Renoprotective effects of neuronal NOS-derived nitric oxide and cyclooxygenase-2 metabolites in transgenic rats with inducible malignant hypertension

Matthew E. Patterson,1 John J. Mullins,2 and Kenneth D. Mitchell1
1Department of Physiology, Tulane University Health Sciences Center, New Orleans, Louisiana; and 2Centre for Cardiovascular Science, The University of Edinburgh Medical School, Edinburgh, United Kingdom

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Patterson ME, Mullins JJ, Mitchell KD. Renoprotective effects of neuronal NOS-derived nitric oxide and cyclooxygenase-2 metabolites in transgenic rats with inducible malignant hypertension. Am J Physiol Renal Physiol 294: F205–F211, 2008. First published October 31, 2007; doi:10.1152/ajprenal.00150.2007.—The present study was performed to determine the effects of neuronal nitric oxide synthase (nNOS) and cyclooxygenase-2 (COX-2) inhibition on blood pressure and renal hemodynamics in transgenic rats with inducible ANG II-dependent malignant hypertension [strain name: TGR(Cyp1a1Ren2)]. Male Cyp1a1-Ren2 rats (n = 7) were fed a normal diet containing indole-3-carbinol (I3C; 0.3%) for 6–9 days to induce malignant hypertension. Mean arterial pressure (MAP) and renal hemodynamics were assessed in pentobarbital sodium-anesthetized Cyp1a1-Ren2 rats before and during intravenous infusion of the nNOS inhibitor S-methyl-L-thiocitrul-line (t-SMTC; 1 mg/h). In hypertensive Cyp1a1-Ren2 rats, t-SMTC increased MAP from 169 ± 3 to 188 ± 4 mmHg (P < 0.01), which was a smaller increase than in noninduced rats (124 ± 9 to 149 ± 9 mmHg, P < 0.01, n = 5). Additionally, t-SMTC decreased renal plasma flow (RPF) to a similar extent (~34 ± 13 vs. ~35 ± 12% in the hypertensive and normotensive rats (4.1 ± 0.2 to 2.7 ± 0.5 and 3.1 ± 0.3 to 2.0 ± 0.3 ml min−1 g−1, respectively, P < 0.01) but did not alter glomerular filtration rate (GFR) in either group. In additional experiments, administration of the COX-2 inhibitor, nimesulide (3 mg/kg iv), during simultaneous infusion of t-SMTC decreased MAP in both hypertensive and noninduced rats (182 ± 2 to 170 ± 3 mmHg and 153 ± 3 to 140 ± 3 mmHg, respectively, P < 0.01). Nimesulide also decreased RPF (1.9 ± 0.2 to 0.8 ± 0.1 ml min−1 g−1, P < 0.01) and GFR (0.9 ± 0.1 to 0.4 ± 0.1 ml min−1 g−1, P < 0.01) in hypertensive rats but did not alter RPF or GFR in noninduced rats. The present findings demonstrate that both nNOS-derived NO and COX-2 metabolites exert pronounced renal vasodilator influences in hypertensive Cyp1a1-Ren2 rats. The data also indicate that the renal vasodilator effects of COX-2-derived prostanoids in hypertensive Cyp1a1-Ren2 rats are not dependent on nNOS activity.

nitric oxide (NO) and cyclooxygenase (COX) metabolites are mediators of various physiological processes and exert pronounced renal vasodilator influences to maintain renal hemodynamics. NO is important in maintaining vascular tone and acute inhibition of its production causes increases in blood pressure and renal vascular tone (27, 39, 47, 60). NO production is mediated by three isoforms of the enzyme NO synthase (NOS): endothelial (eNOS), inducible (iNOS), and neuronal (nNOS). The kidney constitutively expresses nNOS in the macula densa and efferent arterioles (1, 27, 37, 47, 60), and it has been demonstrated that nNOS-derived NO contributes importantly to the regulation of the renal hemodynamics (38, 55, 60, 61). Specifically, it has been shown that nNOS-derived NO acts to counteract tubuloglomerular feedback-mediated preglomerular vasoconstriction (21, 24). In addition, nNOS-derived NO enhances efferent, but not afferent, arteriolar responsiveness to ANG II in juxtamedullary nephrons (23, 38).

