Role of Renal Nerves in Afferent Arteriolar Reactivity in Angiotensin-Induced Hypertension

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Abstract The objective of this study was to determine the contribution of renal nerves to the enhanced afferent arteriolar reactivity observed in angiotensin II (Ang II)-induced hypertension. Uninephrectomized Sprague-Dawley rats were divided into four groups: sham rats, renal-denervated rats, Ang II-infused (at 40 ng/min for 13 days), and Ang II-infused + renal-denervated rats. With the use of an implanted arterial catheter, mean arterial pressure (MAP) was monitored in conscious rats. Ang II infusion resulted in a progressive increase in MAP from 98 ± 4 mm Hg (day 0) to 166 ± 7 mm Hg (day 13). This increase in MAP was attenuated in denervated rats and averaged 136 ± 3 mm Hg on day 13. Kidneys were harvested on day 13 for micromorphological experiments or measurement of intrarenal Ang II levels. Basal afferent arteriolar diameter was similar in all groups, and group averages ranged from 19.6 to 20.7 μm. Chronic Ang II Infusion increased intrarenal Ang II levels. Renal denervation did not alter this effect. Increasing perfusion pressure from 100 to 160 mm Hg reduced afferent arteriolar diameter significantly by 11.2 ± 0.6% in the sham group and by a similar degree in the remaining three groups. Superfusion with Ang II (10 nmol/L) reduced afferent arteriolar diameter by 34.3 ± 2.0% in the sham group. This response was enhanced in Ang II-infused (62.3 ± 3.4%) but not in renal-denervated or Ang II-infused + renal-denervated rats. Additionally, the enhanced afferent arteriolar reactivity to Ang II was not influenced by adrenergic receptor blockade. The afferent arteriolar response to norepinephrine was enhanced in renal-denervated, Ang II-infused, and Ang II-infused + renal-denervated rats compared with sham control. Administration of the calcium ionophore A23187 decreased afferent arteriolar diameter similarly in all four groups. These results indicate that renal nerves contribute to the development of hypertension and to the enhanced afferent arteriolar responsiveness to Ang II elicited by chronic Ang II infusion.

Key Words • afferent arteriolar • angiotensin II • arterial pressure • autoregulation • norepinephrine

Chronic infusion of initially supressor doses of Ang II into uninephrectomized rats produces a slowly developing hypertension that is similar to that observed in two-kidney, one clip rats and leads to an augmentation of intrarenal Ang II content. The mechanisms responsible for the sustained hypertension remain unclear. Some studies suggest that chronic exposure to Ang II doses enhances cardiovascular responsiveness to the octapeptide. Other investigators have postulated that the progressive increases in intrarenal Ang II levels critically important. Intrarenal Ang II directly causes microvascular vasoconstriction, leading to decreases in renal blood flow, glomerular capillary pressure, and glomerular filtration rate. Intrarenal Ang II also stimulates sodium reabsorption by the proximal tubule and enhances the sensitivity of the tubuloglomerular feedback mechanism. The net effect of these actions is increased fluid and electrolyte retention, blunted pressure natriuresis, and elevated arterial pressure.

Renal sympathetic nerves also contribute to the development of hypertension in several experimental models, including the two-kidney, one clip Goldblatt model. Enhanced renal nerve activity has been implicated in the progression of hypertension in Ang II-infused rats. Administration of central sympatholytic drugs inhibits the increase in MAP observed in Ang II-infused hypertension. However, the specific mechanisms by which enhanced sympathetic activity contributes to the sustained increase in arterial pressure remain unclear. Renal nerve activity influences renal hemodynamics and can contribute importantly to the genesis of hypertension. Recent studies, using hydronephrotic rat kidneys, revealed that pregglomerular arterioles exhibited frequency-dependent vasoconstriction in response to renal nerve stimulation. These data demonstrate that acute increases in renal nerve activity can influence renal microvascular function and thereby modulate renal hemodynamics in normotensive animals. However, the effect of chronic renal denervation on renal microvascular responsiveness in Ang II-dependent hypertension remains unclear. Therefore, we performed the present studies to determine the effect of renal denervation on the development of Ang II-induced hypertension. In addition, we performed experiments to determine the influence of renal denervation on the increases in intrarenal Ang II content and enhanced afferent arteriolar responsiveness to Ang II that accompany chronic Ang II infusion.

