Hemodynamic basis for the limited renal injury in rats with angiotensin II-induced hypertension

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ANGIOTENSIN II (ANG II) is thought to play a major role in the development and progression of chronic kidney disease (CKD), and its blockade is recommended as a primary strategy for reducing CKD progression (31, 40, 66). It has been postulated that in addition to causing direct hypertensive (barotrauma-mediated) injury, ANG II also activates other blood pressure (BP)-independent tissue injury pathways such as inflammation, oxidative stress, aberrant O2 utilization, hypoxia, and profibrotic signaling (11, 17, 31, 32, 42, 47, 59, 63). Thus, it is widely believed that susceptibility to renal injury is increased in ANG II excess states, and the ANG II infusion model has been extensively used to investigate such mechanisms. However, while the chronic administration of ANG II has been repeatedly shown to produce sustained hypertension and renal injury in rodents (29, 30, 36, 41, 46, 48, 52, 54, 57, 61, 62, 65), such studies have not directly addressed the issue of susceptibility to renal injury, per se, in ANG II-infused animals. Any increase in the physical pressure (i.e., BP) within the intrarenal vasculature, if of sufficient magnitude and regardless of cause, is expected to result in barotrauma and vascular injury (4, 15, 20, 33, 35). Therefore, the susceptibility to hypertensive-induced renal damage (HIRD) in disease states or models can only be directly assessed by an examination of the BP threshold for renal injury and the slope of the relationship between renal injury and BP (i.e., increase in renal injury/mmHg increase in BP) (5, 10). Such quantitative relationships between directly measured BP and HIRD have still not been defined in ANG II-infused models.

Given the plethora of postulated mechanisms of ANG II-mediated renal injury, the degree of renal injury observed in previous studies (29, 30, 36, 41, 46, 48, 52, 54, 57, 61, 62, 65) appears to be surprisingly modest for reasons that remain unclear. Accordingly, the goal of the present study was to define the quantitative relationships between BP and HIRD in ANG II-infused rats and compare it with another very frequently used model of HIRD (2, 3, 22, 56, 58, 70), the Nω-nitro-L-arginine methyl ester (L-NAME)-induced nitric oxide synthesis inhibition model, for which such relationships have been recently defined (2, 3, 22, 56, 58, 70). Given the importance of hemodynamic factors in determining quantitative BP transmission to the renal microvasculature, we also investigated the effects of ANG II versus L-NAME on BP-renal blood flow (RBF) relationships in conscious rats.

METHODS

Animals. All experiments were performed on male Sprague-Dawley rats (Harlan) weighing 250–350 g and fed standard 1% NaCl Purina chow and provided water ad libitum. All animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals, and all protocols were approved by the Hines Veterans Affairs Institutional Animal Care and Use Committee.

Surgical procedures and experimental design. To investigate the quantitative relationships between BP and HIRD, rats (n = 42) were anesthetized with isoflurane, and a BP radiotransmitter (model TA11PA-C40, Data Sciences, St. Paul, MN) was inserted, via the femoral artery, into the abdominal aorta below the level of the renal arteries for the continuous assessment of BP (sampled for 10 s every 10 min for 24 h/day). After rats had recovered from surgery, baseline

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BP (average of days 5–7 post-surgery), proteinuria (24-h urine collection, sulfosalicylic acid), and serum creatinine levels (colorimetric QuantiChrom Creatinine Assay Kit, BioAssay Systems, Hayward, CA) were assessed. One group of rats (n = 23) was then continuously administered L-NAME via drinking water (500 mg/l) for 4 wk. Water intake in male Sprague-Dawley rats from Harlan is ~100 ml/kg·day~1 in our animal facility (22); therefore, the dose of L-NAME in the present study was ~50 mg·kg~1·day~1. Another group of rats (n = 19) was anesthetized with isoflurane, and an osmotic minipump (2ML4, Alzet) was implanted subcutaneously between the scapulae for the chronic administration of either 300 ng·kg~1·day~1 (n = 8) or 500 ng·kg~1·day~1 (n = 11) ANG II. These doses of ANG II and L-NAME were used because they elicit relatively similar increases in BP, as determined from preliminary studies. As there were no significant differences in the BP response or the extent of HIRD between rats administered 300 or 500 ng·kg~1·day~1 ANG II, these data were combined into a single group. A 24-h urine collection was conducted at 2 and 4 wk of hypertension for the determination of proteinuria. At 4 wk, a blood sample was obtained for a final serum creatinine measurement, and kidneys were perfused fixed with paraformaldehyde-lysine-phosphate for the histological assessment of renal injury.

