Cross talk between the intrarenal dopaminergic and cyclooxygenase-2 systems

Ming-Zhi Zhang,1 Bing Yao,1 James A. McKanna,2 and Raymond C. Harris1

George O’Brien Center for Kidney and Urologic Diseases, and 1Departments of Medicine and 2Cell and Developmental Biology, Vanderbilt University School of Medicine and Department of Veterans Affairs Medical Center, Nashville, Tennessee

Submitted 28 June 2004; accepted in final form 9 December 2004

Zhang, Ming-Zhi, Bing Yao, James A. McKanna, and Raymond C. Harris. Cross talk between the intrarenal dopaminergic and cyclooxygenase-2 systems. Am J Physiol Renal Physiol 288: F840–F845, 2005. First published December 21, 2004; doi:10.1152/ajprenal.00240.2004.—In mammalian kidney, dopamine produced in the proximal tubule (PT) acts as an autocrine/paracrine natriuretic hormone that inhibits salt and fluid reabsorption in the PT. In high-salt-treated animals, PT dopamine activity increases and inhibits reabsorption, leading to increased salt and fluid delivery to the macula densa (MD) and subsequent natriuresis and diuresis. Regulated cyclooxygenase-2 (COX-2) in the MD represents another intrinsic system mediating renal salt and fluid homeostasis. Renal cortical COX-2 is inversely related to salt intake, and decreased extracellular NaCl stimulates COX-2 expression in cultured MD/cortical thick ascending limb cells. The current study investigated interactions between renal dopamine and cortical COX-2 systems.

In rats fed a control diet, the dopamine precursor L-dihydroxyphenylalanine (L-DOPA) or the DA1 receptor agonist SKF-81297 suppressed cortical COX-2 expression. High salt suppressed cortical COX-2 expression, which was attenuated by inhibition of dopamine production with benserazide or the DA1 receptor antagonist, SCH-23390. In contrast, L-DOPA or the dopamine-metabolizing enzyme inhibitor entacapone suppressed low-salt-induced cortical COX-2 expression. Inhibition of PT reabsorption with the carbonic anhydrase inhibitor acetazolamide suppressed cortical COX-2 expression. In contrast, treatment with distally acting diuretics led to elevation of cortical COX-2. These results indicate that dopamine modulates renal cortical COX-2 expression by modifying PT reabsorption.

DOPAMINE IS A MAJOR REGULATOR of mammalian proximal tubule (PT) salt and water reabsorption by inhibiting activity of both apical (e.g., Na/H exchange and chloride bicarbonate exchange and Na-P cotransporter) and basolateral (e.g., Na-K-ATPase and Na-HCO3 cotransporter) transporters (1, 5, 16, 24, 26). The dopamine precursor L-dihydroxyphenylalanine (L-DOPA) is filtered at the glomerulus, absorbed, and converted to dopamine in the PT by amino acid decarboxylase (AADC), which is highly expressed in the PT. In the kidney, dopamine is metabolized by catechol-O-methyltransferase (COMT) and monoamine oxidase A (MAO).

Renal metabolized dopamine receptors are divided into two subclasses: DA1 and DA2 receptors. DA1 receptors (D1 and D5) are coupled to Gs and stimulate adenylate cyclase (AC). DA2 receptors (D2, D3, and D4) are coupled to Gi and inhibit AC. In the kidney, DA1 receptors are localized in the PT and renal vasculature and play a major role in mediating natriuresis. In addition, DA2 receptors are found in both vasculature and tubular structures. In high-salt (HS)-treated animals, dopamine activity in the PT increases; this increased dopamine activity inhibits net salt and fluid reabsorption in this segment, resulting in increased salt and fluid delivery to the cortical thick ascending limbs (cTAL) and macula densa (MD), and subsequent natriuresis (3). In contrast, dopamine activity in the PT decreases in low-salt (LS)-treated animals (2), resulting in increased salt and fluid reabsorption in the PT and reduced delivery of salt and fluid to cTAL and MD, and subsequent antidiuresis.

