Oxidative Stress Causes Renal Dopamine D1 Receptor Dysfunction and Salt-Sensitive Hypertension in Sprague-Dawley Rats

Anees A. Banday, Yuen-Sum Lau, Mustafa F. Lokhandwala

Abstract—Renal dopamine plays an important role in maintaining sodium homeostasis and blood pressure (BP) during increased sodium intake. The present study was carried out to determine whether renal dopamine D1 receptor (D1R) dysfunction contributes to increase in salt sensitivity during oxidative stress. Male Sprague-Dawley rats, divided into various groups, received tap water (vehicle); 1% NaCl (high salt [HS]); L-buthionine sulfoximine (BSO), an oxidant; and HS plus BSO with or without Tempol, an antioxidant, for 12 days. Compared with vehicle, HS intake increased urinary dopamine production and decreased basal renal Na/K-ATPase activity but did not affect BP. BSO-treated rats exhibited oxidative stress and a mild increase in BP. In these rats, D1R expression and G protein coupling were reduced, and SKF38393, a D1R agonist, failed to inhibit Na/K-ATPase activity and promote sodium excretion. Concomitant administration of BSO and HS caused oxidative stress, D1R dysfunction, and a marked increase in BP. Although renal dopamine production was increased, it failed to reduce the basal Na/K-ATPase activity in these animals. Treatment of BSO plus HS rats with Tempol decreased oxidative stress and restored endogenous, as well as exogenous, D1R agonist-mediated Na/K-ATPase inhibition and normalized BP. In conclusion, during HS intake, the increased dopamine production via Na/K-ATPase inhibition prevents an increase in BP. During oxidative stress, D1R function is defective, and there is mild hypertension. However, in the presence of oxidative stress, HS intake causes marked elevation in BP, which results from a defective renal D1R function leading to the failure of dopamine to inhibit Na/K-ATPase and promote sodium excretion. (Hypertension. 2008;51:367-375.)

Key Words: dopamine — G proteins — Na/K-ATPase — L-buthionine sulfoximine — glutathione

The mechanisms by which increased dietary sodium intake raises arterial blood pressure (BP) are not fully understood, but they seem to be related to the inability of the kidneys to excrete the excess amounts of sodium.1,2 The primary role of the kidneys in the regulation of BP was originally proposed by Guyton3 based on some elegant experiments involving the pressure-natriuresis response. The kidney has several regulatory systems involved in the control of sodium homeostasis and BP. In particular, renal dopamine plays a major natriuretic role in the complex physiological network that has evolved to maintain sodium balance and BP.2,4–6

Dopamine synthesized within the renal proximal tubules plays an important role in the regulation of renal sodium excretion.4,5 There are 5 genetically distinct dopamine receptors (D1, D2, D3, D4, and D5), which are expressed in renal proximal tubules.4,5,7–10 Activation of dopamine D1 receptors (D1Rs) results in inhibition of the Na/K-ATPase and Na/H exchanger activities in the proximal tubule, leading to an increase in sodium excretion.11 The ability of endogenous dopamine to maintain sodium homeostasis depends on the state of sodium balance.4,5,11 A regulatory action is observed under conditions of increased sodium intake. Under these conditions, the dopaminergic inhibition of sodium transport is caused by an increase in renal dopamine production.4,5,11

An altered renal D1R function is present in human and rodent genetic hypertension.12,13 There is a decreased natriuretic effect of dopamine and impaired D1R function in Dahl salt-sensitive hypertensive rats.12,14 Interestingly, evidence to date indicates that, in both human and animals models of hypertension, inheritable or salt induced, there is increased oxidative stress that coexists with defective D1R function.4,5,12,13,15–18 In obese Zucker rats, antioxidant supplementations decreases oxidative stress, restores D1R signaling, and lowers BP.15 Collectively, these observations suggest that oxidative stress–mediated renal D1R dysfunction could be responsible for salt-sensitive hypertension. To test this hypothesis, different groups of animals were given tap water supplemented with l-buthionine sulfoximine (BSO), sodium chloride, and BSO plus sodium chloride along with Tempol for 12 days. Oxidative markers, kidney function, D1R function, and BP were studied at the end of treatment.
Methods

Materials
[^3H]SCH23390 hydrochloride (R[+]-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hydrochloride) and [15S^32P]GTP\gamma S (guanosine 5’-γ-thio)triphosphate ([γS]GTP) were purchased from NEN Life Sciences. 8-Isoprostanate enzyme immunoassay kit (catalog No. 516351) and glutathione colorimetric assay kit (catalog No. GSH-420) were obtained from Cayman and OXIS Health, respectively. R (+)-SKF38393 hydrochloride (an active enantiomer of [±]-1-phenyl-2,3,4,5-tetrahydro-[1H]-3-benzazepine-7,8-diol), BSO, Tempol, Inactin, sodium pentobarbital, ouabain, and all of the other reagents were purchased from Sigma-Aldrich.

