Upregulation of Renal Sodium Transporters in D5 Dopamine Receptor–Deficient Mice

Xiaoyan Wang, Yingjin Luo, Crisanto S. Escano, Zhiwei Yang, Laureano Asico, Hewang Li, John E. Jones, Ines Armando, Quansheng Lu, David R. Sibley, Gilbert M. Eisner, Pedro A. Jose

Abstract—D5 dopamine receptor (D5R)-deficient (D5R−/−) mice have hypertension that is aggravated by an increase in sodium intake. The present experiments were designed to test the hypothesis that a dysregulation of renal sodium transporters is related to the salt sensitivity in D5R−/− mice. D5R was expressed in the renal proximal tubule, thick ascending limb, distal convoluted tubule, and cortical and outer medullary collecting ducts in D5R+/+ mice. On a control Na+ diet, renal protein expressions of NKCC2 (sodium-potassium-2 chloride cotransporter), sodium chloride cotransporter, and α and γ subunits of the epithelial sodium channel were greater in D5R−/− than in D5R+/+ mice. Renal renin abundance and urine aldosterone levels were similar but renal angiotensin II type 1 receptor (AT1R) protein expression was increased in D5R−/− mice. An elevated Na+ diet increased further the elevated blood pressure of D5R−/− mice but did not affect the normal blood pressure of D5R+/+ mice. The increased levels of NKCC2, sodium chloride cotransporter, and α and γ subunits of the epithelial sodium channel persisted with the elevated Na+ diet and unaffected by chronic AT1R blockade (losartan) in D5R−/− mice. The expressions of proximal sodium transporters NHE3 (sodium hydrogen exchanger type 3) and NaPi2 (sodium phosphate cotransporter type 2) were increased by the elevated Na+ diet in D5R−/− mice; the increased expression of NHE3 but not NaPi2 was abolished by AT1R blockade. Our findings suggest that the increased protein expression of sodium transporters/channels in distal nephron segments may be the direct consequence of the disruption of D5R, independent of the renin–angiotensin aldosterone system. (Hypertension. 2010;55:1431-1437.)

Key Words: dopamine ■ dopamine receptor ■ knockout mouse ■ hypertension ■ sodium excretion ■ sodium transporters ■ AT1R blockade

Dopamine receptors include the D1-like (D1R and D2R) and D2-like (D2R, D3R, and D4R) receptor subfamilies. All dopamine receptor subtypes are expressed in the kidney.1,2 Lack or dysfunction of one dopamine receptor gene may affect renal tubular sodium transport, which may result in sodium retention and contribute to the development of hypertension. However, the differential nephron segment expression of dopamine receptor subtypes suggests that they may regulate sodium transport and blood pressure via different mechanisms.3

The locus of the human D5R gene, 4p15.1-16.1, and its pseudogenes, 1q21.1 and 2p11.1-p11.2,4 have been linked to human essential hypertension.5–8 Several single nucleotide polymorphisms of the human D5R gene are associated with diminished function.9 Disruption of the D5R gene in mice increases blood pressure that is aggravated by an increase in sodium intake.10,11 It is possible that the salt sensitivity in D5R−/− mice is caused by increased sodium transport in the kidney, but the nephron segment(s) involved has not been established.

