Pressure-Induced Renal Injury in Angiotensin II Versus Norepinephrine-Induced Hypertensive Rats

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Abstract—The susceptibility to renal perfusion pressure (RPP)—induced renal injury was investigated in angiotensin II (Ang II)– versus norepinephrine (NE)-infused hypertensive rats. To determine the magnitude of RPP-induced injury, Sprague-Dawley rats fed a 4% salt diet were instrumented with a servocontrolled aortic balloon occluder positioned between the renal arteries to maintain RPP to the left kidney at baseline levels whereas the right kidney was exposed to elevated RPP during a 2-week infusion of Ang II IV (25 ng/kg per minute), NE IV (0.5, 1.0, and 2.0 μg/kg per minute on days 1, 2, and 3 to 14, respectively), or saline IV (sham rats). Over the 14 days of Ang II infusion, RPP averaged 161.5±8.0 mm Hg to uncontrolled kidneys and 121.9±2.0 mm Hg to servocontrolled kidneys. In NE-infused rats, RPP averaged 156.3±3.0 mm Hg to uncontrolled kidneys and 116.9±2.0 mm Hg to servocontrolled kidneys. RPP averaged 111.1±1.0 mm Hg to kidneys of sham rats. Interlobular arterial injury and juxtaglomerular glomerulosclerosis were largely RPP dependent in both models of hypertension. Superficial cortical glomerulosclerosis was greater and RPP dependent in NE- versus Ang II-infused rats, which was primarily independent of RPP. Outer medullary tubular necrosis and interstitial fibrosis were also primarily RPP dependent in both models of hypertension; however, the magnitude of injury was exacerbated in Ang II-infused rats. We conclude that elevated RPP is the dominant cause of renal injury in both NE- and Ang II-induced hypertensive rats and that underlying neurohumoral factors in these models of hypertension alter the pattern and magnitude of RPP-induced renal injury. (Hypertension. 2009;54:1269-1277.)

Key Words: angiotensin II ■ blood pressure ■ kidney ■ norepinephrine ■ renal injury

Hypertension results in various pathological changes within the kidney, including vascular, glomerular, and tubulointerstitial injuries. The pattern and magnitude of renal injury varies according to the etiology of hypertension and/or the presence of underlying renal disease and remains an important question with respect to the diagnostic accuracy of the progression of renal disease. Although hypertension is the second leading cause of end-stage renal disease, the direct effects of elevated renal perfusion pressure (RPP) in hypertension-induced renal injury remain ambiguous, because the susceptibility to renal injury is known to vary greatly across human populations, as well as in experimental and genetic models of hypertension. The mechanisms responsible for the differences in the susceptibility to RPP-induced renal injury are uncertain but are likely attributable to complex interactions among elevated RPP, altered paracrine and endocrine factors, genetic factors, and/or the presence of underlying renal disease.

We have begun to address these issues in a systematic way by first determining the role of underlying neurohumoral factors in altering the susceptibility to RPP-induced renal injury by comparing the pattern and magnitude of RPP-induced renal injury in norepinephrine (NE)- versus angiotensin II (Ang II)–induced hypertensive Sprague-Dawley rats.

Various forms of hypertension are associated with elevated sympathetic activity and/or inappropriate activation of the renin-angiotensin system, in which both pressure-dependent and pressure-independent patterns of end-organ damage have been described.

The long-standing limitation of drawing conclusions about the contribution of elevated RPP versus the circulating endocrine factors in previous studies has been the inability to distinguish the relative contribution of each because the kidneys are simultaneously exposed to both potentially injurious factors. Using chronic arterial pressure servocontrol techniques adapted to rats, we have determined previously that elevated RPP, per se, accounts for the majority (~80%) of juxtaglomerular glomerular and outer medullary tubulointerstitial injuries in Ang II-infused rats fed a 4% NaCl diet for 14 days. In the current study, we used a chronic servocontrol of RPP technique to enable a more precise analysis and comparison of the magnitude of RPP-dependent and -independent patterns of renal injury in 2 different models of hypertension, one induced by chronic NE infusion and the other by chronic Ang II infusion.