COX metabolizes arachidonic acid to various prostanoids (7, 40, 49, 56) and is generally thought to exist in both a constitutive form (COX-1) and an inducible form (COX-2) (7, 15, 16, 56). However, COX-2 has also been shown to be constitutively expressed in the kidney, specifically in the macula densa cells, the cortical thick ascending limb of Henle, and the intercalated cells of the cortical collecting duct (7, 15–17).

Similar to nNOS-derived NO, metabolites of COX-2 have vasodilatory actions that help to counteract the effects of ANG II-induced vasoconstriction (7, 15–17, 43, 48, 51, 54). The observation that both nNOS and COX-2 are expressed in the macula densa of the kidney has led to a variety of studies evaluating the potential interaction between products of COX-2 and nNOS and their role in regulating renal hemodynamics in normotensive and hypertensive states. However, controversy remains over the exact influences of nNOS and COX-2 on renal hemodynamics in ANG II-dependent forms of hypertension. Furthermore, uncertainty exists regarding the interactive effects of nNOS and COX-2 on renal hemodynamics in ANG II-dependent forms of hypertension, in particular, in ANG II-dependent malignant hypertension.

Malignant hypertension is a severe form of hypertension characterized by rapidly increasing blood pressure, severe renal vasoconstriction and ischemia, activation of the renin-angiotensin system, and renal injury which consists of glomerulosclerosis, inflammation, and cellular proliferation in the cortical vessels and tubulointerstitium, and fibrinoid necrosis (14, 26, 58, 59). A transgenic rat line [strain name: TGR(Cyp1a1Ren2)] was created that allows the induction of ANG II-dependent malignant hypertension (25). This transgenic rat line was generated by inserting the mouse Ren2 renin gene, fused to the cytochrome P-450 1a1 (Cyp1a1) promoter, into the genome of the Fischer 344 rat (25). Induction of the Cyp1a1 promoter by dietary administration of the aryl hydrocarbon, indole-3-carbinol (I3C), drives hepatic expression of the Ren2 renin gene and results in the development of ANG...
II-dependent malignant hypertension (14, 25, 33, 35, 43, 44, 46). We recently demonstrated that COX-2-derived vasoconstrictor prostanoids contribute importantly to the elevated arterial blood pressure in Cyp1a1-Ren2 rats with malignant hypertension (43). These studies also showed that COX-2-derived vasodilatory metabolites play an important role in the maintenance of renal hemodynamics following induction of malignant hypertension in Cyp1a1-Ren2 rats (43). However, little information exists regarding the potential influence of nNOS-derived NO on blood pressure and renal hemodynamics in ANG II-dependent malignant hypertension. Similarly, the interactive effects of nNOS-derived NO and COX-2 metabolites on renal blood pressure and hemodynamics in ANG II-dependent malignant hypertension remain unclear.

The present study was performed to determine the effects of nNOS inhibition on arterial blood pressure and renal hemodynamics during the developmental phase of malignant hypertension in Cyp1a1-Ren2 rats. Given the uncertainty regarding the potential interactive influences of nNOS and COX-2 on renal hemodynamics in ANG II-dependent malignant hypertension, an additional objective was to determine whether the renoprotective effects of COX-2 metabolites are dependent on nNOS activity in Cyp1a1-Ren2 rats with malignant hypertension.

