Intrarenal Dopamine Attenuates Deoxycorticosterone Acetate/High Salt–Induced Blood Pressure Elevation in Part Through Activation of a Medullary Cyclooxygenase 2 Pathway

Bing Yao, Raymond C. Harris, Ming-Zhi Zhang

Abstract—Locally produced dopamine in the renal proximal tubule inhibits salt and fluid reabsorption, and a dysfunctional intrarenal dopaminergic system has been reported in essential hypertension and experimental hypertension models. Using catechol-O-methyl-transferase knockout (COMT−/−) mice, which have increased renal dopamine because of deletion of the major renal dopamine-metabolizing enzyme, we investigated the effect of intrarenal dopamine on the development of hypertension in the deoxycorticosterone acetate/high-salt (DOCA/HS) model. DOCA/HS led to significant increases in systolic blood pressure in wild-type mice (from 115±2 to 153±4 mm Hg), which was significantly attenuated in COMT−/− mice (from 114±2 to 135±3 mm Hg). In DOCA/HS COMT−/− mice, the D1-like receptor antagonist SCH-23390 increased systolic blood pressure (156±2 mm Hg). DOCA/HS COMT−/− mice also exhibited more urinary sodium excretion (COMT−/− versus wild-type: 3038±430 versus 659±102 μmol/L per 24 hours; P<0.01). Furthermore, DOCA/HS-induced renal oxidative stress was significantly attenuated in COMT−/− mice. COX-2–derived prostaglandins in the renal medulla promote sodium excretion, and dopamine stimulates medullary prostaglandin production. Renal medullary COX-2 expression and urinary prostaglandin E2 excretion were significantly higher in COMT−/− than in wild-type mice after DOCA/HS treatment. In DOCA/HS-treated COMT−/− mice, the COX-2 inhibitor SC-58236 reduced urinary sodium and prostaglandin E2 excretion and increased systolic blood pressure (153±2 mm Hg). These studies indicate that an activated renal dopaminergic system attenuates the development of hypertension, at least in large part through activating medullary COX-2 expression/activity, and also decreases oxidative stress resulting from DOCA/HS. (Hypertension. 2009;54:1077-1083.)

Key Words: dopamine ■ hypertension ■ cyclooxygenase 2 ■ prostaglandin E2 ■ oxidative stress ■ kidney

Although dopamine is an essential neurotransmitter, extraneurual dopamine also serves important physiological functions. The kidney possesses a robust intrarenal dopaminergic system that is distinct from any neural dopaminergic input. Circulating concentrations of dopamine in the piconomolar range, whereas dopamine levels in the kidney can reach high nanomolar concentrations.1 The dopamine precursor, L-dihydroxyphenylalanine, is taken up in the proximal tubule after filtration at the glomerulus and is then converted to dopamine by aromatic amino acid decarboxylase.1–4 Renal dopamine is metabolized predominantly by catechol-O-methyltransferase (COMT), with a smaller contribution by monoamine oxidase.

The cellular actions of dopamine are mediated by signaling through G protein–coupled 7 transmembrane receptors. There are 5 known renal dopamine receptors, which are divided into 2 subclasses, D1-like and D2-like receptors. D1-like receptors (D1 and D5) are coupled to Gi, and stimulate adenylate cyclase. D2-like receptors (D2, D3, and D4) are coupled predominantly to Go. In the mammalian kidney, dopamine serves as a major regulator of salt and water reabsorption by inhibiting both proximal and distal solute and water transport, mediated at least in part by the inhibition of the following specific tubule transporter activities: (1) apical (eg, Na/H exchange, chloride-bicarbonate exchange, and Na-P cotransport) and basolateral (eg, Na-K-ATPase and Na-HCO3 cotransport) transporters in the proximal tubule; (2) apical Na-K-2Cl cotransporter in the thick ascending limb; and (3) apical Na+ channel and aquaporins-2 and -4 in the collecting duct.1–5

Alterations in intrarenal dopamine production and/or activity have been reported in essential hypertension.2,3 Decreased intrarenal dopamine production, dysfunctional dopamine signaling in the proximal tubule because of abnormalities in G protein-coupled receptor kinase coupling to D1-like receptors, and decreased D1-like receptors in the medulla have been found in experimental models of hypertension.4–8 Deleting each of the 5 dopamine receptor subtypes leads to hypertension, whereas increased renal dopamine prevents high salt–induced elevation of blood pressure.9 However, the mechanisms underlying dopamine-mediated antihypertensive effects are not fully understood.