Materials and Methods
An expanded Materials and Methods section is available in the online Data Supplement at http://hyper.ahajournals.org.
Experimental Animals
All of the studies were performed on 12-week–old male Sprague-Dawley rats (Harlan) that were provided water ad libitum and provided a 0.4% NaCl AIN-76 rodent diet (Dyets). All of the protocols were approved by the Medical College of Wisconsin Institutional Animal Care and Use Committee.

Experimental Design and Chronic Servocontrol of RPP
To determine the role of neurohumoral factors in altering the susceptibility to RPP-induced renal injury, all of the rats were surgically instrumented with an inflatable vascular occluder positioned on the aorta between the left and right renal arteries through a midsagittal abdominal incision. Three groups of rats were studied: (1) 14-day NE-infused rats (n = 7); (2) 14-day Ang II-infused rats (n = 7); and (3) 14-day saline-infused sham rats (n = 7). Four days after surgery, the diet was switched to a 4.0% NaCl AIN-76 rodent diet (Dyets) for all of the rats for the remainder of the study. Rats were allowed to recover for 10 days, during which 3 stable days of baseline blood pressure were recorded. After baseline blood pressure was obtained, the IV solution was switched to either NE or Ang II, whereas saline was continuously infused for sham-operated rats. During NE or Ang II infusion, the vascular occluder was chronically servocontrolled to maintain RPP at baseline levels to the left kidney, whereas the right kidney was exposed to elevated RPP for 14 days. The vascular occluder cuff was never inflated for saline-infused sham rats. For the Ang II group, 25 ng/kg per minute was infused (IV) continuously for 14 days, as we have reported previously. In a separate group of rats, the dose of NE was increased over the first 3 days, which produced a sustained elevation of mean arterial pressure (MAP) over 14 days that was comparable to the pattern of infusion and continued for the entire duration of the experimental protocol in the NE and Ang II groups.

Histological Analysis
Histological and immunohistochemical analyses were performed to quantify the magnitude of RPP-dependent and -independent superficial cortical and juxtamedullary glomerular injury, interlobular arterial remodeling and injury, and outer medullary tubular necrosis and interstitial fibrosis, as we have described previously, and are provided in detail in the online Data Supplement, available at http://hyper.ahajournals.org.

Statistical Methods
Data are presented as mean ± SE. A 2-way repeated-measures ANOVA, followed by a Tukey post hoc test, was used to determine daily differences in blood pressure and heart rate across groups over the 17-day experimental protocol. A paired t test was used to assess differences in indices of renal injury between servocontrolled and uncontrolled kidneys, whereas an unpaired t test was used to compare servocontrolled and sham kidneys within a group and for the comparison of servocontrolled or uncontrolled kidneys between groups. Pearson correlation and linear regression analysis were performed for sham, servocontrolled, and uncontrolled kidneys within each model to assess the relationship between MAP and indices of renal injury. A P < 0.05 was considered significant.

Results
MAP to Left and Right Kidneys
The average daily MAPs for sham, NE, and Ang II rats are summarized in Figure 1. MAP was significantly elevated over the 14 days of NE and Ang II infusion as compared with the 3 baseline days (P < 0.05). In both NE and Ang II groups, MAP to the uncontrolled kidney remained significantly elevated above the servocontrolled MAP and the MAP of sham rats for days 1 to 14 (P < 0.05). There were no significant differences in MAP between the servocontrolled kidneys and kidneys from sham rats across all of the time points. The MAPs to the uncontrolled kidneys in NE and Ang II groups were similar across the 14 days of infusion, except for day 3 of infusion, in which MAP was significantly (P < 0.05) higher in Ang II- versus NE-infused rats.

Heart Rate, Systolic Blood Pressure, Diastolic Blood Pressure, and Pulse Pressure
The average daily heart rate over the 14 days of drug or saline infusion was not significantly different among sham (416 ± 1 bpm), NE-infused (401 ± 5 bpm), and Ang II-infused (402 ± 5 bpm) rats; however, heart rate was significantly (P < 0.05) lower on days 1 and 2 of drug infusion in both NE- and Ang II-infused rats as compared with sham rats but subsequently approached the level of sham rats on days 3 to 13. No significant differences in heart rate were observed between NE- and Ang II-infused rats.

