Renal outer medullary potassium channel (ROMK) is encoded by the \( KCNJ1 \) (potassium inwardly-rectifying channel, subfamily J, member 1) gene and expressed in the apical membranes of thick ascending limb of Henle and cortical collecting duct cells; ROMK 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. Evidence from the phenotype of humans and rodents with functional ROMK deficiency supports the contention that selective ROMK inhibitors (ROMKi) will represent a novel diuretic with potential of therapeutic benefit for hypertension. ROMKi have recently been synthesized by Merck & Co, Inc. The present studies were designed to examine the effects of ROMKi B on systemic hemodynamics, renal function and structure, and vascular function in Dahl salt-sensitive rats. Four experimental groups—control, high-salt diet alone; ROMKi B 3 mg·kg\(^{-1}\)·d\(^{-1}\); ROMKi B 10 mg·kg\(^{-1}\)·d\(^{-1}\); and hydrochlorothiazide 25 mg·kg\(^{-1}\)·d\(^{-1}\)—were included in prophylactic (from week 1 to week 9 on high-salt diet) and therapeutic studies (from week 5 to week 9 on high-salt diet), respectively. ROMKi B produced sustained blood pressure reduction and improved renal and vascular function and histological alterations induced by a high-salt diet. ROMKi B was superior to hydrochlorothiazide at reducing blood pressure. Furthermore, ROMKi B provided beneficial effects on both the plasma lipid profile and bone mineral density. Chronic ROMK inhibition not only prevented but also reversed the development of hypertension and end-organ damage in Dahl salt-sensitive rats. Our findings suggest a potential utility of ROMKi B as a novel antihypertensive agent, particularly for the treatment of the salt-sensitive hypertension patient population. (Hypertension. 2017;69:00-00. DOI: 10.1161/HYPERTENSIONAHA.116.08358.)

**Online Data Supplement**

**Key Words:** Dahl salt-sensitive rats ■ end-organ protection ■ hydrochlorothiazide ■ hypertension ■ ROMK inhibitor
studies (prophylactic and therapeutic dosing regimens) in Dahl salt-sensitive (Dahl SS) rats, a rodent model of salt-sensitive hypertension.13–15

Materials and Methods
Four experimental groups—control, high-salt diet alone; ROMKi B 3 mg·kg$^{-1}$·d$^{-1}$; ROMKi B 10 mg·kg$^{-1}$·d$^{-1}$; and HCTZ 25 mg·kg$^{-1}$·d$^{-1}$—were included in prophylactic (from week 1 to week 9 on high-salt diet) and therapeutic studies (from week 5 to week 9 on high-salt diet), respectively. The compounds were administrated in feed. Blood pressure was measured by radiotelemetry. Renal function was assessed in metabolic cage studies. Vascular function was assessed using a vascular relaxation assay.

A detailed Materials and Methods section is given in the online-only Data Supplement.

Statistical Analyses
All data are presented as mean±standard error of the mean. A repeated measure analysis of variance was used for time course data analysis. For end point comparisons among all groups, 1-way analysis of variance followed by Newman–Keuls post hoc test was used. P values of <0.05 were considered to be of statistical significance.

Results
Effects of ROMKi B and HCTZ on Blood Pressure and Heart Rate
In the prophylactic study, systolic and diastolic blood pressures were 148±0.9 and 100±1.5 mm Hg, respectively, while the Dahl SS rats were on a control (0.3% NaCl) diet; systolic and diastolic blood pressures increased to 161±0.5 and 110±1.0 mm Hg, respectively, after switching to a high-salt (4% NaCl) diet for 1 week. The systolic and diastolic blood pressures progressively increased to 217±5.7 and 156±4.3 mm Hg, respectively, over the subsequent 8-week period of high salt feeding in the vehicle group, which reflects the salt-sensitive characteristic of this rat strain. The systolic and diastolic blood pressures were significantly reduced in those animals that received ROMKi B at either 3 or 10 mg·kg$^{-1}$·d$^{-1}$ dose and HCTZ at 25 mg·kg$^{-1}$·d$^{-1}$ treatment; the magnitude of systolic blood pressure reduction was 62, 70, and 44 mm Hg and diastolic pressure reduction was 22, 35, and 14 mm Hg, respectively. Heart rate changes at the initiation of the treatment with either ROMKi B or HCTZ significantly increased heart rate (≈10% increase for the first 3 days). Heart rate in the control group was significantly increased after 5 weeks of high salt challenge, which may reflect cardiac dysfunction in the control group (Figure 1).