Animal Treatment
Male Sprague-Dawley (SD) rats (200 to 250 g) were obtained from Harlan. The animals were divided into 8 experimental groups: V, animals maintained on tap water (vehicle); HS, animals receiving high salt (1% NaCl); BSO, animals provided with 30 mmol/L of BSO; HS+BSO, animals receiving both high salt and BSO; T, animals provided with 1 mmol/L of Tempol; HS+T, animals kept on high salt and Tempol; BSO+T, animals receiving BSO and Tempol; and HS+BSO+T, animals receiving high salt, BSO, and Tempol. BSO, an oxidant; high salt; and Tempol, an antioxidant, were provided in drinking water for 12 days. All of the experiments were performed according to the National Institutes of Health and University of Houston guidelines and protocols for care and use of laboratory animals.

Surgical Procedures for Renal Function Studies
Rats were anesthetized with Inactin (100 mg/kg, IP), and tracheotomy was performed to facilitate breathing. To measure the BP and heart rate, the left carotid artery was catheterized with a PE 10 tubing. This tubing was connected to a Statham P23AC pressure transducer. BP and heart rate were continuously recorded on a Grass polygraph (model 7D, Grass Instrument).

Experimental Protocol for Renal Function Studies
The effect of SKF38393 on sodium and water excretion was determined in all of the experimental groups of anesthetized rats prepared for measurement of BP and collection of urine samples from the left ureter as described previously. The protocol consisted of a 45-minute stabilization period after the surgery, followed by 5 consecutive 30-minute collection periods: basal 1, drug, recovery 1, and recovery 2. During basal 1 and drug, a constant infusion of 0.9% NaCl at a rate of 1 mL/h was administered intravenously. Collection of blood samples (replaced by equal amount of saline) were collected at the end of each period. Plasma was separated by centrifugation of blood samples at 1500 g for 15 minutes. Sodium and potassium concentrations were measured by flame photometer 480 (Ciba Corning Diagnostics), and creatinine levels were determined by creatinine analyzer (Model 2, Beckman).

Determination of Dopamine Levels in Urine Samples
Urine samples were spiked with an internal standard epinephrine and extracted with alumina equilibrated with 1.5 mol/L of Tris-HCl and 1% Na2EDTA (pH 8.6). The urine-alumina mixture was centrifuged (10 000g, 4°C) followed by 2 washes with distilled water. Dopamine was eluted with 0.2 mL of 0.2 N perchloric acid, filtered, and injected into a high-performance liquid chromatography (Model 1525, Waters Corporation) equipped with a C18 reverse-phase, 3-μm Luna column (100 × 2 mm, Phenomenex). The sample was eluted by a mobile phase (25 mmol/L of NaH2PO4, 50 mmol/L of Na-citrate, 0.03 mmol/L of EDTA, 10 mmol/L of diethylamine HCl, 2.2 mmol/L of sodium octylsulfate [pH 3.2], 30 mL/L of methanol, and 22 mL/L of dimethylacetamide) at a flow rate of 0.4 mL/min. Dopamine peak was determined by the Coulometric electrochemical detector (Model Coulochem III, ESA, Inc). The amount of dopamine present in the urine sample was calculated from the known standard curve and corrected for sample loss because of extraction based on the recovery of the internal standard epinephrine.

Indices (Biomarkers) of Oxidative Stress
Renal malondialdehyde was determined by measuring the malondialdehyde level in butanol extracts of cell lysate by the method of Mihara and Uchiyama. Urine Samples determination of the D1R G protein-coupling, membrane were incubated with 50 nM [3H]SCH-23390, a D1R antagonist, in 250 μL (final volume) of binding buffer for 120 minutes at 25°C. Nonspecific binding was determined in the presence of 1 μmol/L of unlabeled SCH-23390.

