Cyclooxygenase-2 Modulates Afferent Arteriolar Responses to Increases in Pressure

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Abstract—This study was designed to examine the contribution of cyclooxygenase-2 (COX-2) in the afferent arteriolar autoregulatory responses to increases in perfusion pressure and its relationship with neuronal nitric oxide synthase (nNOS). In rat kidneys, afferent arteriolar diameter responses to increases in perfusion pressure were assessed in vitro with the blood-perfused juxtamedullary nephron technique. Basal afferent arteriolar diameter at 100 mm Hg averaged 21.0±1.2 μm (n=7), and the vasoconstrictor response to increasing perfusion pressure to 160 mm Hg averaged 18.4±1.2%. Superfusion with the COX-2 inhibitor NS398 (10 μmol/L) did not influence basal diameters, but it did significantly enhance the vasoconstrictor response to the increase in perfusion pressure (32.9±4.0%). In contrast to previous findings that the nNOS inhibitor S-methyl-l-thiocitrulline (10 μmol/L) enhanced afferent arteriolar autoregulatory responses in normal rat kidneys, in this study, administration of 10 μmol/L S-methyl-l-thiocitrulline did not further modulate the vasoconstrictor response to increases in perfusion pressure in the NS398-treated kidneys of normal rats (31.8±4.7%). When tubuloglomerular feedback activity was interrupted by papillectomy and the addition of 50 μmol/L furosemide to the blood perfusate (n=5 for each), the afferent arteriolar constrictor responses to increasing perfusion pressure to 160 mm Hg averaged 7.9±0.9% and 10.7±0.7%, respectively, and they were significantly attenuated compared with the responses observed in control kidneys. NS398 treatment did not modulate the afferent arteriolar autoregulatory responses in papillectomized or furosemide-treated kidneys. These results indicate that COX-2–derived metabolites contribute to the nNOS modulation of pressure-mediated afferent arteriolar autoregulatory responses. (Hypertension. 1999;34[part 2]:843-847.)

Key Words: autoregulation • neuronal nitric oxide synthase • macula densa • papillectomy • furosemide

Both nitric oxide (NO) and cyclooxygenase (COX) metabolites are recognized as paracrine factors of renal microvascular function.1 Recent studies demonstrated that NO derived from the activation of neuronal nitric oxide synthase (nNOS), localized to the macula densa cells, exerts a counteracting modulatory influence on tubuloglomerular feedback (TGF)–mediated afferent arteriolar constriction.2,3 Because the TGF mechanism contributes to afferent arteriolar autoregulation,4–6 nNOS also exerts a counteracting modulatory influence on the afferent arteriolar autoregulatory responses to changes in renal perfusion pressure (RPP).7 Thus, the macula densa nNOS seems to be one of the important factors opposing renal vasconstrictor systems. It has been demonstrated that long-term nNOS inhibition generates hypertension in rats8 and that nNOS activity is decreased in the nonclipped kidney of 2-kidney, 1-clip rats9 and the kidneys of rats infused with angiotensin II long term.10

In rat kidneys, nonselective COX inhibition with meclofenamate and indomethacin blunted arterial pressure–mediated increases in papillary blood flow when RPP was elevated.11 Nevertheless, in dog12 and rat13 kidney preparations, nonselective COX inhibition has consistently failed to alter the autoregulatory responses to changes in perfusion pressure. Thus, the contribution of COX-derived metabolites to overall autoregulatory responsiveness remains controversial. Recent immunohistochemical studies have revealed the unique presence of an inducible isoform of COX, COX-2, in the kidney; its constitutive expression is localized to the cells of the ascending loops of Henle14 and the macula densa.15 In addition, we recently demonstrated that renal vasodilator responses to NO are partially mediated by the production of vasodilatory metabolites, which is caused by the increased activity of COX-2.16 It is, therefore, possible that metabolites derived from COX-2, localized to the macula densa segment, mediate the modulating influence of nNOS-derived NO on pressure-mediated afferent arteriolar autoregulatory responses. However, the specific influences of COX-2 on pressure-induced afferent arteriolar autoregulatory responses remain undetermined.
In the present study, we hypothesized that COX-2 modulates afferent arteriolar autoregulatory responses to changes in RPP by interacting with nNOS-derived NO around the macula densa cells. To test this hypothesis, afferent arteriolar autoregulatory responses to increases in RPP were assessed under conditions of normal and inhibited COX-2 activity with the blood-perfused juxtamedullary nephron technique combined with videomicroscopy. We also determined whether nNOS inhibition could modulate afferent arteriolar autoregulatory responses to increases in RPP during COX-2 inhibition. The juxtamedullary nephrons visualized in this preparation give rise to long loops of Henle that extend into the papilla before looping back to the distal tubule and past the macula densa segment, and thus minimizes TGF-dependent vasaconstrictor influences on microvascular function. In the present study, the effects of COX-2 inhibition with NS398 on afferent arteriolar autoregulatory responses to increases in RPP were also assessed when the TGF mechanism was blocked pharmacologically by the addition of furosemide or physically by papillectomy.

