Heterozygous Disruption of Renal Outer Medullary Potassium Channel in Rats Is Associated With Reduced Blood Pressure


See Editorial Commentary, pp 240–241

Abstract—The renal outer medullary potassium channel (ROMK, KCNJ1) mediates potassium recycling and facilitates sodium reabsorption through the Na+/K+/2Cl− cotransporter in the loop of Henle and potassium secretion at the cortical collecting duct. Human genetic studies indicate that ROMK homozygous loss-of-function mutations cause type II Bartter syndrome, featuring polyuria, renal salt wasting, and hypotension; humans heterozygous for ROMK mutations identified in the Framingham Heart Study have reduced blood pressure. ROMK null mice recapitulate many of the features of type II Bartter syndrome. We have generated an ROMK knockout rat model in Dahl salt-sensitive background by using zinc finger nuclease technology and investigated the effects of knocking out ROMK on systemic and renal hemodynamics and kidney histology in the Dahl salt-sensitive rats. The ROMK−/− pups recapitulated features identified in the ROMK null mice. The ROMK−/− rats, when challenged with a 4% salt diet, exhibited a reduced blood pressure compared with their ROMK+/+ littermates. More importantly, when challenged with an 8% salt diet, the Dahl salt-sensitive rats with 50% less ROMK expression showed increased protection from salt-induced blood pressure elevation and signs of protection from renal injury. Our findings in ROMK knockout Dahl salt-sensitive rats, together with the previous reports in humans and mice, underscore a critical role of ROMK in blood pressure regulation. (Hypertension. 2013;62:288-294.) • Online Data Supplement

Key Words: Dahl salt-resistant rats ▪ gene knockout ▪ hypertension ▪ Kcnj1 protein, rat ▪ renal insufficiency, acute ▪ ROMK protein, rat

Hypertension is one of the most common chronic diseases with complex pathogenesis. It is likely that hypertension is a consequence of an interaction between genetic and environmental factors. Although genome-wide association studies have identified multiple genes that are associated with hypertension, a great deal of our current understanding of the molecular mechanisms involved in blood pressure (BP) regulation1 has come from analysis of the genes responsible for monogenetic syndromes of hypertension and hypotension. For example, loss-of-function mutations in the Na+/K+/2Cl− cotransporter (NKCC2, SLC12A1) causes Gitelman syndrome, characterized by salt-sensitive (SS) hypertension, hyperkalemia, and metabolic acidosis.6,7 Loss-of-function mutations in Na+/K+/2Cl− cotransporter (NKCC2, SLC12A1) or renal outer medullary potassium channel (ROMK, KCNJ1) are responsible for Bartter syndrome type I and type II, respectively.6,11 These disorders are characterized by polyuria, salt wasting, hypokalemia, metabolic alkalosis, hypercalciuria, and hypotension. Furthermore, Ji et al12 demonstrated that heterozygous mutations in all 3 of these genes, SLC12A3, SLC12A1, and KCNJ1, are associated with clinically significant BP reduction and protection from development of hypertension. Thus, these syndromes further underscore a key role of renal

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salt handling in BP regulation. Indeed, targeting NCC and NKCC2 by pharmacological inhibition using thiazide and loop diuretics, respectively, has been successfully used to manage hypertension and states of volume overload for several decades. ROMK, since its identification and cloning by Ho et al11 as the founding member of the inward-rectifying K+ channel family, has attracted considerable attention in the field of renal physiology and beyond. Extensive research has verified the pivotal role of ROMK in regulation of salt and potassium homeostasis.14 Of particular note, genetically engineered ROMK-deficient mice exhibit Bartter syndrome type II–like phenotype.15,16 This mouse model has become an extremely valuable tool for investigating the regulation of other renal transporters and the response to various diuretics in the absence of ROMK.17,18 We used zinc finger nuclease (ZFN)19,20 to generate ROMK+/− and ROMK−/− rats on the Dahl SS background, which is a rodent model of SS hypertension and has been widely used to investigate the molecular mechanisms underlying the development of salt-induced hypertension.21–23 In addition to the opportunity to evaluate effects of ROMK deletion on the well-characterized changes in BP and kidney function under challenge with increased salt load, Dahl SS rats offer the advantage of ease of study with radio telemetry devices to enable accurate BP measurement over time. We hypothesize that ROMK deletion in rats would recapitulate Bartter syndrome type II–like phenotype in mice and human genetic data for heterozygosity. To this end, the present study was designed to characterize systemic hemodynamics, renal function, and kidney histology in ROMK knockout Dahl SS rats to further gain insight into the role of ROMK in BP regulation in an SS population. It is believed that the mechanisms responsible for susceptibility to salt-induced hypertension are probably quite similar for both humans and animals.24

Methods

Methods section is available in the online-only Data Supplement.