[^3H]SCH23390 Binding
Fifty micrograms of membrane protein were incubated with 50 nM [3H]SCH-23390, a D1R antagonist, in 250 μL (final volume) of binding buffer for 120 minutes at 25°C. Nonspecific binding was determined in the presence of 1 μmol/L of unlabeled SCH-23390.

Measurement of [35S]Guanosine-γ-Thiophosphate Binding
To determine the D1R G protein-coupling, membrane were incubated with [35S]guanosine-γ-thiophosphate (GTPγS) and stimulated by 1 μmol/L of SKF38393. Nonspecific [35S]GTPγS binding was determined in the presence of 100 μmol/L of unlabeled GTP γS. Specific binding was calculated as the difference between total and nonspecific binding.

Effect of SKF38393 on Na,K-ATPase Activity
Na,K-ATPase activity was determined as reported earlier.

[^3H]SCH23390 and [35S]GTPγS binding was performed to determine 8-Isoprostane and glutathione were determined by a commercially available immunoassay kit and colorimetric assay kit, respectively. As described previously, fluorescence spectrometric assay of O2 production was performed to determine the nitrosonimide-adene dinucleotide (phosphate) oxidase (NAD[P]H oxidase) activity in renal proximal tubules.

Preparation of Renal Proximal Tubular Suspension
Renal proximal tubular suspension was prepared as described earlier.

[^3H]SCH23390 and [35S]GTPγS" binding was determined in the presence of 1 μmol/L of unlabeled SCH-23390.

[^3H]SCH23390 and [35S]GTPγS binding was determined in the presence of 1 μmol/L of unlabeled SCH-23390.

[^3H]SCH23390 and [35S]GTPγS binding was determined in the presence of 1 μmol/L of unlabeled SCH-23390.

[^3H]SCH23390 and [35S]GTPγS binding was determined in the presence of 1 μmol/L of unlabeled SCH-23390.

[^3H]SCH23390 and [35S]GTPγS binding was determined in the presence of 1 μmol/L of unlabeled SCH-23390.

[^3H]SCH23390 and [35S]GTPγS binding was determined in the presence of 1 μmol/L of unlabeled SCH-23390.

[^3H]SCH23390 and [35S]GTPγS binding was determined in the presence of 1 μmol/L of unlabeled SCH-23390.

[^3H]SCH23390 and [35S]GTPγS binding was determined in the presence of 1 μmol/L of unlabeled SCH-23390.

[^3H]SCH23390 and [35S]GTPγS binding was determined in the presence of 1 μmol/L of unlabeled SCH-23390.
increase in urinary dopamine excretion in animals on high salt (HS), HS + BSO (BSO), HS + Tempol (T), and HS plus BSO and Tempol compared with vehicle. BSO or Tempol did not alter the renal dopamine production. HS intake did not affect the BP, whereas BSO-treated rats exhibited a mild increase in BP compared with vehicle. Concomitant treatment with BSO and HS caused a marked increase in BP. Treatment with Tempol normalized BP in BSO and BSO + HS rats. There was no difference in glomerular filtration rate among various experimental groups.

Oxidative Stress
 Compared with vehicle, HS intake did not have any effect on oxidative or antioxidative markers. Treatment with BSO without or with HS caused marked oxidative stress as evidenced by reduced renal glutathione, increased renal malondialdehyde and serum, and urinary excretion of 8-isoprostanone (Table 1). In addition, these animals showed increased activity of NAD(P)H oxidase. Treatment of BSO and BSO + HS rats with Tempol normalized the malondialdehyde and 8-isoprostane levels and NAD(P)H oxidase activity. Although Tempol caused an increase in gluthathione levels of BSO and BSO + HS rats, it remained significantly lower than vehicle. There was no difference in malondialdehyde, 8-isoprostane, and glutathione levels, as well as NAD(P)H oxidase activity, among vehicle, HS, or Tempol groups (Table 1).

