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−/− vs 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, extrarenal 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 are in the picomolar 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 Gs, and stimulate adenylate cyclase. D2-like receptors (D2, D3, and D4) are coupled predominantly to Gi. 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.6–8 Deleting each of the 5 dopamine receptor subtypes leads to hypertension,4 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 antihy-
pertensive effects, and increasing evidence suggests that dopamine also has antioxidant effects, which may contribute to its antihypertensive effects.\textsuperscript{10,11} In addition, dopamine can stimulate prostaglandin production in the renal medulla.\textsuperscript{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.\textsuperscript{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\textsuperscript{−/−} mice on the 129/J/sv background were obtained from Helkamaa et al\textsuperscript{9} of Rockefeller University. All of the mice were genotyped before use with PCR. The COMT primers 5'-GCAGTGATCGGAGTACAG-3' (forward) and 5'-TAGCCGTTTCCAGTGGTC-3' (reverse) generated a 599-bp product in heterozygous (not shown) and wild-type mice (Figure 1A). The COMT-/-Wild type mice have increased dopamine levels in the kidney and urine because of deletion of the major renal dopamine-metabolizing enzyme.\textsuperscript{15} All of the mice were fed normal pelleted rodent chow with 0.29% sodium (wt/wt, Harlan). Mice were divided into 6 groups: control wild-type, DOCA/HS-treated wild-type, control COMT\textsuperscript{−/−}, and DOCA/HS-treated COMT\textsuperscript{−/−} with or without administration of D1-like receptor antagonist or selective cyclooxygenase (COX) 2 inhibitor. DOCA pellets (150 mg, 60-day release; Innovative Research of America) were implanted SC. HS was achieved by adding 1% NaCl in the drinking water. The COX-2 inhibitor SC-58236 (2 mg/kg) was given by daily gastric gavage (a gift from Searle Monsanto). The D1-like receptor antagonist SCH-23390 (Sigma-Aldrich) was given at a dose of 1 mg/kg per day via osmotic minipump (2004; Alzet) implanted SC under sterile conditions and ether anesthesia. To collect 24-hour urine samples, the animals were first acclimated individually in metabolic cages, and then 24-hour urine samples were collected.

**Blood Pressure Measurement Using Tail-Cuff and Carotid Catheterization**

Systolic blood pressure (SBP) was measured with a tail-cuff micromanometer.\textsuperscript{16} Blood pressure was also measured using carotid catheterization. Mice were anesthetized with 80 µg/g of ketamine (Pfizer Laboratories) and 8 µg/g of inactin (BYK) by IP administration. Mice were placed on a temperature-controlled pad. After tracheostomy, phycocyanin 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).\textsuperscript{17}

**Determination of Urinary F\textsubscript{2}-Isoprostane and Metabolite of Prostaglandin E\textsubscript{2}**

Urinary F\textsubscript{2}-isoprostane, a well-accepted marker of systemic oxidative stress, and urinary metabolite of prostaglandin E\textsubscript{2} (PGE\textsubscript{2}; PGE-M), the major metabolite of PGE\textsubscript{2}, were measured by gas chromatography/electrode capture/negative chemical ionization mass spectrometry assay, as described previously.\textsuperscript{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.\textsuperscript{19,20} The kidney sections were immunostained with rabbit anti-uniruine COX-2 antibody (Cayman Chemicals), rabbit anti-antiinflammatory antibody (a marker of oxidative stress; Santa Cruz Biotechnology), and monoclonal rat anti nitrotyrosine antibody (a marker of oxidative stress, AbD Serotec). Western analysis was carried out as described previously.\textsuperscript{21} Antibodies used for Western analysis included COX-2 antibody (Cayman Chemicals) and COMT antibody (Novus Biologicals).

**Quantitative Image Analysis**

Macrophage infiltration and nitrotyrosine immunostaining were quantified using the BIOQUANT image analysis system (R&M Biometrics).\textsuperscript{22}
DOCA/HS-Induced Diuresis and Natriuresis Were Augmented in COMT\textsuperscript{−/−} Mice

Urinary volume and urinary Na (UNa) and K excretions were similar between control wild-type and control COMT\textsuperscript{−/−} mice. DOCA/HS led to significant increases in urine volume, UNa excretion, and urinary K excretion in wild-type mice, but not in control COMT\textsuperscript{−/−} mice, resulting in 3.4-fold increases in COMT\textsuperscript{−/−} mice compared with wild-type mice. Urinary dopamine excretion was significantly higher in DOCA/HS-treated wild-type mice compared with DOCA/HS-treated COMT\textsuperscript{−/−} mice (SBP: 153±4 versus 135±3 mm Hg; P<0.05; n=5).

