Abnormal renal medullary response to angiotensin II in SHR is corrected by long-term enalapril treatment

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Dukacz, Stephen A. W., Ming-Guo Feng, Lu-Fang Yang, Robert M. K. W. Lee, and Robert L. Kline. Abnormal renal medullary response to angiotensin II in SHR is corrected by long-term enalapril treatment. Am J Physiol Regulatory Integrative Comp Physiol 280: R1076–R1084, 2001.—This study tested the hypotheses that renal medullary blood flow (MBF) in spontaneously hypertensive rats (SHR) has enhanced responsiveness to angiotensin (ANG) II and that long-term treatment with enalapril can correct this. MBF, measured by laser Doppler flowmetry in anesthetized rats, was not altered significantly by ANG II in Wistar-Kyoto (WKY) rats, but was reduced dose dependently (25% at 50 ng·kg⁻¹·min⁻¹) in SHR. Infusion of N⁶-nitro-L-arginine methyl ester (L-NAME) into the renal medulla unmasked ANG II sensitivity in WKY rats and that long-term treatment with enalapril can correct this. MBF, measured by laser Doppler flowmetry in anesthetized rats, was not altered significantly by ANG II in Wistar-Kyoto (WKY) rats, but was reduced dose dependently (25% at 50 ng·kg⁻¹·min⁻¹) in SHR. Infusion of N⁶-nitro-L-arginine methyl ester (L-NAME) into the renal medulla unmasked ANG II sensitivity in WKY rats and that long-term treatment with enalapril abolished the responses to ANG II in SHR. In 18–19-wk-old SHR treated with enalapril (25 mg·kg⁻¹·day⁻¹ when 4 to 14 wk old), ANG II did not alter MBF significantly, but sensitivity to ANG II was unmasked after L-NAME was infused into the renal medulla. Endothelium-dependent vasodilation (assessed with aortic rings) was significantly greater in treated SHR when compared with that in control SHR. These results indicate that MBF in SHR is sensitive to low-dose ANG II and suggest that this effect may be due to an impaired counterregulatory effect of nitric oxide. Long-term treatment with enalapril improves endothelium-dependent vascular relaxation and decreases the sensitivity of MBF to ANG II. These effects may be causally related to the persistent antihypertensive action of enalapril in SHR.

endothelial-dependent relaxation; nitric oxide; renal blood flow; blood pressure; vascular hypertrophy

A substantial amount of evidence implicates the kidney as having an integral role in the long-term control of arterial pressure through the mechanism of pressure natriuresis (4, 12). How sodium and water excretion are altered in response to changes in arterial pressure is not totally clear, although evidence has suggested that in rats changes in blood flow to the renal medulla are involved because medullary blood flow (MBF), unlike total renal blood flow (RBF) and glomerular filtration rate, varies directly with alterations in renal perfusion pressure (33). This pressure dependency of MBF appears to involve nitric oxide (NO; 1, 9). Although MBF is a mere 5–10% of total RBF, changes in MBF can significantly affect sodium and water excretion via alterations in renal interstitial hydrostatic pressure and in the medullary osmotic concentration gradient (35). Thus MBF is seen to have an important role in regulation of arterial pressure normally in hypertension (5).

It has been demonstrated that the pressure-natriuresis relationship in the spontaneously hypertensive rats (SHR) is shifted to higher levels of arterial pressure (32). Furthermore, the relationship between renal artery pressure (RAP) and MBF is blunted, as is the relationship between RAP and renal interstitial hydrostatic pressure, when compared with those of normotensive rats (17, 34). The reason for these differences is unknown, although several studies have suggested the possibility of a functionally impaired NO system in the kidney of SHR (14, 19). In addition, it is known that kidneys of SHR are more sensitive to angiotensin (ANG) II, showing greater reductions in RBF and sodium excretion when compared with Wistar-Kyoto (WKY) rats (18, 40). Is it possible that there is a link among these observations that contributes to the attenuated pressure natriuresis and hypertension in SHR?