Table 1. General Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>V</th>
<th>HS</th>
<th>BSO</th>
<th>HS + BSO</th>
<th>T</th>
<th>HS + T</th>
<th>BSO + T</th>
<th>HS + BSO + T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food per day, g</td>
<td>19.2 ± 2.1</td>
<td>17.3 ± 3.1</td>
<td>20.3 ± 1.9</td>
<td>18.4 ± 4.3</td>
<td>20.9 ± 3.2</td>
<td>17.9 ± 3.6</td>
<td>17.6 ± 3.8</td>
<td>18.7 ± 4.1</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>250.0 ± 10.0</td>
<td>255.0 ± 12.0</td>
<td>245 ± 13.0</td>
<td>260.0 ± 15.0</td>
<td>259.0 ± 11.0</td>
<td>247.0 ± 13.0</td>
<td>265.0 ± 10.0</td>
<td>266.0 ± 10.0</td>
</tr>
<tr>
<td>8-Isop-U, pg/mg of creatinine</td>
<td>0.8 ± 0.04</td>
<td>0.84 ± 0.02</td>
<td>1.2 ± 0.08*</td>
<td>1.3 ± 0.1*</td>
<td>0.78 ± 0.03</td>
<td>0.80 ± 0.05</td>
<td>0.84 ± 0.05</td>
<td>0.86 ± 0.02</td>
</tr>
<tr>
<td>8-Isop-P, fg/mg of creatinine</td>
<td>40.1 ± 1.3</td>
<td>42.6 ± 1.9</td>
<td>51.0 ± 1.6*</td>
<td>53.5 ± 2.1*</td>
<td>38.1 ± 1.9</td>
<td>40.2 ± 1.8</td>
<td>44.1 ± 2.0</td>
<td>43.8 ± 1.6</td>
</tr>
<tr>
<td>MDA, mmol/mg of protein</td>
<td>0.20 ± 0.01</td>
<td>0.24 ± 0.02</td>
<td>0.32 ± 0.02*</td>
<td>0.37 ± 0.04*</td>
<td>0.18 ± 0.01</td>
<td>0.19 ± 0.02</td>
<td>0.22 ± 0.01</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>GSH, mmol/mg of protein</td>
<td>1.6 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>0.7 ± 0.02*</td>
<td>0.68 ± 0.08*</td>
<td>1.8 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.1 ± 0.3†</td>
<td>1.0 ± 0.2†</td>
</tr>
<tr>
<td>NA-OX, fluorescence units/min per mg of protein</td>
<td>410.0 ± 30.0</td>
<td>465.0 ± 44.0</td>
<td>601.0 ± 38.0*</td>
<td>655.0 ± 43.0*</td>
<td>381.0 ± 33.0</td>
<td>403.0 ± 32.0</td>
<td>455.0 ± 33.0</td>
<td>488.0 ± 51.0</td>
</tr>
<tr>
<td>GFR, mL/min</td>
<td>0.97 ± 0.1</td>
<td>1.1 ± 0.13</td>
<td>0.87 ± 0.07</td>
<td>0.92 ± 0.08</td>
<td>1.0 ± 0.1</td>
<td>0.97 ± 0.1</td>
<td>0.92 ± 0.1</td>
<td>0.88 ± 0.1</td>
</tr>
<tr>
<td>DA, pmol/min</td>
<td>5.0 ± 0.3</td>
<td>9.2 ± 0.6*</td>
<td>4.8 ± 0.4</td>
<td>8.6 ± 0.5*</td>
<td>5.3 ± 0.3</td>
<td>9.4 ± 0.8*</td>
<td>4.9 ± 0.4</td>
<td>8.9 ± 0.6*</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>103.4 ± 5.0</td>
<td>111.6 ± 7.3</td>
<td>117.2 ± 5.6*</td>
<td>143.4 ± 5.8†</td>
<td>97.3 ± 6.2</td>
<td>105.4 ± 2.4</td>
<td>103.5 ± 5.2</td>
<td>107.4 ± 6.4</td>
</tr>
</tbody>
</table>

Glucose, mg/dL; 8-Isop-U indicates 8-isoprostane-urine; 8-Isop-P, 8-isoprostane-plasma; MDA, malondialdehyde; GSH, glutathione; NA-OX, renal NAD(P)H oxidase; GFR, glomerular filtration rate; DA, dopamine; MBP, mean blood pressure. Data (n = 8 animals) were analyzed by ANOVA followed by posthoc Newman-Keuls multiple comparison test. *P < 0.05 was considered statistically significant.

D1R Expression in Renal Proximal Tubular Membranes
The specific membrane binding of [3H]SCH23390, a D1R antagonist, was significantly reduced in proximal tubules from BSO- and HS + BSO-treated rats. Compared with vehicle, high salt did not produce any effect on ligand binding (Figure 4). Treatment of BSO and HS + BSO rats with Tempol restored D1R expression. Similar D1R binding was observed in V, HS, T, and HS + T animal groups (Figure 4).

