Cyclooxygenase-2 Inhibition Decreases Renin Content and Lowers Blood Pressure in a Model of Renovascular Hypertension

Jun-Ling Wang, Hui-Fang Cheng, Raymond C. Harris

Abstract—It has been proposed that the macula densa participates in the regulation of increased renin expression in renovascular hypertension (RVH) and that prostaglandins may be among the mediators of macula densa function. We have previously shown that in renal cortex, cyclooxygenase-2 (COX-2) expression is localized to the macula densa and surrounding cortical thick ascending limb and increases in high-renin states, such as salt restriction and angiotensin-converting enzyme inhibition. In the present studies, we examined the effect of the selective COX-2 inhibitor SC58236 on plasma renin activity (PRA) and renal renin expression in RVH in rats. The aorta was coarcted between right and left renal arteries, and animals received either SC58236 or vehicle for 1 week. At day 8, vehicle-treated coarcted rats were hypertensive (mean carotid arterial blood pressure: 138±3 versus 87±2 mm Hg in sham-operated controls; n=9 to 11; P<0.001) and exhibited a disparity of kidney size (ratio left/right kidney: 0.78±0.04 versus 1.02±0.02; n=9 to 10; P<0.001). PRA increased significantly (84.6±6.5 versus 9.0±1.4 ng angiotensin I [Ang I] per milliliter per hour; n=8 to 9; P<0.01). In the coarcted rats, neither renin mRNA expression nor renin activity of the right kidney was altered (renin/GAPDH mRNA: 1.12±0.05-fold levels in control rats; n=6; P=NS; renin activity: 23.4±1.8 versus 27.1±3.4 ng Ang I per hour per milligram protein; n=8 to 9; P=NS). However, the renin mRNA of the left kidney increased to 3.0±0.6-fold of control (n=6), and the renin activity increased to 189.0±28.6 ng Ang I per hour per milligram protein (n=6; P<0.01). Expression of COX-2 mRNA and immunoreactive protein increased in the affected left kidney but was not different from control in the unaffected right kidney. SC58236 treatment to coarcted rats did not affect kidney size (ratio left/right kidney: 0.79±0.06; n=9). However, PRA was significantly decreased compared with the vehicle-treated coarcted rats (19.8±2.8 ng Ang I per milliliter per hour; n=9; P<0.01). The left kidney renin mRNA and renin content were also decreased (1.7±0.3-fold control; n=6; P<0.05; and 45.7±7.6 ng Ang I per hour per milligram protein; n=9; P<0.01, respectively), while renin mRNA and renin content of the right kidney were not altered. SC58236 lowered mean arterial blood pressure (122±5 mm Hg; n=14; P<0.05 compared with vehicle). A significant correlation was observed between PRA and mean blood pressure (r=0.75; P<0.01). In summary, these studies indicate that the selective COX-2 inhibitor SC58236 decreases renin production and release in RVH and suggest an important role for COX-2 regulation of the renin-angiotensin system. (Hypertension. 1999;34:96-101.)

Key Words: renin ■ cyclooxygenase ■ hypertension, renal ■ macula densa ■ kidney

Both experimental models and clinical experience have indicated that prostaglandins are involved in the regulation of renal renin expression and release.1–4 In addition to physiological regulation of renin in response to alterations in intravascular volume status, prostaglandins have also been implicated in the mediation of increased renin production by the affected kidney(s) in renovascular hypertension (RVH).5,6 The source of these modulating prostaglandins has been in dispute, in part because of uncertainty about the mechanisms that regulate the abnormal renin production in RVH. Previous studies suggested that baroreceptors or abnormal sympathetic tone or both were the primary mediators of renin production and release in RVH,1 thereby implicating the local release of prostacyclin from afferent arterioles. However, more recent studies have suggested that macula densa signaling may also contribute to increased renin production in RVH.7,8

The conversion of arachidonic acid to prostaglandin H2 by prostaglandin G2/H2 synthase (cyclooxygenase) is a key enzymatic step in the regulation of prostaglandin synthesis.9 Two isoforms of cyclooxygenase (COX) exist: constitutive (COX-1) and inducible (COX-2).10,11 COX-1 is the most prevalent isoform in the mammalian kidney, and immunoreactive COX-1 has been localized to arteries and arterioles, glomeruli, and collecting ducts. However, no immunoreactive

Received November 17, 1998; first decision December 4, 1998; revision accepted February 18, 1999.