Prostaglandins also regulate renal salt and water homeostasis in mammals (12, 28, 31). Cyclooxygenase (COX) is a rate-limiting step for prostaglandin biosynthesis. Two distinct COX isoforms exist: a “constitutive” COX-1 and an “inducible” COX-2 (19, 23). In mammalian kidney, COX-1 is localized to arteries and arterioles, glomeruli, the collecting duct epithelium, and renal medullary interstitial cells (30). COX-2 is localized to macula densa/cTAL in the cortex and to a subset of interstitial cells in the medulla (13, 36–38). COX-2 is proposed to be the major COX isoform contributing to the regulated production of prostaglandins affecting renal vascular tone and salt and water homeostasis. COX-2 has two proposed functions in the cortex: dilation of afferent arterioles and control of renin secretion (12, 14). Renal cortical COX-2 is stimulated by LS, but suppressed by HS (13, 15, 34). In cultured rabbit MD/cTAL cells, COX-2 expression is stimulated by decreased concentrations of extracellular NaCl (9). However, the mechanisms underlying regulation of cortical COX-2 by alterations in dietary salt in vivo remain unclear. If COX-2 is also regulated by luminal NaCl in vivo, a change in PT reabsorption is expected to alter cortical COX-2 expression. As PT reabsorption is regulated by locally produced dopamine activity, the present study was designed to explore interactions between the intrarenal dopaminergic and COX-2 systems in response to alterations in salt intake.

MATERIALS AND METHODS

Animals. Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN). Male rats (4–6 wk old) were used, as renal cortical COX-2 expression is still relatively high compared with adult animals during this period and Na-K-ATPase in the PT is sensitive to dopamine inhibition (11, 39). The dopamine precursor L-DOPA was administered in the drinking water at a dose of 100 mg·kg−1·day−1. The AADC inhibitor benserazide (30 mg·kg−1·day−1), dopamine-metabolizing enzyme MAO inhibitor phenelzine (25 mg·kg−1·day−1), COMT inhibitor entacapone (50 mg·kg−1·day−1), DA1 receptor antagonist SCH-23390 (6 μg·kg−1·day−1), and DA2 receptor antagonist sulpiride (6 μg·kg−1·day−1) were administered via gastric lavage. The DA1 receptor agonist SKF-81297 (100 μg/ml in 0.9% NaCl) at a dose of 6 μg·kg−1·day−1 and proximal diuretic acetazolamide (5 mg/ml in 4.5% NaCl) were administered daily from 0600 to 2100 h via gastric lavage. Animals were fasted overnight, and blood samples were taken at 0800 h by cardiac puncture. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: R. C. Harris, C-3121 Medical Center North, Dept. of Medicine, Vanderbilt Univ., Nashville, TN 37232-4794 (E-mail: ray.harris@vanderbilt.edu).

http://www.ajprenal.org
are explained in RESULTS. To collect 24-h urine, the rats were acclimated with 1% NaCl in tap water. Combinations of the above protocols were performed for the entire image; i.e., no region- or object-specific editing was performed. Contrast and color level adjustment (Adobe Photoshop) were performed. BCA (bicinchoninic acid) protein assay reagent kit was used to determine protein concentration.

Table 1. Effects of l-DOPA, ACZ, HCTZ, and amiloride on UV, urinary Na and K excretion

<table>
<thead>
<tr>
<th></th>
<th>l-DOPA</th>
<th>ACZ</th>
<th>HCTZ</th>
<th>Amiloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV, ml/24 h</td>
<td>3.8±2.3</td>
<td>4.5±0.9</td>
<td>3.9±1.3</td>
<td>4.8±0.2</td>
</tr>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Na, μM/24 h</td>
<td>576±208</td>
<td>688±80</td>
<td>640±128</td>
<td>704±96</td>
</tr>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>K, μM/24 h</td>
<td>1,232±397</td>
<td>1,328±188</td>
<td>1,248±174</td>
<td>1,392±190</td>
</tr>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
</tbody>
</table>

Values are means ± SE. Animals were caged individually for 3 days. Control 24-h urine was collected on day 4. As shown in Table 1, renal cortical COX-2 expression was already decreased 12 h after initiation of treatment, and maximal inhibition was reached after treatment for 5 days. SKF-81297 was dissolved in normal saline. To investigate whether vehicle alone (normal saline) affected cortical COX-2 expression, the rats were treated with vehicle (0.9% NaCl, 0.15 ml each ip, twice a day) or SKF-81297 (0.15 ml each ip, twice a day) for 5 days. As shown in Fig. 1B, bottom, vehicle alone had no effect on cortical COX-2 expression, while SKF-81297 significantly suppressed cortical COX-2 expression.