Methods

Animal Preparation

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass.) were housed in wire cages and maintained in a temperature-controlled room that was regulated on a 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 weight) were anesthetized with sodium pentobarbital (50 mg/kg IP), and the left kidney was removed via a left flank incision. An osmotic minipump (model 2002, Alza Corp) was implanted subcutaneously at the dorsum.
of the neck. Minipumps were selected to deliver a constant infusion for 14 days. Ang II–infused rats received Ang II (Novabiochem) at a rate of 40 ng/min. Sham rats were given a saline infusion as a vehicle control. Half of the rats studied underwent right kidney denervation via a right flank incision. The right renal artery was stripped of adipose and connective tissue before being painted with a 10% phenol solution. Surgically prepared rats were divided into four experimental groups: sham rats infused with 0.9% NaCl vehicle (SH rats, n = 26), renal-denervated rats infused with 0.9% NaCl vehicle (DX rats, n = 24), rats infused with Ang II (ANG rats, n = 28), and renal-denervated rats infused with Ang II (ANG+DX, n = 28).

Measurement of Blood Pressure and Heart Rate

Blood pressure was measured every 3 days in conscious rats by tail-cuff plethysmography (model 32-0338, Harvard Apparatus). Direct determinations of MAP and heart rate were made in six rats from each group by implantation of an indwelling catheter. The catheter (PE-10) was placed in the abdominal aorta via the left femoral artery. The distal end was joined to PE-50 tubing and passed subcutaneously to the dorsum of the neck. Patency was maintained with a heparin lock. MAP and heart rate measurements were performed on day 13 after minipump implantation. Each experiment used two rats from the same treatment group (i.e., SH, DX, ANG, or ANG+DX), with one rat serving as the blood donor and the second as the kidney donor. Rats were anesthetized with sodium pentobarbital (50 mg/kg IP). The right kidney of the blood donor was harvested for measurement of renal Ang II and norepinephrine contents. Blood was collected into a heparinized (500 U) syringe via a carotid arterial cannula. Plasma and erythrocyte fractions were collected and prepared as previously described.15,16 Reconstituted blood was passed through a 5-μm nylon mesh and oxygenated in a closed reservoir with 95% O2/5% CO2 gas mixture.

Assessment of Afferent Arteriolar Reactivity

Afferent arteriolar reactivity was assessed with the in vitro blood-perfused juxtamedullary nephron technique combined with videomicroscopy, as previously described.15,16 Experiments were performed on day 13 after minipump implantation. Each experiment used two rats from the same treatment group (i.e., SH, DX, ANG, or ANG+DX), with one rat serving as the blood donor and the second as the kidney donor. Rats were anesthetized with sodium pentobarbital (50 mg/kg IP). The right kidney of the blood donor was harvested for measurement of renal Ang II and norepinephrine contents. Blood was collected in a heparinized (500 U) syringe via a carotid arterial cannula. Plasma and erythrocyte fractions were collected and prepared as previously described.15,16 Reconstituted blood was passed through a 5-μm nylon mesh and oxygenated in a closed reservoir with 95% O2/5% CO2 gas mixture.

The right kidney of the kidney donor was cannulated and perfused with a Tyrode’s solution (pH 7.4) containing 51% bovine serum albumin and a mixture of l-lysine, l-proline, and l-arginine (Sigma Chemical Co). The kidney was excised and sectioned longitudinally, with the papilla reflected back, and the tissue covering the inner cortical surface was removed. Large arteries not supplying the tissue being studied were ligated, and the inner cortical surface was continuously superfused with a warmed (37°C) Tyrode’s solution containing 1% bovine serum albumin. The Tyrode’s perfusate was replaced with the reconstituted blood. Perfusion pressure was measured at the perfusion cannula tip, and renal perfusion pressure was initially set at 100 mm Hg.