Dopamine-mediated inhibition of salt and water reabsorption in proximal and distal tubules contributes to its antihypertensive effects. 

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pertensive effects, and increasing evidence suggests that dopamine also has antioxidant effects, which may contribute to its antihypertensive effects.10,11 In addition, dopamine can stimulate prostaglandin production in the renal medulla.12,13 Prostaglandins produced in the renal medulla promote sodium and water excretion, and inhibition of prostaglandin production has the potential to increase blood pressure in some individuals.14 In the current studies, we investigated whether intrarenal dopamine can protect against deoxycorticosterone acetate/high-salt (DOCA/HS)-induced hypertension and whether dopamine-mediated stimulation of renal medullary prostaglandin production may contribute to any dopamine-mediated antihypertensive effects.

Methods

Animals

All of the animal experiments were performed in accordance with the guidelines of the institutional animal care and use committee of Vanderbilt University. Wild-type and COMT−/− mice on the 129J/sv background were obtained from Helkamaa et al15 of Rockefeller University. All of the mice were genotyped before use with PCR. The COMT primers 5′-GCAGTGATCCGGAGTACAG-3′ (forward) and 5′-TAGGGTCTTCCAGTGTC-3′ (reverse) generated a 599-bp product in heterozygous (not shown) and wild-type mice (Figure 1A). The neo cassette primers 0IMR013 and 0IMR014 (Jackson Laboratory) generated a 280-bp product in heterozygous (not shown) and homozygous mice (Figure 1A). Deletion of kidney COMT was also confirmed by Western analysis after the animals were euthanized (Figure 1B). COMT was also confirmed by Western analysis after the animals (not shown) and homozygous mice (Figure 1A). Deletion of kidney COMT was also confirmed by Western analysis after the animals were euthanized (Figure 1B).

Figure 1. DOCA/HS-induced elevations of blood pressure were attenuated in COMT−/− mice. A, Genotyping of COMT−/− mice with PCR. B, Western analysis showed deletion of COMT in the COMT−/− mouse kidney. C, Blood pressure was higher in wild-type than in COMT−/− mice after DOCA/HS treatment. *P<0.01 vs control; †P<0.05 vs control COMT−/−; ‡P<0.05 vs DOCA/HS wild-type; §P<0.05 vs DOCA/HS COMT−/−. COX-2 I indicates COX-2 inhibitor SC58236.

Determination of Urinary F₂-Isoprostane and Metabolite of Prostaglandin E₂

Urinary F₂-isoprostane, a well-accepted marker of systemic oxidative stress, and urinary metabolite of prostaglandin E₂ (PGE₂; PGE-M), the major metabolite of PGE₂, were measured by gas chromatography/electron capture/negative chemical ionization mass spectrometry assay, as described previously.18

Immunohistochemistry and Western Blot

The mice were anesthetized with Nembutal (50 mg/kg, IP) and given heparin (1000 U/kg, IP) to minimize coagulation. One kidney was taken out for Western analysis and the other was perfused with 3.7% formaldehyde, 10 mmol/L of sodium m-periodate, 40 mmol/L of phosphate buffer, and 1% acetic acid through the aortic trunk. After fixation, the selected tissues were dehydrated, paraffin embedded, and immunostained, as described previously.19,20 The kidney sections were immunostained with rabbit antimurine COX-2 antibody (Cayman Chemicals), rabbit antiinhibitory tetroxytrosine antibody (a marker of oxidative stress; Santa Cruz Biotechnology), and monoclonal rat antibody to the prostaglandin E₂ metabolite, 15,15-dihydro-15-keto-PGF₂α (Cayman Chemicals). Blood Pressure Measurement Using Tail-Cuff and Carotid Catheterization