Statistical Analyses

All data are presented as means±SEM. A repeated-measure ANOVA was used for time course data analysis. One-way ANOVA, followed by Newman–Keuls post hoc test, was used for comparisons of only 1 time point values in all groups. A P<0.05 was considered to be of statistical significance.

Results

Generation of ROMK Knockout Rats

Injection of ROMK-specific ZFN into Dahl SS embryos resulted in a deletion of 209 bp DNA fragment encoding positions G225 to G433 of the ROMK open reading frame. Furthermore, the ZFN-induced mutation leads to frameshift and premature termination of ROMK open reading frame (Figure 1). A functional ROMK monomer consists of 2 transmembrane domains: a pore loop and cytoplasmic NH2- and COOH-terminal domains. The pore loop region is a major determinant of the ROMK single-channel conductance. Deletion of 209 bp in the ROMK open reading frame results in failure to encode a functional ROMK protein because the premature termination codon is upstream of the first transmembrane domain. We observed that breeding of heterozygous mutant founders generates offspring of ROMK+/−, ROMK−/−, or ROMK+/+ in the expected Mendelian inheritance pattern (data not shown).

Genotype and Molecular Characterization

Polymerase chain reaction analysis of rat tail genomic DNA from 7- to 10-day pups is shown in Figure 2A. Primers amplify a 397-bp product for the knockout allele and a 606-bp product for wild-type allele. A custom-designed Taqman probe detects only wild-type ROMK mRNA but not mutant ROMK mRNA. In ROMK−/− rats, kidney wild-type ROMK mRNA level was reduced by 50%, and no wild-type ROMK mRNA was detected in ROMK−/− rats (Figure 2B). Immunohistochemistry showed a weaker ROMK expression in ROMK−/− rats compared with their wild-type littermates and the absence of ROMK in ROMK−/− rats (Figure 2C). Western blot analysis showed that ROMK−/− rats have 50% ROMK protein compared with the ROMK+/+ rats, whereas no ROMK was detected in membranes prepared from ROMK−/− kidneys (Figure 2D).

Phenotypic Features of ROMK−/− Pups

The survival rate of ROMK−/− pups dramatically declined after postnatal day 14; subcutaneous injection of saline failed to improve the survival rate. To provide a comprehensive analysis of all 3 genotypes, blood and kidney tissues were harvested from pups at postnatal day 13. The body weight of ROMK−/− pups was approximately half of the ROMK+/+ or ROMK+/− pups, and the ROMK−/− pups exhibited severe volume depletion as indicated by poor skin turgor, increased whole blood electrolyte concentration (Na+, K+, and Cl−), and increased hematocrit. The ROMK−/− pups also showed metabolic acidosis and increased blood urea nitrogen (BUN) level (Table), and their respiratory rates were higher to compensate for metabolic acidosis. Kidney histology of ROMK−/− pups showed tubular dilatation, tubular mineralization, tubular epithelial cell degeneration, acute inflammation of the tubules, and dilatation of the pelvis. Based on the location and the pattern of the affected tubules, the renal tubular changes seemed to predominantly affect the distal convoluted tubules and collecting ducts (Figure 2E). These features are similar to those reported for ROMK knockout mice.15,16 In contrast to the ROMK−/− pups, ROMK+/− and ROMK+/+ pups demonstrated no significant difference in appearance, body and organ weights, blood chemistry parameters, and kidney histology.