Results

DOCA/HS-Induced Increase in Blood Pressure

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\textsuperscript{−/−} 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\textsuperscript{−/−} mice after DOCA/HS treatment (SBP: 149±4 versus 131±3 mm Hg of DOCA/HS COMT\textsuperscript{−/−} mice; P<0.05; n=6). To confirm that this attenuated DOCA/HS-induced blood pressure increases in COMT\textsuperscript{−/−} 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\textsuperscript{−/−} mice (SBP: 153±4 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\textsuperscript{−/−} Mice

Vasculature-derived oxidative stress may contribute to DOCA/HS-induced hypertension.\textsuperscript{23–25} Urinary F\textsubscript{2}-isoprostane levels were similar between control wild-type and control COMT\textsuperscript{−/−} mice. DOCA/HS led to significant increases in urinary F\textsubscript{2}-isoprostane excretion in wild-type and COMT\textsuperscript{−/−} mice (wild-type: 1.73±0.26 versus 0.76±0.13 ng/24 hours, P<0.01; COMT\textsuperscript{−/−}: 2.49±0.31 versus 0.55±0.11 ng/24 hours, P<0.01 versus control COMT\textsuperscript{−/−} but P>0.05 versus DOCA/HS-treated wild-type; n=6; Figure 2A).

DOCA/HS-Treated COMT\textsuperscript{−/−} Mice Have Higher Urinary PGE\textsubscript{2} Excretion and Medullary COX-2 Expression

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

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.
tagladin 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).

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 urinary 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 prostaaglandin production. Both COX isoforms, COX-1 and COX-2, are expressed at high levels in the inner medulla/papilla.21,33 In the medulla, prostaaglandins act as diuretic and natriuretic agents by increasing blood flow in the vasa recta, decreasing salt reabsorption in the medullary thick ascending limbs, and reducing vasopressin-stimulated water reabsorption from collecting ducts.34 All of these effects are inhibited by COX-2 inhibitors.14 Inhibition of prostaglandin production by COX-2 inhibitors may cause edema and modest elevations in blood pressure in a minority of subjects and may also exacerbate
pre-existing hypertension. 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 glycyrrhizin acid, whereas COX-1 expression is unaltered. Furthermore, we have reported previously that inhibition of COX-2 activity augmented blood pressure elevations in glycyrrhizin 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/HS-treated 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.
compared with increased blood pressure and vascular oxidative stress in the wild-type mice. In contrast, vascular superoxide anion production did not increase in a hypertension model induced by noradrenaline infusion, suggesting that increased vascular oxidative stress may not be secondary to increased blood pressure, per se.

Previous studies have indicated that activation of D1-like or D2-like receptors can induce antioxidant responses. D2-receptor and D5-receptor knockout mice develop ROS-dependent hypertension. In these mice, renal NADPH activity and expression are increased, and inhibition of NADPH oxidase activity normalizes the blood pressure. COMT is the major intrarenal dopamine metabolizing enzyme, and COMT−/− mice have increased intrarenal dopamine levels because of the absence of COMT metabolism of dopamine. However, plasma dopamine concentrations are similar between wild-type and COMT−/− mice, whereas intrarenal and urinary dopamine levels are significantly higher in COMT−/− mice. After DOCA/HS treatment, systemic oxidative stress (urinary F2-isoprostane excretion) was similar between wild-type and COMT−/− mice, whereas intrarenal oxidative stress was significantly lower in COMT−/− mice than wild-type mice (Figure 2). Western blotting with anti-4-hydroxynonenal antibody, another biomarker of oxidative stress, also demonstrated that DOCA/HS-induced renal oxidative stress was attenuated in COMT−/− mice (unpublished data). That COX-2 inhibition increased blood pressure without an increase in markers of intrarenal oxidative stress in COMT−/− mice suggests that the intrarenal oxidative stress in response to DOCA and/or high salt is not necessarily the result of increased blood pressure and that the ability of dopamine to reduce intrarenal oxidative stress is not necessarily only attributable to increased natriuresis and diuresis.

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; and (4) 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 vasorect 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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Disclosures

None.

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