Systolic blood pressure (SBP) was measured with a tail-cuff micromanometer.16 Blood pressure was also measured using carotid catheterization. Mice were anesthetized with 80 µg/g of ketamine (Ft Dodge Laboratories) and 8 µg/g of inactin (BYK) by IP administration. Mice were placed on a temperature-controlled pad. After tracheostomy, phycoerythrin 10 tubing was inserted into the right carotid artery. The catheter was tunneled under the skin, exteriorized, secured at the back of the neck, filled with heparinized saline, and sealed. The catheterized mouse was housed individually and trained 3 times before the measurement of blood pressure with a Blood Pressure Analyzer (Micro-Med).17

Quantitative Image Analysis

Macrophage infiltration and nitrotyrosine immunostaining were quantified using the BIOQUANT image analysis system (R&M Biometrics).22

Figure 1. DOCA/HS-induced elevations of blood pressure were attenuated in COMT−/− mice. A, Genotyping of COMT−/− mice with PCR. B, Western analysis showed deletion of COMT in the COMT−/− mouse kidney. C, Blood pressure was higher in wild-type than in COMT−/− mice after DOCA/HS treatment. *P<0.01 vs control; †P<0.05 vs control COMT−/−; ‡P<0.05 vs DOCA/HS wild-type; §P<0.05 vs DOCA/HS COMT−/−. COX-2 I indicates COX-2 inhibitor SC58236.
Micrography

Bright-field images from a Leitz Orthoplan microscope with digital video cassette RGB video camera were digitized and saved as computer files. Contrast and color-level adjustment (Adobe Photoshop) were performed for the entire image, that is, no region- or object-specific editing or enhancements were performed.

Statistical Analysis

Values are presented as mean ± SEM. ANOVA and Bonferroni t test were used for statistical analysis, and differences were considered significant when P<0.05.

Results

DOCA/HS-Induced Diuresis and Natriuresis Were Augmented in COMT −/− Mice

Urine volume and urinary Na (UNa) and K excretions were similar between control wild-type and control COMT −/− mice. DOCA/HS led to significant increases in urine volume and UNa in wild-type mice, but even more significant increases in COMT −/− mice, resulting in ~3.4-fold higher levels in urine volume and ~4.6-fold higher levels of UNa in COMT −/− mice than in wild-type mice after DOCA/HS treatment (Table). Urinary dopamine excretion was significantly higher in control COMT −/− mice than in control wild-type mice (7858 ± 1794 versus 4133 ± 670 ng/24 hours of control wild-type mice; P<0.01; n=4), similar to our previous reports.15 DOCA/HS treatment did not affect urinary dopamine excretion appreciably in COMT −/− mice and wild-type mice (6273 ± 608 versus 3015 ± 670 ng/24 hours of DOCA/HS wild-type mice; P<0.01; n=4).

DOCA/HS-Induced Increases in Blood Pressure Were Attenuated in COMT −/− Mice

When blood pressure was measured by tail-cuff microphonic manometer, we found that, although blood pressure was similar between control wild-type and control COMT −/− mice (SBP: 112±4 versus 117±3 mm Hg of wild-type mice; n=6), it was significantly higher in wild-type compared with COMT −/− mice after DOCA/HS treatment (SBP: 149±2 versus 131±3 mm Hg of DOCA/HS COMT −/− mice; P<0.05; n=6). To confirm that this attenuated DOCA/HS-induced blood pressure increases in COMT −/− mice, blood pressures were measured using carotid catheterization in another set of mice. As indicated in Figure 1C, blood pressure was significantly higher in DOCA/HS-treated wild-type mice compared with DOCA/HS-treated COMT −/− mice (SBP: 153±4 versus 135±3 mm Hg; P<0.05; n=5).

