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) rats, 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±1 (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 microcirculatory 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 attenuated intrarenal Ang II levels and averaged 62.3±4% 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 enhanced afferent arteriolar response to norepinephrine was enhanced in renomed, and Ang II-infused, and Ang II-infused + renal-denervated rats compared with sham controls. 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 reactivity to Ang II elicited by chronic Ang II infusion.

Key Words: afferent arteries • angiotensin II • autoregulation • norepinephrine

Chronic infusion of initially subpressor 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 are critically important. Intrarenal Ang II directly causes microvascular vasoconstriction, leading to reductions 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 also 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-induced 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 preganglionic 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 reactivity 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

SAP 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 made with a blood pressure analyzer (model BPA100E, Micro Med, Inc).

### Assessment of Afferent Arteriolar Reactivity

Afferent arteriolar reactivity was assessed with the in vitro blood-perfused juxtanephric nephron technique combined with videomicroscopy, as previously described. Experiments were performed on day 15 after minipump implantation. Each experiment used two rats from the same treatment group (ie, SH, DX, ANG, or ANG+DX), 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). The right kidney of the kidney donor was cannulated and perfused with so&urn pentobarbital (50 mg/kg IP). The right kidney of the blood donor was harvested for measurements of renal Ang II and norepinephrine contents. Blood was collected into a heparinized (500 U/ml) syringe via a carotid arterial cannula. Plasma and erythrocyte fractions were collected and prepared as previously described. Norepinephrine contents were quickly decapsulated and weighed. As previously described, kidneys used for the analysis of intrarenal Ang II and norepinephrine contents were quickly decapsulated and weighed. As previously described, kidneys were placed in ice-cold methanol (10% wt/vol), homogenized with a chilled glass homogenizer, and centrifuged. The supernatants were dried and reconstituted in 4 mL of 50 mmol/L sodium phosphate buffer containing 1% albumin. Reconstituted samples were extracted (Bond-Elut, Analytichem), using a Microsorb Cl 8 chromatography coupled with an electrochemical detector (model LC-44, B Sloan Systems), using a Microvorb C18 column (4.6x150 mm) (Rainin Instruments) and a Perkin-Elmer series 10 HPLC pump. The retention times for norepinephrine averaged 5.2 minutes.

### Experimental Protocols

A 10-minute equilibration period was allowed before the initiation of each assessment of afferent arteriolar reactivity. Autoregulatory capability was assessed by increasing renal perfusion pressure in a stepwise manner from 100 to 130 and 160 mm Hg. The effect of Ang II on afferent arteriolar diameter was examined by exposing the tissue to 0.1 and 10 mmol/L Ang II. The effect of norepinephrine (Levophed bitartrate, Sanofi Winthrop Pharmaceuticals) on afferent arteriolar diameter was examined by superfusing the tissue with 10 mmol/L and 1 μmol/L norepinephrine. The effect of the calcium ionophore A23187 on afferent arteriolar diameter was also determined. After the control period, the tissue was exposed by administration of 1- and 100-nmol/L concentrations of A23187.

To control for the possibility that acute superfusion with Ang II might stimulate neurotransmitter release from sympathetic varicosities in kidneys from nerve-intact SH and ANG rats, we also performed experiments during adrenergic receptor blockade. According to the procedures described above, afferent arteriolar diameter responses to Ang II were assessed before and during superfusion with a combination of adrenergic receptor antagonists (1 μmol/L prazosin, 10 μmol/L propranolol, and 10 μmol/L yohimbine, Sigma). This combination of adrenergic receptor antagonists completely inhibited the afferent arteriolar vasoconstriction elicited by 1 μmol/L norepinephrine. The presence of the adrenergic receptor antagonists, 1 μmol/L norepinephrine decreased afferent arteriolar diameter by 0.2±0.5% compared with control vasoconstruction of 61±5.6% Time control experiments (n=4) performed to determine the reproducibility of afferent arteriolar responses to repeat Ang II administration revealed no significant difference in the responses between the first and second treatments. Ang II concentrations of 0.1 and 10 mmol/L decreased afferent arteriolar diameter by 32±1.7% and 34±2.3%, respectively, in the SH group and by 52±1.6% and 53±1.4% in the ANG group.