BP and Renal Function in Adult ROMK+/− Rats

We first assessed body and organ weight, blood chemistry, and kidney histomorphology in young (6- to 8-week old) ROMK−/− and ROMK+/− rats, and no difference was found. Telemetry studies in adult ROMK−/− and ROMK+/− rats showed that, while on a 0.25% salt diet, systolic BP was 5 mm Hg and 2 to 3 mm Hg lower in ROMK−/− than ROMK+/− in females and males, respectively; diastolic BP was not different between ROMK−/− and ROMK+/− rats. After a 4% salt diet challenge for 4 weeks, both systolic BP and diastolic BP difference between ROMK−/− and ROMK+/− were significantly increased in females and males (Figure 3). However, other parameters assessed were not significantly different between ROMK−/− and ROMK+/− rats; these included food intake, water intake,
urine output, urinary electrolytes (including Na⁺, K⁺, Cl⁻, Ca²⁺, and Mg²⁺), prostaglandin E2 excretion, urinary levels of renal injury biomarkers (including lipocalin-2, osteopontin, kidney injury molecule-1, and renal papillary antigen-1), blood chemistry (Na⁺, K⁺, Cl⁻, pH, Hct, HCO₃⁻, BUN, creatinine, cystatin C), and glomerular filtration rate (as assessed by creatinine clearance). The ROMK⁺⁻ and ROMK⁺⁺ rats also demonstrated similar kidney histological characteristics. To further differentiate BP and renal function response to a higher salt challenge, male ROMK⁺⁻ and ROMK⁺⁺ rats received an

Figure 1. Zinc finger nuclease (ZFN) generates loss-of-function renal outer medullary potassium channel (ROMK) mutation in Dahl salt-sensitive rats. Rat ROMK gene structure and the ZFN targeting site are shown. Rat ROMK gene contains 2 exons and 1 intron. The asterisk indicates the relative location of the ZFN site within the ROMK gene structure and protein domains. Deletion of 209 bp from the ROMK gene leads to frameshift and premature termination of ROMK open reading frame, as shown in the lower part of the figure. The truncated protein lacks most of the ROMK domains shown in the oval circle and will not lead to any functional ROMK protein. WT indicates wild type.

Figure 2. Renal outer medullary potassium channel (ROMK) genotype analysis, ROMK mRNA and protein expression, and kidney histomorphology of ROMK⁺⁻ and ROMK⁺⁺ pups. A, Polymerase chain reaction analysis of rat genomic DNA showed 397-bp and 606-bp fragments from the mutant and wild-type alleles, respectively. Lane 1, ROMK⁺⁺; lane 2, ROMK⁺⁻; lane 3, ROMK⁻⁻. B, A custom-designed Taqman probe detected that wild-type ROMK mRNA level was reduced by 50% in ROMK⁺⁻ rats and was absent in ROMK⁻⁻ rats. C, Immunohistochemistry (IHC) showed a weaker ROMK expression in ROMK⁺⁻ rats, but no detectable ROMK was recognized in ROMK⁻⁻ kidneys. D, Western blot showed that ROMK⁺⁻ rats had 50% ROMK protein reduction compared with ROMK⁺⁺ rats, whereas no ROMK was shown in the ROMK⁻⁻ kidneys. E, ROMK⁺⁻ pups had tubular dilatation, hypertrophy, and mineralization (top); tubular degeneration, inflammation, and mineralization (middle); epithelial cell hypertrophy (bottom). Kidney histomorphology in ROMK⁺⁺ pups was unremarkable.
8% salt diet for 4 weeks in a separate study. Interestingly, under an 8% salt diet, the magnitude of BP difference between ROMK−/− and ROMK+/− was numerically greater than that in 4% salt diet condition. In addition, ROMK−/− rats presented with decreased renal injury biomarkers, such as kidney injury molecule-1 and renal papillary antigen-1 (Figure 3).

Discussion

ROMK deletion using ZFN in Dahl SS rats, confirmed at DNA, mRNA, and protein levels, was successfully generated. Dahl SS ROMK−/− pups recapitulated features identified in the ROMK null mice.15,16 These include severe volume depletion with hydropnephrosis and loss of organization and structure in the medullary area within the kidney, increased BUN, hyperkalemia, and metabolic acidosis. The extremely high BUN in ROMK−/− pups is consistent with decreased renal injury biomarkers, such as kidney injury molecule-1 and renal papillary antigen-1 (Figure 3).