The average daily systolic blood pressure (SBP) and diastolic blood pressure in uncontrolled kidneys of both NE- and Ang II-infused rats were significantly (P < 0.05) elevated as compared with servocontrolled kidneys and kidneys from sham rats over days 1 to 14. Although there were no significant differences in diastolic blood pressure between uncontrolled kidneys of NE- and Ang II-infused rats, SBP was significantly higher in NE-infused rats on days 12 to 14 of drug infusion as compared with Ang II-infused rats. Furthermore the SD of the average daily SBP, used as an index of BP variability, over the 14 days of drug infusion averaged 19.0 ± 0.9 mm Hg in uncontrolled kidneys of NE-infused rats and 13.0 ± 0.6 mm Hg in Ang II-infused rats, which were both significantly higher than the variability.
observed in servocontrolled kidneys and kidneys from sham rats (6.7 ± 0.1 mm Hg). The average daily SBP variability was significantly \( (P < 0.05) \) higher in uncontrolled kidneys of NE- versus Ang II-infused rats on days 5, 8, 10, 11, and 14 of drug infusion, suggesting a greater lability of blood pressure in the NE model of hypertension.

The average daily pulse pressure (PP) over the 14 days of drug infusion was significantly lower in servocontrolled kidneys of NE- (15.0 ± 0.5 mm Hg) and Ang II-infused (12.0 ± 0.7 mm Hg) rats as compared with kidneys from sham rats (26.0 ± 0.4 mm Hg). PP in uncontrolled kidneys from NE- (31.0 ± 2.0 mm Hg) and Ang II-infused (23.0 ± 0.9 mm Hg) rats was significantly higher as compared with their respective servocontrolled kidneys over the 14 days of drug infusion. The average daily PP in uncontrolled kidneys in NE-infused rats was significantly higher as compared with Ang II-infused rats and kidneys from sham rats on days 11 to 14 of drug infusion.

### Interlobular Arterial Remodeling and Injury

The vascular smooth muscle wall:lumen cross-sectional area ratio of interlobular arteries is presented in Figure 2A. The vascular smooth muscle wall:lumen cross-sectional area ratio (A) and injury (B) in 20 randomly selected interlobular arteries in kidneys from 14-day sham, servocontrolled (SC), and uncontrolled (UC) kidneys. A significant elevation in the wall:lumen ratio and injury was observed in interlobular arteries exposed to elevated RPP in both the Ang II- and NE-induced models of hypertension. Values are mean ± SE. \(^*P < 0.05\) vs respective SC kidney.

Figure 2.

In NE-infused rats, elevated RPP contributed to 92% of superficial cortical glomerulosclerosis with the uncontrolled kidneys exhibiting 24.1 ± 2.6% of 30 glomeruli with sclerosis as compared with the servocontrolled kidneys, in which only 5.5 ± 1.5% of 30 glomeruli were sclerosed \( (P < 0.05) \). The direct effects of NE (8% contribution) did not result in significant superficial cortical glomerulosclerosis, as determined by comparison with the kidneys from sham rats.

In Ang II-infused rats, superficial glomerulosclerosis was moderately, but significantly \( (P < 0.05) \), elevated in both servocontrolled (9.8 ± 2.5% of 30 glomeruli) and uncontrolled (13.8 ± 2.7% of 30 glomeruli) kidneys as compared with kidneys from sham rats. There was no difference in superficial glomerulosclerosis between the servocontrolled and uncontrolled kidneys of Ang II-infused rats. Elevated Ang II was responsible for \( \approx 60\% \) of superficial cortical glomerulosclerosis, whereas elevated RPP only contributed \( \approx 40\% \), which indicates that elevated Ang II, per se, was the dominant cause of superficial glomerular injury, which is similar to our previous study. These data indicate that the magnitude of superficial cortical glomerular injury is significantly higher \( (P < 0.05) \) in NE- versus Ang II-infused rats, with the susceptibility to RPP-induced superficial cortical glomerulosclerosis being greater in the presence of elevated levels of circulating NE as compared with Ang II.
Juxtamedullary Glomerulosclerosis

Elevated RPP was responsible for 87% of juxtamedullary glomerulosclerosis in NE-infused rats. Sclerosis was significantly (P<0.05) higher in uncontrolled (37.1±4.7% of 20 glomeruli) versus servocontrolled (9.8±2.2% of 20 glomeruli) kidneys. The direct effects of elevated circulating levels of NE on juxtamedullary glomerulosclerosis were minimal and did not reach statistical significance as compared with kidneys from sham rats.