Active sodium transport along the nephron occurs mainly through transport proteins at the apical membrane: sodium/hydrogen exchanger isoform 3 (NHE3) and the type 2 sodium phosphate cotransporter (NaPi2) in the proximal tubule, the bumetanide-sensitive sodium-potassium-2 chloride cotransporter (BSC1) (or NKCC2) and NHE3 in the thick ascending limb of loop of Henle, the thiazide-sensitive sodium-chloride cotransporter (TSC) (or sodium chloride cotransporter [NCC]) in the distal convoluted tubule and the amiloride-sensitive epithelial sodium channel (ENaC) (subunits α, β, and γ) in the connecting tubule and collecting duct, as well as Na+/K+ ATPase at the basolateral membrane in almost all nephron segments. However, it is not known whether the D5R can regulate the expression of these proteins, in part, because the nephron segments in which the D5R is expressed in mice have not been determined.10–12 It is also not clear whether the
knockout of the D5R gene alters the renal expression of the different dopamine receptor subtypes. Therefore, the present experiments were designed to determine a novel renal mechanism of salt-sensitive hypertension by testing the hypothesis that a dysregulation of renal sodium transporters is related to the salt sensitivity of the blood pressure of D5R−/− mice. We measured blood pressure, urinary sodium and aldosterone excretions, and renal protein expressions of dopamine receptors, the type 1 angiotensin II receptor (AT1R), renin, and sodium transporters/channels in D5R−/− and D5R+/+ mice. We determined whether the increased expression and activity of AT1R is responsible for any alteration in renal sodium transporters in D5R−/− mice, we also measured renal sodium transporters/channels in mice chronically given the AT1R blocker losartan.

Methods

Generation of D5R−/− Mice

Heterozygous mice (D5R+/−) were generated as reported.10,11 The D5R−/− and D5R+/+ (6th generation in C57BL/6/Taconic) mice were identified by DNA genotyping. C57BL/6 Taconic mice were used instead of C57BL/6 Jackson mice because C57BL/6/Taconic mice, unlike C57BL/6 Jackson mice, do not develop elevated blood pressure with an increase in sodium intake.11,14 The studies were conducted in accordance with National Institutes of Health guidelines for the ethical treatment and handling of animals in research and approved by the Institutional Animal Care and Use Committee, initially at Georgetown University, and subsequently at the Children’s National Medical Center.

Sodium Balance Study in Ration-Fed Mice

D5R−/− and D5R+/+ mice (4 to 6 months old, mixed sex, n=5) were housed in metabolic cages. The mice, for each 25 g of body weight, were ration-fed for 10 days with gelled control sodium diet (0.2 to 0.3% Na+) consisting of ground rodent chow (5 g) and melted agar (0.04 g) in 10 mL of distilled water.15–17 Ration feeding of gelled food eliminates the possibility of food crumbs contaminating the urine collections. Blood pressures were measured in the aortae, via the femoral arteries of mice under anesthesia (pentobarbital, 50 mg/kg intravenously).10,11,14 Urine and blood samples were analyzed for electrolytes (E4A Electrolyte system, Beckman, Fullerton, Calif), creatinine (Creatinine Analyzer 2, Beckman), and aldosterone (radioimmunoassay, Diagnostic Products Corp, Los Angeles, Calif).

Semiquantitative Immunoblotting

The kidneys, removed after euthanasia, were homogenized.15–17 Renal plasma membrane-enriched fractions were obtained by centrifugation of kidney homogenates at 17 000 g, 30 minutes at 4°C18; the 17 000 g fraction resulted in the enrichment of Na+K+ ATPase α subunit by more than 60%, relative to whole kidney homogenates (data not shown). Equal amounts of protein were separated by 7.5% or 10% SDS-PAGE and transferred onto nitrocellulose membranes (Bio-Rad, Hercules, Calif). The membranes were probed with the indicated primary antibodies, and then exposed to horseradish peroxidase–conjugated secondary antibodies (Pierce, Rockford, Ill). The primary antibodies were rabbit polyclonal antibodies against NHE3, NaPi2, NKCC2, NCC, ENaCs (gifts from Dr Mark A. Knepper, Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health), D5R, actin (Sigma), and mouse monoclonal antibody to Na+K+ ATPase α subunit (Upstate Biotechnology, Lake Placid, NY). The specificities of these antibodies have been reported.16–26 The specificity of the polyclonal D5R antibody was initially determined by the ability of the antibody to detect human D5R protein heterologously expressed in HEK293 cells (positive control) without and with peptide-preadsorption (negative control).12 In this report, we show that the D5R antibody recognizes D5R in kidneys from mice with D5R gene (D5R+/−) but not in mice without the D5R gene (D5R−/−) (Figure 1). The chemiluminescent signals were quantified by the NIH ImageJ program and normalized against the corresponding actin bands.