Recently, Zou et al. (42) demonstrated that the renal medullary NO system has an important counterregulatory role in protecting the renal medulla from vasoconstriction due to ANG II, i.e., ANG II stimulates NO production in the renal medulla, which offsets the vasoconstrictor activity of ANG II. Thus in normotensive rats, the renal medullary circulation is relatively insensitive to ANG II. However, partial impairment of the NO system by low-dose N⁶-nitro-L-arginine methyl ester (L-NAME) unmasks ANG II vasoconstrictor activity in the medulla (42). Consistent with these findings, it has been shown that MBF is unresponsive to AT₁-receptor blockade in normotensive rats (24). In contrast, we showed that AT₁-receptor blockade increases MBF in SHR (8). Based on these findings, we
hypothesized that an impaired NO system in the SHR kidney would not only blunt the pressure dependency of MBF to changes in RAP, but it would also increase the sensitivity of the medullary circulation to the constrictor effects of ANG II. Both of these effects would in theory contribute to altered MBF regulation and hypertension in SHR.

This study tested the abovementioned hypothesis in two ways: 1) by comparing MBF sensitivity to ANG II in SHR and WKY rats to determine whether SHR did indeed differ from the WKY strain of normotensive rats and 2) by determining how long-term ANG I converting enzyme (ACE) inhibition influences the MBF response to ANG II in SHR. The rationale for the latter studies was that ACE inhibitors have been shown to improve endothelium-dependent vascular relaxation in SHR (2, 3, 16), and our own previous results with ACE inhibition showed a persistent antihypertensive effect associated with a normalization of the pressure-dependency of MBF in SHR (7). These observations are consistent with the abovementioned hypothesis and suggest that ANG II sensitivity of the medullary circulation would be decreased after ACE inhibitor treatment in SHR.

METHODS

All experimental protocols followed guidelines of the Canadian Council on Animal Care and were approved by the University of Western Ontario Council on Animal Care.

Series 1. Effect of ANG II on MBF in SHR and WKY Rats

The purpose of this experiment was to determine whether a difference exists in the sensitivity of the renal medullary circulation to ANG II between SHR and WKY rats. Male SHR and WKY rats (Harlan, Minneapolis, MN) were purchased at 13–14 wk of age and used 1–2 wk after arrival. The rats were housed in the animal care facility, which was maintained at 21°C, and a 12:12-h light-dark cycle. Rats were provided with standard rat chow (Pro Lab RMH 3000; Agway, St. Mary’s, OH) and tap water ad libitum.

Experimental preparation. The basic preparation used to measure RBF and MBF was described previously (7, 8). On the day of the experiment, rats were anesthetized with Inactin (100 mg/kg body wt ip; Research Biochemicals International, Natick, MA) and ketamine (30 mg/kg body wt im; MTC Pharmaceuticals, Cambridge, Ontario, Canada). Body temperature was monitored by a thermistor and maintained at 37 ± 1°C by use of a controller, a heat lamp, and a warming pad. A tracheotomy was done to facilitate breathing (PE-240). The femoral artery was cannulated (pulled PE-50 tubing), with the tip of the cannula advanced 4.5 cm to just below the left renal artery, to measure mean arterial pressure and RAP during manipulation of an aortic balloon cuff located proximal to the left renal artery. The left external jugular vein was cannulated (2 cannulas, pulled PE-50). One cannula was used for the infusion of a 5% albumin in 0.9% NaCl for 30 min (330 μl·kg body wt⁻¹·min⁻¹) to replace fluids lost during surgery. The other cannula was used for the infusion of 1% albumin in 0.9% NaCl (330 μl·kg body wt⁻¹·min⁻¹) for the entire duration of the experiment. With use of this protocol, animals would be expected to be slightly volume expanded. The 1% albumin solution also served as the vehicle for ANG II infusions during the experiment. A midline abdominal incision was made, and the right kidney was removed. The remaining kidney was denervated by separating the renal artery and renal vein and stripping away any fibers and adventitia, followed by application of a 10% phenol in alcohol solution to the renal artery and vein.