From the George M. O’Brien Kidney and Urologic Diseases Center and Division of Nephrology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tenn.

Correspondence to R.C. Harris, MD, Division of Nephrology, S 3322, MCN, Vanderbilt University School of Medicine, Nashville, TN 37232.

E-mail Ray.Harris@mcmail.vanderbilt.edu

© 1999 American Heart Association, Inc.

Hypertension is available at http://www.hypertensionaha.org
COX-1 is expressed in the cortical thick ascending limb of Henle (cTALH) or macula densa. In previous studies, we determined that COX-2 expression in the renal cortex was localized to occasional cells of the cTALH in the region of the macula densa. With salt depletion, COX-2 expression increased significantly in cTALH and in the macula densa. More recently, we have determined that administration of angiotensin-converting enzyme (ACE) inhibitors to animals on a normal diet also increased cTALH/macula densa COX-2 expression, and we have determined that a selective inhibitor of COX-2 decreased the increases in plasma and kidney renin activity and renal renin mRNA expression that result from ACE inhibition. Similarly, Harding et al have determined that COX-2 inhibitors inhibited renin increases in response to salt depletion. Recent studies by Hartner et al have indicated that COX-2 expression increased in the affected kidney in a model of 2-kidney, 1 clip hypertension and was associated with coordinate increases in renin expression. Therefore, the present studies were designed to determine whether, in an experimental model of RVH, inhibition of COX-2 activity altered renin expression and affected the associated hypertension.

Methods
Sprague-Dawley rats were from Harlan (Indianapolis, Ind). Anti–COX-2 antibodies were from Cayman. The selective COX-2 inhibitor SC58236, which exhibits a COX-2/COX-1 selectivity of 1780-fold, was a gift from Searle Monsanto Co. 32 P-CTP (3000 Ci/mmol) was from Amersham, angiotensin I (Ang I) (125) radioimmunoassay was from NEN, and rat renin cDNA was a gift of Kevin Lynch, University of Virginia. Other reagents were purchased from Sigma Chemical Co.

Animal Model and Experimental Procedures
Male Sprague Dawley rats, weighing 150 to 200 g, were divided into 3 groups: sham-operated rats were used as control; coarctated rats were gavaged with SC58236 (10 mg/kg body wt per day in 1% methylcellulose) or vehicle only for 7 days, beginning on the day after surgery. All experiments were performed within the guidelines of the Institutional Animal Care and Use Committee of Vanderbilt University School of Medicine. Surgical procedures were performed with the use of ketamine hydrochloride (Rampum) (9:1) anesthesia. RVH was produced by partial subdiaphragmatic aortic constriction modified from previously described techniques. Briefly, after median laparotomy, the abdominal aorta was isolated between the right and left exits of the renal artery. Cotton thread was used to constrict the aorta, with the extent of narrowing limited by a blunted 19-gauge needle (1 mm OD). At day 7, the left carotid artery was cannulated under ketamine hydrochloride/xylazine anesthesia, and blood pressure was determined with a blood pressure analyzer (Digi-Med) 1 hour after recovery from anesthesia. Mean blood pressure (MAP) was calculated as the average of 10 measurements. After measurement of MAP, blood was collected from the catheter for plasma renin activity (PRA) measurements, rats were killed, both kidneys were weighed separately, and tissue was processed as indicated below.