The tissue was transilluminated on the fixed stage of a Nikon Eclipse microscope equipped with long-working-distance objectives. Video images obtained by a Newvicon camera (model NC-67M; Dage-MTI) were electronically enhanced (MFJ-1452, MFJ Enterprises), displayed on a monitor (Conrac Display System), and recorded on videotape for later analysis (Super VHS videocassette recorder, Panasonic). Afferent arteriolar inside diameter was measured with an image-shearing monitor (Instrum-
Repetitive measures on one factor and Fisher's protected least-paired and panel t tests, respectively. Two-way ANOVA with squares difference test were also used for determination of afferent arteriolar diameter. A value of \( P < 0.05 \) was considered significant. Data are presented as mean±SE.

### Results

#### Effect of Renal Denervation on Arterial Pressure in Ang II-Infused Rats

As illustrated in Fig 1A, MAP averaged 100±1 and 98±1 mm Hg in SH and DX rats, respectively, and it remained at these levels throughout the 13-day infusion period. Chronic Ang II infusion in ANG rats led to progressive increases in MAP from a basal level of 98±1 to 166±7 mm Hg by day 13. In ANG+DX rats, the increase in MAP during chronic Ang II infusion was attenuated and averaged 136±3 mm Hg on day 13, but MAP was still significantly greater than the starting MAP of SH rats after 13 days of vehicle infusion. Heart rate averaged 421±5, 419±8, 426±5, and 418±8 beats per minute in SH, DX, ANG, and ANG+DX rats (n=6 in each group), respectively, on day 0 and did not change in any group during the 13 days of study.

On day 13, body weights averaged 312±2 and 317±4 g for the SH (n=15) and DX (n=15) rats, respectively, and 297±4 and 299±3 g for the ANG (n=19) and ANG+DX (n=21) rats, respectively. Ang II infusion but not denervation significantly attenuated weight gain compared with the SH rats. These results are consistent with a recent study that reported a stunted growth rate during Ang II infusions. SAP from 121±2 to 188±3 mm Hg in ANG rats during the 13-day infusion period, and this increase was significantly attenuated in ANG+DX rats, averaging 156±2 mm Hg on day 13. In SH rats, SAP averaged 122±2 mm Hg on day 0 and did not change in response to vehicle infusion. In DX rats, SAP was 122±2 mm Hg on day 0 and decreased only slightly on days 3, 7, and 10 of vehicle infusion.

#### Effects of Ang II Infusion and Renal Denervation on Intrarenal Contents of Ang II and Norepinephrine

We assessed intrarenal Ang II levels to determine whether renal denervation altered intrarenal Ang II content. Intrarenal Ang II contents did not differ between the SH (153±39 fmol/g, n=6) and DX (163±22 fmol/g, n=6) groups. ANG II infusion for 13 days increased (\( P < 0.05 \)) intrarenal Ang II content similarly in the ANG (307±20 fmol/g, n=7) and ANG+DX (287±26 fmol/g, n=7) groups. These results are consistent with previous studies. However, although intrarenal Ang II content was significantly increased by chronic Ang II infusion as previously reported, renal denervation did not modify this increase.

Intrarenal norepinephrine levels averaged 135±12 and 129±6 ng/g tissue in the SH (n=5) and ANG (n=5) groups, respectively. These values were greater than those in kidneys from the DX (35±4 ng/g, n=5) and ANG+DX (37±4 ng/g, n=5) groups and demonstrate the effectiveness of the denervation procedure. Nevertheless, intrarenal Ang II infusion did not significantly influence norepinephrine content in either renal-denervated (ANG+DX versus DX, \( P = 0.51 \)) or sham-denervated (ANG versus SH, \( P = 0.71 \)) groups.