To investigate whether activation of the D1-like receptor contributed to the attenuated DOCA/HS-induced elevation of blood pressure in COMT −/− mice, a subset of DOCA/HS-treated COMT −/− mice was treated with the D1-like receptor antagonist SCH-23390. SCH-23390 treatment increased blood pressure in DOCA/HS-treated COMT −/− mice (SBP: 156±2 versus 135±3 mm Hg; P<0.05; n=5).

DOCA/HS-Induced Increases in Oxidative Stress and Macrophage Infiltration in the Kidney Were Attenuated in COMT −/− Mice

Vasculature-derived oxidative stress may contribute to DOCA/HS-induced hypertension.23–25 Urinary F2-isoprostane levels were similar between control wild-type and control COMT −/− mice. DOCA/HS led to similar increases in urinary F2-isoprostane excretion in wild-type and COMT −/− mice (wild-type: 1.73±0.26 versus 0.76±0.13 ng/24 hours, P<0.01; COMT −/−: 2.49±0.31 versus 0.55±0.11 ng/24 hours, P<0.01 versus control COMT −/− but P>0.05 versus DOCA/HS-treated wild-type; n=6; Figure 2A). Dopamine has been proposed to exert antioxidant effects in the kidney.10,11 Renal oxidative stress was evaluated via nitrotyrosine immunostaining. Nitrotyrosine immunostaining was primarily found in the renal medulla. As shown in Figure 2B and 2C, DOCA/HS led to significant increases in nitrotyrosine immunostaining in wild-type mice but only minimal increases in COMT −/− mice. Macrophage infiltration also increases in response to increased oxidative stress.26 As shown in Figure 3, DOCA/HS led to significant increases in macrophage infiltration in the cortex and medulla in wild-type mice (cortex: 41.2±2.7 versus 21.0±1.4 cells per field, P<0.01; medulla: 73.9±3.3 versus 39.0±2.2 cells per field, P<0.01; n=6) but less significant increases in COMT −/− mice (cortex: 24.8±1.4 versus 18.7±1.3 cells per field, P<0.05; medulla: 52.9±2.7 versus 45.8±1.6 cells per field, P<0.05; n=6).

DOCA/HS-Treated COMT −/− Mice Have Higher Urinary PGE2 Excretion and Medullary COX-2 Expression

Prostaglandins promote renal sodium excretion,21 and dopamine has been reported to stimulate medullary pro-

Table. Effects of DOCA/HS on Urine Volume, UNa Excretion, and Urinary K Excretion in Wild-Type and COMT −/− Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Wild-Type Mice</th>
<th>COMT −/− Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DOCA/HS</td>
</tr>
<tr>
<td>24-H urine volume, mL</td>
<td>1.1±0.1</td>
<td>6.4±1.6*</td>
</tr>
<tr>
<td>24-H UNa excretion, μM</td>
<td>123±10</td>
<td>659±102†</td>
</tr>
<tr>
<td>24-H urinary K excretion, μM</td>
<td>164±20</td>
<td>134±19</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Animals were caged individually for 24 hours, and urine sample was collected. n=6 in each group. HS indicates 1% NaCl in drinking water; C2I, COX-2 inhibitor SC58236.

*P<0.01 vs control.
†P<0.01 vs DOCA/HS wild-type mice.
‡P<0.01 vs DOCA/HS COMT −/− mice.
coX-2 inhibition increased blood pressure in doCA/Hs-treated coMt⁻/⁻ mice

