Intrarenal Dopaminergic System Regulates Renin Expression

Ming-Zhi Zhang, Bing Yao, Xiaofeng Fang, Suwan Wang, James P. Smith, Raymond C. Harris

Abstract—Dopamine is a major regulator of proximal tubule salt reabsorption and is a modulator of renin release. Dopamine has been reported to stimulate renin release in vitro through activation of D1-like receptors. However, previous studies investigating dopamine regulation of renin release in vivo have provided contradictory results, indicating stimulation, inhibition, or no effect. We have reported previously that macula densa cyclooxygenase-2 (COX-2) is suppressed by dopamine. Because macula densa COX-2 stimulates renal renin expression, our current studies investigated dopamine regulation of renal renin release and synthesis in vivo. Acute treatment with a D1-like receptor agonist, fenoldopam, significantly inhibited renin release, as did acute inhibition of proximal tubule salt reabsorption with acetazolamide. In catechol-O-methyl transferase knockout (COMT−/−) mice, which have increased kidney dopamine levels because of deletion of the major intrarenal dopamine metabolizing enzyme, there was attenuation in response to a low-salt diet of the increases of renal cortical COX-2 and renin expression and renin release. A high-salt diet led to significant decreases in renal renin expression but much less significant decreases in COMT−/− mice than wild type mice, resulting in higher renal renin expression in COMT−/− mice. In high salt–treated wild-type mice or COX-2 knockout mice on a normal salt diet, fenoldopam stimulated renal renin expression. These results suggest that dopamine predominantly inhibits renal renin expression and release by inhibiting macula densa COX-2, but suppression of renal cortical COX-2 activity reveals a contrasting effect of dopamine to stimulate renal renin expression through activation of D1-like receptors. (Hypertension. 2009;53:564-570.)

Key Words: dopamine ■ renin ■ kidney ■ COX-2 ■ salt reabsorption ■ proximal tubule ■ macula densa

Although dopamine is an essential neurotransmitter, it also serves important physiological functions in the mammalian kidney. Dopamine is a major regulator of mammalian proximal tubule salt and water reabsorption.1 In the mammalian kidney, dopamine is primarily produced in the proximal tubule. The dopamine precursor l-dihydroxyphenylalanine (l-DOPA) is filtered at the glomerulus and is taken up by the proximal tubule via luminal transporters and converted to dopamine by aromatic L-amino acid decarboxylase, which is highly expressed in the proximal tubule. In the kidney, dopamine is metabolized predominantly by catechol-O-methyl transferase (COMT), with a smaller contribution by monoamine oxidase. Through activation of D1-like receptors, locally produced dopamine in the proximal tubule acts as an autocrine/paracrine natriuretic hormone by inhibiting activity of both apical (eg, Na/H exchange and chloride-bicarbonate exchange and Na-P cotransport) and basolateral (eg, Na-K-ATPase and Na-HCO3 cotransport) transporters.2–5

The renin-angiotensin system plays a pivotal role in the control of electrolyte and fluid balance and blood pressure. Under normal physiological conditions, renal renin release and synthesis are tightly regulated. In the kidney, renin is primarily expressed in the juxtaglomerular cells of afferent arterioles, and its release and expression are regulated by numerous factors, including variations in dietary salt intake, renal arteriolar tone, macula densa–derived signals, sympathetic nervous system activity, and hormones.6,7 The macula densa regulates afferent arteriolar tone and renin production and release by sensing alterations in luminal NaCl.8,9

Intrarenal dopamine has been proposed to be a modulator of renal renin release, but the effects of dopamine on renal renin release are still incompletely understood. Dopamine has been shown to increase renin release in vitro in cultured juxtaglomerular cells or kidney cortical sections through activation of D1-like receptors.10–12 However, the effect of dopamine on renin release in vivo is far from resolved. Different in vivo studies have reported that dopamine can increase, decrease, or have no effect on renin release.13–19 The contradictory effects of dopamine on renin release in vivo may be related to differences in experimental design. Theoretically, dopamine could regulate renal renin release in vivo through ≥3 different mechanisms: (1) direct stimulation via activation of D1-like receptors as demonstrated in the in vitro studies;10–12 (2) indirect stimulation by decreasing blood pressure and/or afferent arteriolar tone;20,21 or (3) indirect...
inhibition because of inhibiting proximal tubule salt reabsorption, with a subsequent increase of tubular NaCl delivery to the macula densa.8,9