Renin Activity
At the time of death, blood was collected on ice in EDTA (1 mg/mL blood). The plasma was separated and frozen at −20°C until assayed. For renal tissue renin measurement, the kidneys were homogenized in 0.1 mol/L Tris-HCl, pH 7.4, containing 3.4 mmol/L 8-hydroxyquinolone sulfate, 0.25 mmol/L EDTA, 0.1 mmol/L phenylmethylsulfonyl fluoride, 1.6 mmol/L dimeracrol, 5 mmol/L sodium tetrathionate, and 0.1% Triton X-100. The concentration of protein was determined with a BCA protein assay kit (Pierce). After centrifugation of the homogenate, the supernatant was incubated for 1 hour with excess exogenous renin substrate (rat plasma obtained from rats nephrectomized 48 hours before collection). Renin was analyzed by radioimmunoassay with a (125)Ang I kit (NEN).

RNA Extraction and Northern Blotting
Renal cortex RNA was extracted by the acid guanidinium thiocyanate–phenol chloroform method, as described previously. RNA samples were electrophoresed in a denatured agarose gel and transferred to nitrocellulose membranes and hybridized with a 1.4-kb 32P-labeled BamHI/HindIII cDNA fragment of rat renin. The membranes were then stripped and rehybridized with GAPDH.

Immunoblotting
Renal cortex was homogenized in 30 mmol/L Tris-HCl, pH 8.0, and 100 μmol/L 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. The microsomes were resuspended in SDS-sample buffer and heated to 100°C for 5 minutes, and the protein was separated on 8% SDS gels under reducing conditions and transferred to Immobilon-P transfer membranes (Millipore). The blots were blocked overnight with 100 mL Tris-HCl, pH 7.4, containing 5% nonfat dry milk, 3% albumin, and 0.5% Tween-20, followed by incubation for 16 hours with rabbit anti-murine polyclonal antiserum to COX-2 (Cayman) at 2.5 μg/mL dilution. The second reagent, biotinylated goat anti-rabbit antibody, was detected with the use of avidin and biotinylated horseradish peroxidase (Pierce) and exposed on film with the use of enhanced chemiluminescence Western blotting detection reagents (ECL, Amersham).

Statistical Analysis
All values are presented as mean±SEM. ANOVA, Bonferroni t tests, and simple regression were used for statistical analysis, and differences were considered significant at P<0.05.

Results
After coarctation, the wet weight of the left kidney of the vehicle-treated animals decreased and the right kidney increased, with a resultant ratio of left kidney weight/right kidney weight of 0.78±0.04 (n=10). Treatment with the COX-2–specific inhibitor SC58236 did not affect this alteration in kidney weight (0.79±0.06; n=9; P=NS compared with vehicle) but was different from control (1.02±0.02; n=9; P<0.01) (Figure 1).

Renal cortical COX-2 expression was determined on day 7 after aortic coarctation. COX-2 mRNA expression in the affected left kidney, normalized to the housekeeping gene, GAPDH, was 180% of that in control animals (n=2), while COX-2 mRNA expression in the unaffected right kidney was 90% of control (Figure 2, top). Similarly, immunoreactive COX-2 protein expression was increased to 175% of control in the left kidney but was unchanged in the right kidney (n=2) (Figure 2, bottom). Of interest, treatment with the selective COX-2 inhibitor SC58236 partially blunted increases in COX-2 expression in the affected left kidney (Figure 2).

On day 7 after surgery, PRA was significantly increased in the vehicle-treated coarcted rats compared with controls (control versus coarctation: 9.0±1.4 versus 84.6±6.5 ng Ang I per milliliter per hour; n=8 to 9; P<0.001). Treatment of coarcted rats with SC58236 significantly decreased PRA compared with the vehicle-treated animals (19.8±2.8 Ang I
Renin activity was significantly elevated in the left kidney after coarctation compared with control (189.0 ± 28.6 versus 27.1 ± 3.4 ng Ang I per hour per milligram protein; n = 8 to 9; P < 0.01) and was numerically but not statistically significantly decreased in the right kidney (23.4 ± 1.8 ng Ang I per hour per milligram protein; P = NS). SC58236 treatment significantly decreased renin activity in the left kidney compared with the vehicle-treated rats (45.7 ± 7.6 ng Ang I per hour per milligram protein; n = 9; P < 0.01), although the levels were still significantly greater than those of controls (P < 0.01 compared with control). Right

Figure 1. Ratio of wet kidney weight (left/right) after 1 week of aortic coarctation. The experimental groups are sham operated (control), coarctation + vehicle, and coarctation + the COX-2 inhibitor SC58236; n = 9 to 10. **P < 0.01 compared with control.

