td>8</td>
<td>157.6±2.8</td>
<td>3.97±0.08</td>
<td>115.3±3.0</td>
<td>7.23±0.04</td>
<td>21.9±1.1</td>
<td>49.3±1.3(^*)</td>
</tr>
</tbody>
</table>

N, number of animals; PNa, PK, PCI, PHCO\(_3\), plasma Na\(^+\), K\(^+\), Cl\(^-\), HCO\(_3\) concentrations; HCT, hematocrit. \(^*\)Significant difference from same sex wild-type mice (\(P < 0.05\)).

![Fig. 4. Liquid retention rates (LRRs) in male and female wild-type (WT-M, WT-F, \(n = 12\) for both groups) and ROMK knockout (KO-M, KO-F, \(n = 36, 40\), respectively) mice kidneys. Each symbol indicates the ratio of liquid and kidney weight.](http://ajpregu.physiology.org/)

![Fig. 5. Hydronephrosis development in male and female ROMK knockout mice. Hydronephrosis of each mouse was scaled based on the liquid retention rate (LRR) in kidney. The mice with LRR \(\leq 10\%\) were classified as normal, \(10 < \text{LRR} \leq 25\%\) as slight hydronephrosis, \(\text{LRR} > 25\%\) as severe hydronephrosis. \(n = 18\) for male, \(20\) female.](http://ajpregu.physiology.org/)
ing limb cells (19), and reduces IMCD Na\(^{+}\)-K\(^{+}\)-ATPase activity (16). Given the condition of salt wasting and dehydration, inhibition of Na\(^{+}\)/H\(^{+}\) absorption by PGE\(_{2}\) would enhance the severity of salt wasting and diuresis in Bartter’s syndrome, an outcome that likely contributes to the incidence and magnitude of hydronephrosis in ROMK-null mice.

Previous studies reported that both PGE\(_{2}\) and TXB\(_{2}\) production increased in a hydronephrosis model induced by unilateral ureteral obstruction in rat (20), by renal vein constriction in rabbit (34), and in human congenital obstructive uropathy (22). These studies indicated that PGE\(_{2}\) and TXB\(_{2}\) are important pathophysiological regulators in response to increased hydrostatic pressure and to reduced renal blood flow. However, it is not clear whether higher PGE\(_{2}\) and TXB\(_{2}\) level is secondarily induced by hydronephrosis. To investigate this mechanism, we first examined urinary concentrations of both PGE\(_{2}\) and TXB\(_{2}\) in wild-type and ROMK-null mice and compared the differences between sexes. Our data show that both PGE\(_{2}\) and TXB\(_{2}\) excretion were significantly higher in female than in male ROMK-null mice. The increment of urinary TXB\(_{2}\) excretion was significantly higher in female than in male null mice. * and # represent same significant difference as in A; **Significant difference compared with the wild-type mice. ##Significant difference between male and female (P < 0.01).

The reasons for significantly higher production/excretion of PGE\(_{2}\) in female ROMK-null mice are still under investigation. However, it has been found that estrogen increases PGE\(_{2}\) concentrations and AT-1 receptor expression in the renal cortex and medulla in mice (1). Estrogen also upregulates PGE\(_{2}\) synthesis and downregulates PGE\(_{2}\) degradation by inhibiting 15-PGDH activity (10). It has also been established that ANG II stimulates PGE\(_{2}\) (14, 37) and TXB\(_{2}\) excretion (46), and the renin-ANG II system is elevated in Bartter’s syndrome (21). Given the fact that both ANG II and estrogen increase the level of PGE\(_{2}\), the additive effect of ANG II and estrogen on PGE\(_{2}\) production could result in more severe renal functional deficiencies in female than in male Bartter’s. Studies of sex differences in spontaneously hypertensive rats demonstrated that PGE\(_{2}\) and TXB\(_{2}\) respond to sex hormones differently (38). These studies found enhanced urinary excretion of PGE\(_{2}\) and TXB\(_{2}\) excretions failed to exhibit correlation with LRR in male mice, and the LRR and TXB\(_{2}\) excretion seemed to be negatively correlated in female null mice. It should be noted that greater numbers of animals are needed to measure PGE\(_{2}\), TXB\(_{2}\), and hydronephrosis, especially in animals with higher levels of LRR, as shown in Fig. 7. However, in view of a lower survival rate of female knockout mice and a very small percentage of knockout mice having 60% of LRR (Fig. 4), from present data, we are not able to conclude that PGE\(_{2}\) is directly causing the hydronephrosis.

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TXB2 with increased COX-2 expression in female compared to male spontaneous hypertensive rats (SHR). Orchidectomy was associated with increased PGE2 metabolite excretion and microsomal PGE synthase protein expression, suggesting that testosterone inhibits PGE2 synthesis, but TXB2 excretion was not affected by gonadectomy in either male or female SHR (38).

Several lines of evidence from previous studies support the hypothesis that increased PGE2 in ROMK-null mice is induced by higher renin-angiotensin system (RAS) activity. First, studies in rats with chronic ANG II infusion increased PGE2, PGF2 production and also COX-2 expression at the mRNA and protein levels in isolated glomeruli and in mesangial cells (17). Second, chronic administration of furosemide, which causes effects similar to Bartter’s syndrome, increased PGE2 metabolite excretion and was associated with increased PGE2 excretion and COX-2 expression in the cTAL and the macula densa (5). The relative role of ANG II in regulation of COX-2 in glomeruli, mesangial cells, and in the macula densa and cTAL needs to be investigated.

The mechanism of the hydronephrosis in ROMK-null mice is not clear since higher urine volume should not increase the hydrostatic pressure in the kidney unless there is obstruction of urine output downstream of the kidney. In our renal clearance experiments with 40 plus ROMK-null mice of both sexes, we observed that bladders of knockout mice were always full of urine before the cannulation, and the sizes of the bladders were estimated to be 2 to 3 times larger than those in WT mice. Although we were unable to measure the pressure in the bladder, it is reasonable to assume that the pressure was higher, since the bladder muscle wall always looks very thin in the ROMK null mice compared with WT. Such large amounts of urine accumulated in the bladder may increase the renal hydrostatic pressure, which, in turn, may result in hydronephrosis. Why large amounts of urine accumulate in the bladder of ROMK null mouse and whether the knockout of the ROMK channel alters the KATP channel activity in the bladder need to be investigated.

Perspectives and Significance

Previously, most observations in experimental animals and in humans (including ageing, diabetes, and polycystic kidney disease, acute and chronic ischemic renal failure) have shown that female hormone and/or supplementary treatments with estradiol were found to attenuate the progression of renal disease (27, 32, 35). However, we have found in ROMK Barter’s mouse models that female ROMK Barter’s mice exhibit a lower GFR, a more severe hydronephrosis, a higher fractional Na+ excretion, and a greater reduction in growth and survival rates. We have suggested that elevated PGE2 and TXB2 production in female ROMK null mice may be one of the contributing factors for the more severe phenotype in this sex. It remains unclear, however, why higher PGE2 and TXB2 are exhibited and how they contribute to accelerated renal failure in female ROMK null mice.

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REFERENCES


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SEX DIFFERENCES IN ROMK BARTTER’S MICE