METHODS

The experimental protocol used in this investigation conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee of Tulane University Health Sciences Center. Experiments were performed on four groups of age-matched male Cyp1a1-Ren2 transgenic rats, weighing between 250 and 350 g, bred at Tulane University School of Medicine from stock animals supplied from the University of Edinburgh, Scotland. In two of the groups, male Cyp1a1-Ren2 rats (n = 7) were fed normal rat food containing the aryl hydrocarbon I3C (0.3% wt/wt; diet TD 00554, Harlan-Teklad, Madison, WI) for 6–9 days to induce malignant hypertension (14, 25, 33, 35, 43, 44). In the other two groups (n = 5), male Cyp1a1-Ren2 rats fed normal rat food (diet TD 90229, Harlan-Teklad), which did not contain I3C, served as normotensive controls. Renal clearance experiments were performed on all rats. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and placed on a thermostatically controlled surgical table to maintain body temperature at 37°C. A tracheostomy was performed and the animals were allowed to breathe air enriched with oxygen, which has been shown to improve the stability of arterial blood pressure of pentobarbital anesthetized rats (34–36). This procedure does not induce oxidative stress in anesthetized normotensive rats surgically prepared for renal clearance experiments (46). The left femoral artery was cannulated to allow continuous monitoring of arterial blood pressure. Blood pressure was monitored with a Statham pressure transducer (model P23 DC) and recorded using a computerized data-acquisition system (MP100 system; BIOPAC Systems, Santa Barbara, CA) with the Acqknowledge software package (version 3.2.4, BIOPAC). The left external jugular vein was cannulated to allow intravenous infusion of solutions and additional anesthesia. The rats were infused intravenously, at a rate of 1.2 ml/h, with isotonic saline containing 6% albumin (bovine; Calbiochem, San Diego, CA) during the surgery and thereafter with isotonic saline containing 1% albumin, 7.5% polyfructosan (Inustet, Lentz, Austria), and 1.5% para-aminophenipic acid (PAH; Merck, Whitehouse Station, NJ). A suprapubic incision was made and the bladder was exposed by blunt dissection through the abdominal wall. The bladder was catheterized to allow timed urine collections to be made. Following the surgery, the rats were allowed to stabilize for 1 h.

In the first series of experiments with one group of control animals (n = 5) and one group of rats induced with I3C (n = 7), the experimental protocol consisted of two 30-min control urine collections after which the selective nNOS inhibitor, S-methyl-l-thiocitruline (l-SMTC; Sigma, St. Louis, MO), was infused intravenously at a rate of 1 mg/h. l-SMTC has been shown to be ~17-fold more selective for nNOS compared with eNOS (13). The dose of l-SMTC used in the present study was chosen to approximate the dose (10 μM) that was found to elicit pronounced and selective blockade of nNOS in the in vitro blood-perfused juxtamedullary nephron preparation (20–24). Following a 30-min stabilization period, two additional 30-min urine collections were obtained. In a second series of experiments with a group of noninduced control rats (n = 5) and a group of I3C-induced rats (n = 7), after the surgical preparation and a 60-min stabilization period, animals were infused with l-SMTC at 1 mg/h for ~30 min, followed by two 30-min urine collections. Subsequently, during continued administration of l-SMTC, the rats received a single intravenous bolus of the selective COX inhibitor, nimesulide (3 mg/kg; Sigma). Nimesulide has been shown to be highly selective for the COX-2 enzyme (18, 48, 52) and we previously demonstrated that this dose of nimesulide elicits substantial blockade of COX-2 in Cyp1a1-Ren2 rats with malignant hypertension (43). After a 30-min equilibration period, two additional 30-min urine collections were obtained. In all experiments, the rate of intravenous fluid administration was maintained constant at 1.2 ml/h. Arterial blood samples (~300 μl) were obtained after the second and fourth urine collection to allow determination of whole kidney hemodynamics. At the end of each experiment both kidneys were removed, decapsulated, blotted dry, and weighed.

Urine volume was determined gravimetrically. Inulin and PAH concentrations in both urine and plasma were measured by standard spectrophotometry. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were estimated from the clearance of inulin and PAH, respectively. Renal blood flow was calculated as RPF/(1 – hematocrit). Renal vascular resistance (RVR) was determined from the quotient of mean arterial pressure (MAP) and calculated renal blood flow. Statistical analyses were performed using one-way ANOVA, one-way repeated-measures ANOVA, and two-way ANOVA with one-factor repeated followed by Tukey’s test when appropriate. Statistical significance was defined as P < 0.05. All data are expressed as means ± SE.