A Silastic balloon cuff was placed around the aorta distal to the celiac artery and proximal to the superior mesenteric artery. The cuff allowed for the maintenance of a constant RAP during the infusion of slightly pressor doses of ANG II. The kidney was freed from the surrounding tissue and placed in a gauze-lined, stainless steel kidney cup to reduce movement of the kidney. A renal medullary interstitial catheter, consisting of pulled PE-10 tubing attached to PE-50 tubing, was inserted 4–5 mm into the kidney on the lateral pole, parallel with the renal artery (22). This catheter was fixed in place using cyanoacrylate adhesive, connected to a syringe pump (Harvard Apparatus, South Natick, MA), and used to infuse 0.9% NaCl, t-NAME (1.4 μg·kg⁻¹·min⁻¹) in 0.9% NaCl (42) or l-arginine (320 μg·kg⁻¹·min⁻¹) in 0.9% NaCl (25), into the renal medullary region at a flow rate of 0.5 ml/h. The intra renal medullary infusions of t-NAME and L-arginine were shown previously not to alter resting MBF in normotensive rats (25, 42). The infusion was started at the end of surgery and was continued throughout the entire experiment. To measure MBF a fiberoptic strand (500 μm diameter; Edmund Scientific, Barrington, NJ) was inserted into the lower pole of the kidney through a hole made in the capsule using a 26-gauge needle. The strand was advanced parallel to the aorta to a depth of 6–7 mm to the medulla of the kidney. The fiberoptic strand was fixed in place using cyanoacrylate adhesive and connected to a laser-Doppler flowmeter (model PF3; Perimed, Stockholm, Sweden) via a master probe coupler (model 318; Perimed). To ensure a good optical connection, fused silica matching liquid (Cargille Laboratories, Cedar Grove, NJ) was used for the connection between the master-probe coupler and the fiberoptic strand. Total RBF was determined using a transit-time ultrasonic flowmeter (model T206; Transonic Systems, Ithaca NY), coupled to a flow probe (model 1RB) that was placed around the left renal artery. Lubricating jelly was used as an acoustic coupler between the flow probe and the vessel. RAP, RBF, and MBF were recorded on a polygraph (Grass, Quincy, MA).

Experimental protocol. Control RAP, MBF, and RBF readings were taken for 10 min after the 1-h equilibration period, before starting the ANG II infusion. Three doses of ANG II were used in this study: 5, 15, and 50 ng·kg⁻¹·min⁻¹ iv. Each dose, starting with the lowest, was given for 10–15 min, which was adequate time to allow readings to stabilize. The balloon cuff was used to maintain RAP at the level observed during the control period. After completion of the final ANG II infusion (50 ng·kg⁻¹·min⁻¹) the renal artery was tied off to obtain a zero-flow MBF reading, and this number was subsequently subtracted from each MBF reading for the particular rat. Using this technique, we and others have shown that laser Doppler measurements in the renal medullary circulation can be used to compare both within and between group measurements (e.g., 7–9, 21, 24, 34). After completion of the experiment, the rat was killed by an intravenous injection of MgSO₄·KCl solution. The kidney was removed, cut in half, blotted dry, and weighed. The location of the optical fiber and the intramedullary catheter were examined and confirmed to be in the outer medulla. Changes in MBF were expressed as percent change from baseline.
Series II. Effect of Long-Term Enalapril Treatment in SHR

Male SHR were purchased at 3–4 wk of age and were individually housed in wire mesh steel cages in the animal facility. SHR were divided into two groups: one untreated controls and the other treated with the ACE inhibitor enalapril maleate (25–30 mg·kg⁻¹·day⁻¹) in the drinking water, from 4- to 14-wk-old, as described before (6, 7). The experiments described were done 4–5 wk after stopping the drug treatment, when rats were 18–19 wk old.