Recent studies have suggested that cyclooxygenase-2 (COX-2) may represent another locally regulated system mediating renal salt and water homeostasis.9 In adult kidney cortex, COX-2 is predominantly restricted to the macula densa and adjacent cortical thick ascending limbs. Macula densa COX-2 stimulates renal renin expression and release.6,20,22

Cross-talk exists between intrarenal dopaminergic and COX-2 systems. Our previous studies suggested that intrarenal dopamine tonically suppresses COX-2 expression in the macula densa via modulation of salt and fluid reabsorption in the proximal tubule.23 Therefore, our current studies were undertaken to investigate dopamine regulation of renal renin release and synthesis in vivo and the potential role of macula densa COX-2 in dopamine regulation of renal renin synthesis and release. Our results suggest that dopamine predominantly inhibits renal renin expression and release, but there is a residual stimulation of renal renin expression through activation of D1-like receptors when renal cortical COX-2 activity is suppressed.

Methods

Animals

All of the animal experiments were performed in accordance with the guidelines of the Vanderbilt University Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (4 to 6 weeks old) were used, because renal cortical COX-2 expression is still relatively high at this age compared with adult animals.24 COX-2+/− mice on the 129/B6 background were originally generated by Dinchuk et al,25 and heterozygous breeding pairs were obtained from Jackson Laboratories (Bar Harbor, Maine). Mice were genotyped by PCR as noted in our previous reports.26 Wild-type and COMT−/− mice on the 129Isv background were obtained from Dr Maria Karayiorgou at Rockefeller University.27 Animals on low-salt diets received a single injection for 1-hour treatment or at a dose of 2 mg/kg per day via IP injection for 1-week treatment.28 In addition, a osmotic minipump (2001; Alzet) implanted SC under sterile conditions for molecular neuroscience research.29

Immunohistochemistry

At the termination of an experiment, 1 kidney from each animal was removed for Western blot analysis, and the other was perfused with fixative in situ for histology. Under deep anesthesia with Nembutal (70 mg/kg IP), the animal was first exsanguinated with heparinized saline (0.9% NaCl, 2 U/mL of heparin, and 0.02% sodium nitrite) through a transcardiac aortic cannula and then fixed with 3.7% formaldehyde in an acidic solution (pH 4.5) containing phosphate, periodate, acetate, and sodium chloride, as described previously.30

The fixed kidney was dehydrated through a graded series of ethanol, embedded in paraffin, sectioned (4 μm), and mounted on glass slides. Internal controls and comparisons were facilitated by creating compound blocks with multiple specimens that were sectioned and stained together. The kidney sections were immunostained with affinity-purified rabbit antiserum COX-2 antibody (160126, Cayman Chemicals) diluted to 2.5 μg/mL or with rabbit antirenin antiserum (1:6000 dilution, a generous gift from Prof T. Inagami, Vanderbilt University). Vectastain ABC-Elite was used to localize the primary antibodies with a chromogen of oxidized diaminobenzidine, followed by a light toluidine blue counterstain.

Quantitative Image Analysis

Based on the distinctive density and color of renin immunoreactivity in video images, the number, size, and position of stained cells were quantified using the BIOQUANT true-color windows system (R&M Biometrics) equipped with digital stage encoders that allow high-magnification images to be mapped to global coordinates throughout the whole section.24 Whole renal cortices from each section were quantified at ×160 magnification. Sections from ≥3 regions of each kidney were analyzed, and their average immunoreactive renin area/cortex area was used as data from 1 animal sample.