RESULTS

Chronic administration of 0.3% I3C in Cyp1a1-Ren2 rats (n = 7) for 7–9 days resulted in the development of severe hypertension (169 ± 3 vs. 124 ± 9 mmHg, P < 0.01; Fig. 1A). The development of hypertension was associated with a 17% reduction in body weight (from 266 ± 14 to 222 ± 14 g, P < 0.01), and rats induced with I3C exhibited reduced body weight compared with noninduced rats (222 ± 14 vs. 294 ± 12 g, P < 0.01). As previously observed (14, 25, 33, 35, 43, 44, 46), the hypertensive rats also demonstrated severe lethargy, polyuria, and adoption of hunched posture, which are clinical manifestations of malignant hypertension in the rodent (26, 33, 58, 59). These observations confirm that dietary administration of 0.3% I3C induced malignant hypertension in the Cyp1a1-Ren2 transgenic rats.

MAP responses to the intravenous administration of the nNOS inhibitor, l-SMTC, are shown in Fig. 1A. Intravenous administration of l-SMTC (1 mg/h) elicited an increase in MAP in both induced and noninduced Cyp1a1-Ren2 rats. In hypertensive Cyp1a1-Ren2 rats, l-SMTC increased MAP from
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Fig. 1. A: effects of 5-methyl-L-thiocitrulline (L-SMTC; 1 mg/h) on mean arterial pressure of noninduced Cyp1a1-Ren2 transgenic rats (filled bars) and Cyp1a1-Ren2 rats induced with 0.3% indole-3-carbinol (13C) for 6-9 days (hatched bars). *P < 0.01 vs. control. †P < 0.01 vs. noninduced. B: effects of nimesulide (3 mg/kg) on mean arterial blood pressure in noninduced (filled bars) and induced Cyp1a1-Ren2 rats (hatched bars) infused with L-SMTC (1 mg/h). *P < 0.01 vs. L-SMTC alone. †P < 0.01 vs. noninduced.

169 ± 3 to 188 ± 4 mmHg (P < 0.01, n = 7), which was a significantly smaller (P < 0.05) increase than in the noninduced normotensive control rats (124 ± 9 to 149 ± 9 mmHg, P < 0.01, n = 5; Fig. 1A). Figures 2A and 3A illustrate the GFR and RPF responses to intravenous administration of L-SMTC. As shown in Fig. 2A, baseline GFR was similar between the normotensive and hypertensive rats (1.19 ± 0.12 vs. 0.96 ± 0.12 ml min⁻¹ g⁻¹) and was not altered during L-SMTC administration in either group. In contrast, hypertensive Cyp1a1-Ren2 rats had a lower basal RPF than noninduced rats (3.1 ± 0.39 vs. 4.1 ± 0.2 ml min⁻¹ g⁻¹, P < 0.01; Fig. 3A). As shown in Fig. 3A, intravenous infusion of L-SMTC reduced RPF in both normotensive (4.1 ± 0.2 to 2.7 ± 0.5 ml min⁻¹ g⁻¹, P < 0.05) and hypertensive rats (3.1 ± 0.3 to 2.0 ± 0.3 ml min⁻¹ g⁻¹, P < 0.05). L-SMTC reduced RPF to a similar extent in the hypertensive and normotensive rats (~34 ± 13 vs. ~35 ± 12%). Consistent with previous findings (33, 43, 46) and as shown in Fig. 4A, the hypertensive rats exhibited a markedly elevated RVR (26 ± 3 vs. 13 ± 1 mmHg ml⁻¹ min⁻¹ g⁻¹, P < 0.01). Figure 4A also illustrates the effects of intravenous administration of L-SMTC on RVR in hypertensive and normotensive rats. L-SMTC markedly increased RVR in both normotensive (13 ± 1 to 33 ± 6 mmHg ml⁻¹ min⁻¹ g⁻¹, P < 0.01) and hypertensive rats (26 ± 3 to 47 ± 6 mmHg ml⁻¹ min⁻¹ g⁻¹, P < 0.01).