### Effects of Chronic Ang II Infusion and Renal Denervation on the Responsiveness of Afferent Arterioles to Increasing Perfusion Pressure

We performed in vitro experiments using the juxtamedullary nephron preparation to assess afferent arteriolar reactivity. At a perfusion pressure of 100 mm Hg, afferent arteriolar diameters were similar in all four groups and averaged 19.9±0.9 (SH), 20.1±0.7 (DX), 19.5±0.7 (ANG), and 20.7±0.6 (ANG+DX) \( \mu \)m. The site along the afferent arteriole where luminal diameters were measured averaged between 78 and 83 \( \mu \)m from the glomerulus for each of the four groups.

Fig 2 illustrates the effects on afferent arteriolar diameter of increasing perfusion pressure. In kidneys from SH rats, increasing perfusion pressure from 100 to 130 and 160 mm Hg significantly reduced afferent arteriolar diameter from 19.9±0.9 to 18.8±1.0 and 17.7±0.9 \( \mu \)m, respectively. Similar results were obtained in the other three groups (Fig 2A). Increasing perfusion pressure from 100 to 160 mm Hg significantly decreased afferent arteriolar diameter in ANG and ANG+DX rats, but not in SH and DX rats. These results are consistent with recent studies that reported a stunted growth rate during Ang II infusions.

#### Statistical Analysis

Between- and within-group analyses were performed with unpaired and paired \( t \) tests, respectively. Two-way ANOVA with repeated measures on one factor and Fisher's protected least-paired and panel t tests, respectively. Two-way ANOVA with squares difference test was also used for determination of differences between groups for measurements of MAP, SAP, and afferent arteriolar diameter. A value of \( P < 0.05 \) was considered significant. Data are presented as mean±SE.
Effects of Chronic Ang II Infusion and Renal Denervation on the Responsiveness of Afferent Arterioles to Norepinephrine

As shown in Fig 4A, norepinephrine significantly reduced afferent arteriolar inside diameter from 19.3 ± 1.3 to 11.7 ± 1.0 μm in the SH groups. Chronic renal denervation significantly enhanced the responsiveness of afferent arterioles to norepinephrine, indicating denervation hypersensitivity. Superfusion of norepinephrine (1 μmol/L) significantly decreased afferent arteriolar diameter from 20.2 ± 0.8 to 7.1 ± 0.4 μm in DX rats. Chronic Ang II infusion also enhanced the afferent arteriolar vasoconstrictor responses to norepinephrine. Norepinephrine (1 μmol/L) significantly reduced afferent arteriolar diameter from 19.4 ± 0.9 to 6.8 ± 0.4 μm in ANG rats. The magnitude of this response was significantly greater than that observed in SH rats (Fig 4B, P < 0.05). This enhanced response to norepinephrine in the Ang II–infused rats was not altered by chronic renal denervation since the response in the ANG+DX group was similar to that observed in the ANG group (20.5 ± 0.7 to 6.7 ± 0.3 μm). Thus, no significant differences in norepinephrine-evoked afferent arteriolar vasoconstriction were observed among the DX, ANG, and ANG+DX groups.

Effects of Chronic Ang II Infusion and Renal Denervation on the Responsiveness of Afferent Arterioles to Ang II

Baseline afferent arteriolar diameters were similar in each group and averaged 19.8 ± 1.1, 20.2 ± 0.7, 19.1 ± 0.9, and 20.6 ± 0.6 μm in the SH, DX, ANG, and ANG+DX groups, respectively (Fig 3A). Superfusion with Ang II significantly decreased afferent arteriolar diameter in all four rat groups (Fig 3A, P < 0.05 versus respective control diameter), however, afferent arteriolar responsiveness to Ang II was significantly enhanced in the ANG group (Fig 3B). Interestingly, renal denervation (ANG+DX) completely prevented the enhanced reactivity to Ang II elicited by chronic Ang II infusion. Afferent arteriolar responsiveness to Ang II superfusion in the ANG+DX rats was not significantly different from that in the SH or DX control rats. Denervation alone did not attenuate the different arteriolar responses compared with those observed in SH rats.