Renal Immunocytochemistry

In separate studies, kidneys from anesthetized D5R+/− and D5R−/− mice were perfused with PBS (pH 7.4) and fixed with Histochoice (Amresco, Solon, Okla). Three-micron sections were cut from paraffin-embedded tissues for hematoxylin/eosin staining and indirect immunoperoxidase labeling.12 Immunoreactivity was visualized with the Vip kit (Vector, Burlingame, Calif) and bright-field microscopy, under the same conditions and magnifications (Eclipse E600, Nikon, Tokyo, Japan). The experiments were performed at least 3 times.

Chronic Salt Loading

In additional studies, D5R+/− and D5R−/− mice were fed an elevated sodium (2% to 3% Na+) diet for 3 weeks. After euthanasia, the kidneys were harvested for immunoblotting, as above.
**Telemetry**

D5\(^{+/+}\) and D5\(^{-/-}\) mice were fed a control sodium diet (0.2 to 0.3% Na\(^+\)) for 3 to 4 weeks, then switched to an elevated sodium (2% to 3% Na\(^+\)) diet for 2 weeks. The mice had free access to food and water. Blood pressures were monitored by telemetry, via preimplanted transmitters in conscious mice.\(^{11,15}\) Mice were acclimated to the apparatus and the environment for a total of 7 days before measurements were taken.

**Losartan Treatment**

D5\(^{+/+}\) and D5\(^{-/-}\) mice were fed with an elevated sodium (2% to 3% Na\(^+\)) diet for 3 weeks. In the last 5 to 7 days of the study, the AT1R blocker losartan was injected intraperitoneally (20 mg [100 μL/L] per day, for 5 to 7 days).\(^{26}\) After euthanasia, the kidneys were harvested for immunoblotting, as above.

**Statistical Analysis**

Data are expressed as means±SE and as percentages of D5\(^{+/+}\) mice for immunoblotting. Unpaired Student’s t test was used for a 2-group comparison and ANOVA, Holm–Sidak test for multigroup comparison. \(P<0.05\) was considered significant.

**Results**

**Distribution of D5R Along the Nephron**

Whole kidney homogenates immunoblotted with the polyclonal antibody to D5R showed a 55-kDa band in D5\(^{+/+}\) but not in D5\(^{-/-}\) mice. Strong immunostaining was observed in kidneys from D5\(^{+/+}\) mice but not in D5\(^{-/-}\) mice (Figure 1A). No immunostaining was observed when the antibody was blocked by preadsorption with the immunizing peptide (not shown). These studies confirmed the specificity of the D5R antibody and the disruption of the D5R gene in D5\(^{-/-}\) mice.

D5R was expressed in the proximal convoluted and straight tubules (including brush border membranes), thick ascending limb of Henle, distal convoluted tubule, and cortical and outer medullary collecting ducts. Minimal staining was observed in the juxtaglomerular cell, glomerulus, macula densa, afferent arteriole, and thin descending limb of Henle (Figure 1B).

**Effect of the Disruption of D5R Gene (D5\(^{-/-}\)) on Some Physiological Parameters in Mice Fed a Control Sodium Diet**