SKF38393-Induced D1R G Protein Coupling in Renal Proximal Tubular Membranes
SKF-38393 elicited D1R G protein coupling as evidenced by increased [35S]GTPγS binding in proximal tubular membranes from V and HS rats (Figure 5). However, SKF-38393 failed to stimulate [35S]GTPγS membrane binding in proximal tubules from BSO- and HS + BSO-treated rats (Figure 5). Treatment of BSO- and HS + BSO-supplemented animals with Tempol restored the D1R G protein coupling (Figure 5). The basal [35S]GTPγS binding (pmol/mg protein) was similar in all of the animal groups (V, 4.3 ± 0.4; BSO, 3.9 ± 0.5; HS, 4.1 ± 0.3; HS + BSO, 4.7 ± 0.6; T, 4.0 ± 0.3; BSO + T, 5.1 ± 0.6; HS + T, 4.8 ± 0.5; and BSO + HS + T, 4.8 ± 0.6).
SKF38393-Induced Adenylyl Cyclase Activation in Renal Proximal Tubules

Incubation of proximal tubular membranes from V and HS animals with SKF38393 caused a significant increase in cAMP accumulation. However, SKF-38393 failed to increase cAMP levels in membranes from BSO- and HS/BSO-treated rats. Tempol treatment restored the SKF38393-induced adenylyl cyclase activation in these animals. Basal cAMP levels and forskolin, a direct activator of adenylyl cyclase-induced cAMP accumulation, were similar in all of the experimental groups (Table 2).

SKF38393-Induced Na/K-ATPase Inhibition in Renal Proximal Tubular Membranes

In an effort to investigate the effect of endogenous dopamine on Na/K pump, we first determined the basal Na/K-ATPase activity in renal proximal tubules. As shown in Figure 6A, the basal Na/K-ATPase activity was significantly lower in HS-treated rats compared with vehicle. On the other hand, in animals treated with BSO or BSO+HS, there was no decrease basal Na/K-ATPase activity. Tempol supplementation resulted in a decrease of basal Na/K-ATPase activity in animals given HS plus BSO (Figure 6A). However, Tempol did not affect the basal Na/K-ATPase activity in vehicle, BSO, or HS rats (Figure 6A).

Because increased dopamine production failed to decrease basal Na/K-ATPase activity in HS+BSO rats, it may reflect the failure of D1R activation to inhibit Na/K-ATPase activity in renal proximal tubules. Therefore, we incubated proximal tubules with D1R agonist SKF38393 and determined the Na/K-ATPase activity. Interestingly, in V and HS rats, the incubation of proximal tubules with SKF38393 caused significant inhibition of Na/K-ATPase activity (Figure 6B). However, SKF38393 failed to inhibit Na/K-ATPase activity in proximal tubules from BSO-and HS+BSO-treated animals (Figure 6B). Treatment of these animals with Tempol restored the SKF-38393-induced Na/K-ATPase Inhibition (Figure 6C).

Discussion

Our results show that in normotensive SD rats HS intake increased urinary dopamine production and sodium excretion and decreased basal renal proximal tubular Na/K-ATPase activity. HS did not affect BP or the ability of SKF-38393, the
D1R agonist, to inhibit Na/K-ATPase and promote sodium excretion. Treatment of SD rats with BSO, an inhibitor of \[\text{[35S]}\text{GTP}\]H9253, a key enzyme in glutathione biosynthesis, reduced renal glutathione levels and increased oxidative stress, which was accompanied by D1R downregulation and uncoupling from G protein. There was no change in renal dopamine production, these animals exhibited mild increases in BP, and SKF-38393 failed to stimulate proximal tubular adenyl cyclase, inhibit Na,K-ATPase activity, or promote sodium excretion. On the other hand, co-supplementation of BSO with HS caused a marked increase in BP along with increased oxidative stress and defective D1R function, despite increased renal dopamine production. Tempol supplementation of BSO and BSO+HS rats reduced oxidative stress, restored D1R function, and normalized BP. These results demonstrate the importance of dopamine as a natriuretic hormone in that it inhibits sodium transporters, increases sodium excretion, and contributes to maintenance of BP during HS intake. More importantly, these data also identify the role of oxidative stress in causing renal D1R defects and provide an unequivocal support to the significance of D1R dysfunction in contributing to salt-sensitive hypertension in the presence of oxidative stress.