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Effects of Chronic Ang II Infusion and Renal Denervation on the Responsiveness of Afferent Arterioles to A23187

Responses of afferent arterioles to superfusion with the calcium ionophore A23187 are shown in Fig 5. In response to A23187, afferent arteriolar diameter decreased significantly from 20.3±0.4 to 19.1±0.4 μm (1 nmol/L) and 17.6±0.5 μm (100 nmol/L) in SH rats (Fig 5A). The magnitudes of the responses to A23187 were similar in all four rat groups (Fig 5B). The afferent arteriolar response to A23187 was unaffected by renal denervation, chronic Ang II infusion, or the combination of renal denervation and chronic Ang II infusion.

Effects of Adrenergic Receptor Blockers on the Enhanced Responsiveness of Afferent Arterioles to Ang II

The effect of adrenergic receptor blockade on the afferent arteriolar vasoconstrictor response to Ang II is illustrated in Fig 6. Adrenergic receptor blockers did not affect basal afferent arteriolar diameter in any of the groups. During adrenergic receptor blockade, afferent diameter decreased by 35.5±3.0% in response to 10 nmol/L Ang II in SH rats. This decrease was similar to that (34.6±2.9%) observed before adrenergic receptor blockade (Fig 6A). In kidneys from ANG rats, Ang II (10 nmol/L) significantly reduced afferent arteriolar inside diameter by 56.4±2.5% before adrenergic blockade. This decrease was greater than that observed in SH rats (P<0.05) but was not altered by adrenergic receptor blockade (57.4±2.9%). Thus, adrenergic receptor blockade did not alter basal afferent arteriolar diameter or the diameter response to Ang II in SH or ANG rats.

Discussion

Both direct and indirect measurements of arterial pressure confirmed previous reports that chronic infusions of initially subpressor doses of Ang II lead to a slowly developing hypertension. Renal denervation attenuated the increase in MAP in our model. In both groups, arterial pressure increased without significantly changing heart rate. This is consistent with previous studies in rabbits in which Ang II infusion has been shown to reset the baroreceptors to maintain a higher arterial pressure without adjusting heart rate. Since resetting of the baroreceptors is mediated via central α1-adrenergic receptors, limiting the denervation to include only the renal nerves does not influence resetting of the baroreceptors during chronic Ang II infusion.

In the present study, afferent arteriolar responsiveness to increasing perfusion pressure was not significantly altered by chronic Ang II infusion in either innervated or denervated rats. In contrast, afferent arteriolar responsiveness to Ang II and norepinephrine was enhanced in rats chronically infused with Ang II compared with SH rats. During the development of Ang II-infused hypertension, pressor responses to acute administration of Ang II are...
The enhanced reactivity to Ang II was prevented by renal denervation, suggesting that enhanced afferent arteriolar reactivity to Ang II requires intact renal nerves. Several possible explanations could be considered for renal nerve involvement in the enhanced afferent arteriolar responsiveness to Ang II. First, since Ang II is known to facilitate sympathetic neurotransmission, acute administration of Ang II might stimulate neurotransmitter release from intact sympathetic nerve varicosities in the microvasculature of kidneys harvested from ANG rats. This acute, Ang II–mediated neurotransmitter release could enhance the afferent arteriolar vasoconstriction through an adrenergic receptor-mediated mechanism. However, since afferent arteriolar reactivity to Ang II was not affected by adrenergic receptor blockade in kidneys from ANG rats, this explanation seems unlikely unless Ang II is stimulating release of a nonadrenergic neurotransmitter. Second, although renal denervation does not affect the intrarenal angiotensinogen mRNA levels in rats, renal nerves might contribute to the intrarenal accumulation of Ang II during chronic Ang II infusions and enhance the afferent arteriolar responsiveness to acutely administered Ang II. However, the elevated intrarenal Ang II content in ANG+DX rats did not differ from that observed in ANG rats. Another possibility is that chronic Ang II infusion might continuously modulate sympathetic neurotransmission by directly enhancing renal nerve activity or by enhanced stimulation of the central nervous system sympathetic activity. This chronically modulated neurotransmission might alter the responsiveness of afferent arteriolar smooth muscle cells to Ang II. Finally, it may be that the ability of renal denervation to prevent enhancement of afferent arteriolar responsiveness to Ang II may not occur through a direct effect of renal nerves in microvascular reactivity. Rather, the effect of renal denervation on afferent arteriolar responsiveness could be mediated by the associated reduction in MAP and thus a reduction in the microvascular compensation that occurs with chronically elevated arterial pressure.