In the therapeutic study, systolic and diastolic blood pressures were increased to 198±2.7 and 140±4.6 mm Hg after 5 weeks of 4% NaCl diet in all rats. Four weeks of treatment with ROMKi B at 3 or 10 mg·kg$^{-1}$·d$^{-1}$ and HCTZ at 25 mg·kg$^{-1}$·d$^{-1}$ lowered systolic blood pressure by 33, 51, and 23 mm Hg and diastolic blood pressure by 22, 35, and 14 mm Hg, respectively. Heart rate changes at the initiation of the treatment were similar to those in the prophylactic study, namely, a transient increase in the higher dose of ROMKi B treatment group (Figure 1).

Effects of ROMKi B and HCTZ on Renal Excretory Function and Kidney Injury Biomarkers
Food intake or body weight was not different among all groups during the studies. Water intake and urine output were in balance. Neither ROMKi B nor HCTZ had a significant effect on urinary excretion of Na$^+$, K$^+$, Cl$^-$, Mg$^{2+}$, and PO$_4^{3-}$ (data not shown). However, ROMKi B at 10 mg·kg$^{-1}$·d$^{-1}$ caused a significant urinary loss of Ca$^{2+}$, while animals that received HCTZ demonstrated a retention of Ca$^{2+}$ in both the prophylactic and therapeutic studies (Figure S2 in the online-only Data Supplement).

Proteinuria is a biomarker of glomerular and tubular injury. Urinary protein excretion was markedly and progressively increased in Dahl SS rats on the 4% NaCl diet, which was significantly attenuated in animals that received either ROMKi B or HCTZ, with a greater beneficial effect in the high dose of ROMKi B group in both prophylactic and therapeutic studies (Figure 2). Lipocalin-2 is a biomarker of renal tubular epithelial injury, and kidney injury molecule-1 is a protein that is specially expressed in proximal tubules and is a sensitive biomarker for proximal tubular injury. Both ROMKi B and HCTZ significantly decreased urinary excretion of lipocalin-2 and kidney injury molecule-1 in both prophylactic and therapeutic studies (Figure S3). Other kidney injury biomarkers, such as osteopontin and renal papillary antigen 1, had similar changes as lipocalin-2 and kidney injury molecule-1 (data not shown).

Plasma Electrolytes, Creatinine, Cystatin C, Lipids, and Compound Concentrations
Plasma electrolytes (Na$^+$, K$^+$, Cl$^-$, Mg$^{2+}$, and Ca$^{2+}$) were not different among all groups at the end of each study (data not shown). Both ROMKi B and HCTZ significantly decreased plasma creatinine and serum cystatin C levels in the prophylactic but not the therapeutic study (Figure S4). Interestingly, prophylactic and therapeutic treatment with ROMKi B or HCTZ also decreased plasma cholesterol and low-density lipoprotein levels (Figure S5). Plasma concentrations of ROMKi B at 3 or 10 mg·kg$^{-1}$·d$^{-1}$ and HCTZ at 25 mg·kg$^{-1}$·d$^{-1}$ groups were ≈0.23, 0.66, and 1.39 μmol/L, respectively. Of note, the IC$_{50}$ of ROMKi B is ≈20 mmol/L; the plasma concentrations achieved in the present study provide a >90% ROMKi inhibition. Concentrations of ROMKi B and HCTZ were also monitored in feed at weeks 0, 4, and 8 and confirmed that both compounds were stable in the diet.