In Ang II-infused rats, elevated RPP was responsible for 80% of juxtamedullary glomerulosclerosis, because sclerosis was significantly (P<0.05) higher in uncontrolled (35.7±9.6% of 20 glomeruli) versus servocontrolled (13.2±2.0% of 20 glomeruli) kidneys. The direct effects of elevated circulating levels of Ang II (20%) on juxtamedullary glomerulosclerosis were modest but did reach statistical significance as compared with kidneys from sham rats (P<0.05). These data indicate that elevated RPP is the dominant cause of juxtamedullary glomerulosclerosis in both NE- and Ang II-induced hypertensive rats.

Outer Medullary Tubular Injury and Fibrosis

Tubular Injury

As shown in Figure 4A, elevated RPP increased outer medullary tubular injury in both NE- (P<0.05) and Ang II-infused (P<0.05) rats. The magnitude of tubular injury present in the uncontrolled kidneys of Ang II-infused rats was significantly (P<0.05) greater as compared with the uncontrolled kidneys of NE-infused rats. Furthermore, the difference in outer medullary tubular injury between the uncontrolled and servocontrolled kidneys of rats receiving Ang II (1.0±0.3) was significantly greater (P<0.05) when compared with rats receiving NE (0.28±0.1). Elevated Ang II, per se, was directly responsible for 10% of outer medullary tubular injury (P<0.05 as compared with sham) and resulted in more renal injury (P<0.05) as compared with the direct effects of NE (0% contribution to injury). A significant (P<0.05) correlation between outer medullary tubular injury and the 14-day average MAP was found in both NE- (r=0.81) and Ang II-infused (r=0.89) rats. Representative images, obtained with a ×10 objective lens, depicting the pattern of outer medullary tubular injury, are shown in Figure 4A. These results suggest that elevated RPP is responsible for the majority of outer medullary tubular injury in both models of hypertension; however, Ang II greatly increases the susceptibility to RPP-induced outer medullary tubular injury as compared with NE.

Interstitial Fibrosis

The percentage of positive α-smooth muscle actin (SMA) regions within the outer medulla was used as an index of...
outer medullary fibrosis (see the online Data Supplement), as we have described previously.14,16 As shown in Figure 4B, elevated RPP was responsible for 78% of outer medullary fibrosis in NE- (P<0.09) and 65% in Ang II-infused (P<0.05) rats. Outer medullary interstitial fibrosis was significantly (P<0.05) greater in the uncontrolled kidneys of Ang II-infused rats as compared with the uncontrolled kidneys of NE-infused rats. Furthermore, the difference in outer medullary fibrosis between the uncontrolled and servocontrolled kidneys of rats receiving Ang II (2.1±0.8) was higher (P<0.06) as compared with rats receiving NE (0.4±0.2). Ang II directly resulted in 35% of outer medullary fibrosis, which was significantly (P<0.05) greater as compared with the direct effects of NE (22%). A significant correlation between outer medullary fibrosis and the 14-day average MAP was found in both NE- (r=0.43) and Ang II-infused (r=0.70) rats. As shown in Figure 4B, the dark-brown regions are positive for α-SMA and tended to be localized to only vasa recta capillaries in kidneys with minimal interstitial fibrosis. In kidneys with significant fibrosis, α-SMA staining was more diffuse, with more intense staining in both vasa recta capillaries and tubules. Thus, elevated RPP was responsible for the majority of outer medullary interstitial fibrosis, and Ang II increased the susceptibility of the kidney to RPP-induced outer medullary fibrosis.

Discussion

Results of the present study show that elevated RPP was the dominant cause of renal damage in both NE- and Ang II-induced hypertensive rats; however, the pattern and magnitude of RPP-induced injury varied between the models. Injury of the superficial cortical glomeruli was greater in NE-infused rats and largely RPP dependent, whereas, in comparison, the elevation of RPP did little damage to cortical glomeruli in Ang II-infused rats. In contrast, outer medullary tubular injury and interstitial fibrosis were greatly exacerbated by elevations of RPP in Ang II-infused rats. Values are mean±SE. *P<0.05 NE servocontrolled. †P<0.05 vs sham and NE SC. ‡P<0.05 vs NE UC. Calibration bar=200 μm.