Figure 2. COX-2 expression in renal cortex after 1 week of aortic coarctation. Representative experiments of COX-2 mRNA (top) and COX-2 immunoreactive protein (bottom) are presented. Lanes: 1, sham-operated control; 2, aortic coarctation, right kidney; 3, aortic coarctation, left kidney; 4, aortic coarctation + SC58236, right kidney; 5, aortic coarctation + SC58236, left kidney. Relative expression of GAPDH mRNA expression is provided for comparison (top).

Figure 3. Top, PRA after 1 week of aortic coarctation; n = 8 to 9. **P < 0.01. Middle, Renal renin activity after 1 week of aortic coarctation. Striped bars indicate the unaffected right kidney; solid bars, affected left kidney; n = 8 to 9. *P < 0.01. Bottom, Renal renin mRNA after 1 week of aortic coarctation; n = 6. **P < 0.01; *P < 0.05. Inset, Representative photo. Lane 1, control; lane 2, coarctation, right kidney; lane 3, coarctation, left kidney; lane 4, coarctation + SC58236, right kidney; lane 5, coarctation + SC58236, left kidney.
renal renin activity of SC58236-treated animals was unchanged (22.0±3.0 ng Ang I per hour per milligram protein; n=9; P=NS) (Figure 3, bottom).

To determine whether alterations in renal renin mRNA expression coincided with the observed alterations in renal renin activity and PRA, RNA was extracted from each kidney and hybridized with a rat renin cDNA probe and normalized to GAPDH mRNA expression. In the vehicle-treated coarcted rats, renal renin mRNA increased in the left kidney (3.0±0.6-fold control; n=6; P<0.01), while renin mRNA in right kidney did not change significantly (1.1±0.1-fold control; P=NS). Administration of SC58236 decreased renin mRNA levels in the left kidney to 1.7±0.6-fold control (n=6; P<0.05 compared with untreated), without modifying expression in the right kidney (1.2±0.1-fold control; P=NS) (Figure 3, middle).

Seven days after surgery, aortic coarctation led to significant increases in carotid MAP compared with control animals (138±3 versus 87±2 mm Hg; n=9 to 11; P<0.01). Administration of SC58236 significantly decreased MAP compared with the vehicle-treated coarcted rats (122±5 mm Hg; n=14; P<0.05) (Figure 4, top). Regression analysis of the 3 groups of animals indicated a significant correlation between MAP and PRA (r=0.75, P<0.01) (Figure 4, bottom).

**Discussion**

A role for prostaglandins in the mediation of hypertension has long been recognized, but initial observations concerning their potential mechanisms were somewhat contradictory. It is well recognized that in humans with essential hypertension, administration of aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs) can sometimes aggravate the hypertension. In contrast, chronic infusion of prostaglandin E2 in dogs led to renin-dependent hypertension. In dogs with significant renal artery stenosis, prostaglandin E2 production increased selectively from the stenotic kidney, and aspirin administration inhibited prostanoid synthesis and suppressed stimulation of renin release. In the aortic coarctation model of RVH in rats, the nonspecific cyclooxygenase inhibitor indomethacin also decreased PRA and decreased systemic blood pressure. Similarly, in humans with RVH, intravenous aspirin significantly reduced systemic blood pressure, while blood pressure was increased in patients with essential hypertension. Intravenous aspirin also decreased PRA in the subset of hyperreninemic essential hypertensives. Therefore, these data suggest that while NSAIDs may aggravate hypertension in non-renin-dependent hypertension, possibly by increasing salt retention, in renin-dependent hypertension, COX inhibition will decrease renin levels and ameliorate hypertension. The present studies demonstrate that similar effects are observed in RVH with selective inhibition of COX-2 activity and strongly suggest that the prostaglandins involved in regulation of increased renin production originate at least in part from the macula densa.