An additional group of male Sprague-Dawley rats from Harlan (n = 15) was chronically instrumented with a BP radiotransmitter and a RBF transducer (model 1RB, Transonic Systems, Ithaca, NY), which was placed on the left renal artery and packed in Dacron mesh to ensure proper alignment of the transducer and vessel (9, 24). The transducer cable was secured to the back muscles, routed subcutaneously, exteriorized at the back of the neck, and connected to a flowmeter (model T106, Transonic Systems) during RBF recordings. Rats recovered for 1 wk after the implantation of the BP transmitter and RBF probe. BP and RBF were then obtained (200 Hz) for 3 h on one to three separate occasions at 24-h intervals in conscious rats. After these baseline BP-RBF measurements, one group of rats (n = 8) was anesthetized and implanted with osmotic minipumps to chronically deliver ANG II (500 ng·kg~1·day~1) for 2 wk, whereas another group (n = 7) was administered L-NAME via drinking water (50 mg·kg~1·day~1) for 2 wk. Three days after the induction of hypertension, BP and RBF recordings were again obtained at 200 Hz for a 3-h period and repeated every 2–3 days for 2 wk.

Assessment of renal injury. Renal injury was assessed in a blinded fashion by one of the investigators (M. Picken) using 4-3-h period and repeated every 2–3 days for 2 wk. The transducer cable was secured to the back muscles, routed subcutaneously, exteriorized at the back of the neck, and connected to a flowmeter (model T106, Transonic Systems) during RBF recordings. Rats recovered for 1 wk after the implantation of the BP transmitter and RBF probe. BP and RBF were then obtained (200 Hz) for 3 h on one to three separate occasions at 24-h intervals in conscious rats. After these baseline BP-RBF measurements, one group of rats (n = 8) was anesthetized and implanted with osmotic minipumps to chronically deliver ANG II (500 ng·kg~1·day~1) for 2 wk, whereas another group (n = 7) was administered L-NAME via drinking water (50 mg·kg~1·day~1) for 2 wk. Three days after the induction of hypertension, BP and RBF recordings were again obtained at 200 Hz for a 3-h period and repeated every 2–3 days for 2 wk.

Assessment of renal injury. Renal injury was assessed in a blinded fashion by one of the investigators (M. Picken) using 4-μm-thick sections stained with periodic acid-Schiff. As previously described (16, 23, 25, 27), glomerular injury was expressed as the total percentage of 100 glomeruli exhibiting lesions of either glomerulosclerosis/necrosis and/or ischemic glomerulosclerosis, as previously described (16). We have previously shown that assessment of the severity of glomerular injury using this method compared with a semiquantitative glomerular injury score based on the percentage of glomerular surface involved yielded comparable results (26). Tubulointerstitial injury and fibrosis were assessed on a semiquantitative scale of 0–4. The vascular injury score was calculated as the number of interlobular or afferent arterioles exhibiting acute disruptive injury (lesions of necrosis, thrombosis, aneurysmal dilatation, and/or onion skinning) per 100 glomeruli.

Ambient renal hemodynamics. As previously described (22, 55), mean arterial pressure (MAP), renal vascular resistance (RVR), and RBF were averaged for each rat over the two to three separate 3-h recordings at baseline and over the six to seven separate 3-h recordings during ANG II and L-NAME administration.

Table 1. Body weight, serum creatinine levels, and kidney weight in ANG II- and L-NAME-infused rats

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<th>Body Weight, g</th>
<th>Serum Creatinine, mg/dl</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>Final</td>
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<tr>
<td>ANG II</td>
<td>272 ± 5</td>
<td>332 ± 10*</td>
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<tr>
<td>L-NAME</td>
<td>265 ± 4</td>
<td>294 ± 12*</td>
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Values are expressed as means ± SE; n = 19 ANG II (300–500 ng·kg~1·min~1) hypertensive rats and 23 Nω-nitro-L-arginine methyl ester (L-NAME; 50 mg·kg~1·min~1) hypertensive rats. *P < 0.05, maximum vs. baseline; †P < 0.05, maximum vs. ANG II.

RESULTS

Body weight, kidney weight, and serum creatinine levels. Baseline body weight and serum creatinine levels were not significantly different between groups (Table 1). Rats administered ANG II gained a significantly greater amount of body weight compared with rats administered L-NAME over the 4-wk protocol. Absolute kidney weight was significantly less in rats administered L-NAME (1.3 ± 0.05 g) versus ANG II (1.6 ± 0.05 g) at the completion of the study; however, no differences were observed when kidney weight was normalized to body weight (Table 1). Final serum creatinine values were significantly greater in the L-NAME versus ANG II model at the completion of the study, suggesting a greater degree of renal impairment (Table 1).