Immunoblotting

Renal cortex was Homogenized in 30 mmol/L of Tris-HCl (pH 8.0) and 100 mmol/L of phenylmethylsulfonyl fluoride (1:9 wt/vol). After a 10-minute centrifugation at 10 000g, the supernatant was centrifuged for 60 minutes at 110 000g to prepare microsomes, as described previously.31 The microsomes were resuspended in sodium dodecyl sulfate-sample buffer and heated to 100°C for 5 minutes, and the protein was separated on 8% SDS-PAGE gels under reducing conditions and transferred to Immobilon-P transfer membranes. The blot was blocked overnight with 100 mmol/L of Tris-HCl (pH 7.4), containing 5% nonfat dry milk and 0.1% Tween-20, followed by incubation for 16 hours with 1 μg/mL of affinity-purified rabbit antiserum COX-2 antibody. The primary antibodies were detected with peroxidase-labeled goat antirabbit IgG (Santa Cruz Biotechnology) and exposed on film by using enhanced chemiluminescence (Amersham).

Blood Collection and Determination of Plasma Renin Activity

Blood was taken from conscious mice via the femoral vein in the morning between 9 AM and 11 AM and collected into a microvette containing 2 μL of 125-mmol/L EDTA in its tip. The plasma was separated and frozen at −80°C until assayed. Plasma renin activity (PRA) was determined by radioimmunoassay (Gammacoat, Dia-Sorin) as the generation of angiotensin I (Ang I).32 Plasma samples were incubated for 1 hour with excess exogenous renin substrate (plasma from rats nephrectomized 48 hours before collection) to generate Ang I.

Micrography

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

Statistical Analysis

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

Acute Fenoldopam Treatment Inhibits Renin Release

To investigate dopamine regulation of renal renin release, adult mice (male, 129/B6) were treated with vehicle (water) or the D1-like receptor agonist fenoldopam (1 mg/kg, IP) for 1 hour, and then blood samples were collected for measurement of PRA. As shown in Figure 1A, acute fenoldopam treatment significantly reduced renal renin release (PRA: 1405±230 versus 2579±455 ng of Ang I/mL per hour of vehicle; P<0.05; n=5). Acute fenoldopam treatment also inhibited renin release in mice with low-salt diets for a week (PRA [ng of Ang I/mL per hour]: normal salt: 2363±473; low salt: 5853±1825, P<0.05 versus normal salt; low salt plus fenoldopam: 3295±232, P<0.05 versus normal salt and low salt; n=4; Figure 1B). One potential mechanism of fenoldopam-mediated inhibition of renin release is inhibition of proximal tubule salt reabsorption. In this regard, acute inhibition of proximal tubule salt reabsorption was induced by administering acetazolamide. As shown in Figure 1A, acute acetazolamide treatment (1 hour, 20 mg/kg, IP) mimicked fenoldopam to reduce renin release (PRA: 1236±400 versus 2579±455 ng of Ang I/mL per hour of vehicle; P<0.05; n=4). To investigate whether fenoldopam or acetazolamide had additive effects, a subset of mice was treated with acetazolamide (20 mg/kg) and fenoldopam (1 mg/kg). As shown in Figure 1A, acetazolamide and fenoldopam reduced renin release to similar levels seen in mice treated with acetazolamide or fenoldopam alone (PRA: 1325±153 ng of Ang I/mL per hour; P<0.05 versus acetazolamide- or fenoldopam-treated mice; n=4). In addition, inhibition of COX-2 activity with SC58236 (2 mg/kg) did not affect acetazolamide- or fenoldopam-induced inhibition of renin release (data not shown).

COMT−/− Mice Have Increased Endogenous Dopamine Levels in the Kidneys

In contrast to dopamine regulation of renin release, dopamine regulation of renal renin expression has been largely ignored. In our previous studies, we determined that either administration of the dopamine precursor, L-DOPA, or the D1-like agonist, SKF-81297, would inhibit macula densa COX-2 expression in the rats. Similar to COX-2 inhibition, our pilot studies indicated that renal renin expression was also inhibited in the rats treated with L-DOPA or SKF-81297 (data not shown). However, we were not able to rule out potential systemic or off-target actions of these pharmacological manipulations. COMT−/− mice, in which the major intrarenal

Figure 1. Acute fenoldopam treatment inhibited PRA. A, Acute treatment with fenoldopam or acetazolamide alone or both of them inhibited PRA similarly. *P<0.05 vs vehicle. B, Acute fenoldopam treatment also inhibited PRA in low-salt–treated animals. *P<0.05 vs normal salt; †P<0.05 vs low salt.

