Interactive Nitric Oxide–Angiotensin II Influences on Renal Microcirculation in Angiotensin II–Induced Hypertension

Atsuhiro Ichihara, John D. Imig, Edward W. Inscho, L. Gabriel Navar

Abstract—The present study was conducted to determine the contribution of nitric oxide to angiotensin II (Ang II) reactivity of afferent and efferent arterioles from Ang II–infused hypertensive rats. Experiments were performed in vitro with the blood-perfused juxtamedullary nephron technique in kidneys harvested from hypertensive Sprague-Dawley rats (181 ± 1 mm Hg) that had received 60 ng/min Ang II subcutaneously for 13 days. Superfusion with 0.1, 1, and 10 nmol/L Ang II reduced afferent arteriolar diameter (18.1 ± 0.6 μm; n = 12) by 10.0 ± 0.7%, 28.1 ± 1.7%, and 52.8 ± 1.9%, respectively, and efferent arteriolar diameter (17.2 ± 1.4 μm; n = 8) decreased by 9.3 ± 0.7%, 27.0 ± 1.2%, and 50.4 ± 1.6%, respectively. Nitric oxide synthase inhibition with 100 μmol/L Nω-nitro-L-arginine (NLA) reduced resting afferent and efferent arteriolar diameters to 14.7 ± 0.4 and 14.3 ± 1.2 μm, respectively, and enhanced afferent but not efferent arteriolar reactivity to Ang II. The enhanced afferent arteriolar reactivity to Ang II was eliminated by addition of the nitric oxide donor S-nitroso-N-acetylpenicillamine (SNAP, 10 μmol/L), which reversed the NLA-induced decrease in diameter. Addition of 10 μmol/L SNAP, without NLA, blunted efferent but not afferent arteriolar reactivity to Ang II. Afferent (n = 7) and efferent arteriolar diameters (n = 6) decreased by 48.5 ± 2.2% and 41.0 ± 1.9%, respectively, in response to 10 nmol/L Ang II. These results suggest that in this model of hypertension, maintained nitric oxide production in afferent arterioles counteracts the enhanced afferent arteriolar reactivity that occurs in Ang II–induced hypertension. (Hypertension. 1998;31:1255–1260.)

Key Words: rats ■ kidney ■ arterioles ■ nitric oxide ■ angiotensin II

Rats infused with initially suppressor doses of Ang II slowly develop progressive hypertension similar to that observed in two-kidney, one clip Goldblatt hypertension.1 In these models of hypertension, renal Ang II levels are increased and afferent arteriolar reactivity to Ang II is enhanced.2 Because afferent arterioles are the segment that accounts for the major fraction of preglomerular vascular resistance, an alteration in the vascular reactivity of this segment may exert a strong influence on the regulation of renal blood flow and glomerular filtration rate. Nevertheless, in the nonclipped kidney of two-kidney, one clip Goldblatt hypertensive animals and in kidneys of Ang II–infused hypertensive rats, renal blood flow and glomerular filtration rate are either not reduced or only slightly diminished.3–5 Although decreases in the number of intrarenal Ang II receptors might occur as a consequence of the increased renal Ang II levels,6 afferent arteriolar responsiveness to Ang II is actually enhanced in this model of hypertension.7 Therefore, the mechanisms responsible for the maintenance of near normal renal blood flow and glomerular filtration rate in this model of hypertension remain undetermined.

In vivo studies have suggested that NO exerts an important role in maintaining renal hemodynamics near the normal range in kidneys with elevated Ang II levels.7,8 A recent study showed that urinary excretion of nitrates and nitrites did not increase but was maintained in rats infused chronically with Ang II,9 suggesting that NO formation is not diminished in this model of hypertension. Furthermore, in normotensive rat kidneys, both afferent and efferent arteriolar responsiveness to Ang II are modulated by NO.10 Because preglomerular and postglomerular vascular tone contribute to renal vascular resistance and glomerular filtration rate,11 the relative degree to which NO affects the afferent and efferent arteriolar responsiveness to Ang II significantly affects renal function. Such interactions may be further enhanced in hypertensive conditions characterized by elevated Ang II levels and enhanced microvascular reactivity to Ang II.