Hypertension and renal injury. The average BP at baseline and during the 4-wk of ANG II (n = 19) and L-NAME (n = 23) administration (presented as 2-day averages) is shown in Fig. 1A. While both ANG II and L-NAME led to sustained increases in BP over the 4-wk protocol, BP was significantly greater between days 4 and 15 in ANG II-infused rats. However, BP levels plateaued in ANG II-infused rats after ~2 wk but continued to rise in L-NAME-infused rats such that significantly higher values were achieved over the last 2 days compared with ANG II-infused rats. The average systolic BP...
over the entire 4-wk protocol was significantly \((P < 0.05)\) higher in ANG II \((191.1 \pm 3.2 \text{ mmHg})\) versus L-NAME \((179.9 \pm 2.5 \text{ mmHg})\) hypertensive rats.

As shown in Fig. 1B, ANG II and L-NAME led to similar increases in proteinuria by 2 wk; however, proteinuria reached significantly greater levels at 4 wk in rats administered L-NAME despite the higher 4-wk average systolic BP observed in ANG II-infused rats. Moreover, the similar level of proteinuria observed at 2 wk of hypertension coincided with a significantly lower average systolic BP in L-NAME- versus ANG II-infused rats \((180 \pm 3 \text{ vs. } 197 \pm 1 \text{ mmHg}), \text{ respectively, averaged over days 12–16 of hypertension}).

The pattern and magnitude of HIRD in ANG II and L-NAME hypertensive rats are shown in Fig. 2. In both models of hypertension, a pattern of malignant nephrosclerosis was observed with acute disruptive vascular injury, glomerular injury \(\sim 75\% \text{ glomerulosclerosis/necrosis and } \sim 25\% \text{ ischemic glomerulosclerosis)}, and tubulointerstitial injury. Total glomerular injury (sum of glomerulosclerosis/necrosis and ischemic glomerulosclerosis) was significantly higher in L-NAME versus ANG II hypertensive rats. While both types of glomerular injury were higher in L-NAME-administered rats, the individual differences did not reach statistical significance.

The magnitude of tubulointerstitial injury paralleled the level of glomerular injury in both models of hypertension and was 2.3-fold greater \((P < 0.05)\) in rats administered L-NAME versus ANG II. In contrast, both the magnitude (average number of vessels exhibiting vascular injury per 100 glomeruli evaluated) and incidence of acute disruptive vascular injury were similar between L-NAME \((2.1 \pm 0.5, \text{ 12 of 23 rats})\) and ANG II \((2.0 \pm 0.7, \text{ 10 of 19 rats})\) hypertensive rats. In summary, these data demonstrate a very modest and significantly lower level of glomerular and tubulointerstitial injury in ANG II versus L-NAME hypertensive rats, despite a higher average systolic BP in the ANG II model.

The quantitative relationships between BP and glomerular injury are shown in Fig. 3A. Significant correlations between BP and renal injury were observed in L-NAME \((r^2 = 0.29, P < 0.01)\) and ANG II \((r^2 = 0.21, P < 0.05)\) hypertensive rats. The slope of the relationship between BP and renal injury, while higher in rats administered L-NAME \((y = 0.31x - 45.21)\), was not statistically different to that observed in rats administered ANG II \((y = 0.19x - 28.88)\). However, the \(x\)-intercept of the relationship between BP and renal injury was significantly higher in ANG II versus L-NAME hypertensive rats, indicating an increased BP threshold for the development of renal injury. The increased BP threshold for hypertension was also noted in ANG II hypertensive rats. The average percent glomerular injury plotted within 10-mmHg systolic BP bins corresponding to the average systolic BP achieved during the duration of hypertension.

Similar differences in the threshold for vascular injury were also noted. For example, the average systolic BP of the rats in which vascular injury was observed was 198.3 \(\pm 2.9\) mmHg in the ANG II model \((10 \text{ of 19 rats})\) and 183.1 \(\pm 3.8\) in the L-NAME model \((12 \text{ of 23 rats})\). The average systolic BP in rats in which no evidence of vascular injury was found was 183.4 \(\pm 2.6\) mmHg in ANG II and 178.1 \(\pm 2.9\) mmHg in L-NAME hypertensive rats. Collectively, these data clearly demonstrate a very modest level of HIRD and the higher BP threshold required for the development of such injury in the ANG II versus L-NAME model of hypertension in rats.