Figure 2. COMT−/− mice exhibited increased endogenous dopamine levels. Compared with age- and sex-matched wild-type mice, COMT−/− mice had increased kidney dopamine levels (A) and urinary dopamine excretion (B) but decreased renal levels of the dopamine metabolite 3-MT (C). Plasma dopamine concentrations were similar among wild-type and COMT−/− mice (D). *P<0.01 vs wild-type.
dopamine-metabolizing enzyme has been deleted, provide an alternative experimental system. As shown in Figure 2, COMT−/− mice had increased renal dopamine levels (184±14 ng/mg of protein of wild-type; \( P<0.01; n=6 \)) and increased urinary dopamine excretion (713±999 versus 381±1038 ng/24 hours of wild-type; \( P<0.01; n=6 \)), along with decreased renal levels of 3-MT, the major dopamine metabolite in the kidney (12±4 versus 35±8 ng/mg of protein of wild-type; \( P<0.01; n=6 \)). Plasma dopamine concentrations, however, were comparable between wild-type and COMT−/− mice (0.43±0.11 versus 0.38±0.10 pg/mL of wild-type; \( P>0.05; n=6 \)). Therefore, the observed elevations of renal dopamine levels in COMT−/− mouse kidney are the result of absent COMT metabolism of dopamine in the kidney.

### Low Salt–Induced Renal Cortical COX-2

#### Elevation Is Attenuated in COMT−/− Mice

COX-2 was not detectable by immunostaining in kidney cortex in adult control wild-type and COMT−/− mice on a normal-salt diet, consistent with previous reports. After low-salt treatment for 3 weeks, macula densa COX-2 expression was significantly higher in low-salt–treated wild-type mice than in low-salt–treated COMT−/− mice (Figure 3A), consistent with our previous report that low-salt–induced cortical COX-2 elevation was attenuated by increased intrarenal dopamine activity in the rats. Immunoblotting confirmed that renal cortical COX-2 expression was higher in low-salt–treated wild-type than in low-salt–treated COMT−/− mice (Figure 3B).

#### Low-Salt–Induced Increases in Renal Renin Synthesis and Release Are Attenuated in COMT−/− Mice

Renal renin expression was determined by immunostaining and was quantified with an image analysis system. As indicated in Figure 4A and 4C, in mice on a normal salt diet, renal renin expression was significantly lower in COMT−/− than in age- and gender-matched wild-type mice (renin-positive area/cortex area \( \times 10^{-3} \): 2.84±0.22 versus 3.84±0.29 of wild-type; \( P<0.05; n=4 \)), indicating that renal renin expression may be tonically suppressed by the intrarenal dopaminergic system. Low-salt treatment for 3 weeks led to significant increases in expression of renal renin (250%) in wild-type mice (renin-positive area/cortex area \( \times 10^{-3} \):...
Renin Expression in COX-2 Activation of D1-Like Receptor Stimulates Renal Renin

In further studies, wild-type mice on a normal salt diet or wild-type mice on a high-salt diet for the second week were treated with fenoldopam for 7 days. As indicated in Figure 5B, fenoldopam treatment inhibited renal renin expression in mice on a normal salt diet but increased renal renin expression in high-salt–treated mice (renin-positive area/cortex area ×10⁻³: control: 6.04±0.24; fenoldopam: 3.22±0.32, P<0.05 versus control; high salt: 1.50±0.36, P<0.05 versus control; high salt plus fenoldopam: 3.32±0.24, P<0.05 versus control and high salt; n=4), suggesting that dopamine may stimulate renal renin expression via activation of the D1-like receptor in volume expanded conditions. Immunoreactive renal cortical COX-2 was undetectable in both control and fenoldopam-treated animals on the high-salt diet (data not shown).