**HS Intake and BP**

Several lines of evidence suggest that intrarenally formed dopamine plays a role in the regulation of sodium excretion and that this effect is, at least in part, mediated by a direct action of the hormone on proximal tubule sodium reabsorption.4–6,11–13 Dopamine, via activation of D1R, exerts its effect on sodium reabsorption by inhibiting proximal tubule Na/K-ATPase activity.4–6,11–13 To study the physiological relevance of these findings, the present study evaluated the role of the renal dopamine system in regulating sodium balance and BP during increased dietary sodium intake. Our findings demonstrate that change in sodium intake regulates both renal dopamine formation and the dopamine-induced inhibition of Na/K-ATPase in renal proximal tubules. We observed that in SD rats, HS intake increased urinary dopamine excretion, generally considered to reflect intrarenal dopamine production. The increased dopamine formation was accompanied by increased sodium excretion and decreased Na/K-ATPase activity. In addition, the exogenous D1R agonist also inhibited Na/K-ATPase activity, leading to an increase in sodium excretion. Taken together, the results imply that HS intake increases renal dopamine formation, which inhibits proximal tubular Na/K-ATPase activity and promotes sodium excretion. This effect contributes to increased sodium excretion and maintenance of normal BP.

**Figure 3.** Urine flow before, during, and after 1 μg/kg per minute of SKF38393 in A. Basal indicates values before drug administration; Drug, values during drug administration; Recovery, values after drug infusion was terminated. All of the time intervals (basal, drug, and recovery) were 30 minutes. Two basal and 2 recovery collections were averaged and shown. A, *P<0.05 vs basal V; #P<0.05 vs basal BSO (1-way ANOVA); $P<0.05 drug vs respective basal; ¥P<0.05 drug vs respective recovery (repeated-measure ANOVA). B, *P<0.05 vs basal T; #P<0.05 vs basal BSO+T (1-way ANOVA); $P<0.05 drug vs respective basal; ¥P<0.05 drug vs respective recovery (repeated-measures ANOVA). Both 1-way and repeated-measures ANOVAs were followed by posthoc Newman-Keuls multiple comparison test. P<0.05 was considered statistically significant (n=8 animals).

**Figure 4.** \([\text{3H}]\text{SCH23390 binding in renal proximal tubular membranes. *P<0.05 vs V; #P<0.05 vs BSO; and $P<0.05 vs HS+BSO (1-way ANOVA followed by posthoc Newman-Keuls multiple comparison test). P<0.05 was considered statistically significant (n=8 animals).}\)
Oxidative Stress and BP

Recent studies have indicated that both hypertensive humans and animals have decreased antioxidant capacity and produce excessive amounts of ROS.16,17,35–40 Although renal D1R function was accompanied by a mild increase in BP. There also exists in vivo evidence for renal dopamine D1R function in prohypertensive DS rats.42,43 In the present study we found that, in BSO-treated rats with Tempol decreased oxidative stress, restored D1R function, and normalized BP, indicating the role of D1R dysfunction in the mild elevation of BP associated with oxidative stress.

Oxidative Stress, HS Intake, and BP

The novel observation of this study was that oxidative stress–induced renal D1R dysfunction contributes to salt-sensitive hypertension in SD rats. HS intake alone for the same amount of time did not result in increased BP when renal D1R function was normal, thus suggesting a role of D1R in salt sensitivity. Abnormalities in the renal dopaminergic system, in view of its potential natriuretic actions, have been suggested to contribute to the development of salt-sensitive hypertension.12,14,41,42 Several lines of evidence indicate that hypertension in Dahl salt-sensitive rats results from the inability to excrete sodium adequately in response to salt challenge.12,14,41,42 Reports also suggest that the ability of the D1 agonist fenoldopam to decrease renal proximal sodium transport is reduced in salt-sensitive hypertensive humans compared with salt-resistant hypertensive or control normotensive subjects.4,5,12

Table 2. SKF38393 (100 μm) and Forskolin (10 μmol/L)-Induced Adenylyl Cyclase Activation