Effect of ANG II on RBF and MBF in SHR. Surgical preparation for series 2 was identical to that for series 1. The right kidney was removed and used for morphometric analysis as described below. Four groups were used for intramedullary infusions of 0.9% NaCl or L-NAME (1.4 μg·kg⁻¹·min⁻¹) in 0.9% NaCl: 1) control plus saline, 2) control plus L-NAME, 3) treated plus saline, and 4) treated plus L-NAME. The experimental protocol was identical to that used in series 1. At the end of the ANG II infusion protocol, sections of the aorta were removed from the control and treated rats that had not received L-NAME to evaluate endothelial function in vitro.

Evaluation of endothelium-dependent relaxation using aortic rings. The thoracic aorta was removed, cleaned of connective tissue, and cut into rings ~3 mm in width. Four rings were used from each rat: two with an intact endothelium and two denuded by inserting the tip of a small forceps into the lumen and rolling gently on a Krebs-soaked paper towel. The rings were then suspended between wire stirrups in a jacketed 10-ml organ bath that was maintained at 37°C. The stirrups were connected to a force transducer (model FT03; Grass Instruments, Quincy, MA) and a micrometer, allowing for the adjustment of passive tension. The rings were equilibrated for 1 h in Krebs-Henseleit solution, which was continuously aerated with 95% oxygen-5% carbon dioxide. The Krebs-Henseleit solution was composed of (in mmol/l) 4.7 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄, 2.5 CaCl₂, 118 NaCl, 25 NaHCO₃, 11 glucose, and 0.03 Na₂-EDTA, and had a pH of 7.4. The rings were slowly stretched to a resting tension of 2 g (the optimal resting tension for maximal strength of contraction as determined in pilot studies) and equilibrated for 1 h. During the equilibration period, the Krebs solution in the organ bath was replaced every 20 min. After the first hour, the rings were tested for viability by the addition of 60 mM phenylephrine. Statistical analysis was done using a one-way ANOVA for unpaired observations. For morphometric studies, data were analyzed with a one-tailed t-test because a priori we expected to see evidence for decreased vascular hypertrophy in treated rats. Calculated F and t values were considered to be significant at P < 0.05.

RESULTS

Series I. Effect of ANG II on MBF in SHR and WKY Rats

Mean arterial pressure under Inactin anesthesia was significantly greater for the SHR (160 ± 3 mmHg, n = 22) when compared with that for WKY rats (103 ± 3 mmHg, n = 21). Baseline values for arterial pressure measured after the equilibration period remained significantly higher in SHR than in WKY rats (Table 1). Infusion of L-arginine or L-NAME into the renal medulla during the equilibration period did not significantly alter arterial pressure, total RBF, or MBF in either SHR or WKY rats (Table 1).

Total RBF in WKY rats decreased dose-dependently with intravenous infusion of ANG II, whether or not

<table>
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<tr>
<th>Group</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>RBF, ml·min⁻¹·g kwt⁻¹</th>
<th>MBF, Perfusion Units</th>
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<td>WKY</td>
<td></td>
<td></td>
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<tr>
<td>Saline</td>
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<td>100 ± 6</td>
<td>9.0 ± 0.8</td>
<td>39 ± 3</td>
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<td>7</td>
<td>93 ± 5</td>
<td>7.3 ± 0.6</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>7</td>
<td>93 ± 5</td>
<td>7.8 ± 0.6</td>
<td>39 ± 3</td>
</tr>
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<td>SHR</td>
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<tr>
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<td>6</td>
<td>143 ± 4*</td>
<td>8.8 ± 1.0</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>L-NAME</td>
<td>8</td>
<td>148 ± 4*</td>
<td>6.4 ± 0.9</td>
<td>33 ± 6</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>8</td>
<td>139 ± 4*</td>
<td>6.4 ± 0.7</td>
<td>43 ± 4</td>
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</tbody>
</table>