The present study was performed to evaluate further the influence of NO on afferent and efferent arteriolar function in Ang II–infused hypertensive rats. We hypothesized that intrarenal NO differentially influences afferent and efferent arteriolar tone as well as the microvascular responsiveness to Ang II in Ang II–induced hypertension. To test this hypothesis, we used the in vitro blood-perfused juxtamedullary nephron technique12,13 and determined the effects of NO blockade and of exogenously supplied NO on the responsiveness of afferent and efferent arterioles to Ang II in chronically Ang II–infused hypertensive rats.

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Methods

Preparation of Animals
Male Sprague-Dawley rats (Charles River Labs, Wilmington, Mass) were housed in wire cages and maintained in a temperature-controlled room that was regulated on a 12/12-hour light-dark cycle. Rats had free access to water and standard rat chow (Ralston Purina). All experimental protocols were approved by the Tulane University Animal Care and Use Committee. Rats (175 to 200 g body wt) were anesthetized with sodium pentobarbital (50 mg/kg IP). An osmotic minipump (model 2002, Alza Corp) was implanted subcutaneously at the dorsum of the neck. Ang II-infused rats received Ang II (Novabiochem) at a rate of 60 ng/min for a period of 13 days. Systolic blood pressure was measured every 3 days in conscious rats by tail-cuff plethysmography (model 52-0338; Harvard Apparatus).

Assessment of Renal Arteriolar Reactivity
Afferent and efferent arteriolar reactivity were assessed with the in vitro blood-perfused juxtamedullary nephron technique combined with videomicroscopy, as previously described. Experiments were performed on day 13 after minipump implantation. Each experiment used two rats from the same treatment group with one rat serving as the blood donor and the second rat as the kidney donor. Rats were anesthetized with sodium pentobarbital (50 mg/kg IP), and a cannula was inserted into the left carotid artery of the blood donor. Donor blood was collected into a heparinized (500 U) syringe via the carotid arterial cannula and centrifuged to separate the plasma and cellular fractions. Theuffy coat was removed and discarded. Plasma oncotic pressure was adjusted to 18 mm Hg by the addition of bovine serum albumin (Sigma Chemical Co). After sequential passage of the plasma through 5- and 0.22-μm filters (Gelman Sciences), erythrocytes were added to achieve a hematocrit of 33%. This reconstituted blood was passed through a 5-μm nylon mesh and thereafter stirred continuously in a closed reservoir that was pressurized with a 95% O₂/5% CO₂ gas mixture. The right kidney of the kidney donor was perfused by a cannula advanced into the right renal artery via the mesenteric artery. The perfusate was a Tyrode’s solution (pH 7.4) containing 5.1% bovine serum albumin and a mixture of L-amino acids (Sigma Chemical Co). The kidney was excised and sectioned longitudinally, retaining the papilla intact in the perfused dorsal two thirds of the organ. Small incisions of the lateral fornices allowed the papilla to be reflected back, thus exposing the pelvic mucosa and tissue covering the inner cortical surface. Overlying connective tissue was removed, and the veins were cut open to reveal the tubules, glomeruli, and related vasculature of the juxtamedullary nephrons. The arterial supply of the exposed microvasculature was isolated by ligating the large branches of 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. Perfusion pressure was monitored by a pressure cannula centered in the perfusion cannula and maintained at 100 mm Hg during all experimental protocols. Perfusion pressure was regulated by adjusting the rate of gas inflow into the blood reservoir. The perfusion chamber was warmed, and the inner cortical surface was continuously superfused with a warmed (37°C) Tyrode’s solution containing 1% bovine serum albumin.