A few ROMK−/− rats have survived to adulthood; we are currently attempting to generate an ROMK−/− colony in our laboratory. It will be intriguing to understand and probe the mechanisms that favor the survival of ROMK−/− rats.

ROMK null mice mimic human Bartter II syndrome; however, there is minimal information on the phenotype of heterozygous mice.15,16 In our studies, ROMK−/− and ROMK+/− rats (pups or adults, male or female) are indistinguishable in appearance, body and organ weights, blood chemistry, renal function, and kidney histomorphology. On a low-salt diet, BP was slightly but not statistically significantly lower (2 to 5 mm Hg) in ROMK−/− compared with ROMK+/− rats. When challenged with a 4% salt diet, the ROMK−/− rats exhibited a reduced BP compared with their ROMK+/− littermates for both males and females. More strikingly, when challenged with a higher salt load (8% salt diet), the ROMK−/− Dahl SS rats showed greater resistance to salt-induced BP elevation and signs of protection from renal injury, as demonstrated by decrease in renal injury biomarkers, compared with the ROMK−/− littermates. In humans identified from Framingham heart study,12 individuals with ROMK heterozygous mutation have reduced SBP (<6.3 mm Hg) and DBP (<3.4 mm Hg), with 60% reduction in risk of developing hypertension at 60 years of age. Our findings in ROMK−/− rats are the first, to our knowledge, to recapitulate the BP phenotype in humans with ROMK heterozygosity. Although the BP reduction in humans with ROMK heterozygous mutation is not inevitably associated with salt challenge, it is appreciated that Western diet has high sodium content. Thus, our data further demonstrated that 50% reduction in ROMK activity could result in BP reduction.

The mechanism by which either genetically deleting NKCC2 or blockade of NKCC2 with loop diuretics lowers BP remains to be elucidated.5,31–33 Similarly, the mechanism of BP reduction by ROMK disruption is not entirely understood. ROMK is expressed in the apical membranes of thick ascending limb of Henle and cortical collecting duct; it mediates potassium recycling and facilitates sodium reabsorption through NKCC2 in the thick ascending limb and potassium secretion in the cortical collecting duct.14 Because ROMK plays a critical role in the regulation of

Table. ROMK−/− Pups Exhibited Severe Volume Depletion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Genotype</th>
<th>Genotype</th>
<th>Genotype</th>
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<tbody>
<tr>
<td></td>
<td>ROMK+/−</td>
<td>ROMK+/+</td>
<td>ROMK−/−</td>
</tr>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>29.5±0.4</td>
<td>26.6±1.4</td>
<td>14.3±0.2†</td>
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<tr>
<td>Kidney weight, g</td>
<td>0.25±0.03</td>
<td>0.23±0.01</td>
<td>0.19±0.01</td>
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<tr>
<td>Kidney/body weight, mg/g</td>
<td>8.6±1.1</td>
<td>8.8±0.4</td>
<td>13.4±0.6†</td>
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<tr>
<td>Blood Na+, mmol/L</td>
<td>129.3±0.6</td>
<td>127.7±0.6</td>
<td>148.2±1.7†</td>
</tr>
<tr>
<td>Blood K+, mmol/L</td>
<td>4.8±0.1</td>
<td>4.7±0.2</td>
<td>6.4±0.4†</td>
</tr>
<tr>
<td>Blood Cl−, mmol/L</td>
<td>99.3±0.8</td>
<td>98.0±0.6</td>
<td>130.4±2.3†</td>
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<tr>
<td>Blood HCO3−, mmol/L</td>
<td>29.2±0.9</td>
<td>31.6±0.8</td>
<td>21.6±1.2†</td>
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<td>Blood pH</td>
<td>7.3±0.04</td>
<td>7.3±0.02</td>
<td>7.4±0.05</td>
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<tr>
<td>Hematocrit (proportion of 1)</td>
<td>0.18±0.009</td>
<td>0.17±0.014</td>
<td>0.34±0.032†</td>
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<tr>
<td>BUN, mmol/L</td>
<td>8.75±1.14</td>
<td>10.10±0.71</td>
<td>49.98±0.0†</td>
</tr>
</tbody>
</table>

Data are mean±SEM (n=5–6 in each group). Animal age: 13 days. No significant difference for all parameters between ROMK+/− and ROMK+/+. ROMK indicates renal outer medullary potassium channel.