<table>
<thead>
<tr>
<th>Group</th>
<th>V</th>
<th>BSO</th>
<th>HS</th>
<th>HS+BSO</th>
<th>T</th>
<th>BSO+T</th>
<th>HS+T</th>
<th>HS+BSO+T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal*</td>
<td>62.2±3.4</td>
<td>57.2±2.9</td>
<td>65.0±2.2</td>
<td>59.1±2.9</td>
<td>63.1±3.1</td>
<td>65.0±4.1</td>
<td>57.2±4.0</td>
<td>59.1±5.1</td>
</tr>
<tr>
<td>SKF-38393†</td>
<td>84.2±3.9†</td>
<td>64.1±3.1</td>
<td>86.0±3.6‡</td>
<td>65.3±3.8</td>
<td>82.2±3.8‡</td>
<td>82.1±2.9‡</td>
<td>86.0±4.0‡</td>
<td>79.1±2.7‡</td>
</tr>
<tr>
<td>Forskolin§</td>
<td>187.2±11.3‡</td>
<td>205.0±18.6‡</td>
<td>206.6±15.1‡</td>
<td>210.2±19.9‡</td>
<td>198.2±10.3‡</td>
<td>207.0±15.3‡</td>
<td>189.6±14.1‡</td>
<td>203.2±16.9‡</td>
</tr>
</tbody>
</table>

The basal values from all the groups (data, mean±SE of 6 to 8 different animals) were analyzed by ANOVA and posthoc Newman-Keuls multiple comparison test, whereas, basal versus SKF-38393 and basal versus forskolin were analyzed by t test. P<0.05 was considered statistically significant.

* cAMP was expressed as picomoles per minute per milligram of protein.
†Membranes were incubated with 100 μmol/L of SKF38393, a D1R agonist.
‡Significantly different from respective basal.
§Membranes were incubated with 10 μmol/L of forskolin, a direct activator of adenylyl cyclase.
Dopamine D1 Receptors and Salt Sensitivity

Banday et al

In conclusion, we found that animals with functional renal D1R maintain sodium homeostasis by increasing renal dopamine formation and inhibiting Na/K-ATPase activity during HS intake. Oxidative stress causes D1R dysfunction and a mild increase in BP. In conditions associated with oxidative stress, the increased HS intake results in development of hypertension. Antioxidant Tempol decreases oxidative stress, restores D1R function, and mitigates salt sensitivity, provid-

Oxidative Stress and D1R Dysfunction

In an effort to understand the mechanisms responsible for oxidative stress–mediated D1R dysfunction, we studied the D1R expression and coupling to G protein. We observed that oxidative stress decreased D1R expression, and SKF-38393 failed to stimulate G protein coupling and increase cAMP accumulation, suggesting uncoupling from effector complex. The functional consequence of D1R downregulation and uncoupling was blunting of SKF38393-induced Na/K-ATPase inhibition in renal proximal tubules and subsequent failure of the D1R agonist to cause an increase in sodium excretion. Treatment of these animals with Tempol restored D1R expression and coupling. These results are in agreement with our previous studies showing that, in aging and diabetes, oxidative stress contributes to D1R dysfunction, as antioxidant Tempol ameliorated the oxidative stress and restored D1R function. Similar to our findings, defective coupling of the D1R is reported in spontaneously hypertensive rats and DS rats: D1-like receptor agonists were unable to stimulate G protein coupling and increase cAMP accumulation, suggesting uncoupling from effector complex. The functional consequence of D1R downregulation and uncoupling was blunting of SKF38393-induced Na/K-ATPase inhibition in renal proximal tubules and subsequent failure of the D1R agonist to cause an increase in sodium excretion. Treatment of these animals with Tempol restored D1R expression and coupling. These results are in agreement with our previous studies showing that, in aging and diabetes, oxidative stress contributes to D1R dysfunction, as antioxidant Tempol ameliorated the oxidative stress and restored D1R function. Similar to our findings, defective coupling of the D1R is reported in spontaneously hypertensive rats and DS rats: D1-like receptor agonists were unable to stimulate adenylyl cyclase in proximal tubules of kidneys from DS rats, whereas forskolin, a direct adenylyl cyclase activator, stimulated the enzyme in both DS and DR rats. Although the detailed mechanism responsible for BSO-induced D1R G protein uncoupling was not studied, in a recent study we have reported that exposure of renal epithelial cells to H2O2, an oxidant, increased protein kinase C activity, translocated GRK-2 to the cell membrane, and increased basal D1R serine phosphorylation, resulting in uncoupling of D1R from G proteins. Therefore, we can speculate that BSO-induced oxidative stress, via increased protein kinase C activity, may cause D1R hyperphosphorylation, leading to receptor G protein uncoupling.