The tissue was transilluminated on the fixed stage of a Leitz Laborlux-12 microscope equipped with a 100-W halogen lamp (Leica Inc) and a water-immersion objective (×40, Zeiss). Video images of the microvessels were obtained by a Newvicon camera (model NC-67M, Dage-MTI), passed through a time-date generator (model WJ-810, Panasonic) and a video contrast enhancer (model 605, Javelin Electronics), and recorded on videotape for later analysis (videocassette recorder HR-VP618U, JVC). Afferent and efferent arteriolar inside diameters were measured at 12-second intervals with a calibrated digital image-shearing monitor (Instrumentation for Physiology and Medicine) that yielded diameter measurements reproducible within 0.5 μm. Afferent arteriolar diameters were measured at sites 75 to 120 μm upstream from the glomerulus, and efferent arteriolar diameters were measured at sites within 75 μm of the glomerulus, before the first branch. 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. All agents were administered by addition to the bathing solution.

Influences of Endogenous and Exogenous NO on Afferent and Efferent Arteriolar Vascular Diameters
The contribution of endogenous NO production to microvascular diameter was determined in Ang II-infused hypertensive rats. Afferent and efferent arteriolar diameters were measured under control conditions and during exposure to increasing concentrations (1 to 1000 μmol/L) of the NO synthase inhibitor NLA (Aldrich Chemical Co). In a second series of experiments, we examined the effect of exogenously administered NO on microvascular diameter by measuring afferent and efferent arteriolar diameters before and during exposure to the NO donor SNAP (Sigma Chemical Co). Microvascular diameter responses were determined under control conditions and during exposure to increasing concentrations (0.1 to 100 μmol/L) of SNAP.

Roles of Endogenous and Exogenous NO in Afferent and Efferent Arteriolar Responsiveness to Ang II
The role of endogenous NO production on Ang II–mediated microvascular vasocostriction was determined by evaluating the decreases in afferent and efferent arteriolar diameters in response to 0.1, 1, and 10 nmol/L Ang II (Calbiochem-Novabiochem Corp) before and during the addition of 100 μmol/L NLA. A second series of experiments was performed to determine the effect of exogenously administered NO on the microvascular response to Ang II. Afferent and efferent arteriolar diameters were measured in response to 0.1, 1, and 10 nmol/L Ang II before and after the addition of 10 μmol/L SNAP. Finally, experiments were performed to determine whether Ang II stimulation of NO synthesis contributes to endogenous NO modulation of afferent and efferent arteriolar reactivity to Ang II. In these experiments, ambient NO concentration was fixed at a constant level with 10 μmol/L SNAP, and afferent and efferent arteriolar reactivity to the same concentrations of Ang II were examined in the presence and absence of 100 μmol/L NLA. We previously determined the reproducibility of arteriolar responses to repeated Ang II administration and found no significant difference in the responses between the first and second treatments.

Statistical Analysis
Differences in mean values between treatment groups were evaluated with a two-way ANOVA for repeated measures. Comparisons between afferent and efferent arteriolar diameter responses to different treatments and comparisons of percent changes in the Ang II response between treatments were made by a Fisher’s protected least-squares difference test followed by Bonferroni’s correction for multiple comparisons. Comparisons of the Ang II response expressed in micrometers between treatments were made by means of a paired t test with Bonferroni’s correction for multiple comparisons. A value of P<0.05 was considered significant. Data are presented as mean±SEM.
Results

Chronic Ang II infusion significantly increased systolic blood pressure from 121 ± 6 mm Hg on day 0 to 181 ± 6 mm Hg on day 13 (n = 52). The increase in systolic blood pressure in the Ang II–infused rats is similar to that observed previously and is greater than that measured previously in the saline vehicle–infused control rats.

Effect of NLA on Afferent and Efferent Arterioles

The dose-response effects of NLA on afferent and efferent arteriolar diameters were determined in Ang II–infused hypertensive rats. Control diameter of the afferent arteriole averaged 18.7 ± 0.7 μm (n = 6). As illustrated in Figure 1A, superfusion with 1, 10, 100, and 1000 μmol/L NLA reduced afferent arteriolar diameter by 5.9 ± 0.6%, 13.2 ± 0.3%, 20.9 ± 1.5%, and 20.5 ± 1.6%, respectively. Efferent arteriolar diameter averaged 17.6 ± 0.4 μm (n = 6) under control conditions and decreased by 4.4 ± 0.4%, 9.7 ± 1.1%, 16.6 ± 1.6%, and 16.3 ± 1.5%, respectively, in response to the same concentrations of NLA. The afferent arteriolar vasoconstriction to 10 μmol/L NLA was significantly greater than the vasoconstrictor response of efferent arterioles.