### Measurement of Renal Ang II and Norepinephrine Contents

Kidneys collected for measurement of intrarenal Ang II and norepinephrine contents were quickly decapsulated and weighed. As previously described, kidneys for the analysis of intrarenal Ang II were placed in ice-cold methanol (10% wt/vol), homogenized with a chilled glass homogenizer, and centrifuged. The supernatants were dried and reconstituted in 4 mL of 50 mmol/L sodium phosphate buffer containing 1% albumin. Reconstituted samples were extracted (Bond-Elut, Analytichem), and the eluates were evaporated to dryness and reconstituted in Ang II assay buffer. Ang II content was measured by radioimmunoassay using rabbit anti-Ang II antisera (Arnel) as previously reported. The sensitivity of the Ang II assays was 1±0.3 fmol/g during 90% maximal binding. Specific binding was 36±2.8%, and nonspecific binding was 9±0.1% for the Ang II assays.

The remaining kidneys were used for measurement of intrarenal norepinephrine content. Each kidney was rapidly frozen in 1 mL of 0.1 mol/L perchloric acid and stored at −70°C until analyzed. The frozen samples were homogenized in 10 mL ice-cold 0.3 mol/L perchloric acid and centrifuged at 20 000g for 20 minutes at 4°C. Norepinephrine content in the supernatant of the renal homogenate was determined by high-performance liquid chromatography coupled with an electrochemical detector (model LC-44, Bioanalytical Systems), using a Microvorb C18 column (4.6x150 mm) (Rainin Instruments) and a Perkin-Elmer series 10 HPLC pump. The retention times for norepinephrine averaged 5.2 minutes.

### Selected Abbreviations and Acronyms

- Ang II = angiotensin II
- MAP = mean arterial pressure
- SAP = systolic arterial pressure
- SHR = spontaneously hypertensive rat(s)
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-.significance difference test were 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.

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.18 SAP values of rats used for analysis of afferent arteriolar reactivitites are shown in Fig 1B. In agreement with direct pressure measurements, chronic Ang II infusion significantly increased SAP from 121 ± 1 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. Thus, although intrarenal Ang II content was significantly increased by chronic Ang II infusion as previously reported,13 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 (33 ± 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) μm. The site along the afferent arteriole where luminal diameters were measured averaged between 78 and 83 μm 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 μm, 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 from 19.9 ± 0.9 to 18.7 ± 1.0 μm in ANG+DX rats but not in DX rats.

### Notes

2. Statistical Analysis
3. Effects of Chronic Ang II Infusion and Renal Denervation on the Responsiveness of Afferent Arterioles to Increasing Perfusion Pressure
4. *A* = Ang II–Infused and renal-denervated rats
5. *B* = Ang II–Infused and sham-denervated rats
6. *C* = Ang II–Infused rats
7. *D* = Ang II–Infused and renal-denervated rats
8. *E* = Ang II–Infused and sham-denervated rats
9. *F* = Ang II–Infused and sham-denervated rats
10. *G* = Ang II–Infused and sham-denervated rats
11. *H* = Ang II–Infused and renal-denervated rats
12. *I* = Ang II–Infused and sham-denervated rats
13. *J* = Ang II–Infused and sham-denervated rats
14. *K* = Ang II–Infused and sham-denervated rats
15. *L* = Ang II–Infused and sham-denervated rats
16. *M* = Ang II–Infused and sham-denervated rats
17. *N* = Ang II–Infused and sham-denervated rats
18. *O* = Ang II–Infused and sham-denervated rats
19. *P* = Ang II–Infused and sham-denervated rats
20. *Q* = Ang II–Infused and sham-denervated rats
21. *R* = Ang II–Infused and sham-denervated rats
22. *S* = Ang II–Infused and sham-denervated rats
23. *T* = Ang II–Infused and sham-denervated rats
24. *U* = Ang II–Infused and sham-denervated rats
25. *V* = Ang II–Infused and sham-denervated rats
26. *W* = Ang II–Infused and sham-denervated rats
27. *X* = Ang II–Infused and sham-denervated rats
28. *Y* = Ang II–Infused and sham-denervated rats
29. *Z* = Ang II–Infused and sham-denervated rats