All physiological data are summarized in Table 1. We have reported that the blood pressures are higher in D5\(^{-/-}\) mice than D5\(^{+/+}\) littersmates when measured by telemetry in the conscious state or when measured from the aorta via the femoral artery in the anesthetized state.\(^{10,11}\) The systolic and diastolic blood pressures were measured under anesthesia in this group of mice in the present studies. The higher blood pressure in D5\(^{-/-}\) compared to D5\(^{+/+}\) mice was confirmed (Table 1). There were no differences in heart rate, body weight, and food/water intake between 2 groups. Serum Na\(^+\), K\(^+\), Cl\(^-\) and creatinine concentrations and creatinine clearance were also similar in the two mouse strains. No differences were found in daily urinary sodium and water excretion between the two mouse strains (only the data on the last day of the balance study are shown in Table 1). The higher blood pressure in D5\(^{-/-}\) mice than in D5\(^{+/+}\) littersmates was not associated with a greater urinary sodium, suggesting that a state of sodium retention may have already been achieved by the time the variables were measured. Mice were found in the expression of the NCC, a sodium transporter in the distal convoluted tubule, AT1R, and renin in D5 knockout mice. The renal protein expression of all D2-like dopamine receptors, AT1R, and renin in mice are shown in Figure 2A through 2C. NHE3 expression did not differ between D5\(^{-/-}\) and D5\(^{+/+}\) mice, whereas NaPi2 protein was decreased in D5\(^{-/-}\) relative to D5\(^{+/+}\) mice. Expressions of NKCC2, a sodium transporter in the thick ascending limb of Henle, and NCC, a sodium transporter in the distal convoluted tubule, were increased in D5\(^{-/-}\) relative to D5\(^{+/+}\) mice (Figure 2), although the nephron segment distribution of NCC (Figure 3) was not altered in D5\(^{-/-}\) mice. The α and γ subunits of ENaC were also increased in D5\(^{-/-}\) mice, but no differences were found in the expression of the β subunit of ENaC and the α subunit of Na\(^+\) K\(^+\) ATPase (Figure 2B and 2C) between the 2 groups.

**Effect of the Disruption of D5R Gene (D5\(^{-/-}\)) on Renal Dopamine Receptors, AT1R, and Renin in Mice Fed a Control Sodium Diet**

The renal protein expression of all D2-like dopamine receptors was similar in D5\(^{-/-}\) and D5\(^{+/+}\) mice, but the renal protein expression of D1R was lower in D5\(^{-/-}\) than in D5\(^{+/+}\) mice (Figure 4A), indicating that there was no compensatory upregulation of the other dopamine receptor subtypes in the kidney as a consequence of the deletion of the D5R gene.

Renal AT1R protein was increased in D5\(^{-/-}\) mice, in agreement with our previous report,\(^{13,26}\) but renal renin protein was similar in D5\(^{-/-}\) and D5\(^{+/+}\) mice (Figure 4B). The specificity of the antibodies against AT1R (AT1R, N-10, Santa Cruz Biotechnology, Santa Cruz, Calif) and renin were measured under pentobarbital anesthesia.

Table 1. Physiological Data of D5\(^{-/-}\) and D5\(^{+/+}\) Mice Ration-Fed a Control Sodium Diet

<table>
<thead>
<tr>
<th>Mouse Group</th>
<th>D5(^{+/+})</th>
<th>D5(^{-/-})</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>93±2</td>
<td>122±5(^*)</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>78±3</td>
<td>101±2(^*)</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>347±8</td>
<td>328±14</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>26±0.9</td>
<td>25±0.2</td>
</tr>
<tr>
<td>Serum Na(^+), mmol/L</td>
<td>152±1</td>
<td>148±2</td>
</tr>
<tr>
<td>Serum K(^+), mmol/L</td>
<td>4.3±0.4</td>
<td>4.5±0.8</td>
</tr>
<tr>
<td>Serum Cl(^-), mmol/L</td>
<td>124±0.4</td>
<td>126±0.9</td>
</tr>
<tr>
<td>Serum creatinine, μmol/L</td>
<td>26.4±3.5</td>
<td>31.7±6.2</td>
</tr>
<tr>
<td>Gelled-food intake, g/day</td>
<td>14.7±0.2</td>
<td>13.2±0.7</td>
</tr>
<tr>
<td>Water intake, mL/day</td>
<td>0.39±0.01</td>
<td>0.35±0.02</td>
</tr>
<tr>
<td>Urine output, mL/day</td>
<td>2.9±0.3</td>
<td>3.1±0.9</td>
</tr>
<tr>
<td>Na(^+) excretion, mmol/day</td>
<td>0.31±0.03</td>
<td>0.29±0.07</td>
</tr>
<tr>
<td>Creatinine clearance, mL/day</td>
<td>159±33</td>
<td>120±32</td>
</tr>
</tbody>
</table>