Effect of SNAP on Afferent and Efferent Arterioles

The dose-response effects of SNAP on afferent and efferent arteriolar diameters are shown in Figure 1B. Superfusion with 0.1, 1, 10, and 100 μmol/L SNAP increased afferent arteriolar diameter from a control of 19.0 ± 0.8 μm (n = 6) by −0.2 ± 0.6%, 5.3 ± 0.2%, 12.0 ± 1.2%, and 8.2 ± 0.9%, respectively. Efferent arteriolar diameters averaged 17.9 ± 1.1 μm (n = 6), and the same concentrations of SNAP increased efferent arteriolar diameters by 0.1 ± 0.2%, 3.6 ± 0.7%, 6.4 ± 0.8%, and 6.6 ± 0.7%. The afferent arteriolar vasodilation to 10 μmol/L SNAP was significantly greater than efferent arteriolar response.

Effect of NLA on Afferent and Efferent Arteriolar Responses to Ang II

The effects of endogenously formed NO on afferent and efferent arteriolar reactivity to Ang II are depicted in Figures 2 and 3. Afferent arteriolar diameter averaged 18.1 ± 0.6 μm (n = 12) under control conditions and decreased by 10.0 ± 0.7%, 28.1 ± 1.7%, and 52.8 ± 1.9% during superfusion with 0.1, 1, and 10 nmol/L Ang II, respectively. After the recovery of afferent arteriolar diameter from Ang II–induced vasoconstriction, NO synthesis inhibition with 100 μmol/L NLA decreased basal afferent arteriolar diameter by 18.4 ± 1.2% to 14.7 ± 0.4 μm. In the presence of NLA, afferent arteriolar responsiveness to Ang II was significantly enhanced (Figure 2B). During addition of NLA, afferent arteriolar diameters decreased by 19.8 ± 2.0%, 48.1 ± 2.8%, and 79.2 ± 3.8% in response to the same Ang II concentrations. At all concentrations of Ang II, the percent decreases were significantly greater than those observed before addition of NLA (Figure 2B).

Figure 1A illustrates that efferent arteriolar diameter averaged 17.2 ± 1.4 μm (n = 8) under control conditions and decreased by 9.3 ± 0.7%, 27.0 ± 1.2%, and 50.4 ± 1.6% during superfusion with 0.1, 1, and 10 nmol/L Ang II, respectively. After the recovery of efferent arteriolar diameter, NLA (100 μmol/L) decreased basal efferent arteriolar diameter by 16.8 ± 0.5% to 14.3 ± 1.2 μm. However, NLA did not influ-
ence efferent arteriolar responsiveness to Ang II, and the relative efferent arteriolar diameters were similarly reduced to 9.3 ± 0.8%, 28.1 ± 1.4%, and 50.3 ± 2.4% in response to the same Ang II concentrations (Figure 3B).

Effect of SNAP on Afferent and Efferent Arteriolar Responses to Ang II
To examine whether the NO donor SNAP attenuates renal arteriolar responsiveness to Ang II, afferent and efferent arteriolar diameter responses to Ang II were examined before and during SNAP administration in Ang II–infused hypertensive rats. Figure 4A demonstrates that afferent arteriolar diameter averaged 18.9 ± 0.4 μm (n=7) under control conditions and decreased by 12.5 ± 1.5%, 25.1 ± 1.3%, and 51.2 ± 0.9% during superfusion with 0.1, 1, and 10 nmol/L Ang II, respectively. The addition of 10 μmol/L SNAP increased resting afferent arteriolar diameters by 11.1 ± 1.5% to 21.0 ± 0.6 μm, but the relative constrictor responses to Ang II were not significantly altered. In the presence of SNAP, afferent arteriolar diameters decreased by 11.7 ± 1.7%, 22.6 ± 1.9%, and 48.5 ± 2.2% in response to 0.1, 1, and 10 nmol/L Ang II (Figure 4B).