To further investigate whether increased medullary cox-2 expression/activity contributed to the protection against doCA/Hs-induced elevation of blood pressure in coMt⁻/⁻ mice, a subset of doCA/Hs-treated coMt⁻/⁻ mice was treated with the selective cox-2 inhibitor SC-58236. Cox-2 inhibition increased blood pressure in doCA/Hs-treated coMt⁻/⁻ mice (SBP: 153 ± 2 versus 135 ± 3 mm Hg; P < 0.05; n = 5; Figure 1C) and decreased urine volume, UNa (Table), and urine PGE-M excretion (2.2 ± 0.4 versus 17.2 ± 4.2 ng/24 hours, P < 0.01 versus control and doCA/Hs-treated coMt⁻/⁻ mice; n = 5; Figure 4A) but had no effect on urinary F2-isoprostane excretion (2.4 ± 0.5 versus 2.5 ± 0.3 ng/24 hours; n = 6) or expression of the intrarenal oxidative stress marker nitrotyrosine (Figure 2). The cox-2 inhibitor significantly decreased doCA/Hs-induced macrophage infiltration in the renal cortex (15.7 ± 1.7 versus 24.8 ± 1.4 cells per field; P < 0.01; n = 6) and renal medulla (40.6 ± 2.4 versus 52.9 ± 2.7 cells per field; P < 0.01; n = 6; Figure 3B), consistent with the anti-inflammatory effect of cox-2 inhibition.

Discussion

The current studies investigated the effects of increased renal dopamine on the development of doCA/Hs-induced hypertension. The major findings include the following: (1) in coMt⁻/⁻ mice, which have increased intrarenal dopamine levels,15 doCA/Hs-induced elevation of blood pressure was attenuated; (2) coMt⁻/⁻ mice exhibited augmented diuresis and natriuresis in response to doCA/Hs; (3) doCA/Hs-induced increases in renal oxidative stress and macrophage infiltration were attenuated in coMt⁻/⁻ mice; and (4) doCA/Hs-treated coMt⁻/⁻ mice had increased urinary PGE₂ excretion and medullary cox-2 expression/activity. of note, administration of a cox-2 inhibitor to doCA/Hs-treated coMt⁻/⁻ mice led to increases in blood pressure and decreases in urinary sodium excretion but did not increase markers of renal and systemic oxidative stress or decrease renal macrophage infiltration. Taken together, these results indicate that an activated intrarenal dopaminergic system may attenuate doCA/Hs-induced elevation of blood pressure by promoting diuresis and natriuresis by increased medullary cox-2 expression/activity. In addition, doCA and/or high salt may induce intrarenal oxidative stress directly rather than secondary to increased blood pressure,27 and dopamine inhibits the oxidative stress directly rather than as a result of decreasing blood pressure and/or increasing sodium excretion.28–32

cox is a rate-limiting step in prostaglandin production.12,13 As shown in Figure 4A, urinary PGE-M excretion was numerically but not significantly increased in control coMt⁻/⁻ mice compared with control wild-type mice. doCA/Hs led to a modest increase in urinary PGE-M excretion in wild-type mice (6.8 ± 0.5 versus 4.5 ± 0.4 ng/24 hours; P < 0.05; n = 5) but a significant increase in coMt⁻/⁻ mice (17.2 ± 4.2 versus 6.7 ± 1.5 ng/24 hours; P < 0.01; n = 5). Immunoblotting indicated that medullary cox-2 expression was higher in control coMt⁻/⁻ mice than in control wild-type mice. doCA/Hs stimulated medullary cox-2 expression to a greater extent in coMt⁻/⁻ mice than in wild-type mice (Figure 4B). Immunostaining confirmed higher cox-2 expression in medullary interstitial cells after doCA/Hs treatment in coMt⁻/⁻ mice than in wild-type mice (Figure 4C).
Renal medullary COX-2 expression is stimulated by the activation of mineralocorticoid receptors by administration of DOCA or inhibition of 11β-hydroxysteroid dehydrogenase-2 activity with glycyrrhizic acid, whereas COX-1 expression is unaltered. Furthermore, we have reported previously that inhibition of COX-2 activity augmented blood pressure elevations in glycyrrhizic acid/HS-treated animals, whereas inhibition of COX-1 activity had no effect.