Data are means±SE. \(^*P<0.05\) vs D5\(^{+/+}\) mice (4–6 months old, mixed sex, n=5/group), Student’s t test. DBP indicates diastolic blood pressure; MAP, mean arterial blood pressure; SBP, systolic blood pressure. Blood pressures were measured under pentobarbital anesthesia.

retain sodium for several hours and then achieve sodium balance by the first or second day after a high salt load.\(^{28,29}\)

The renal protein expression of all D2-like dopamine receptors, AT1R, and renin in mice are shown in Figure 2A through 2C. NHE3 expression did not differ between D5\(^{-/-}\) and D5\(^{+/+}\) mice, whereas NaPi2 protein was decreased in D5\(^{-/-}\) relative to D5\(^{+/+}\) mice. Expressions of NKCC2, a sodium transporter in the thick ascending limb of Henle, and NCC, a sodium transporter in the distal convoluted tubule, were increased in D5\(^{-/-}\) relative to D5\(^{+/+}\) mice (Figure 2), although the nephron segment distribution of NCC (Figure 3) was not altered in D5\(^{-/-}\) mice. The α and γ subunits of ENaC were also increased in D5\(^{-/-}\) mice, but no differences were found in the expression of the β subunit of ENaC and the α subunit of Na\(^+\) K\(^+\) ATPase (Figure 2B and 2C) between the 2 groups.

The renal protein expression of all D2-like dopamine receptors was similar in D5\(^{-/-}\) and D5\(^{+/+}\) mice, but the renal protein expression of D1R was lower in D5\(^{-/-}\) than in D5\(^{+/+}\) mice (Figure 4A), indicating that there was no compensatory upregulation of the other dopamine receptor subtypes in the kidney as a consequence of the deletion of the D5R gene. Renal AT1R protein was increased in D5\(^{-/-}\) mice, in agreement with our previous report,\(^{13,26}\) but renal renin protein was similar in D5\(^{-/-}\) and D5\(^{+/+}\) mice (Figure 4B). The specificity of the antibodies against AT1R (AT1R, N-10, Santa Cruz Biotechnology, Santa Cruz, Calif) and renin were measured under pentobarbital anesthesia.
The increased protein levels of renal distal sodium transporters, channels, and pump in D5+/− mice were also increased in D5−/− mice, relative to D5+/+ mice. The β subunit of ENaC was similar in the 2 mouse strains (Table 2).

**Effects of Losartan on Renal Sodium Transporters in D5−/− and D5+/+ Mice**

To determine whether the increased AT1R expression in the kidney contributed to the alterations in sodium transporters, channels, and pump in D5−/− mice, we studied mice treated with the AT1R blocker losartan, and fed the elevated sodium diet (Figure 6A through 6C). We have reported that renal AT1R is increased13,26 and losartan treatment normalized the high blood pressure of D5−/− mice.26

The increased protein expression of the distal sodium transporters (NKCC2, NCC, α and γ subunits of ENaC) (Figure 6B and 6C) in D5−/− mice on the control and elevated sodium diets, respectively, persisted in the losartan-treated D5−/− mice. There were 2 bands for γENaC, a higher band (90 kDa) and a lower band (75 kDa), which is believed to be the active form of γENaC, following the proteolysis or deglycosylation of the larger, inactive form (90 kDa).21 The higher band was absent and the density of the lower band was increased in D5−/− mice, relative to their D5+/+ littermates. We do not know the reason for the absence of the higher band in the D5−/− mice, but it is possible that all the “inactive” forms are converted to the “active” forms in these mice. Losartan abrogated the increased expression of NHE3 but not the increased expression of NaPi2 and Na+K+ ATPase α subunit observed in D5−/− mice (Figure 6 and Table 2); losartan also decreased the expression of the β subunit of ENaC.