In the mammalian kidney, the macula densa consists of a cluster of morphologically unique tubular epithelial cells localized at the distal end of the thick ascending limb of Henle's loop. The macula densa participates in regulation of renin secretion and tubuloglomerular feedback. At low flow rates (functional volume depletion), sodium concentrations at the distal end of the ascending loop of Henle tubular fluid fall as low as 20 mEq/L, while at high flow rates, sodium concentrations may be as high as 60 to 80 mEq/L. By monitoring the salt concentration of the tubular fluid at the distal end of the loop of Henle, the macula densa thereby monitors glomerular filtration rate and absolute proximal reabsorption. Under physiologic conditions, glomerular filtration rate and absolute proximal reabsorption are dependent on, and reflections of, effective circulating volume, and the macula densa is situated to serve as both a sensor and an effector of total salt and volume homeostasis; however, pathophysiological decreases in renal blood flow secondary to renal artery stenosis may also be sensed by the macula densa as a decrease in effective circulating volume. Although the underlying mechanisms for the elevated renin activity in these states were previously attributed solely to baroreceptor activation of renin, recent studies by Kurtz and associates have shown that macula densa-mediated renin release is also involved in mediating the increased renin expression in the 2-kidney, 1 clip model of RVH. In addition, these investigators have demonstrated an important role for prostanoids in mediating the increased renin expression in this model.

In rat kidney, COX-2 expression is localized to scattered cTALH cells in the region of the macula densa, with occasional macula densa cells also expressing immunoreactive enzyme. In chronically salt-depleted animals, COX-2 expression in the macula densa and surrounding cTALH cells increases significantly. Recent studies in a 2-kidney, 1 clip model of RVH have also demonstrated increases in macula densa/cTALH cell expression of COX-2; in these studies, glomeruli with increased macula densa COX-2 expression...
also demonstrated increased juxtaglomerular renin expression.\textsuperscript{18} Similarly, in the present studies, aortic coarctation led to significant increases in renal cortical COX-2 mRNA and protein expression in the affected kidney, and selective inhibition of COX-2 significantly inhibited increases in PRA and renal renin activity and decreased renal renin mRNA expression. In addition, there was a partial but significant amelioration of the hypertension in response to COX-2 inhibition. As indicated in Figure 2, there also appeared to be a trend for SC58236 treatment to decrease COX-2 expression in the affected left kidney; further studies will be required to determine the mechanism underlying this observation.

In other studies in our laboratory, we have determined that administration of either an ACE inhibitor or an angiotensin type 1 receptor antagonist led to increases in macula densa and peri–macula densa COX-2 expression, suggesting that angiotensin II may downregulate cTALH/macula densa-derived prostanoids in the regulation of renin release in response to inhibition of angiotensin II production.\textsuperscript{16} In similar studies, Harding et al\textsuperscript{17} have determined that administration of NS398, a selective COX-2 antagonist, significantly inhibited increases in renin mRNA expression and renal renin activity in response to a low salt diet. Therefore, these studies indicate that prostanoids generated from COX-2 localized in the region of the macula densa play an important role in both the physiological and pathophysiological regulation of renal renin expression and release.

In our previous studies, we demonstrated that SC58236 decreased prostaglandin production in isolated glomeruli from remnant kidneys.\textsuperscript{23} Therefore, although we assume that renal COX-2 activity in vivo was also inhibited, actual demonstration of inhibition is somewhat problematic, because the usually accepted means of determination of inhibition of renal cyclooxygenase activity, measurement of urinary prostaglandins, undoubtedly measures predominantly distal nephron (COX-1) activity.