Vascular Hypertrophy and Injury

The vascular wall:lumen ratio in interlobular arteries was significantly elevated in uncontrolled kidneys exposed to elevated RPP in both the NE and Ang II models of hyper-
tension. Remodeling and injury in small renal resistance vessels are common in individuals with primary hypertension and in most experimental and genetic models of hypertension. Hypertrophy of resistance vessels in response to chronic elevations in RPP is a physiological adaptation to reduce the amount of vascular wall stress and contributes to maintaining a relatively constant renal blood flow and glomerular filtration rate in the face of elevated systemic pressures. Over time, however, with excessive vascular hypertrophy, autoregulation is impaired as the diffusion distance of oxygen across the smooth muscle wall increases, which can result in injury to both the vasculature and downstream structures. In both the NE and Ang II models of hypertension, elevated RPP, per se, accounted for 90% of the total amount of vascular hypertrophy in superficial cortical vessels. This is consistent with our previous servocontrol study in Ang II-infused rats and indicates that elevated RPP, per se, is the primary stimulus for hypertrophy of interlobular arteries in both NE- and Ang II-induced models of hypertension.

Our data indicate that elevated RPP results in significant interlobular arterial injury in both NE- and Ang II-induced models of hypertension. Interestingly, elevation of circulating NE or Ang II, per se, at the concentrations used in this study, did not directly stimulate significant vascular remodeling or injury. Although previous studies suggest that vascular injury is in part attributable to direct consequences of elevated Ang II, many of these studies have been performed in cell culture models or with the use of various antihypertensive drugs administered to different groups of animals, making it difficult to control for the contributions of elevated RPP, as was done in the present study. Importantly, our data demonstrate that elevated RPP was the dominant cause of interlobular arterial remodeling and injury in both NE and Ang II models of hypertension. That is, the normalization of arterial blood pressure prevented most of the vascular complications arising as a result of hypertension in these rats.

Glomerulosclerosis

Superficial Cortical Glomerulosclerosis

Superficial cortical glomerular injury in hypertension is typically modest unless there are severe elevations in blood pressure, such as in malignant nephrosclerosis, or in models of hypertension associated with impaired renal blood flow autoregulation or underlying renal disease. In the present study, superficial glomeruli of Ang II-infused rats were greatly protected from elevated RPP, as we have observed previously. The increase in superficial cortical glomerulosclerosis, albeit relatively small, could be attributed to the direct effects of elevated circulating Ang II, as predicted on the basis of evidence that Ang II directly stimulates free radical production, extracellular matrix production, inflammation, and transforming growth factor-β production.

NE-infused rats exhibited greater RPP-dependent levels of superficial glomerulosclerosis than that elicited by the direct effects of Ang II. Although it has been reported previously that 8 weeks of chronic phenylephrine infusion in rats resulted in only a relatively small degree of glomerular injury, it is difficult to compare the magnitude of glomerular injury between studies. Specifically, the present study produced higher levels of arterial pressure for a shorter duration and could be expected to exhibit greater levels of renal injury. The goal of our study was to produce the same levels of hypertension with both Ang II and NE.

Superficial cortical glomerular injury that could be attributed to the direct effects of circulating NE was minimal. These observations suggest that the greater degree of NE-versus Ang II-related RPP-induced glomerulosclerosis was a consequence of relatively less preglomerular vasoconstriction in the NE model of hypertension. The data of previous studies are unclear on this issue with isolated arterioles from rats suggesting preferential postglomerular constriction with Ang II compared with NE, whereas others suggest, using micropuncture techniques in acutely anesthetized rats, that Ang II results in greater constrictor actions (independent of RPP) on afferent arterioles. Others have found that glomerular filtration rate does not decrease after chronic administration of NE, whereas a similar level of hypertension produced by Ang II resulted in a decrease in glomerular filtration rate. Thus, the lack of RPP-induced superficial glomerulosclerosis in the Ang II model of hypertension may be a result of increased preglomerular vasoconstriction, which could confer protection from RPP-induced glomerular injury. Furthermore, the small, RPP-independent pattern of superficial cortical glomerulosclerosis in the Ang II-induced model of hypertension may have been a consequence of ischemia because of increased preglomerular vasculature constriction.