The second series of experiments evaluated the effects of administration of the selective COX-2 inhibitor, nimesulide (3 mg/kg), in induced and noninduced Cyp1a1-Ren2 rats pretreated with the nNOS inhibitor L-SMTC. In these experiments, rats receiving intravenous infusion of L-SMTC exhibited similar values for MAP, GFR, RPF, and RVR to those observed in the first series of experiments. As shown in Fig. 1B, subsequent administration of nimesulide reduced MAP in both noninduced and induced rats (from 153 ± 3 to 140 ± 3 and from 182 ± 2 to 170 ± 3 mmHg, respectively, P < 0.01 in both cases). As shown in Figs. 2B and 3B, administration of nimesulide during continued nNOS blockade elicited substantial decreases in GFR (0.9 ± 0.1 to 0.4 ± 0.1 ml min⁻¹ g⁻¹, P < 0.01) and RPF (1.9 ± 0.2 to 0.8 ± 0.1 ml min⁻¹ g⁻¹, P < 0.01) in hypertensive Cyp1a1-Ren2 rats, but not in noninduced normotensive rats. As shown in Fig. 4B, subsequent administration of nimesulide to L-SMTC-treated rats increased RVR from 44 ±
The present study examined the effects of inhibition of nNOS and COX-2 on blood pressure and renal function in Cyp1a1-Ren2 transgenic rats with ANG II-dependent malignant hypertension. As previously observed, induction of the Ren2 renin gene by dietary administration of 0.3% I3C for 6–9 days resulted in the development of malignant hypertension and elevated RVR (33, 43, 46). Administration of the nNOS inhibitor, L-SMTC, caused pronounced increases in MAP and RVR in both hypertensive and noninduced normotensive Cyp1a1-Ren2 rats. L-SMTC also reduced RPF to a similar extent in the hypertensive and normotensive rats. Administration of the COX-2 inhibitor, nimesulide, during continued nNOS blockade decreased MAP in both normotensive and hypertensive rats. Additionally, COX-2 inhibition following nNOS blockade decreased RPF and GFR and increased RVR in hypertensive rats but not in normotensive rats.

Despite the markedly elevated arterial pressure, hypertensive Cyp1a1-Ren2 transgenic rats exhibited normal values of GFR. The hypertensive rats also exhibited reduced RPF and increased RVR. These findings are consistent with our previous observations that GFR and RPF are maintained within or slightly below the normal range and confirm that preglomerular vascular resistance is significantly elevated in Cyp1a1-Ren2 transgenic rats with malignant hypertension (33, 35, 43, 46). The present study does not allow determination of the relative contribution of the direct pre- and postglomerular vasoconstrictor effects of ANG II and the autoregulatory response to the increased arterial pressure to the maintenance of normal GFR and the reduced RPF in Cyp1a1-Ren2 rats with malignant hypertension.

Administration of the selective nNOS inhibitor, L-SMTC, elicited pronounced increases in MAP in both hypertensive and noninduced normotensive rats. However, the magnitude of the L-SMTC-induced increase in MAP was smaller in the hypertensive rats than in the normotensive rats. This indicates that the influence of nNOS-derived NO to counteract the prohypertensinogenic actions of ANG II to increase arterial pressure is significantly reduced in Cyp1a1-Ren2 rats with malignant hypothesi0s.
hypertension. Although it is not generally recognized that nNOS-derived NO plays an important systemic vasodilatory role, it has been demonstrated that nNOS-derived NO suppresses sympathetic outflow and that inhibition of nNOS causes elevated MAP by removing this inhibition on sympathetic tone (28, 41, 53). In addition, it has been shown that ANG II inhibits expression of nNOS in the brain (4, 63). Thus, in a setting of severe ANG II-dependent hypertension, blockade of nNOS could further increase sympathetic nerve activity which could lead to further elevations in blood pressure. Furthermore, inhibition of nNOS has been shown to alter cerebral blood flow (50, 62), which could also influence sympathetic tone and induce alterations in blood pressure. Therefore, it is possible that the L-SMTC-induced elevations in MAP could have resulted, at least in part, from removal of the effects of nNOS-derived NO to suppress sympathetic outflow. Regardless of the specific mechanism, however, the present findings indicate that the peripheral vasodilatory influence of nNOS-derived NO is reduced following induction of malignant hypertension in Cyp1a1-Ren2 transgenic rats. Such a reduced vasodilatory influence of nNOS-derived NO would likely act to exacerbate the peripheral vasoconstrictor effects of ANG II and, thereby, contribute to the hypertension.