As depicted in Figure 5A, efferent arteriolar diameter averaged 16.4 ± 0.8 μm (n=6) and decreased by 9.8 ± 0.4%, 17.7 ± 0.6%, and 53.3 ± 0.9% during superfusion with 0.1, 1, and 10 nmol/L Ang II, respectively. The addition of 10 μmol/L SNAP significantly increased resting efferent arteriolar diameter by 5.1 ± 0.7% to 17.2 ± 0.9 μm, and this increase was similar to that observed in the series shown in Figure 1B. The addition of 10 μmol/L SNAP significantly blunted the vasoconstrictor response to Ang II. In the presence of SNAP, efferent arteriolar diameter decreased by 7.6 ± 0.5%, 11.4 ± 1.0%, and 41.0 ± 1.9% during superfusion with the same concentrations of Ang II, and the percent decreases were significantly smaller than those observed under control conditions (Figure 5B).

Effect of NLA Combined With SNAP on Afferent and Efferent Arteriolar Responses to Ang II
Afferent arteriolar diameter averaged 17.6 ± 0.9 μm (n=6) under control conditions and decreased by 9.0 ± 0.6%, 27.2 ± 1.5%, and 48.6 ± 2.8% in response to 0.1, 1, and 10 nmol/L Ang II, respectively. After the recovery of afferent arteriolar diameter from Ang II–induced vasoconstriction, addition of 100 μmol/L NLA significantly decreased basal afferent arteriolar diameter by 19.8 ± 0.7% to 14.1 ± 0.7 μm. Then 10 μmol/L SNAP increased afferent arteriolar diameter to 17.3 ± 0.8 μm. Thus, afferent diameter during combined treatment with SNAP+NLA was similar to the control diameter. Therefore, afferent arteriolar diameter declined by 9.3 ± 0.9%, 23.8 ± 2.9%, and 40.5 ± 3.2% in response to 0.1, 1, and 10 nmol/L Ang II, which was similar to that observed under control conditions.

Efferent arteriolar diameter averaged 16.1 ± 0.7 μm (n=5) under control conditions and decreased by 9.2 ± 1.1%, 26.4 ± 2.0%, and 51.1 ± 2.1% during superfusion with 0.1, 1, and 10 nmol/L Ang II, respectively. After the recovery of efferent arteriolar diameter from Ang II–induced vasoconstriction, addition of 100 μmol/L NLA decreased basal efferent arteriolar diameter by 16.6 ± 0.8% to 13.5 ± 0.6 μm.
arteriolar reactivity to increases in perfusion pressure or to the calcium ionophore A23187 is not enhanced in Ang II–infused hypertension. We have previously demonstrated that NLA decreased afferent and efferent arteriolar diameters to the same extent in enalapril-treated normotensive control rats and untreated normotensive control rats. The present results in Ang II–infused hypertensive rats indicate that the maximal response of afferent arterioles to NLA (a 20.9 ± 1.5% decrease) in Ang II–infused hypertensive rats is greater than that (a 16.7 ± 0.4% decrease) observed in untreated normotensive control rats, but that efferent arteriolar maximal response to NLA (a 16.6 ± 1.6% decrease) in Ang II–infused hypertensive rats is similar to that (a 16.6 ± 0.6% decrease) observed in untreated normotensive control rats.

In addition, exogenous NO derived from SNAP increased afferent and efferent arteriolar diameters, and the increase in diameter with 10 μmol/L SNAP was significantly greater in afferent than efferent arterioles, suggesting that afferent arterioles have a greater capacity for NO to vasodilate compared with efferent arterioles. This relationship between afferent and efferent arteriolar reactivity to SNAP is similar to that observed previously in enalapril-treated normotensive control rats. Therefore, chronic Ang II infusion does not modify the relatively greater capacity of NO to modulate microvascular tone in afferent versus efferent arterioles.