Another possible explanation for the greater RPP-induced superficial cortical glomerulosclerosis in NE-infused rats could be related to the sensitivity of the tubuloglomerular feedback mechanism. Previous studies have shown that Ang II, but not NE, serves as an important modulator of tubuloglomerular feedback sensitivity, resulting in higher levels of afferent arteriolar constriction in the presence of elevated distal tubule flow rates or increased distal NaCl delivery. NE may fail to sensitize tubuloglomerular feedback–mediated afferent arteriolar constriction in response to an RPP-induced increase in distal NaCl delivery.

In addition, the superficial cortical glomerulosclerosis in NE-infused rats could have occurred as a result of the differences observed in the arterial pulse waves and variability of pressure with NE-induced hypertension. It has been demonstrated that SBP, PP, and blood pressure variability can influence the magnitude of hypertension-induced renal injury. Although the average MAP was similar between the uncontrolled kidneys of NE and Ang II models of hypertension, the PP and both the magnitude and variability of SBP were significantly higher in NE-infused rats over the second week of drug infusion as compared with Ang II-infused rats. The myogenic mechanism of renal blood flow autoregulation intrinsic to the preglomerular vasculature is thought to protect glomeruli from RPP-induced injury; however, the higher SBP, as well as the greater magnitude of SBP fluctuations, in NE-infused rats may have resulted in a greater fraction of systemic pressure being transmitted to the glomerular capillaries.
**Juxtamedullary Glomerulosclerosis**

In the present study, juxtamedullary glomerulosclerosis was prominent in both NE- and Ang II-infused rats and was almost entirely a direct consequence of elevated RPP. Hypertension-induced juxtamedullary glomerulosclerosis is typically more severe compared with superficial cortical glomerulosclerosis and is often observed in the early phases of hypertension. The increased susceptibility to RPP-induced injury in juxtamedullary glomeruli is thought to be attributable to the larger diameters and shorter lengths of the preglomerular vessels, thereby exposing these glomeruli to elevated capillary pressures and, ultimately, to barotrauma-related injury.

Our results stand in strong contrast to the commonly accepted belief that the direct effects of Ang II are largely responsible for the glomerular and interstitial injury through the downstream activation of various pathways, such as the production of reactive oxygen species and transforming growth factor-β, in an RPP-independent manner. Clearly, these pathways do contribute to the injury, as supported by observations that inhibitors of the renin-angiotensin system are found to be superior to other antihypertensive treatments because of their RPP-independent renal protection.

The results of this study, however, suggest that the majority of glomerular, primarily juxtamedullary, damage observed in Ang II-infused rats is a direct result of elevated RPP, an idea that is gaining strength.

**Outer Medullary Tubulointerstitial Injury**

A major finding from this study is that the susceptibility to RPP-induced outer medullary tubulointerstitial injury and fibrosis is substantially greater in Ang II-infused as compared with NE-infused hypertensive rats despite a similar magnitude of hypertension in both groups. We have previously demonstrated an RPP-dependent pattern of outer medullary injury in Ang II-infused rats and have proposed that this could be explained by the poor autoregulation of blood flow in the region of the outer medulla. The attenuation of RPP-induced outer medullary injury in NE-infused rats does not appear to be explained by greater preglomerular constriction, because juxtamedullary glomerular RPP-induced injury was as great in NE-infused rats as that observed in Ang II-infused rats.

The mechanisms responsible for the reduced susceptibility to RPP-induced outer medullary injury in NE-infused hypertension remains unclear, but recent studies provide some possible explanations. It is recognized that increased RPP results in decreased reabsorption of NaCl in the proximal tubule, which increases distal tubule flow rate and NaCl delivery. We have found recently that acute elevations of RPP increase the production of superoxide in the medullary thick ascending limb and interstitial hydrogen peroxide levels in the outer medulla, responses that appear to be mediated through stimulation of superoxide production via NADPH oxidase within the medullary thick ascending limb. Because studies have shown that Ang II, but not NE, increases the expression of NADPH oxidase, the increased NaCl delivery to the medullary thick ascending limb associated with elevated RPP may stimulate greater amounts of superoxide and hydrogen peroxide in the Ang II model of hypertension, resulting in greater tissue injury and fibrosis of the outer medulla.