**Methods**

**Assessment of Afferent Arteriolar Diameter**

The experiments were performed in accordance with the guidelines and practices established by the Tulane University Animal Care and Use Committee. Afferent arteriolar diameter was assessed in vitro with the blood-perfused juxtamedullary nephron technique combined with videomicroscopy, as previously described. Briefly, each experiment used 2 male Sprague-Dawley rats (Charles River Labs, Wilmington, Mass) weighing 350 to 400 g. One rat served as the blood donor, and the second rat was the kidney donor. Rats had free access to water and standard rat chow (Ralston-Purina) before each experiment, and they were anesthetized with sodium pentobarbital (50 mg/kg IP). Donor blood was collected into a heparinized syringe and centrifuged to separate the plasma and cellular fractions. Plasma oncotic pressure was adjusted to 18 mm Hg by the addition of BSA (Sigma). After processing the plasma through filters, erythrocytes were added to achieve a hematocrit of 33%. This reconstituted blood was stirred continuously in a closed reservoir that was pressurized with a 95% O2/5% CO2 gas mixture.

The right renal artery of the kidney donor was cannulated and perfused with Tyrode’s solution (pH 7.4) containing 5.1% BSA and a mixture of L-amino acids (Sigma Chemical Co). The kidney was excised and prepared as previously described such that the vasculature of the juxtamedullary nephrons was visualized directly and the papilla remained intact. The arterial supply of the exposed microvasculature was isolated by ligating the large branches of the renal artery with fine suture (nylon black monofilament, 10-0; Vanguard Surgical System).

After the dissection was completed, the Tyrode's perfusate was replaced with the reconstituted blood. RPP was monitored by a pressure cannula centered in the tip of the perfusion cannula and regulated by adjusting the rate of gas inflow into the blood reservoir. RPP was initially set at 100 mm Hg. The perfusion chamber was warmed, and the inner cortical surface of the kidney was continuously superfused with warmed (37°C) Tyrode’s solution containing 1% BSA.

The tissue was transilluminated on the fixed stage of a Leitz Laborlux-12 microscope. Video images of the microvessels were transferred by a Newivicon camera (model NC-67 mol/L, Dage-MTI) through an image enhancer (MF-1452, MFJ Enterprises) and recorded on videotape for later analysis (Videocassette Recorder HR-VP618U; JVC). Afferent arteriolar inside diameters were measured at 12-s intervals using a calibrated digital image-shearing monitor (Institutional for Physiology and Medicine) that yielded diameter measurements reproducible within 0.5 μm. Afferent arteriolar diameters were measured at sites within 100 μm upstream from the glomerulus. A minimum 10-minute equilibration period was allowed before the initiation of each experimental procedure. The average diameter during the final 1 minute of each 3-minute treatment period was used for statistical analysis of steady-state responses.

**Afferent Arteriolar Responses to Increases in RPP During Inhibition of COX-2 and nNOS**

Afferent arteriolar autoregulatory responses were examined by increasing RPP in a stepwise manner from 100 to 130 and 160 mm Hg. After restoration of RPP to 100 mm Hg and recovery of afferent arteriolar diameter, 10 μmol/L NS398 (Cayman Chemical Co) was added to the superfusate to inhibit COX-2 specifically. After 20 minutes of stabilization, the responses to increases in RPP during COX-2 inhibition were determined. After the assessment of afferent arteriolar autoregulatory responses during COX-2 inhibition, 10 μmol/L S-methyl-L-thiocitrulline (L-SMTC; Alexis Co) was added to the superfusate to inhibit nNOS selectively. We previously showed that 10 μmol/L L-SMTC does not influence the acetylcholine-induced vasodilation of juxtamedullary afferent arterioles that can be blocked by nonspecific NO synthase inhibition. After 5 to 10 minutes of stabilization, the responses to increases in RPP during inhibition of COX-2 and nNOS were determined.