Bone Mineral Density
Because the high dose of ROMKi B induced increased urinary calcium loss, we evaluated whether this would affect bone mineral density (BMD). The bone dual-energy X-ray absorptiometry results demonstrated that prophylactic treatment with either ROMKi B or HCTZ significantly increased femur and lumbar spine BMD, and ROMKi B had no effect on femur and lumbar spine BMD in the therapeutic study (Figure S6).
Vascular Function

The vasorelaxant response of the thoracic aorta from all groups to acetylcholine or sodium nitroprusside (SNP) is summarized in Figure 3. Our data show that both ROMKi B and HCTZ shift the acetylcholine and SNP dose–response curve to the left, which indicates an increased acetylcholine-induced nitric oxide–mediated vasorelaxation and increased sensitivity to nitric oxide donation by SNP in aorta, demonstrating preserved endothelium-dependent and -independent responses.

Organ Weights and Histopathologic Findings

Total kidney weight/body weight and heart weight/body weight ratios are summarized in Table. Representative light microscopic findings in kidneys and hearts from the prophylactic study and renal and cardiac histopathologic scores from the therapeutic study are shown in Figures 4 and 5 and Figures S7 and S8. In brief, treatment with either ROMKi B or HCTZ significantly decreased kidney and heart weight, while there were no body weight changes. Microscopic findings in the kidney exhibited severe focal-segmental or global glomerulosclerosis, tubular dilation and protein cast formation, inflammatory cell infiltration, and perivascular fibrosis. Findings in the heart showed myocardial degeneration, interstitial inflammatory cell infiltration, and arteriolar hyalinization in the vehicle group. Both ROMKi B and HCTZ significantly improved the above-described histopathological changes and...
Discussion

Our results demonstrate that chronic ROMK inhibition completely blocked the development of hypertension in a rodent model of salt-sensitive hypertension, with no evidence of tolerance developing during the 8-week chronic study. The blood pressure lowering effect of HCTZ was blunted over time. Hence, ROMKi B is superior to that of HCTZ in blood pressure reduction. Chronic ROMK inhibition and HCTZ treatment also prevented end organs from high salt–induced damage as demonstrated by preserved glomerular (plasma creatinine and cystatin C levels were used as estimators of glomerular filtration rate) and renal tubular function, vascular function, renal and cardiac histopathology in the compound treatment groups. Moreover, chronic ROMK inhibition lowers blood pressure in established hypertension,ameliorates glomerular and renal tubular injury, slows down the progression of proteinuria, and improves vascular endothelial and smooth muscular function, as well as renal and cardiac histopathologic changes. Thus, chronic ROMK inhibition not only prevents but also reverses the development of hypertension and end-organ damage in Dahl SS rats.

We have previously performed a pilot study by orally dosing ROMKi B or HCTZ for 3 days to assess natriuresis/diuresis and blood pressure–dose response relationship in Dahl SS rats. We found that both compounds evoked an acute (0–4 hour) but not prolonged (0–24 hour) natriuretic/diuresis response in a dose-dependent manner; ROMKi B at 3 mg·kg$^{-1}$·d$^{-1}$ and HCTZ at 25 mg·kg$^{-1}$·d$^{-1}$ had an equivalent blood pressure–lowering efficacy (data not shown). Based on this information, we chose the above tested doses plus a high dose (10 mg·kg$^{-1}$·d$^{-1}$) of ROMKi B in our present studies. No 24-hour natriuretic/diuretic response to ROMKi was detected by weekly monitoring of renal excretory function in the present chronic studies, which is consistent with the findings from the previous pilot oral dosing study. Because compounds were administered via medicated diet, it is difficult to determine postdosing time period, and no 0–4 hour urine collection was taken in the present studies. Nevertheless, our data clearly demonstrated that ROMKi B significantly lowers blood pressure in the absence of 24-hour natriuresis/diuresis. The underlying mechanism responsible for blood pressure reduction by ROMK inhibition is not clear, but it could be associated with pressure–natriuresis response alteration. It has been shown that Na$^+/K^+\cdot2Cl^-$ cotransporter and ROMK expression is elevated, and chloride and water reabsorption in the loop of Henle is enhanced in Dahl SS rats. This intrinsic inability to excrete salt and water results in abnormal function.