Alternately, in parallel with the release of reactive oxygen species, Ang II may have resulted in greater ischemia in the outer medulla as compared with NE. Schachinger et al. found in humans that acute administration of Ang II, but not NE, resulted in decreases in renal oxygen tension despite similar increases in blood pressure. Thus, the outer medullary injury caused by the direct actions of Ang II, independent of RPP, observed in the present study could have resulted from ischemia because of increased preglomerular constriction, as discussed previously. Furthermore, it is also possible that Ang II sensitizes the myogenic response of the afferent arteriole, which could produce excessive levels of vasoconstriction and ischemia in response to elevated RPP, and exacerbate the magnitude of outer medullary ischemia. It is recognized that hypoxia can be both a cause and a consequence of renal injury, i.e., RPP-induced injury, and additional studies will be required to determine the specific role of ischemia in Ang II-induced hypertensive renal injury.

In conclusion, it is recognized that the extent and pattern of renal injury differ widely in subjects with hypertension and in various animal models, and the contribution of elevated RPP, per se, to the injury is poorly understood. The insight provided by this study is the demonstration that neurohumoral factors can influence the pattern and magnitude of RPP-induced injury. Having for the first time precisely controlled RPP to 1 kidney while exposing the contralateral kidney to hypertension, we can, with confidence, conclude that elevated RPP significantly contributes to renal injury in both NE and Ang II models of hypertension. The magnitude of RPP-induced injury varies between models with the extent of outer medullary damage being considerably greater in Ang II-infused rats, whereas superficial cortical glomerular injury is more severe in NE-infused rats.

**Perspectives**

The incidence of end-stage renal disease has risen enormously over the last 2 decades, with hypertension recognized as the second leading cause of end-stage renal disease. It is likely that the variation in the susceptibility to RPP-induced injury in different human populations is related to the genetic heterogeneity and the complex etiologies among subjects. Assuming that the rat data are relevant to humans, the results of the present study indicate that excess adrenergic activity associated with hypertension would influence the pattern and magnitude of RPP-induced injury differently than subjects with inappropriately elevated Ang II. As similar studies are carried out in strains of various congenic and transgenic inbred rats with controlled genetic backgrounds, the genomic and functional pathways that determine susceptibility to RPP-induced injury may be elucidated. This would provide a better understanding of the RPP-dependent and independent mechanisms of injury and guide the implemen-
tation and optimization of antihypertensive therapies, which could also minimize renal injury.

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Disclosures
None.

References


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TITLE: PRESSURE-INDUCED RENAL INJURY IN ANGIOTENSIN II VERSUS NOREPINEPHRINE-INDUCED HYPERTENSIVE RATS


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RUNNING TITLE: Susceptibility to pressure-induced renal injury.

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Surgical Preparation
Following an overnight fast, rats were anesthetized with a mixture of ketamine (30 mg/kg i.m.) and acepromazine (2 mg/kg i.p.). Surgery was performed under sterile conditions while rats were maintained on a 37°C warming board. As described previously (1,2), indwelling catheters were implanted in the right carotid and left femoral artery and vein. To allow for chronic servocontrol of left RPP, an inflatable silastic vascular occluder (1.5 mm lumen diameter, 2.5 mm outer diameter; Kent Scientific Corp., Torrington, CT) was positioned around the aorta between the left and right renal arteries through a midsagittal abdominal incision. The vascular occluder cuff was attached to an 80 cm length of flexible Tygon tubing (0.76 mm lumen diameter, 2.29 mm outer diameter). The catheters and Tygon tubing were exteriorized at the back of the neck and the vascular occluder cuff was connected to a custom-built infusion pump driven by a stepper motor (MDrive 23, Intelligent Motion Systems, Inc., Marlborough, CT) for chronic servocontrol of left RPP (3). Sham rats underwent identical surgical and implantation procedures; however, the vascular occluder cuff was never inflated.