**Effects of COX-2 Inhibition on Afferent Arteriolar Responses to Increases in RPP in Papillectomized Kidneys**

The afferent arteriolar autoregulatory responses to increases in RPP were determined under control conditions and during superfusion of the selective COX-2 inhibitor NS398. Transection of the loops of Henle by papillectomy was performed to eliminate the TGF mechanism by interrupting the flow of tubular fluid to the distal nephron, including the macula densa segment. The papilla was cleanly severed near the corticomedullary junction by a single cut, preventing damage to the adjacent tissue. After a 10-minute stabilization period, afferent arteriolar responses to increasing RPP from 100 to 130 and 160 mm Hg were determined. After the control assessment of afferent arteriolar responses to increases in RPP, the superfusate was changed to one containing 10 μmol/L NS398. After a 20-minute stabilization period, the afferent arteriolar responses to increases in RPP were determined during NS398 treatment.

**Effects of COX-2 Inhibition on Afferent Arteriolar Responses to Increases in RPP in Furosemide-Treated Kidneys**

The afferent arteriolar responses to increases in RPP from 100 to 130 and 160 mm Hg were determined under control conditions and during superfusion of NS398 in kidneys treated with the addition of 50 μmol/L furosemide to the blood perfusate. After the control assessment of afferent arteriolar responses to increases in RPP, the superfusate was changed to one that contained 10 μmol/L NS398. After a 20-minute stabilization period, the afferent arteriolar responses to increases in RPP were determined during NS398 treatment.

**Statistical Analysis**

Analyses of changes in afferent arteriolar diameters with increases in RPP and treatments were performed using 1-way ANOVA for repeated
Results

Afferent Arteriolar Responses to Increases in RPP During Inhibition of COX-2 and nNOS

Figure 1 illustrates the afferent arteriolar responses to increases in RPP from 100 to 130 and 160 mm Hg under control conditions and during treatments with NS398 alone and NS398 combined with L-SMTC. Under control conditions, the basal afferent arteriolar diameter averaged 21.0 ± 1.2 μm (n = 7) at an RPP of 100 mm Hg; it significantly decreased by 9.9 ± 0.8% and 18.4 ± 1.2% in response to increasing RPP to 130 and 160 mm Hg, respectively. During COX-2 inhibition with NS398 treatment, the afferent arteriolar diameter averaged 20.7 ± 1.2 μm at an RPP of 100 mm Hg, and it was similar to that observed under control conditions. However, afferent arteriolar constrictor responses to increasing RPP to 130 and 160 mm Hg averaged 22.6 ± 2.7% and 32.9 ± 4.0%, respectively, and they were significantly greater than those observed under control conditions. During NS398 plus L-SMTC treatment, afferent arteriolar diameter averaged 18.1 ± 1.1 μm at an RPP of 100 mm Hg; it was significantly decreased compared with values observed under control conditions and during NS398 treatment alone. Afferent arteriolar constrictor responses to increasing RPP to 130 and 160 mm Hg averaged 19.5 ± 2.6% and 31.8 ± 4.7%, respectively, and they were similar to those determined during NS398 treatment alone.

Effects of COX-2 Inhibition on Afferent Arteriolar Responses to Increases in RPP in Papillectomized Kidneys

Figure 2 describes the afferent arteriolar responses to increases in RPP from 100 to 130 and 160 mm Hg under control conditions and during COX-2 inhibition with NS398 in papillectomized kidneys. Under control conditions, the basal afferent arteriolar diameter averaged 21.6 ± 2.0 μm (n = 5) at an RPP of 100 mm Hg; it significantly decreased by 5.2 ± 0.9% and 7.9 ± 0.9% in response to increases in RPP to 130 and 160 mm Hg, respectively. The decreases in diameter with the increases in RPP observed in papillectomized kidneys were significantly attenuated compared with those observed in papilla-intact kidneys. During NS398 treatment, the afferent arteriolar diameter averaged 21.4 ± 1.9 μm at an RPP of 100 mm Hg; it was similar to that determined under normal conditions. Afferent arteriolar constritor responses to increases in RPP to 130 and 160 mm Hg averaged 5.9 ± 0.9% and 8.8 ± 0.9%, respectively, and they were also similar to those observed under normal conditions.