Table. Total KW/BW and HW/BW Ratios in all Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total KW/BW, g/kg</th>
<th>HW/BW, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prophylactic study</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>10.3±0.2</td>
<td>4.8±0.1</td>
</tr>
<tr>
<td>ROMKi B 3 mg·kg$^{-1}$·d$^{-1}$</td>
<td>8.7±0.2$^*$</td>
<td>3.9±0.1$^*$</td>
</tr>
<tr>
<td>ROMKi B 10 mg·kg$^{-1}$·d$^{-1}$</td>
<td>8.6±0.1$^*$</td>
<td>3.7±0.1$^*$</td>
</tr>
<tr>
<td>HCTZ 25 mg·kg$^{-1}$·d$^{-1}$</td>
<td>9.1±0.2$^*$</td>
<td>4.0±0.1$^*$</td>
</tr>
<tr>
<td><strong>Therapeutic study</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>6.8±0.1</td>
<td>3.5±0.1</td>
</tr>
<tr>
<td>Baseline</td>
<td>9.6±0.1</td>
<td>4.2±0.1</td>
</tr>
<tr>
<td>Vehicle</td>
<td>10.3±0.3</td>
<td>4.7±0.1</td>
</tr>
<tr>
<td>ROMKi B 3 mg·kg$^{-1}$·d$^{-1}$</td>
<td>8.3±0.1$^*$</td>
<td>4.1±0.1$^*$</td>
</tr>
<tr>
<td>ROMKi B 10 mg·kg$^{-1}$·d$^{-1}$</td>
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<td>8.2±0.3$^*$</td>
<td>4.0±0.1$^*$</td>
</tr>
</tbody>
</table>

Data are mean±SEM. BW indicates body weight; HCTZ, hydrochlorothiazide; HW, heart weight; KW, kidney weight; and ROMKi, renal outer medullary potassium inhibitor.

$^*$P<0.05 vs vehicle group.
renal hemodynamics, abnormal pressure–natriuresis relationship, and development of hypertension. Blood pressure is increased to compensate for this intrinsic renal excretory inability to maintain sodium and water balance. With ROMK inhibition, less salt and water are reabsorbed from the loop of Henle, facilitating an increased amount of salt and water being excreted from the kidney. Blood pressure is consequently decreased, and the pressure–natriuresis curve is shifted to the left. That is to say, ROMK inhibition evoked sodium excretion, which may offset this intrinsic inability of sodium excretion from the kidney in Dahl SS rats, ultimately leading to blood pressure reduction.

ROMK plays a critical role in the regulation of renal sodium reabsorption and the body’s potassium homeostasis by facilitating sodium reabsorption through Na+/K+/2Cl− cotransporter in thick ascending limb of Henle and mediating potassium secretion in cortical collecting duct. Therefore, ROMK inhibition could be potassium sparing while causing natriuresis/diuresis, which is a favorable feature for a novel diuretic and would differentiate ROMKi from conventional loop diuretics such as furosemide that cause hypokalemia. Indeed, in our present studies, ROMKi B did not cause increased urinary K+ excretion and had no effect on plasma K+ concentration. However, it is of note that ROMK-mediated K recycling in thick ascending limb of Henle also generates positive potential in the lumen side, driving Ca2+ and Mg2+ reabsorption through a paracellular pathway. Thus, ROMK inhibition could lead to increased urinary Ca2+ and Mg2+ loss.

In our studies, the high dose of ROMKi B caused significant urinary loss of Ca2+; in contrast, HCTZ induces hypocalciuria that is suggested to result from enhancement of passive Ca2+ reabsorption in proximal tubules or stimulation of active

Figure 4. Representative light microscopic findings (10×) in renal histopathology from the prophylactic study. Vehicle group (A) exhibited severe focal-segmental or global glomerulosclerosis, tubular dilation and protein cast formation, inflammatory cell infiltration, and perivascular fibrosis. These lesions were significantly improved by either renal outer medullary potassium inhibitor (ROMKi) B (B and C) or hydrochlorothiazide (HCTZ; D) treatment.