Dopamine and cortical COX-2 in HS. Renal cortical COX-2 expression is suppressed by HS. Dopamine activity in the PT increases in HS-treated rats, and natriuresis induced by HS is attenuated by administering the AADC inhibitor benzerazide to reduce dopamine production (3). To test whether increased dopamine activity in the PT is involved in HS-induced renal cortical COX-2 suppression, the DA1 receptor antagonist SCH-23390, the DA2 receptor antagonist sulpiride, or the AADC inhibitor benzerazide was administered to HS rats. As shown in Table 1, renal cortical COX-2 expression was decreased progressively after l-DOPA treatment. As shown in Fig. 1A, renal cortical COX-2 expression was already decreased 12 h after initiation of treatment, and maximal inhibition was reached after treatment for 5 days. SKF-81297 was dissolved in normal saline. To investigate whether vehicle alone (normal saline) affected cortical COX-2 expression, the rats were treated with vehicle (0.9% NaCl, 0.15 ml each ip, twice a day) or SKF-81297 (0.15 ml each ip, twice a day) for 5 days. As shown in Fig. 1B, bottom, vehicle alone had no effect on cortical COX-2 expression, while SKF-81297 significantly suppressed cortical COX-2 expression.

Dopamine and cortical COX-2 in HS. Renal cortical COX-2 expression is suppressed by HS. Dopamine activity in the PT increases in HS-treated rats, and natriuresis induced by HS is attenuated by administering the AADC inhibitor benzerazide to reduce dopamine production (3). To test whether increased dopamine activity in the PT is involved in HS-induced renal cortical COX-2 suppression, the DA1 receptor antagonist SCH-23390, the DA2 receptor antagonist sulpiride, or the AADC inhibitor benzerazide was administered to HS rats. As shown in Table 1, renal cortical COX-2 expression was decreased progressively after l-DOPA treatment. As shown in Fig. 1A, renal cortical COX-2 expression was already decreased 12 h after initiation of treatment, and maximal inhibition was reached after treatment for 5 days. SKF-81297 was dissolved in normal saline. To investigate whether vehicle alone (normal saline) affected cortical COX-2 expression, the rats were treated with vehicle (0.9% NaCl, 0.15 ml each ip, twice a day) or SKF-81297 (0.15 ml each ip, twice a day) for 5 days. As shown in Fig. 1B, bottom, vehicle alone had no effect on cortical COX-2 expression, while SKF-81297 significantly suppressed cortical COX-2 expression.

Dopamine and cortical COX-2 in HS. Renal cortical COX-2 expression is suppressed by HS. Dopamine activity in the PT increases in HS-treated rats, and natriuresis induced by HS is attenuated by administering the AADC inhibitor benserazide to reduce dopamine production (3). To test whether increased dopamine activity in the PT is involved in HS-induced renal cortical COX-2 suppression, the DA1 receptor antagonist SCH-23390, the DA2 receptor antagonist sulpiride, or the AADC inhibitor benserazide was administered to HS rats. As shown in Table 1, renal cortical COX-2 expression was decreased progressively after l-DOPA treatment. As shown in Fig. 1A, renal cortical COX-2 expression was already decreased 12 h after initiation of treatment, and maximal inhibition was reached after treatment for 5 days. SKF-81297 was dissolved in normal saline. To investigate whether vehicle alone (normal saline) affected cortical COX-2 expression, the rats were treated with vehicle (0.9% NaCl, 0.15 ml each ip, twice a day) or SKF-81297 (0.15 ml each ip, twice a day) for 5 days. As shown in Fig. 1B, bottom, vehicle alone had no effect on cortical COX-2 expression, while SKF-81297 significantly suppressed cortical COX-2 expression.
shown in Fig. 2, HS-mediated cortical COX-2 suppression was attenuated by either an AADC inhibitor benserazide (BEN) or DA1 receptor inhibitor SCH-23390 (DA1 I), but not by DA2 receptor inhibitor sulpiride (DA2 I). A and B: representative Western blots. C: quantitative data. *P < 0.01 vs. control (CTRL). #P < 0.01 vs. HS, n = 6 in each group.

Fig. 3. Photomicrographs of renal cortical COX-2 immunostaining. A and B: renal cortical COX-2 expression was much higher in rats treated with HS plus an AADC inhibitor benserazide (B) than in rats treated with HS alone (A). C and D: high levels of cortical COX-2 expression in LS-treated animals (C) were significantly suppressed by adding L-DOPA (D; width: 400 μM, A and B; 160 μM, C and D).
COX-2 suppression by L-DOPA supplementation in LS-treated animals was attenuated by the DA1 receptor antagonist SCH-23390, but not by the DA2 receptor antagonist sulpiride [Re-nocortical COX-2 was expressed as COX-2-ir area/cortex area ($\times 10^{-3}$)]; LS: 4.45 ± 0.77; LS + L-DOPA: 0.47 ± 0.06, $P < 0.01$ vs. LS; LS + L-DOPA + SCH-23390: 1.73 ± 0.14, $P < 0.01$ vs. LS + L-DOPA; LS + L-DOPA + sulpiride: 0.40 ± 0.02; NS vs. LS + L-DOPA, n = 5 in each group).