Renal hemodynamics in conscious rats administered ANG II and L-NAME. The average MAP, RBF, and calculated RVR obtained from BP-RBF recordings in conscious rats before and during each ANG II \((n = 7)\) or L-NAME \((n = 8)\) administration are shown in Fig. 4. The average BP increase over the 2-wk protocol was significantly greater in rats administered...
ANG II versus l-NAME (56% vs. 38% increase from baseline, respectively; Fig. 4A), similar to the different BP responses observed during the first 2 wk of ANG II and l-NAME administration in the rats shown in Fig. 1A. However, despite the higher BP, ANG II led to a greater reduction in RBF compared with l-NAME (−41% vs. −23% reduction from baseline, respectively; Fig. 4C). Clearly, this reduction in RBF was due to greater increases in RVR in ANG II versus l-NAME hypertension (171% vs. 82% increase from baseline, respectively; Fig. 4B). Indeed, when BP was between 150 and 180 mmHg during ANG II infusion, RBF was significantly lower than values observed when BP fell within the corresponding 10-mmHg BP range. Values are expressed as means ± SE. *P < 0.05 vs. ANG II.

**Fig. 4.** Hemodynamic effects of l-NAME (50 mg·kg⁻¹·day⁻¹, n = 8) and ANG II (500 ng·kg⁻¹·day⁻¹, n = 7) in conscious chronically instrumented rats. ANG II led to a significant ~1.5-fold greater increase in mean arterial pressure (MAP; A) and ~2-fold greater increase in renal vascular resistance (RVR; B), which resulted in an ~1.5-fold greater decrease in renal blood flow (RBF; C). Values are expressed as means ± SE. *P < 0.05 vs. the respective baseline; #P < 0.05 vs. l-NAME.

**DISCUSSION**

A surprisingly modest degree of renal injury was seen in ANG II-infused rats despite the severe hypertension and presumed activation of the plethora of BP-dependent and BP-independent deleterious mechanisms that have been postulated in ANG II-mediated renal damage (11, 17, 31, 32, 42, 47, 59, 63). Although somewhat counterintuitive, these results are nevertheless consistent with several previous studies that have reported similar modest levels of renal injury in ANG II-infused rodents even with higher levels of NaCl intake than used in the present study (29, 30, 36, 41, 46, 48, 52, 54, 57, 61,
Fig. 5. Effects of l-NAME and ANG II on BP-RBF relationships in conscious chronically instrumented rats. A–D: representative BP-RBF recordings at baseline (A and B) and during l-NAME (C) or ANG II (D). No differences were seen in BP-RBF relationships during baseline recordings in both groups, so these data were averaged. Both l-NAME and ANG II led to significant reductions in RBF (expressed as percent changes from RBF when MAP was within 100–100 mmHg) compared with baseline values (E). However, the pattern of changes in RBF during spontaneous fluctuations in BP was markedly different during ANG II and l-NAME infusion. Whereas l-NAME led to a similar ~25% reduction in RBF across the entire range of BP fluctuations, RBF further decreased during episodes of BP elevations in rats administered ANG II. Values are expressed as means ± SE. *P < 0.05 vs. RBF values when BP was within 100–110 and 110–120 mmHg during ANG II administration.
served in ANG II-infused rats would be expected to reduce BP degree of renal microvasculature BP transmission. Consistent to differences in renal hemodynamic factors that govern the levels of ischemia-induced tubulointerstitial injury compared observed during ANG II infusion may have led to greater one possibility is that the extensive renal vasoconstriction nevertheless significant, increase in outer medullary tubulointerstitial injury (54). It should also be emphasized that a modest, but never-
conscious rats administered sympathomimetic agents (43, 55) renal vascular segments associated with ANG II infusion may (13), suggested that the extreme vasoconstriction in upstream pioneering investigators of hypertensive organ damage, Byrom vasoconstrictive effect on the preglomerular vasculature, at least in the exogenously infused ANG II model, as has indeed been shown by a large number of laboratories (18, 34, 37, 44, 50). The significantly lower level of proteinuria, a robust marker of glomerular capillary pressure (69), observed in ANG II versus L-NAME hypertensive rats at 4 wk is also consistent with a greater preglomerular vasconstrictor effect.

The dominant role played by preglomerular tone and autoregulatory ability in determining the pattern and severity of HIRD is also evident when the ANG II model is compared with the 5/6 renal ablation model. The preglomerular vasodilation and impaired autoregulatory ability characteristic of this model result in a greatly reduced BP threshold for HIRD (~125 mmHg) and a much steeper slope of the relationship between BP and HIRD (6, 7, 25–27). However, perhaps a more relevant comparison is with another model of HIRD with intact renal with norepinephrine. Indeed, we have previously suggested that reduced barotrauma-mediated but increased ischemia-mediated renal injury may be a feature of hypertensive models associated with significant renal vasoconstriction (10, 22). Nevertheless, the overall protective importance of renal vasoconstriction is dramatically illustrated by the severe consequences of ANG II infusion in Notch3-deficient mice, which have impaired vasconstrictor responses (12).