### Discussion

Under normal circumstances, an increase in blood pressure activates renal mechanisms to increase sodium excretion to maintain a normal blood pressure (pressure–natriuresis phenomenon).32 In the present report, the sodium intake and urinary sodium excretion on the control sodium diet are similar in D5−/− mice and D5+/+ littermates, indicating the achievement of sodium balance. However, this was achieved at the expense of higher blood pressure in the D5−/− mice.
that reported in normotensive rats; these transporters are decreased by the elevated sodium diet in wild-type C57BL/6 mice, the genetic background of D₅⁻/- mice. NCC expression, which is decreased by an elevated sodium diet in the rat in one study, but not in another, is also decreased by the elevated sodium diet in SJL mice but not in C57BL/6 wild-type mice (X. Wang, I. Armando, C. Escano, L. Asico, P. Jose, unpublished data, 2009). In contrast, the elevated sodium diet in C57BL/6 mice with deleted D₅R increases NCC expression (Table 2). The disruption of the D₅R gene in mice results in the upregulation of renal sodium transporters/channels in all of the distal nephron segments. The increased expression of these distal tubule sodium transporters/channels in D₅⁻/- mice may have attenuated the magnitude of the natriuresis that would have normally accompanied the increase in systemic blood pressure.

Using a specific antibody against D₅R, we find D₅R expression in the thick ascending limb, distal convoluted tubule, as well as the cortical and outer medullary collecting ducts, the nephron segments that express the sodium transporter/channels that are increased in D₅⁻/- mice fed either control or elevated sodium diet. These data suggest that the D₅R may have a direct inhibitory effect on the expression of renal sodium transporters and channels in the distal nephron segments.

Table 2. Results of the Immunoblotting of Na⁺ Transporters/Channels in Mice Fed an Elevated Sodium Diet

<table>
<thead>
<tr>
<th>Mouse Group</th>
<th>D₅⁺/⁺ (n=3)</th>
<th>D₅⁻/- (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHE3</td>
<td>100±6</td>
<td>287±62*</td>
</tr>
<tr>
<td>NaPi2</td>
<td>100±9</td>
<td>177±19*</td>
</tr>
<tr>
<td>NKKCC2</td>
<td>100±3</td>
<td>174±15*</td>
</tr>
<tr>
<td>NCC</td>
<td>100±39</td>
<td>705±141*</td>
</tr>
<tr>
<td>αENaC</td>
<td>100±7</td>
<td>227±13*</td>
</tr>
<tr>
<td>βENaC</td>
<td>100±27</td>
<td>94±5</td>
</tr>
<tr>
<td>γENaC</td>
<td>100±5</td>
<td>454±110*</td>
</tr>
<tr>
<td>αNKA</td>
<td>100±7</td>
<td>322±27*</td>
</tr>
</tbody>
</table>

Data are means±SE, % of D₅⁺/⁺ mice, corrected by actin. *P<0.05 vs D₅⁻/-, Student’s t test. αNKA indicates Na⁺ K⁺ ATPase, α subunit.

indicating a blunting of the pressure-natriuresis relationship with the disruption of the D₅R gene.

The natriuresis associated with increased sodium intake and blood pressure, thought to be an example of aldosterone escape, has been reported to cause by a decrease in the protein abundance/activity of NCC in the renal distal convoluted tubule. It is possible that the impaired pressure-natriuresis mechanism in D₅⁻/- mice may be attributable to increased renal expression of NCC. In the present study, D₅⁻/- mice not only have increased protein expression of NCC, but also one of the sodium transporters in the thick ascending limb (NKKCC2) and the α and γ subunits of ENaC in the collecting duct. The increase in renal sodium transporters with the elevated sodium diet in D₅⁻/- mice is in agreement with the effect of high salt intake in normotensive rats. There may be species and strain differences because an elevated sodium diet for one week does not affect NHE3 or NKKCC2 expression in SJL mice (X. Wang, I. Armando, C. Escano, L. Asico, P. Jose, unpublished data, 2009), similar to...
segments. An effect of Dsr on distal sodium transporter and channel expression could also be indirect, as a result of increased AT1R expression. Dsr promotes the degradation of the AT1R,36 and renal AT1R expression is increased in Dsr/−/− mice,13,26 confirmed in the present report. Both NCC and αENaC are regulated by AT1R.34,35 The protein abundance of NCC and αENaC is decreased in AT1A-null mice34 and in rats treated with AT1R antagonists but increased in rats treated with angiotensin II.35