**Diuretics and cortical COX-2.** To investigate whether pharmacological inhibition of PT reabsorption also regulates cortical COX-2 expression, rats were treated with acetazolamide, a carbonic anhydrase inhibitor that inhibits bicarbonate reabsorption and therefore sodium chloride and fluid reabsorption in the PT. For comparison, other animals were treated with either hydrochlorothiazide or amiloride, diuretics whose sites of action are distal to the macula densa (in distal convoluted tubule and cortical collecting duct, respectively). Urine volume and sodium excretion increased comparably in animals treated with each of the three diuretics (Table 1). However, as shown in Fig. 1, acetazolamide inhibited renal cortical COX-2 expression to a similar extent as L-DOPA, whereas hydrochlorothiazide and amiloride both stimulated cortical COX-2 expression (Fig. 1D).

**DISCUSSION**

In addition to its importance as a neurotransmitter, increasing evidence indicates that dopamine also has important biological effects in peripheral tissues, especially in the kidney. Hypotension, one of the most common side effects resulting from long-term treatment of Parkinson's disease with L-DOPA, is in large part secondary to dopamine's actions in the kidney (1, 4, 5, 16, 24). Locally produced dopamine in the proximal tubule acts via autocrine/paracrine DA1 receptor signaling to inhibit net renal salt and water reabsorption. Recent studies suggested that cortical COX-2 may represent another locally regulated system that mediates renal salt and water homeostasis via mediating afferent arteriole resistance and renin release and biosynthesis (12). The current study investigated interactions of renal dopamine and COX-2 systems and found that alteration of renal dopaminergic activity modulates renal cortical COX-2 expression both under control conditions and in responses to alterations in dietary salt intake.

Renal cortical COX-2 levels are inversely related to salt intake. In cultured MD/cTAL cells, COX-2 expression is stimulated by low medium NaCl (9). If low luminal NaCl reaching MD/cTAL in vivo also modulates cortical COX-2 expression, alterations of PT reabsorption and concomitant variations in luminal NaCl will be expected to modulate cortical COX-2 expression in MD/cTAL cells. In the kidney, locally produced dopamine inhibits net NaCl and fluid reabsorption in the PT (1, 5, 16, 24). Our model predicts that cortical COX-2 expression would be suppressed in HS-treated animals, because dopaminergic activity in the PT increases, resulting in decreased net NaCl and fluid reabsorption in the PT and increased luminal NaCl delivery to MD/cTAL. As shown in Figs. 2 and 3, HS-induced cortical COX-2 suppression was attenuated by administration of the AADC inhibitor benserazide to reduce dopamine production or the DA1 receptor antagonist SCH-23390. Conversely, cortical COX-2 expression is predicted to be stimulated in LS-treated animals because dopaminergic activity in the PT decreases, resulting in increased net salt and fluid reabsorption in the PT and decreased luminal NaCl delivery to MD/cTAL. As shown in Figs. 3 and 4, LS-induced cortical COX-2 expression was reversed by elevation of dopaminergic activity through administration of L-DOPA or a COMT inhibitor entacapone. LS-induced cortical COX-2 elevation was reversed by L-DOPA primarily via a DA1 signaling pathway. L-DOPA or the DA1 receptor agonist also suppressed basal cortical COX-2 expression in rats fed a normal diet. These results suggest that dopamine modulates cortical COX-2 expression by regulating proximal tubule reabsorption through a DA1 receptor-mediated pathway in response to alterations in dietary salt.