In addition to the ambient tone of the preglomerular vasculature, its ability to appropriately and proportionately respond to BP changes (i.e., RBF autoregulation) is expected to be the other major hemodynamic determinant of BP transmission (5). Recent studies showing that ANG II impairs RBF autoregulation have been interpreted as providing a mechanism for enhancing HIRD in ANG II excess states (29, 30, 36, 50, 61, 65). However, such interpretations are difficult to reconcile with the paucity of renal damage seen in ANG II-infused rats in both the present study and previous studies. An explanation for this apparent discrepancy may relate to the nature of renal autoregulatory impairment that has been observed in the vasoconstricted vasculature of anesthetized ANG II-infused rats and in ex vivo renal preparations of ANG II-infused rats (29, 30, 36, 50, 65). While these studies indeed demonstrated an impairment of renal autoregulatory function (i.e., a failure of the renal vasculature to respond to changes in BP) after 1 or 2 wk of ANG II infusion in rats, the autoregulatory impairment was manifest as a reduced ability to vasodilate as renal perfusion pressure was lowered. The implications of impaired autoregulation of this nature are completely opposite to impaired autoregulation in a vasodilated renal vasculature that fails to appropriately vasconstrict when renal perfusion pressure increases (e.g., the remnant kidney). Whereas the latter impairment increases the susceptibility to barotrauma-mediated injury, the former impairment would be expected to reduce microvascular BP transmission and possibly increase the susceptibility to ischemia-mediated injury (5, 10, 51, 55).

Ambient efferent arteriolar resistance is an additional hemodynamic factor that may impact the severity of glomerular hypertension and injury in hypertensive models. While both ANG II and L-NAME (3, 18, 49) are known to cause efferent vasoconstriction, ANG II is thought to be a more selective and potent efferent vasconstrictor and would have been expected to cause greater glomerular hypertension and injury. The rather modest levels of glomerular injury actually observed in ANG II-infused rats is accordingly more consistent with a greater vasoconstrictive effect on the preglomerular vasculature, at least in the exogenously infused ANG II model, as has indeed been shown by a large number of laboratories (18, 34, 37, 44, 50). The significantly lower level of proteinuria, a robust marker of glomerular capillary pressure (69), observed in ANG II versus L-NAME hypertensive rats at 4 wk is also consistent with a greater preglomerular vasconstrictor effect.

The dominant role played by preglomerular tone and autoregulatory ability in determining the pattern and severity of HIRD is also evident when the ANG II model is compared with the 5/6 renal ablation model. The preglomerular vasodilation and impaired autoregulatory ability characteristic of this model result in a greatly reduced BP threshold for HIRD (~125 mmHg) and a much steeper slope of the relationship between BP and HIRD (6, 7, 25–27). However, perhaps a more relevant comparison is with another model of HIRD with intact renal
mass, the salt-supplemented stroke-prone spontaneously hypertensive rat. More modest levels of renal injury as well as a smaller slopes of the relationship between BP and HIRD were observed in ANG II, as well as L-NAME, hypertensive rats (slope: 0.19 ± 0.09 and 0.31 ± 0.11 change in renal injury/change in mmHg, respectively) compared with that recently reported in salt-supplemented stroke-prone spontaneously hypertensive rats with a similar duration and magnitude of hypertension (slope: 1.2 ± 0.2 change in renal injury/change in mmHg) (28). This differential susceptibility to HIRD may also be explained by differences in ambient renal vascular tone (10, 45) given that renal vasoconstriction, similar to that observed in rats administered ANG II and L-NAME, is not observed in salt-supplemented stroke-prone spontaneously hypertensive rats (1).

The morphological pattern of HIRD observed in the present study provides additional insights regarding the pathogenesis of such injury (5, 28). We have suggested that a malignant nephrosclerosis pattern of HIRD, as seen in the present study, supports a barotrauma-mediated pathogenesis and develops when the magnitude of hypertension exceeds both the vascular threshold for acute disruptive injury and autoregulatory capacity to protect glomerular capillaries (5, 8, 23, 28). The evidence that the most severe cases of HIRD were observed primarily in those rats whose average 4-wk systolic BP exceeded the threshold for acute disruptive injury and autoregulatory capacity when the magnitude of hypertension exceeds both the vascular

REFERENCES