Effects of COX-2 Inhibition on Afferent Arteriolar Responses to Increases in RPP in Furosemide-Treated Kidneys

Figure 3 demonstrates afferent arteriolar responses to increases in RPP from 100 to 130 and 160 mm Hg in the kidneys treated with 50 μmol/L furosemide. Experiments were performed under control conditions and during COX-2 inhibition with NS398 alone and NS398 combined with papillectomy. Under control conditions, the basal afferent arteriolar diameter averaged 20.4 ± 1.7 μm (n = 5) at an RPP of 100 mm Hg; it significantly decreased by 6.5 ± 0.7% and 10.7 ± 0.7% in response to increases in RPP to 130 and 160 mm Hg, respectively. The decreases in diameter with the increases in RPP observed in furosemide-treated kidneys were significantly attenuated compared with those observed in control kidneys. During NS398 treatment, afferent arteriolar diameter averaged 20.2 ± 1.5 μm at an RPP of 100 mm Hg, and it was similar to that determined under normal conditions. Afferent arteriolar constrictor responses to
Discussion

The present study evaluated the contribution of COX-2 activity to afferent arteriolar autoregulatory responses to increases in RPP and its relationship with nNOS in juxtamedullary nephrons. COX-2 inhibition with NS398 did not influence basal afferent arteriolar diameters, but it did significantly enhance afferent arteriolar autoregulatory responses to increases in RPP. We previously reported that nNOS inhibition with 10 μmol/L L-SMTC significantly enhanced afferent arteriolar autoregulatory responses to increases in RPP. However, during COX-2 inhibition with NS398, L-SMTC did not further influence afferent arteriolar autoregulatory responses to increases in RPP. These results suggest that in juxtamedullary nephrons, COX-2–derived metabolites significantly modulate pressure-mediated afferent arteriolar autoregulatory responses and that nNOS-derived NO modulates afferent arteriolar autoregulatory responses to changes in RPP through its interactions with COX-2 in and around the macula densa cells. In addition, at an RPP of 100 mm Hg, nNOS inhibition with L-SMTC decreased basal afferent arteriolar diameters, whereas COX-2 inhibition with NS398 had no effect on basal afferent arteriolar diameters. These results suggest that basal nNOS activity influences afferent arteriolar tone and that COX-2 is stimulated directly or indirectly as a result of nNOS activation. Therefore, COX-2 activity may substantially depend on nNOS activity to modulate afferent arteriolar autoregulatory responses to increases in RPP, but it is likely that the net effect of nNOS on afferent arteriolar tone is not completely dependent on COX-2 activity.

Studies using the juxtamedullary nephron preparation have demonstrated that the afferent arteriolar diameter responses to changes in RPP are mediated, at least in part, through the TGF mechanism.4–6 A recent study demonstrated that TGF-mediated afferent arteriolar constriction is counteracted by the interactive influences of nNOS-derived NO and COX-2–derived vasodilator metabolites.16 These results suggest that COX-2 modulation of the pressure-induced afferent arteriolar autoregulatory responses may depend on the TGF mechanism. The present study demonstrates that COX-2 inhibition with NS398 does not influence afferent arteriolar autoregulatory responses to increases in RPP when the TGF mechanism is interrupted by papillectomy or furosemide treatment. The results suggest that COX-2, present in and around the macula densa cells, contributes to the TGF mechanism by exerting an important counteracting modulatory influence on afferent arteriolar constriction responses to increases in RPP. Because afferent arteriolar autoregulatory responses to increases in RPP were similar in the papillectomized and furosemide-treated kidneys, it is unlikely that COX-2 localized to the papilla contributes to the modulation of afferent arteriolar autoregulatory responses. In addition, papillectomy did not influence afferent arteriolar autoregulatory responses to increases in RPP in furosemide-treated kidneys. This result excluded the possibility that papillectomy might influence afferent arteriolar function through a nonspecific effect other than interrupting tubular flow to distal nephron segments.

Although there is controversy in the literature regarding the effects of nonselective COX inhibition on overall autoregulatory responsiveness,11–13 the present study clearly demonstrated that selective COX-2 inhibition significantly enhances afferent arteriolar constrictor responses to increases in RPP. Because COX produces both vasoconstrictor and vasodilator metabolites, depending on which types of renal cells generate the COX metabolites, it may be difficult to determine the overall effects of COX metabolites on renal vasculatures. However, the products generated from COX-2, presumably formed in or around the macula densa segment, seem to exert a counteracting modulation of the pressure-induced afferent arteriolar constriction.

In conclusion, COX-2 inhibition with NS398 significantly enhanced afferent arteriolar constrictor responses to increases in RPP, and this enhancement by NS398 was prevented when the TGF mechanism was interrupted. These results suggest that COX-2, localized to the macula densa segment, has a buffering effect on the afferent arteriolar constriction elicited by increases in perfusion pressure. In addition, nNOS inhibi-
tion failed to enhance the afferent arteriolar constrictor responses to increases in RPP during COX-2 inhibition. It is, therefore, suggested that during increases in perfusion pressure, the increased level of NO derived from the macula densa nNOS stimulates the activity of COX-2 localized to the macula densa segment to generate vasodilatory metabolites, which contribute to buffering the pressure-mediated afferent arteriolar constriction.

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**References**