To elucidate further the interactions between PT reabsorption and cortical COX-2 expression, we compared the effects of diuretics that act by inhibiting sodium reabsorption in different nephron segments. Acetazolamide acts as a diuretic primarily due to its inhibition of carbonic anhydrase activity in the PT, resulting in inhibition of PT reabsorption and increased NaCl delivery to the MD/cTAL. Hydrochlorothiazide and amiloride inhibit sodium reabsorption distal to the MD/cTAL, in distal convoluted tubules and in connecting tubules and collecting tubules, respectively. As indicated in Fig. 1, acetazolamide suppressed cortical COX-2 expression (Fig. 1C), while both hydrochlorothiazide and amiloride stimulated cortical COX-2 expression (Fig. 1D), similar to spironolactone, another diuretic acting mainly in the collecting duct (37). Although loop diuretics may increase cortical COX-2 expression, in part, through direct inhibition of MD/cTAL Na-K-2Cl cotransport (21), the increased cortical COX-2 expression seen with hydrochlorothiazide, amiloride, or spironolactone presumably result from systemic volume depletion. As shown in Table 1, urine volume and sodium excretion increased comparably in acetazolamide-, hydrochlorothiazide-, and amiloride-treated animals, indicating comparable volume depletion. Therefore, in the acetazolamide-treated animals, the stimulating effect of volume depletion on cortical COX-2 expression appears to have been counteracted by increased luminal NaCl delivery to MD/cTAL due to inhibition of PT reabsorption.
The overall suppressive effect of acetazolamide on cortical COX-2 expression indicates the importance of PT salt and fluid reabsorption in the regulation of cortical COX-2 expression.

We suggest that the interactions between the intrarenal dopamine and cortical COX-2 may comprise important physiological and pathophysiological feedback regulation in responses to variations of salt intake. It has been reported that dopamine antagonizes the stimulatory effects of ANG II, a potent stimulator of proximal tubule salt and water reabsorption, via negative interaction between DA1 receptors and AT1 receptors (7, 35). Renal cortical COX-2 metabolites stimulate renin biosynthesis and release, leading to an increase in ANG II production (12, 27). The current finding that increased dopamine in the PT in HS-treated rats leads to downregulation of cortical COX-2 expression describes a pathway for dopamine to indirectly antagonize the renin-angiotensin system.

The effects of dopamine on renal renin release are complicated. Although the stimulatory effects of dopamine and DA1 receptor agonists are consistently demonstrable in studies in vitro, the effect of dopamine on renin secretion in vivo is far from resolved (33). Dopamine has been reported to increase, decrease, or not affect renin secretion in vivo. The apparent contradictory effects of dopamine on renin secretion in vivo may be related to differences in experimental design. Although our current results suggest that dopamine produced in the proximal tubule might indirectly antagonize renin secretion by inhibiting COX-2 expression, other direct effects on juxtaglomerular cells to stimulate renin release by dopamine of extrarenal origin cannot be ruled out.

Endogenous renal dopamine has been thought to affect largely, if not exclusively, tubule sodium reabsorption, but not renal vascular function (29). In the kidney, prostaglandins increase afferent arteriolar blood flow. The current finding that renal cortical COX-2 expression is modulated by dopamine activity in the PT suggests that endogenous dopamine may also play an indirect role in regulating afferent arteriolar blood flow. In HS-treated animals, increased dopamine activity in the PT results in a decrease in cortical COX-2 expression, and this reduced cortical COX-2 may allow the vasoconstrictive effect of tubuloglomerular feedback to prevent excessive salt and water excretion (12, 28). In LS-treated animals, reduced dopamine activity in the PT results in an increase in cortical COX-2 expression, and prostaglandins from this increased cortical COX-2 may counteract vasoconstrictors to maintain afferent arteriolar blood flow. Therefore, the intrarenal dopaminergic system and COX-2 system may act coordinately to maintain afferent arteriolar blood flow in response to variations of salt intake.

Recent studies suggested that abnormalities in the intrarenal dopaminergic system may be an underlying contributing factor to abnormal renal salt and water homeostasis in essential hypertension and in diabetes mellitus (1, 5, 16, 20, 22, 24, 32). Abnormalities in dopamine production and receptor function accompany a high percentage of human essential hypertension and several forms of rodent genetic hypertension (1, 5, 16, 24). Diabetes is characterized by elevated glomerular filtration rate and sodium retention due to reduced luminal NaCl delivery to the MD (20, 32). Reported impairment of the intrarenal dopaminergic system in diabetic patients and streptozotocin-induced diabetic rats could contribute to reduced luminal NaCl delivery at the MD (6, 20, 22, 32). The elevation of renal cortical COX-2 in diabetic patients and diabetic rats (8, 17, 18) is consistent with an impaired renal DA system and reduced luminal NaCl delivery to the MD (32). As renal cortical COX-2 expression appears to be tonically suppressed by the renal dopaminergic system, the interactions of the intrarenal dopamine and cortical COX-2 and their possible roles in the development of hypertension and diabetes mellitus are worthy of further investigation.

GRANTS

This work was supported by the Vanderbilt George O’Brien Kidney and Urologic Diseases Center, National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-39261 and DK-62794, and by funds from the Department of Veteran Affairs.

REFERENCES