Chronic Servocontrol of Renal Perfusion Pressure
Rats were placed in a bidirectional turntable cage system (Rodent Workstation with Raturn system; Bioanalytical Systems, West Lafayette, IN) for several days prior to surgery, as described previously (2). Following surgery, rats were returned to their cages and arterial pressure above (carotid artery) and below (femoral artery) the vascular occluder cuff was monitored 24 hours / day throughout the study. A continuous infusion of saline or drug was administered i.v. at a rate of 6.9 µL/min throughout the study. For chronic servocontrol of left RPP, the vascular occluder cuff was connected to a computerized servocontrol pump that adjusted the inflation of the aortic occluder cuff in response to changes in femoral arterial pressure. During infusion of drug, femoral MAP (left kidney) was maintained within ± 10 mmHg of the control pressure.

Experimental Design
At the end of each protocol, rats were euthanized by excess sodium pentobarbital (100 mg/kg) and the kidneys were quickly removed, decapsulated, and immediately immersion-fixed in 10% neutral buffered formalin and paraffin embedded. To determine the direct role of chronically elevated NE or AngII on renal injury and whether the susceptibility to RPP-induced renal damage was different in AngII vs. NE, we performed semiquantiative histological and immunohistochemical analysis on servocontrolled, uncontrolled, and sham kidneys following 14 days of drug or saline infusion. The extent to which elevated RPP versus circulating NE or AngII was responsible for renal damage (as assessed by semiquantiative analysis described below) was determined as we have described previously (2).

Histological Analysis
Kidney sections (3 um) were mounted on slides and stained with Hematoxylin and Eosin (H&E) to evaluate tubular, glomerular, and vascular pathology. The arterial smooth muscle wall to lumen cross-sectional area (CSA) ratio was used to assess vascular remodeling in interlobular arterioles located within the superficial cortex (25 -
50 µM luminal diameter). Arterial wall/lumen ratio was determined in 20 randomly
selected interlobular vessels spanning the entire cortex by calculating the cross
sectional area (CSA) of the vessel wall and lumen using Metamorph image analysis
software (Molecular Devices, Downington, PA). Vascular injury was assessed by
determining the presence of interlobular arterial hyalinosis or fibrinoid necrosis. Arterial
cross sections were examined using a 20x objective lens and data are presented as the
percentage of vessels exhibiting either hyalinosis or fibrinoid necrosis. Glomerular
injury was determined in 30 randomly selected superficial cortical and 20 randomly
selected juxtamedullary glomeruli spanning the entire cortex per kidney section.
Glomeruli lying in the deep cortex along the corticomedullary border were categorized
as juxtamedullary while glomeruli lying in the outer superficial cortex were categorized
as superficial. Glomeruli were evaluated with a 40x objective lens for the presence of
glomerulosclerosis and are presented as the percentage of glomeruli exhibiting
glomerulosclerosis. All glomeruli were scored twice in a blinded fashion and the
average used for analysis. For quantification of outer medullary tubular injury, the area
percentage of outer medullary tubular protein cast was quantified using Metamorph
image analysis software, as described previously (4). Outer medullary interstitial
fibrosis was determined by immunostaining with an antibody for α-SMA (Dako
Cytomation) using a robotic DAKO autostainer (S3400; Dako Cytomation, Carpinteria,
CA). α-SMA was detected with an Envision/HRP detection kit (Dako Cytomation).
Interstitial myofibroblasts are recognized as a major fibrogenic cell type within the
kidney that can mediate the progression of interstitial fibrosis in several models of renal
injury (5). Myofibroblasts stain positive for α-SMA and are characterized by their ability
to synthesize a variety of extracellular matrix proteins that can result in tubulointerstitial
fibrosis (6-8). The percent α-SMA positive region in the outer medulla was determined
in 20 randomly chosen frames spanning the entire outer medulla using a 20x objective
lens (2,9). All images were captured with a Nikon E400 (Nikon Instruments, Melville,
NY) microscope equipped with a Spot Insight color CCD camera (Diagnostic
Instruments, Sterling Heights, MI). All analyses were blinded to treatment.
References