Afferent arteriolar responsiveness to Ang II was not different between SH and DX rats. Since renal denervation alone did not influence afferent arteriolar responsiveness to Ang II, basal sympathetic tone does not seem to be sufficient to enhance afferent arteriolar responsiveness to Ang II. In contrast, the enhanced afferent arteriolar responsiveness to Ang II observed in ANG rats was prevented in ANG+DX rats. These data suggest that sympathetic neurotransmission modulated by chronic Ang II infusion contributes to the enhanced afferent arteriolar responsiveness to Ang II. Although intrarenal norepinephrine content did not differ between SH and ANG rats, intrarenal norepinephrine content is not supposed to reflect dynamic neurotransmitter activity but only to indicate the effectiveness of renal denervation.

In the present study, we also performed experiments to examine whether chronic Ang II infusion enhanced the calcium sensitivity of arteriolar vascular smooth muscle, thereby enhancing microvascular reactivity to a given stimulus. We examined afferent arteriolar reactivity to direct elevation of intracellular calcium using the calcium ionophore A23187, which acts as a freely mobile carrier to translocate ionic calcium from the extracellular medium to the cytosol via a receptor-independent mechanism. The present results indicate that A23187 caused a nearly identical vasoconstriction among all four groups and suggest that the calcium-induced contractile response is not affected by chronic infusion of Ang II or renal denervation. This, the renal nerve–dependent mechanism by which chronic Ang II infusion enhances afferent arteriolar responsiveness to acute Ang II does not involve modulation of the calcium sensitivity of afferent arteriolar smooth muscle cells.

Norepinephrine and Ang II are thought to utilize similar intracellular signal transduction pathways to elicit vasoconstriction. Therefore, norepinephrine and Ang II could exert synergistic effects on vascular smooth muscle cells and juxtaglomerular cells. A threshold dose of norepi-
nephrine enhanced the Ang II–induced vasocostriction or inhibition of renin release through α1-receptor–mediated activation of calcium channels. Since renal nerve stimulation elicits afferent arteriolar vasocostriction in a frequency-dependent manner, modulation of neurotransmission by chronic Ang II infusion could continuously prime intracellular calcium mobilization pathways at postsynaptic sites. A recent study indicated that β-adrenergic agonists and nephrine increased mRNA expression of the α1-subunit of L-type calcium channels and increased the number of L-type calcium channels in myocytes.

Therefore, in the present study, modulated neurotransmission by chronic Ang II infusion could enhance the expression of functional calcium channels in afferent arteriolar smooth muscle cells and result in the enhanced responsiveness to acute administration of Ang II.

Increased renal nerve activity not only causes afferent and efferent arteriolar vasocostriction through activation of α1-adrenergic receptors but also stimulates postsynaptic β-adrenergic receptors to increase renin secretion. It has been suggested that nephrine directly modulates Ang II receptor density. High concentrations of norephinephrine increase renin secretion. It has been suggested that norephrine directly modulates synaptic sites. A recent study indicated that β-adrenergic receptors stimulate intracellular calcium mobilization pathways at postsynaptic sites. A recent study indicated that β-adrenergic receptors stimulate intracellular calcium mobilization pathways at postsynaptic sites. Therefore, in the present study, modulated neurotransmission by chronic Ang II infusion could enhance the expression of functional calcium channels in afferent arteriolar smooth muscle cells and result in the enhanced responsiveness to acute administration of Ang II.

In conclusion, in Ang II–infused hypertensive rats, MAP increases, and afferent arteriolar responsiveness to Ang II is enhanced. Renal denervation attenuates the development of hypertension and inhibits the enhanced afferent arteriolar reactivity. Enhanced afferent arteriolar reactivity cannot be explained on the basis of enhanced sensitivity of the vascular smooth muscle contractile apparatus for calcium.

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