Figure 5. Renal histopathologic scores from the therapeutic study. Data are mean±SEM (n=8 for each group, except for n=6 in the control group and 4% NaCl baseline group). Both renal outer medullary potassium inhibitor (ROMKi) B and hydrochlorothiazide (HCTZ) significantly decreased glomerular (A), tubular (B), interstitial (C), and vascular (D) scores in kidneys. Vehicle group had a remarkably greater score in each of the above components than the control group. *P<0.05 vs vehicle; †P<0.05 vs HCTZ.
Ca\textsuperscript{2+} reabsorption in distal convoluted tubules.\textsuperscript{22} Because of the concern for the potential effect of increased Ca\textsuperscript{2+} loss on bones, we measured femur and lumbar spine BMD by bone dual-energy X-ray absorptiometry, and interestingly, BMD was increased in the prophylactic study and was unaffected in the therapeutic study in ROMKi B 3 mg·kg\textsuperscript{-1}·d\textsuperscript{-1} and 10 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}-treated groups. We hypothesized that ROMK inhibition could improve regional hemodynamics and organ function by decreasing systemic blood pressure; thereby, Ca\textsuperscript{2+} reabsorption from the digestive system could be enhanced, and ultimately BMD could be unaffected or even improved. Obviously, this presumption needs to be tested in a separate study. It is also intriguing that decreased plasma cholesterol and low-density lipoprotein levels were observed with both prophylactic and therapeutic treatment with ROMKi B or HCTZ, which again might be associated with blood pressure reduction and subsequent improved organ function and lipid metabolism.

ROMKi B at 3 mg·kg\textsuperscript{-1}·d\textsuperscript{-1} and HCTZ at 25 mg·kg\textsuperscript{-1}·d\textsuperscript{-1} had similar beneficial effects on organ protection. Although the blood pressure–lowering effect of HCTZ was blunted over time, the blood pressure in this group remained significantly lower than that in the vehicle group. It is of note that ROMKi B at the high dose of 10 mg·kg\textsuperscript{-1}·d\textsuperscript{-1} had a greater effect on mitigating proteinuria in the prophylactic study and a superior effect in improving myocardial scores in the therapeutic study compared with that of HCTZ at 25 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}. The beneficial organ protective effects of ROMKi B or HCTZ are most likely attributed to an improvement of systemic hemodynamics. ROMKi B at the high dose of 10 mg·kg\textsuperscript{-1}·d\textsuperscript{-1} almost normalized blood pressure, and it provided the greatest benefits in renal and vascular function and renal and cardiac histopathology.

This is the first report to demonstrate that ROMKi lowers blood pressure and provides end-organ protection in a hypertensive rat model. Compared with the phenotype of genetically engineered ROMK-deficient mice,\textsuperscript{8,9} pharmacological inhibition of ROMK did not adversely affect the animal’s survival rate, electrolytes and acid–base balance, kidney function, and structure. In contrast, ROMKi delivered favorable outcomes.

In conclusion, chronic ROMK inhibition not only prevents but also reverses the development of hypertension and end-organ damage in Dahl SS rats. Our findings suggest a potential utility of ROMKi as novel antihypertensive agents, particularly in treating the salt-sensitive hypertension patient population.

**Perspectives**

We previously reported that heterozygous disruption of ROMK in Dahl SS rats exhibited reduced blood pressure and protection from renal injury, which underscores a critical role of ROMK in blood pressure regulation. We further demonstrated in the present study that pharmacological inhibition of ROMK using a small molecule not only prevented but also reversed development of hypertension and end-organ damage in Dahl SS rats. Our findings suggest a potential utility of ROMKi as novel antihypertensive agents, particularly in treating the salt-sensitive hypertension patient population.

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**Disclosures**

All authors are or were employees at Merck & Co, Inc, and may hold stock or stock options of Merck & Co.

**Reference**


What Is New?

- This is the first report to demonstrate that pharmacological inhibition of renal outer medullary potassium using a small molecule lowers blood pressure and provides end-organ protection in a hypertensive rat model.
- Renal outer medullary potassium inhibitor B was tested in prophylactic as well as therapeutic studies in chronic settings.

What Is Relevant?

- Our findings suggest a potential utility of renal outer medullary potassium inhibitor as novel antihypertensive agents, particularly in treating the salt-sensitive hypertension patient population.

Summary

We demonstrated that pharmacological inhibition of renal outer medullary potassium using a small molecule not only prevented but also reversed development of hypertension and end-organ damage in Dahl salt-sensitive rats.