Dopamine has been shown to stimulate prostaglandin production in isolated rabbit kidney and microsomes isolated from rabbit kidney medulla. Infusion of dopamine or the D1-like receptor agonist fenoldopam stimulated renal prostaglandin production in normal volunteers. Dopamine has also been reported to stimulate PGE2 production through activation of D2-like receptors in cultured inner medulla collecting duct cells. In the present studies, renal medullary COX-2 expression and urinary PGE2 excretion were significantly higher with DOCA/HS treatment of COMT−/− mice compared with wild-type mice, and these increases were blocked by a highly selective COX-2 inhibitor. These studies suggest that dopamine stimulates prostaglandin production through increasing medullary COX-2 expression and activity and that the increased COX-2 expression and activity are involved in the increased natriuresis seen in the COMT−/− mice with DOCA/HS, but definitive proof of such integrated regulation and interaction will require inhibition of the intrarenal dopaminergic system.

Although blood pressure was similar in DOCA/HS-treated wild-type and DOCA/HS plus COX-2 inhibitor–treated COMT−/− mice, sodium excretion was different (Table), suggesting that COX-2 inhibition–induced hypertension is not entirely related to renal sodium handling. Rodriguez et al found that selective COX-2 inhibition led to a decreased glomerular filtration rate and sodium excretion and increased blood pressure in dogs. Therefore, decreased glomerular filtration rate may also contribute to COX-2 inhibition–induced blood pressure elevation in the current studies.

Activation of NADPH oxidase and xanthine oxidase and inactivation of Cu/Zn superoxide dismutase all appear to contribute to increased superoxide anion generation in DOCA/HS hypertensive models. Similarly, in cultured human renal proximal tubular cells, aldosterone activated mitochondrial oxidative stress. Vascular and intrarenal oxidative stress accompany DOCA/HS-induced elevations in blood pressure, although the role of oxidative stress in the development or maintenance of hypertension remains controversial. In mice with genetic deletion of the NADPH oxidase subunit gp91phox, DOCA/HS treatment did not lead to increases in blood pressure and vascular oxidative stress.

Figure 3. DOCA/HS-induced renal macrophage infiltration was attenuated in COMT−/− mice. A, Representative photomicrographs of F4/80-positive macrophages in renal cortex and medulla (original magnification: ×250). B, There was significantly less macrophage infiltration in COMT−/− mice than in wild-type mice, and COX-2 inhibition reduced macrophage infiltration further in DOCA/HS-treated COMT−/− mice. *P<0.01 vs control; †P<0.05 vs control; ‡P<0.01 vs DOCA/HS-treated wild-type mice. §P<0.01 vs DOCA/HS-treated COMT−/− mice.
Abnormalities in dopamine production and receptor function accompany a high percentage of human essential hypertension and several forms of rodent genetic hypertension. A general characteristic of essential hypertension is a relative defect in renal sodium and water handling. Intrarenal dopamine may act to protect the kidney from hypertension-induced injury through the following possible mechanisms: (1) inhibition of tubular salt reabsorption, whereby dopamine directly inhibits net NaCl and fluid reabsorption in the proximal and distal tubules; (2) interaction with angiotensin II, whereby intrarenal dopamine antagonizes angiotensin II–induced salt reabsorption in the proximal tubule through decreasing angiotensin II type 1 receptor expression; (3) interaction with intrarenal renin, whereby intrarenal dopamine indirectly inhibits renal renin expression through inhibition of COX-2 expression in the macula; (4) an antioxidant effect; and (5) stimulation of medullary prostaglandin production.

**Clinical Perspectives**

A Guytonian view of hypertension posits that dysfunctional salt and water excretion by the kidney ultimately underlies the development and maintenance of hypertension. Normally functioning kidneys respond to increased intravascular volume by inducing pressure natriuresis, by which increased renal perfusion pressure is transmitted to inhibit tubule reabsorption and to increase vasa recta capillary pressure and blood flow, leading to both increased hydrostatic pressure and medullary interstitial osmotic gradient washout. Using gene targeting plus a renal cross-transplantation technique, Crowley et al. found that deletion of renal angiotensin II type 1 receptors alone is sufficient to reduce blood pressure and that Ang II causes hypertension primarily through its effects on angiotensin II type 1 receptors in the kidney associated with reduced urinary sodium excretion. Our present studies also point out the importance of intrarenal dopamine and renal medullary COX-2 in protecting against the development of hypertension.

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None.

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