P<0.05 vs ROMK+/−.
†P<0.05 vs ROMK+/+.
Figure 3. ROMK$^{−/−}$ rats had reduced blood pressure (BP) compared with ROMK$^{+/+}$ littermates while on a 4% or 8% salt diet and showed less renal injury in response to an 8% salt challenge. A, Study of 4% salt diet in female and male rats. B, Study of 8% salt diet in male rats. BP was recorded by radio telemetry system; data are daily mean±SEM. BP showed significant difference between ROMK$^{+/−}$ and ROMK$^{+/+}$ rats after 1 to 2 weeks of high-salt diet. $^*P<0.05$ vs ROMK$^{+/+}$. Animals were maintained on a low-salt (0.25% salt) diet before the study and switched to a high-salt (4% or 8% salt) diet at day 0. Age at the beginning of the experiment: 17-week old (females) and 11-week old (males). Kidney injury molecule-1, a biomarker for proximal tubules injury. Renal papillary antigen-1, a biomarker for renal papillary injury. DBP indicates diastolic blood pressure; HS, high salt; LS, low salt; ROMK, renal outer medullary potassium channel; and SBP, systolic blood pressure.
renal sodium reabsorption and body’s potassium homeostasis, its disruption would limit NKCC2 function, consequentially leading to decreased Na+ uptake in thick ascending limb and subsequent natriuresis and diuresis. It has been hypothesized that kidney has a central role in BP control, and abnormal pressure-natriuresis exists in all forms of chronic hypertension.34,35 Dahl SS rats is a well-established rodent model of SS hypertension. It has been demonstrated that high salt elevates NKCC2 and ROMK expression and increases/enhances chloride and water reabsorption in the loop of Henle.36,37 This intrinsic inability to excrete salt and water causes abnormal renal hemodynamics and pressure-natriuresis relationship and hypertension development.19 However, in the present study, ROMK+/− rats failed to show natriuresis/diuresis response. We postulate that pressure-natriuresis could have been reset when assessment was performed. Clearly, future studies aimed at assessing Na balance would help us to further understand the role of ROMK in sodium and water homeostasis by using the ROMK knockout rat model. Nevertheless, the present study sheds light on the role of ROMK in BP regulation; the Dahl ROMK+/− rats provide a valuable research tool for investigating the physiological role of ROMK.

Perspectives

We generated an ROMK knockout in Dahl SS rat model using ZFN and demonstrated that the ROMK−/− pups recapitulated features identified in the ROMK null mice. More importantly, the ROMK+/− rats, when challenged with a high-salt diet, exhibited reduced BP and protection from renal injury compared with their ROMK+/+ littermates. Our findings in ROMK knockout Dahl SS rats, together with the previous reports in humans and mice, underscore a critical role of ROMK in BP regulation and further support the promise of ROMK as a target in the treatment of hypertension.

Acknowledgments

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Disclosures

None.

References


What Is New?
- This is the first report characterizing renal outer medullary potassium channel (ROMK) knockout Dahl salt-sensitive rats.
- We generated the first ROMK knockout rat model, which is easier to study and more accurately measure blood pressure than using the mouse model.
- New zinc finger nuclease technology has been used to knockout ROMK in rats.

What Is Relevant?
- Gaining an understanding of the role of ROMK in blood pressure regulation via genetically modifying the ROMK gene.
- The ROMK knockout rat model provides a valuable tool for investigating ROMK function.