We recently demonstrated that Cyp1a1-Ren2 rats with malignant hypertension exhibit elevated urinary excretion of 8-isoprostan e and that systemic administration of tempol decreased arterial blood pressure and the urinary excretion of 8-isoprostane in hypertensive but not normotensive rats (46). These findings indicate that Cyp1a1-Ren2 rats with malignant hypertension exhibit pronounced oxidative stress and that increased superoxide anion levels contribute to the elevated arterial blood pressure. In these experiments, systemic administration of the nonselective NOS inhibitor, nitro-L-arginine (NLA), attenuated the decrease in arterial blood pressure induced by tempol administration in the hypertensive rats (46). This indicates that the elevated arterial blood pressure in Cyp1a1-Ren2 rats with malignant hypertension is due in part to a superoxide anion-mediated reduction in NO bioavailability. Thus it seems likely that enhanced degradation of NO by elevated superoxide anion levels contributed to the diminished blood pressure responses to NOS inhibition in Cyp1a1-Ren2 rats with malignant hypertension.

Previous studies reported that overall intrarenal NOS expression and NO production are increased in various models of hypertension, including renovascular hypertensive rats, spontaneously hypertensive rats, and ANG II-infused hypertensive rats (11, 19, 57). In addition, it has been demonstrated that enhanced NO formation counteracts the vasoconstrictor actions of ANG II in ANG II-dependent forms of hypertension (10, 38). Similarly, it has been shown that there is increased immunoreactivity of nNOS in the kidney of spontaneously hypertensive rats (12) and ANG II-dependent hypertensive rats (9, 38). However, despite an increased overall intrarenal NO production, ANG II-infused hypertensive rats exhibit an impaired ability to produce NO by nNOS (5, 22). In addition, it has been demonstrated that during the development phase of hypertension, TGR(mRen2)27 transgenic rats exhibit an impaired renal vascular responsiveness to blockade of nNOS with L-SMTC despite enhanced expression of nNOS mRNA in the renal cortex (6). Furthermore, it has been demonstrated that nNOS expression is elevated in the renal cortex of ANG II-infused hypertensive rats (9, 38) despite the fact that renal vascular responsiveness to selective nNOS blockade is attenuated in ANG II-infused hypertensive rats (5, 20). Collectively, such findings have demonstrated that renal vascular responsiveness to nNOS-derived NO can be impaired even under conditions in which expression of nNOS in the kidney is increased, and they indicate that impaired renal vasodilator influence of nNOS-derived NO contributes to the development of ANG II-dependent hypertension. In the present study, however, inhibition of nNOS with L-SMTC led to marked increases in RVR and comparable decreases in RPF in hypertensive and normotensive Cyp1a1-Ren2 transgenic rats. Thus the current findings confirm previous observations that nNOS-derived NO exerts important renal vasodilator effects under basal normotensive conditions. However, in contrast to previous studies, the present data demonstrate that the renal vasodilator influence of nNOS-derived NO is maintained in Cyp1a1-Ren2 transgenic rats with ANG II-dependent malignant hypertension. Such a maintained vasodilator influence of nNOS-derived NO exerts a renoprotective influence that likely acts to prevent excessive renal vasoconstriction and maintain renal hemodynamics during the development of ANG II-dependent malignant hypertension in Cyp1a1-Ren2 rats.