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*Fig 1. Effects of chronic Ang II infusion and renal denervation on MAP (A) and SAP (B) in sham-operated rats infused with 0.9% NaCl vehicle (SH) (□, n = 6 for MAP, n = 9 for SAP), renin-denervated rats infused with 0.9% NaCl vehicle (DX) (▲, n = 9 for MAP, n = 9 for SAP), and Ang II–infused rats (ANG) (○, n = 6 for MAP, n = 15 for SAP). Statistical symbols in B are as defined in A.*
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±1.0 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 the 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 vs 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 afferent 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 ± 0.4 μm to 19 ± 0.4 μm (1 nmol/L) and 17 ± 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 ± 3.0% in response to 10 nmol/L Ang II in SH rats. This decrease was similar to that (34 ± 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 ± 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 (37 ± 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 suprpressor 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 α-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 enhancedafferent 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, 

potentiated, this is called autopotentiation. 

This increased sensitivity has also been documented in the SHR model, i.e., an enhanced increase in renal vascular resistance to intravenous infusion of Ang II occurs early in the development of SHR hypertension. This enhanced reactivity in SHR is specific to the renal vasculature since the hindquarter, carotid, and mesenteric arteries responsive to Ang II were similar in SHR and Wistar-Kyoto rats. Unlike the SHR, an increased responsiveness to Ang II has also been demonstrated in the isolated mesenteric vasculature and aorta in the early stages of Ang II–infused hypertension.

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, acutely administered 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 suggests that the calcium–induced contractile response is unaffected by chronic infusion of Ang II or renal denervation. Thus, 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 vasoconstriction or inhibition of renin release through α1-receptor–mediated activation of calcium channels.39,40 Since renal nerve stimulation elicits afferent arteriolar vasoconstriction in a frequency-dependent manner,41 modulation of neurotransmission by chronic Ang II infusion could continuously prime intracellular calcium mobilization pathways at post-synaptic sites. A recent study indicated that β-adrenergic agonists and norepinephrine increased mRNA expression of the α1-subunit of L-type calcium channels and increased the number of L-type calcium channels in myocytes.31 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 vasoconstriction through activation of α1-adrenergic receptors but also stimulates postsynaptic β-adrenergic receptors to increase renin secretion.32 It has been suggested that norepinephrine directly modulates Ang II receptor density. High concentrations of norepinephrine to increase Ang II receptor number in neuronal cell cultures.33 In addition, β-adrenergic receptor activation stimulates and α2-adrenergic receptor activation inhibits adenylyl cyclase, thereby modulating intracellular cAMP levels.34 Since an increase in cAMP upregulates the Ang II receptor mRNA,35 the possibility remains that renal nerve transmission may upregulate Ang II receptors through β-receptor–mediated activation of cAMP.

Few studies have investigated the effects of renal nerve activity on the acute renal microvascular responsiveness to Ang II. Exclusion of β1-adrenergic influences or basal tonic of renal nerves did not affect Ang II–mediated afferent arteriolar vasoconstriction.36,37 This observation is consistent with the current findings that no difference in afferent arteriolar reactivity to Ang II was noted between SH and DX rats. However, the present data suggest that altered renal neurotransmission modulated by chronic Ang II infusion does influence the acute responsiveness to Ang II.

Chronic Ang II infusion also enhanced afferent arteriolar reactivity to norepinephrine, and this augmentation was not inhibited by renal denervation. Therefore, renal nerve–independent enhancement of preglomerular reactivity seems to involve a direct effect of chronic Ang II infusion on afferent arteriolar smooth muscle cells. In other studies, Ang II amplifies the norepinephrine-induced vasoconstriction through the activation of protein kinase C in isolated arteries of rabbits.38 Chronic infusion of Ang II increases intrarenal Ang II content, which may in turn potentiate afferent arteriolar smooth muscle cell contraction in response to norepinephrine. Afferent arteriolar reactivity to norepinephrine was also enhanced by renal denervation alone. Denervation is known to induce catecholamine supersensitivity mediated by adrenergic receptor upregulation and an increase in receptor affinity for norepinephrine.39 Therefore, denervation-induced enhancement of afferent arteriolar reactivity to norepinephrine may be mediated by the modulation of specific adrenergic receptors.

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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