Summary
We generated an ROMK knockout rat model and demonstrated that ROMK plays a critical role in blood pressure regulation.
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Heterozygous Disruption of ROMK in Rats Is Associated with Reduced Blood Pressure

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Supplemental Materials

Expanded Materials and Methods

Animals

Dahl SS rats were purchased from Harlan Labs. Inc. (Indianapolis, IN, USA) and housed in a temperature and humidity-controlled room with a 12:12-hour dark-light cycle with food and water provided ad libitum. All animal procedures were approved in advance by the Institutional Animal Care and Use Committee at Merck Research Laboratories, Rahway, NJ, USA. All studies were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Generation of ROMK knockout rats

Zinc Finger Nuclease (ZFN) was utilized to knockout ROMK function. ZFN constructs targeting the rat ROMK gene were designed and purchased from Sigma Aldrich (St. Louis, MO, USA). The targeting site sequence is CTCAAGTGACCATAGGTTACGgattcaGGTTTGTGAC (lower case represents the ZFN cleavage site). ROMK knockout founders were generated by microinjection of ZFN mRNAs into single-cell rat embryos as described previously.1

Genotype and molecular characterization

Pups were genotyped at postnatal day 7~10 by PCR using DNA extracted from tail biopsies. Kidney tissues were harvested at the completion of the study. ROMK mRNA and protein expression in kidneys were assessed by Taqman and western blot and/or immunohistochemistry (IHC), respectively. Founders harboring the knockout alleles were identified by Cel-1 assay or regular PCRs amplifying the DNA fragment flanking the ZFN target site. The sequences of PCR primers to identify knockout founders are CCGAAGATGGTTTATCACTCAC (forward) and ATGTGGCTGCCAATCAGAAG (reverse).

Quantitative RT-PCR

RNA was isolated from kidney tissues of wild type, ROMK+/− and ROMK−/− rats with six animals per group. The tissues (~20 mg) were homogenized in 600 uL RLT lysis buffer (Qiagen, Hilden, Germany) containing 0.1% (vol/vol) β-mercaptoethanol using the Tissue Lyser (Qiagen, Hilden, Germany). Total RNA was extracted from the homogenized kidney tissues using Qiagen Robot following the manufacturer’s protocol. RNA quality was checked in Qiaxcel instrument (Qiagen, Hilden, Germany). RNA concentration was measured in Greiner half 96-well UV plate (Waltham, Massachusetts, US) by Bio-Tek plate reader (DTX880). 2μg total RNA from each sample was used in 50μl cDNA reaction by using high capacity archive cDNA kit (Applied Biosystems, Foster City, CA). cDNA reaction was setup in Biomek FX liquid handling system. Real-time PCR was performed on the 7900HT PCR System (Applied Biosystems, Foster City, CA) with 2x SYBR Fast PCR Master Mix (SABiosciences-Qiagen) and 2ul cDNA for each reaction. 10ul PCR reaction was loaded into each well of 384-well format custom designed PCR array (SABiosciences-Qiagen) using Biomek FX liquid handling system. The expression levels of mRNA for ROMK were normalized to the average of mouse Actb, Gapdh, Gusb, Hprt1, Ppia and Rp113a in each sample.

Western blot

Membrane preps for immunoblot analysis were made by the method described earlier.2 Frozen whole kidney fragments were crushed on dry ice and further homogenized using a glass dounce homogenizer in ice-cold isolation buffer (20 mM HEPES pH 7.3, 125 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 1% Triton X-100, 0.5% SDS) and 1:50 Protease Inhibitor Cocktail (104 mM AEBSF, 80 uM Aprotinin, 4 mM Bestatin, 1.4 mM E-64, 2 mM Leupeptin, 1.5 mM Pepstatin A, Sigma, St. Louis, MO, USA). The homogenate was subjected to a 1 hour
solubilization while rotating at 4°C. The samples were subsequently centrifuged at 15,000 rpm for 10 minutes to pellet the non-solubilized particulate fraction. The protein concentration of the supernatant was determined by bicinechinonic acid (BCA) kit (Pierce). Membrane proteins (60 μg) were denatured in Laemmli buffer for 40 minutes at ambient temperature, electrophoresed on 4 – 12 % Bis-Tris gels, and transferred to a PVDF membrane using iBlot blotting system (Life Technologies, Grand Island, NY, USA). The blots were probed with the ROMK specific antibody. Expression level was estimated by densitometry measurements using infrared dye-labeled secondary antibodies and Odyssey near infrared imager (Li_COR Biosciences, Lincoln, NE, USA) on the basis of Na⁺/K⁺ ATPase protein loading control.