Several studies demonstrated a role for constrictor prostanoids in the development of ANG II-dependent hypertension (32, 45). It has been demonstrated that ANG II stimulates the release of vasoconstrictor prostanoids such as TxA2 and PGH2 (29, 30, 42, 45) and that treatment with a TxA2 receptor blocker lowers blood pressure in ANG II salt-induced hypertensive rats (32). Similarly, chronic administration of ANG II has been shown to stimulate TxB2 synthesis as evidenced by increased urinary excretion of TxB2 in ANG II-dependent hypertension (31). More recently, we demonstrated that COX-2 blockade with nimesulide normalized arterial blood pressure of Cyp1a1-Ren2 rats with malignant hypertension (43). Subsequent administration of the nonselective COX inhibitor, meclofenamate, further reduced blood pressure of the hypertensive rats indicating that the elevated arterial blood pressure in Cyp1a1-Ren2 rats with malignant hypertension is mediated, in part, by vasoconstrictor prostanoids generated by both the COX-1 and COX-2 enzymatic pathways (43). The present finding that administration of nimesulide, during continued nNOS blockade with L-SMTC, decreased MAP in hypertensive Cyp1a1-Ren2 rats confirms that COX-2-derived vasoconstrictor prostanoids contribute importantly to the elevated arterial pressure in this form of ANG II-dependent malignant hypertension.

The administration of the selective COX-2 inhibitor, nimesulide, decreased RPF and GFR and increased RVR in hypertensive rats but not in normotensive rats. Opay et al. (43) obtained similar findings when comparing the effects of COX-1 and COX-2 blockade on renal hemodynamics in Cyp1a1-Ren2 rats with malignant hypertension. In that study, COX-2 blockade elicited pronounced reductions in RPF and GFR and increases in RVR. Subsequent administration of the nonselective COX inhibitor, meclofenamate, did not lead to any further alterations in renal hemodynamics (43). These results, together with the findings of the current study, suggest that COX-2, not COX-1, is the predominant isoform responsible for producing vasodilatory prostanoids responsible for...
maintaining renal hemodynamics in the setting of ANG II-dependent malignant hypertension.

Given that both nNOS and COX-2 have been shown to be expressed in the kidney, specifically in the macula densa (1, 7, 15–17, 27, 37, 46, 60), it is likely that the products of these enzymes influence renal hemodynamics, and in doing so may interact with one another. Indeed, previous studies indicated an interaction between the activities of nNOS and COX-2 in the regulation of renal function. One such study demonstrated that nNOS provides a positive stimulus to COX-2 expression in high renin states (8). Another study showed that pretreatment with 1-SMTC prevented the afferent arteriolar constrictor response to COX-2 inhibition (21), suggesting COX-2 activity is dependent on nNOS. However, Beierwaltes (3) showed that COX-2 blockade elicited renal vasoconstriction only after nonselective NO inhibition but did not alter renal hemodynamics after selective blockade of nNOS. Similarly, Baylis et al. (2) demonstrated that nonselective COX inhibition induces renal vasoconstriction only after nonselective NO inhibition, but did not elicited alterations in renal hemodynamics under normal conditions, indicating that prostanoid-mediated renal vasodilation occurred only in the absence of NO. These studies suggest that eNOS-derived NO is the primary influence affecting the renal vasodilator effects of COX-2-derived prostanoids. In the present study, nimesulide administration during continued nNOS blockade markedly decreased RPF and GFR in hypertensive rats but not normotensive rats and led to pronounced increases in RVR in hypertensive but not normotensive rats. These findings extend our previous observations (43) and indicate that COX-2 metabolites act to prevent excessive renal vasoconstriction independently of the influence of nNOS-derived NO in Cyp1a1-Ren2 transgenic rats with inducible ANG II-dependent malignant hypertension. In essence, the results of the present study indicate that COX-2-derived vasodilator metabolites play an integral role in maintaining renal hemodynamics independently of nNOS activity in Cyp1a1-Ren2 rats with ANG II-dependent malignant hypertension.

In summary, the present findings demonstrate that nNOS-derived NO and COX-2-derived prostanoids exert a pronounced renal vasodilator influence after induction of malignant hypertension in Cyp1a1-Ren2 transgenic rats. Furthermore, the renal vasodilator effects of COX-2 metabolites in hypertensive Cyp1a1-Ren2 rats are not dependent on nNOS activity. Such maintained renoprotective influences of nNOS-derived NO and COX-2 metabolites would act to prevent excessive renal vasoconstriction and, thereby, help maintain renal hemodynamics following induction of malignant hypertension in Cyp1a1-Ren2 transgenic rats.

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