In conclusion, we found that animals with functional renal D1R maintain sodium homeostasis by increasing renal dopamine formation and inhibiting Na/K-ATPase activity during HS intake. Oxidative stress causes D1R dysfunction and a mild increase in BP. In conditions associated with oxidative stress, the increased HS intake results in development of hypertension. Antioxidant Tempol decreases oxidative stress, restores D1R function, and mitigates salt sensitivity, provid-

Figure 6. Basal (A) and 1 μmol/L of SKF38393-induced inhibition of Na,K-ATPase activity (B and C) in renal proximal tubules. A: *P<0.05 vs V; #P<0.05 vs HS; $P<0.05 vs HS+T; and ¥P<0.05 vs HS+BSO+T (1-way ANOVA followed by posthoc Newman-Keuls multiple comparison test). B, $P<0.05 vs other basal values. C, §§P<0.05 vs T and BSO+T basal values. B and C, **P<0.05 SKF38393 vs respective basal (Student’s t test). P<0.05 was considered statistically significant (n=8 animals).

rats, the endogenous dopamine failed to inhibit Na/K-ATPase activity. The lack of Na/K-ATPase inhibition in these animals cannot be attributed to the failure of the tubules to increase dopamine levels in response to HS intake, because similar levels of dopamine were observed in normotensive HS rats and hypertensive BSO+HS animals. Therefore, it is possible that oxidative stress–induced D1R dysfunction was responsible for the inability of dopamine to inhibit Na/K-ATPase. This is further supported by the observation that the exogenous D1R agonist SKF38393 failed to inhibit Na/K-ATPase and promote natriuresis. Finally, treatment of BSO and HS rats with Tempol reduced oxidative stress and normalized BP. Similar to HS rats, these animals showed decreased basal Na/K-ATPase activity and exhibited normal responsiveness to D1R agonists, providing strong evidence that renal D1 dysfunction contributes to salt-sensitive hypertension.

We found that oxidative stress caused a similar defect in D1R function in BSO- and BSO-HS-treated rats, yet the later group exhibited a marked increase in BP, whereas the former showed a mild increase. However, it is noteworthy that the natriuretic effect of dopamine is prominent only after salt loading and small or negligible during normal salt intake or salt depletion. Under conditions of moderate sodium excess, locally generated dopamine via D1R acts on renal tubules and decreases sodium transport. Under these circumstances renal D1R is responsible for >50% of incremental sodium excretion. In addition, although kidneys play a primary role in sodium homeostasis, the extrarenal effects can also contribute to salt sensitivity. Vaziri and colleagues have shown that chronic consumption of HS or BSO could independently cause the downregulation of various NO synthase isoforms, as well as reduction in NO bioavailability, which contributes to the development and maintenance of hypertension in animals. Studies from our laboratory also showed that, during oxidative stress, HS intake attenuated the vasodilator responses to endothelial-dependent and -independent vasoactive compounds despite NO synthase upregulation. Taken together, these data show that the increased salt sensitivity in conditions associated with oxidative stress may be contributed by both renal and extrarenal signaling molecules responsible for sodium homeostasis and vascular tone.
ing support for the role of renal D1R in salt-sensitive hypertension during oxidative stress.

**Perspectives**

Our findings identify a role for endogenous renal dopamine in maintaining sodium homeostasis and BP during increases in sodium intake under normal physiological condition. More importantly, they demonstrate that a small perturbation in the system, such as an increase in oxidative stress, causes renal dopamine receptor dysfunction, but the BP increase is modest. However, in conditions associated with oxidative plus HS intake, a phenomenon observed in various pathological situations, the compromise in renal dopamine receptor function contributes to hypertension. Therefore, lowering oxidative stress and/or salt intake will be beneficial in protecting against salt-induced hypertension.

**Acknowledgment**

We are thankful to Dr Ran Xu for performing high-performance liquid chromatography.

**Source of Funding**

This study was supported by National Institutes of Health grant AG-25056 from the National Institute of Aging.

**Disclosures**

None.

**References**


Oxidative Stress Causes Renal Dopamine D1 Receptor Dysfunction and Salt-Sensitive Hypertension in Sprague-Dawley Rats
Anees A. Banday, Yuen-Sum Lau and Mustafa F. Lokhandwala

Hypertension. 2008;51:367-375; originally published online December 24, 2007; doi: 10.1161/HYPERTENSIONAHA.107.102111
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/51/2/367

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/