Activation of D1-Like Receptor Stimulates Renal Renin Expression in COX-2⁻/⁻ Mice

The observation that fenoldopam stimulates renal renin expression in the setting of suppressed COX-2 expression and

13.16±0.77; P<0.01 versus control wild type; n=4), but less significant increases (30%) in COX-2⁻/⁻ mice (renin-positive area/cortex area ×10⁻³: 4.30±0.30; P<0.05 versus control COX-2⁻/⁻ and P<0.01 versus low-salt–treated wild-type; n=4), resulting in a 270% increase in renal renin expression in low-salt–treated wild-type mice (Figure 4A through 4C). In wild-type mice, low-salt–induced increases in renal renin expression were attenuated by fenoldopam treatment for a week (renin-positive area/cortex area ×10⁻³: 8.25±0.48; P<0.05 versus control and low-salt–treated wild type; n=4). Similarly, low-salt treatment also led to significant increases in plasma renin levels (340%) in wild-type mice (PRA: 5703±1177 versus 1303±475 ng of Ang I/mL per hour of control; P<0.01; n=6) but less significant increases (130%) in COMT⁻/⁻ mice (PRA: 2744±747 versus 1203±309 ng of Ang I/mL per hour of control; P<0.01 versus control COMT⁻/⁻ and low-salt–treated wild-type; n=6; Figure 4D). Therefore, low-salt–induced increases in renal renin synthesis and release, as well as increases in renal cortical COX-2 expression, were attenuated in COMT⁻/⁻ mice.

Intrarenal Dopamine Stimulates Renal Renin Expression in High-Salt–Treated Animals

To investigate whether dopamine regulation of renal renin expression is influenced by dietary salt intake, COMT⁻/⁻ and wild-type mice were treated with a high-salt diet for 2 weeks, and renal renin expression was measured. High-salt treatment led to significant decreases in renal renin expression in wild-type mice (renin-positive area/cortex area ×10⁻³: 0.86±0.03 versus 3.84±0.29 of control wild-type; P<0.05; n=4) but less significant decreases in COMT⁻/⁻ mice (renin-positive area/cortex area ×10⁻³: 1.51±0.25 versus 2.84±0.22 of control COMT⁻/⁻; P<0.05; n=4), resulting in a 75% increase in renal renin expression in high-salt–treated COMT⁻/⁻ mice versus high-salt–treated wild-type mice (Figure 5A).
activity (high-salt treatment) was investigated further using COX-2−/− mice. As shown in Figure 5C, renal renin expression was much lower at baseline in COX-2−/− than corresponding wild-type mice on a normal salt diet. However, renal renin expression was significantly stimulated by fenoldopam in COX-2−/− mice, although levels were still significantly lower compared with those seen in wild-type mice (renin-positive area/cortex area × 10−3: wild-type: 8.61 ± 0.89; fenoldopam: 5.6 ± 1.0, P < 0.05 versus wild type; COX-2−/−: 0.27 ± 0.12, P < 0.05 versus wild-type; fenoldopam-treated COX-2−/−: 2.34 ± 0.21, P < 0.05 versus wild-type and untreated COX-2−/−; n = 4). To minimize any volume-depleting effect of fenoldopam, which could stimulate renin release,20 another group of fenoldopam-treated COX-2−/− mice had 0.5% NaCl added to the drinking water; this did not alter the fenoldopam-induced stimulation of renal renin expression.

Discussion

The current studies were undertaken to investigate the effects of the intrarenal dopaminergic system on renal release and synthesis. The major findings include the following: (1) acute treatment with the D1-like agonist fenoldopam inhibited renin release; (2) increases in renal cortical COX-2 and renin expression and renin release in response to a low-salt diet were attenuated in COMT−/− mice, which have increased intrarenal dopamine levels; (3) renal renin expression was higher in COMT−/− than in wild-type mice after high-salt treatment; and (4) the D1-like agonist fenoldopam stimulated renal renin expression in high-salt–treated wild-type mice or in COX-2−/− mice on a normal salt diet. Therefore, these results indicate that dopamine predominantly inhibits renal renin synthesis and release through its effects to inhibit COX-2 expression and activity, but previous inhibition of renal cortical COX-2 activity uncovers a smaller effect of dopamine to stimulate renin expression and release.