**Immunohistochemistry**

Frozen kidney sections (5μm) were fixed in cold Methanol for 10 mins. Endogenous peroxidase activities were blocked with 0.3% H2O2 in Methanol for 20 mins. Sections were incubated with Protein Block Serum-Free solution (DAKO, Carpinteria, CA, USA) to block non-specific binding. Primary antibody ROMK (R80) diluted in PBS containing 0.5% Casein was incubated 1hr at RT and detected with HRP Polymer conjugated anti-Rabbit secondary antibody (Life Technology, Grand Island, NY, USA). DAB chromogen (Thermo Scientific, Hanover Park, IL, USA) was used as peroxidase substrate. Slides were counterstained with hematoxylene, dehydrated, and mounted under glass coverslips.

**Blood chemistry and kidney histological analysis in pups**

At postnatal day 13, pups (ROMK⁺/⁺, ROMK⁺/-, and ROMK⁻/-) were anesthetized with 2% isoflurane, blood was collected into lithium-heparinized tubes for immediate analysis of blood chemistry using iSTAT (Abaxis, Union City, CA, USA). Kidneys were harvested for ROMK mRNA, protein expression (western blot and IHC, see above for detailed methods), and histomorphology. For the latter, kidney tissues were fixed in 10% formalin and then paraffin embedded. Tissue sections were stained with hematoxylin and eosin and examined under a light microscope.

**BP, renal function, and renal injury biomarkers assessment in adult rats**

Rats at 8 weeks (males) or 14 weeks (females) of age were anesthetized with 2% isoflurane and pre-medicated with buprenorphine (0.03 mg/kg, s.c.: Reckitt Benckiser healthcare Ltd., Hull, England) prior to surgery. Telemetry devices (TA11PA-C40, Data Sciences International, DSI, St. Paul, MN, USA) were aseptically placed in a subcutaneous pocket on one side of the body with the catheter inserted into the descending aorta via the femoral artery. Penicillin G (150,000 U/kg, s.c. Bimeda Inc., Irwindale, CA, USA) was administered at the end of surgery. Rats were allowed to recover for 2 to 3 weeks prior to experimentation. Radiotelemetry signals were collected and analyzed using DSI Dataquest system version 4.1 (Data Sciences International, DSI, St. Paul, MN). Systolic and diastolic arterial BP and heart rate were determined on a beat by beat basis. During the experiment, rats were housed in metabolic cages. A twenty-four hour food and water intake and urine output were monitored once a week. Urinary electrolytes (including Na⁺, K⁺, Cl⁻, Ca²⁺, and Mg²⁺), creatinine, and protein concentration were assessed by a Roche Modular Chemistry System (Roche Diagnostics, Indianapolis, IN, USA). Urinary PGE₂ was measured using competition-based enzyme-linked immunoassay from Arbor Assays (Ann Arbor, MI, USA) following manufacturer's protocol with minor modifications to enhance sensitivity. Briefly, urine samples were thawed out, diluted in assay buffer, pipetted onto the assay microtiter plates together with controls and detection reagents. Then, following overnight incubation at 4°C, the plates were washed and TMB reagent added to react with bound PGE₂-peroxidase conjugate for 30 minutes at room temperature. The reaction was stopped by the
addition of 1 M HCl and plates were read at 450 nm. Urinary levels of kidney injury biomarkers including lipocalin-2 (LPN), osteopontin (OPN), kidney injury molecule-1 (KIM-1), and renal papillary antigen 1 (RPA-1) were detected using Kidney Injury Panel 1(rat) kit and Argutus AKI (rat) kit from Meso Scale Discovery, LLC. (Gaithersburg, MA, USA). LPN and OPN are biomarkers for proximal and distal tubules, whereas KIM-1 and RPA-1 are biomarkers for proximal tubule and renal papilla, respectively. These biomarkers have been validated in animal studies to reflect damage to known renal toxins. Blood chemistry and kidney histomorphology were assessed at the completion of the study using the same method as described in the above section. Male and female ROMK+/- rats and their ROMK+/*+ littermates were utilized for comparing their BP and renal function. All rats were maintained on a low salt diet (Harlan Teklad diet #7034 containing 0.25% salt). After recording BP and heart rate data for one week, rats received a high salt diet (either TD92034 containing a 4% salt or TD 92012 containing an 8% salt) for a period of 4 weeks during the study.

Reference (for Expanded Materials and Methods)