During the early stages of experimental RVH (1 to 2 weeks after surgery), hypertension is renin dependent, and nonselective NSAIDs have been reported to ameliorate the hypertension when administered at this time.\textsuperscript{5,20} In contrast, at later times, the hypertension is not solely or even predominantly renin dependent, and nonselective NSAIDs either had no effect on blood pressure or actually exacerbated the hypertension,\textsuperscript{32} similar to the effect of NSAIDs in the deoxycorticosterone acetate/salt model of volume expansion hypertension.\textsuperscript{20} Of interest, however, when 8-week 2-kidney, 1 clip costerone acetate/salt model of volume expansion hyperten-

production.\textsuperscript{16} In similar studies, Harding et al\textsuperscript{17} have determined that administration of NS398, a selective COX-2 inhibitor, significantly inhibited increases in renin mRNA expression and renal renin activity in response to a low salt diet. Therefore, these studies indicate that prostanoids generated from COX-2 localized in the region of the macula densa play an important role in both the physiological and pathophysiological regulation of renal renin expression and release.

In our previous studies, we demonstrated that SC58236 decreased prostaglandin production in isolated glomeruli from remnant kidneys.\textsuperscript{23} Therefore, although we assume that renal COX-2 activity in vivo was also inhibited, actual demonstration of inhibition is somewhat problematic, because the usually accepted means of determination of inhibition of renal cyclooxygenase activity, measurement of urinary prostaglandins, undoubtedly measures predominantly distal nephron (COX-1) activity.

used) decrease plasma renin in experimental RVH.\textsuperscript{5,20} However, unlike the findings of Jackson et al\textsuperscript{5} and Lin et al,\textsuperscript{20} who both reported that 7 days after aortic coarctation, indomethacin decreased PRA, Schricker et al\textsuperscript{3} reported that 48 hours after unilateral renal artery clamp, increases in renin mRNA in the affected kidney were inhibited by both indomethacin and meclofenamate, but PRA was not significantly decreased. It should be noted, however, that there was a trend for decreases in both PRA and blood pressure with meclofenamate, which is a relatively equipotent inhibitor of both COX-1 and COX-2, with an IC\textsubscript{50} for COX-2 of 9.7 μmol/L, but not with indomethacin, which has a reported IC\textsubscript{50} for COX-2 of 100 to >1000 μmol/L.\textsuperscript{34,35}

To date, published studies in humans have not localized renal cortical COX-2 to the macula densa region but to glomerular podocytes.\textsuperscript{35} However, studies with the selectively COX-2 inhibitors flurbiprofen and meloxicam have found that these compounds inhibited furosemide-stimulated renin release in normal volunteers, suggesting that in humans COX-2 is also responsible for prostaglandin synthesis mediating renin release.\textsuperscript{36,37} In this regard, furosemide-stimulated renin release is thought to be mediated primarily by inhibition of macula densa Na/K/2Cl cotransport.\textsuperscript{38}

In summary, the present studies indicate that in experimental RVH, administration of a selective inhibitor of COX-2 significantly decreased renal renin production and secretion. The decreases in plasma renin correlated with concomitant decreases in systemic blood pressure. These studies indicate that altered COX-2 expression may be an important mediator of renal renin generation under both physiological and pathophysiological conditions.

Acknowledgments
This work was supported by the Vanderbilt George O’Brien Kidney and Urologic Diseases Center (National Institutes of Health grant DK 39261), by a grant from Searle Monsanto (St Louis, Mo), and by funds from the Department of Veterans Affairs.

References
Cyclooxygenase-2 Inhibition Decreases Renin Content and Lowers Blood Pressure in a Model of Renovascular Hypertension
Jun-Ling Wang, Hui-Fang Cheng and Raymond C. Harris

*Hypertension*. 1999;34:96-101
doi: 10.1161/01.HYP.34.1.96

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/34/1/96

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

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

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