Dopamine receptors 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, whereas D2-like receptors (D2, D3, and D4) are coupled to Gi and inhibit adenylate cyclase.2–5 Previous studies indicated that dopamine could increase renin release in cultured juxtaglomerular cells or renal cortical sections through activation of D1-like receptors.10–12 However, the role of dopamine in the regulation of renin release in vivo is contradictory. Acute fenoldopam treatment has been reported to increase renin release in volunteers and in anesthetized dogs.19,21 In these experiments, increased renin release in response to acute fenoldopam treatment was associated with decreased blood pressure, suggesting that decreased blood pressure after acute fenoldopam treatment contributes to increased renin release. In contrast, in normal volunteers, acute gludopa (synthetic dipeptide γ-L-glutamyl-L-DOPA) treatment led to dramatic increases in urinary dopamine and sodium excretion without affecting blood pressure and led to significant suppression of renin release.13 Gludopa is enzymatically converted to L-DOPA locally by γ-glutamyltranspeptidase, an abundant enzyme in the brush border membrane of proximal tubule, and the L-DOPA is transported across renal brush border membrane and then further converted to dopamine by l-amino acid decarboxylase.34

Our previous studies indicated that macula densa COX-2 expression is suppressed by the intrarenal dopaminergic system.23 It is also noteworthy that we have reported previously that acetazolamide treatment inhibits macula densa COX-2 expression, even in the face of significant natriuresis and diuresis.21 In the current studies, macula densa COX-2 expression was also found to be significantly lower after low-salt treatment in COMT−/− mice than in wild-type mice. Acetazolamide’s diuretic actions are because of inhibition of carbonic anhydrase activity in the proximal tubule, which results in decreases in the proximal tubule salt reabsorption and increases in tubular NaCl delivery to the macula densa. Because acute acetazolamide treatment inhibited renin release, similar to what was observed with fenoldopam and in the COMT−/− mice, the overall inhibitory effect on renin release in mice with increased intrarenal dopamine production or by administration of a selective D1-like agonist underscores the importance of dopamine-mediated inhibition of proximal tubule salt reabsorption in renin release and indicates that dopamine inhibits renal renin expression by inhibiting macula densa COX-2 expression.

In contrast, in response to a high-salt diet, COMT−/− mice had significantly less inhibition of renal renin compared with wild-type mice, leading to a higher renal renin expression in high-salt–treated COMT−/− mice than in high-salt–treated wild-type mice. In high-salt–treated wild-type mice, fenoldopam also stimulated renal renin expression. Furthermore, fenoldopam stimulated renal renin expression in COX-2−/− mice on a normal salt diet. These studies indicate that dopamine can stimulate renal renin expression under conditions in which renal cortical COX-2 activity is already inhibited.

Therefore, our data suggest that the intrarenal dopaminergic system may regulate renal renin expression through ≥2 different mechanisms: indirect inhibition via decreasing proximal salt reabsorption and modulating macula densa COX-2 expression and activity23 and direct stimulation via activation of D1-like receptors.10–12 The overall effect of dopamine on renal renin expression may be a balance between the indirect inhibitory effects and direct stimulatory effects, with indirect inhibition being predominant in normal or volume-depleted conditions but a small direct stimulation becoming evident in volume-expanded conditions.

Clinical Perspectives

These studies point to the complexity and the tightly integrated control of renal regulation of net salt and water excretion and the renin-angiotensin II system. The predominant effect of the intrarenal dopaminergic system is to depress the renin-angiotensin system secondary to decreased proximal reabsorption and inhibition of macula densa–derived signals for renin expression and release. However, these studies elucidated a second effect of dopamine to stimulate renin, presumably by direct activation of juxtaglomerular renin production and release that is masked under normal conditions. Thus, the intrarenal effects of dopamine are, in many ways, a mirror image to those of angiotensin II, which
stimulate proximal reabsorption and, therefore, decrease NaCl delivery to the macula densa but also directly feed back on the juxtaglomerular apparatus to inhibit renin.

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Disclosures

None.

References

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