Renal renin abundance and urinary aldosterone levels are not increased in Dsr/−/− mice. That the Dsr may not regulate renin and indirectly, aldosterone secretion, is in keeping with the absence of Dsr immunostaining in the juxtaglomerular apparatus, including the juxtaglomerular cell and macula densa. These data suggest that the Dsr, unlike the Dsr,1,3,36 may not regulate the secretion/release of renin. High salt intake suppresses renin secretion in kidney. Renin secretion is probably suppressed by salt intake to a similar extent in both Dsr+/++ and Dsr/−/− mice because there is no difference in renal renin level between the mouse strains placed on high salt diet.26 It is, therefore, unlikely that alterations in angiotensin II levels are responsible for the differential expression of distal sodium transporters in Dsr/−/− and Dsr+/++ mice. Nevertheless, we determined whether the increased expression and activity of the AT1R is responsible for the increased expression of distal tubule sodium transporters/channel subunits in Dsr/−/− mice by blocking AT1R function with losartan. In spite of AT1R blockade, the increase in renal distal sodium transporters/channel subunits observed in Dsr/−/− mice fed the elevated sodium diet persisted, suggesting that the increased expression of distal renal sodium transporters/channel subunits in Dsr/−/− mice is independent of the AT1R. The increase in NHE3, but not NaPi2, is abolished by AT1R blockade, suggesting an additional effect of AT1R on NHE3 expression in Dsr/−/− mice. The absence of Dsr seems to enhance the renal tubular effects of AT1R when sodium intake is increased because losartan treatment abolishes not only the increase in NHE3 expression, but also the salt sensitivity in Dsr/−/− mice.26 Endothelin is probably not involved in the hypertension of Dsr/−/− mice because the endothelin A and B receptor antagonists BQ61037 and BQ788,38 respectively, do not affect the arterial blood pressure of Dsr/−/− or Dsr+/++ mice.10 The role of increased sympathetic tone10 and reactive oxygen species production11 in the increased expression of renal distal tubule transporters in Dsr/−/− mice was not evaluated.8

In summary, relative to Dsr+/++ littermates, Dsr/−/− mice have higher blood pressure and greater renal protein expressions of NCC and α and γ subunits of ENaC on control and elevated sodium diet which is not abrogated by AT1R blockade. The expression of the proximal sodium transporters NHE3 and NaPi2 is increased only on elevated sodium diet, and the increase in NHE3 but not NaPi2 is abolished by AT1R blockade in Dsr/−/− mice. We suggest that the increased renal protein expression of these transporters/channels may play an important role in the pathogenesis of salt-sensitive hypertension that occurs with the disruption of the Dsr gene in mice.

Perspectives
Several single nucleotide polymorphisms of the human Dsr gene are associated with diminished function of the receptor.9 The increased renal distal sodium transporters and channels (NCC, NCC, and ENaC) suggest that inhibitors of distal sodium transport may have a greater antihypertensive effect in humans with impaired function of the Dsr.

Acknowledgments
We thank Dr Mark A. Knepper (Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health) for providing the antibodies against the sodium transporters.

Sources of Funding
This work was supported by NIH grants HL068686, HL023081, HL074940, HL092196, and DK039308.

Disclosures
None.

References


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_Hypertension_. 2010;55:1431-1437; originally published online April 19, 2010; doi: 10.1161/HYPERTENSIONAHA.109.148643

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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