Values are means ± SE; n = no. rats studied. Saline or drugs were infused into the renal medulla at 0.5 ml/h beginning after surgery. See text for doses of N⁶-nitro-L-arginine methyl ester (L-NAME) and L-arginine. Gkw, grams kidney weight; MAP, mean arterial pressure; SHR, spontaneous hypertensive rat; WKY, Wistar-Kyoto. *P < 0.05, significant difference between SHR and WKY rats. L-NAME and L-arginine had no significant effect on the baseline values for renal blood flow (RBF) and medullary blood flow (MBF; 2-way ANOVA).
the rats received L-NAME or L-arginine infused into the renal medulla. The decrease in RBF was 25–35\% for the ANG II dose of 50 ng·kg\(^{-1}\)·min\(^{-1}\) (Fig. 1). In SHR the high dose of ANG II produced a 45\% reduction in RBF in rats receiving intrarenal medullary saline. SHR receiving intrarenal medullary L-NAME had a significantly greater reduction (60\%) in RBF, whereas those receiving intrarenal medullary L-arginine did not differ from those receiving intrarenal medullary saline (Fig. 1).

MBF for the WKY receiving either saline or L-arginine intrarenal medullary was not significantly reduced by any of the ANG II doses used (Fig. 2). However, MBF for WKY receiving intrarenal medullary L-NAME was decreased significantly (25\%) by 50 ng·kg\(^{-1}\)·min\(^{-1}\) of ANG II. In contrast, ANG II infusions of 15 and 50 ng·kg\(^{-1}\)·min\(^{-1}\) reduced MBF by 12 and 26\%, respectively, in SHR receiving intrarenal medullary saline (Fig. 2). MBF for SHR receiving intrarenal medullary L-NAME showed a further enhanced sensitivity to ANG II at doses of 15 (30\% reduction) and 50 ng·kg\(^{-1}\)·min\(^{-1}\) (40\% reduction). MBF for the SHR receiving intrarenal medullary L-arginine was not significantly altered by any of the ANG II doses used.

**Series II. Effect of Long-Term Enalapril in SHR**

**Effect of ANG II on RBF and MBF in SHR.** Mean arterial pressure in 18- to 19-wk-old SHR under Inactin anesthesia was 151 ± 3 mmHg (n = 20) for the control rats and 112 ± 4 mmHg (n = 20) for the rats treated with enalapril when 4 to 14 wk old (P < 0.05). After surgery and the 1-h equilibration period, mean arterial pressure for the control groups remained significantly higher than that in treated groups and there was no significant effect of intrarenal medullary L-NAME in either group. No significant difference between any of the groups was observed for RBF or MBF (Table 2).

As in series I total RBF in control SHR receiving intrarenal medullary saline was decreased dose-dependently by ANG II and this effect was enhanced by intrarenal medullary L-NAME (Fig. 3). In SHR treated with enalapril and receiving intrarenal medullary saline, the decrease in RBF at 0.05 ml/h (see text for doses). Renal artery pressure was held constant with a balloon cuff on the abdominal aorta during ANG II infusion. *P < 0.05, significant effect of ANG II at the dose indicated. **P < 0.05, significantly different compared with saline group in same strain.

**Series II. Effect of Long-Term Enalapril in SHR**

**Effect of ANG II on RBF and MBF in SHR.** Mean arterial pressure in 18- to 19-wk-old SHR under Inactin anesthesia was 151 ± 3 mmHg (n = 20) for the control rats and 112 ± 4 mmHg (n = 20) for the rats treated with enalapril when 4 to 14 wk old (P < 0.05). After surgery and the 1-h equilibration period, mean arterial pressure for the control groups remained significantly higher than that in treated groups and there was no significant effect of intrarenal medullary L-NAME in either group. No significant difference between any of the groups was observed for RBF or MBF (Table 2).