The endogenous NO levels existing in the Ang II–infused rats are sufficient to influence the afferent arteriolar reactivity to Ang II, but the efferent responses are just shifted but not enhanced. With further increases in NO activity above the endogenous levels, however, afferent arteriolar reactivity is not influenced further but efferent arteriolar reactivity to Ang II is significantly diminished. NO stimulates soluble guanylate cyclase, and the consequent increase in cyclic GMP can exert a vasodilatory effect on vascular smooth muscle cells through inhibiting phosphatidylinositol hydrolysis and Ca\(^{2+}\) release from sarcoplasmic reticulum without involving Ca\(^{2+}\) influx from extracellular fluid or repletion of intracellular Ca\(^{2+}\) stores. Ang II–evoked vasoconstriction of the efferent arteriole is highly dependent on Ca\(^{2+}\) release from sarcoplasmic reticulum, while the afferent arteriolar response is due to Ca\(^{2+}\) influx from extracellular fluid as well as Ca\(^{2+}\) release from intracellular stores. Accordingly, efferent arteriolar reactivity to Ang II may be much more sensitive to increased levels of NO compared with the afferent arteriolar reactivity. Thus, the addition of SNAP to increase local NO concentrations blunted the Ang II response in efferent arterioles. However, the endogenous NO activity was apparently not sufficient to alter the efferent arteriolar reactivity to Ang II. In afferent arterioles that have maintained NO synthesis activity, Ang II responsiveness was already modified by the prevailing endogenously generated NO, and exogenous NO did not provide any additional effects on Ang II responsiveness, although it did shift the level of operation. These results suggest a subtle but significant shift in the quantitative interactions between NO and Ang II responsiveness in the afferent and efferent arterioles.

Endogenous levels of NO could also modulate afferent arteriolar responsiveness to Ang II through cyclic GMP–independent mechanisms. NO can relax vascular smooth
muscle by stimulating membrane hyperpolarization, possibly through inhibition of 20-HETE production. 20-HETE is an endogenous vasoconstrictor of the afferent arteriole, and it has been suggested that this vasoconstriction occurs via 20-HETE-mediated inhibition of vascular smooth muscle K+ channels and subsequent activation of voltage-gated Ca2+ channels. In addition, afferent arteriolar responsiveness to Ang II has also been reported to involve activation of phospholipase A2, resulting in the release of arachidonic acid from membrane phospholipids. Since 20-HETE is one of the arachidonic acid metabolites in afferent arterioles, NO inhibition of 20-HETE production predominantly at the afferent arteriole could be responsible for modulation of vascular responsiveness to Ang II.

NLA enhancement of afferent arteriolar responsiveness to Ang II was eliminated when NO levels were fixed by the addition of SNAP. This result is similar to that observed previously in enalaprilat-treated normotensive rats and suggests that locally generated NO in afferent arterioles of the hypertensive rat kidney blunts afferent arteriolar reactivity to Ang II without involving an actual increase in NO synthesis activity in response to acute Ang II administration. The results further confirm that the influence of NO on afferent arteriolar function is maintained during the development of Ang II–infused hypertension.

In conclusion, in Ang II–infused hypertensive rats, the vasoconstrictor responses to NLA and the vasodilatory responses to SNAP were greater in afferent than efferent arterioles, indicating that NO synthesis activity and reactivity are relatively maintained in afferent arterioles. NO synthesis inhibition with NLA enhanced afferent but not efferent arteriolar responsiveness to Ang II in Ang II–infused hypertensive rats, and the enhancement was abolished by a fixed concentration of NO. Therefore, NO modulation of Ang II responsiveness is maintained in afferent but not efferent arterioles. In addition, SNAP administration blunted efferent but not afferent arteriolar responsiveness to Ang II, suggesting that the Ang II–evoked efferent arteriolar vasoconstriction is more sensitive to increased levels of NO than afferent arterioles. The maintained NO-dependent tone and function in afferent arterioles may contribute to maintaining renal hemodynamics during the development of Ang II–dependent hypertension.

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