As in series I total RBF in control SHR receiving intrarenal medullary saline was decreased dose-dependently by ANG II and this effect was enhanced by intrarenal medullary L-NAME (Fig. 3). In SHR treated with enalapril and receiving intrarenal medullary saline, the decrease in RBF in response to ANG II was not significantly different from that seen in control SHR. The RBF response to ANG II in treated rats receiving intrarenal medullary L-NAME, although appearing to be enhanced when compared with that in treated SHR receiving intrarenal medullary saline, was not statistically significant.
As also seen in series 1, MBF in control SHR receiving intrarenal medullary saline was decreased significantly (25%) by the high dose of ANG II (Fig. 3). The response to ANG II was enhanced by giving intrarenal medullary L-NAME. MBF in SHR previously treated with enalapril and receiving intrarenal medullary saline, was not altered significantly by any of the doses of ANG II. When intrarenal medullary L-NAME was given to treated rats, MBF was reduced by 37% in response to the high dose of ANG II. This response was not significantly different from that seen in control SHR treated with intrarenal medullary L-NAME.

Evaluation of endothelium-dependent relaxation using aortic rings. Treatment with enalapril did not affect the contractile response of aortic rings from WKY rats or SHR to 60 mM KCl or to increasing doses of phenylephrine (data not shown). Endothelium-dependent and endothelium-independent responses of phenylephrine-preconstricted rings to increasing doses of ACh are shown in Fig. 4. The extent of relaxation was significantly greater for aortic rings taken from treated SHR with intact endothelium when compared with that in the control group with intact endothelium. Aortic rings without endothelium showed similar responses to ACh in both groups. In contrast to the differences observed between groups with ACh, no significant differences in the relaxation response to SNP existed between any of the groups, regardless of treatment or presence or lack of endothelium (Fig. 4).

Table 2. Baseline values 1 h after surgery for MAP, RBF, and MBF in 18- to 19-wk-old control SHR and SHR previously treated at 4 to 14 wk of age with enalapril (25 mg·kg⁻¹·day⁻¹)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>RBF, ml·min⁻¹·g kwt⁻¹</th>
<th>MBF, Perfusion Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control SHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>10</td>
<td>143 ± 6</td>
<td>7.1 ± 0.9</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>L-NAME</td>
<td>10</td>
<td>142 ± 5</td>
<td>7.1 ± 0.5</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>Treated SHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>13</td>
<td>106 ± 3*</td>
<td>6.8 ± 0.5</td>
<td>45 ± 4</td>
</tr>
<tr>
<td>L-NAME</td>
<td>7</td>
<td>106 ± 4*</td>
<td>5.6 ± 0.5</td>
<td>53 ± 5</td>
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Values are means ± SE; n = no. rats studied. Saline or drugs were infused into the renal medulla at 0.5 ml/h beginning after surgery. See text for dose of L-NAME. *P < 0.05, significant effect of previous treatment with enalapril. L-NAME had no significant effect on the baseline values for RBF and MBF (2-way ANOVA).

Fig. 3. Effect of ANG II on renal blood flow (RBF; A) and renal medullary blood flow (MBF; B) in 18- to 19-wk-old control SHR and SHR previously treated at 4 to 14 wk old with enalapril (25 mg/kg per day). Values are means ± SE. Solid bars, control plus saline, n = 10 (RBF) and 10 (MBF); crosshatch bars, control plus L-NAME, n = 10 (RBF) and 10 (MBF); open bars, treated plus saline, n = 13 (RBF) and 13 (MBF); hatch bars, treated plus L-NAME, n = 7 (RBF) and 7 (MBF). Control and treated SHR received saline or L-NAME infused into the renal medulla at 0.05 ml/h (see text for dose). Renal artery pressure was held constant with a balloon cuff on the abdominal aorta during ANG II infusion. *P < 0.05, significant effect of ANG II at the dose indicated. #P < 0.05, significant effect of L-NAME compared with the saline group.

Fig. 4. Relaxation responses of aortic rings from 18- to 19-wk-old control SHR and SHR previously treated at 4 to 14 wk old with enalapril (25 mg/kg per day). Responses to acetylcholine (ACh; A) and sodium nitroprusside (SNP; B). Values are means ± SE. Aortic rings were previously constricted with phenylephrine (PE). Intact, endothelium was intact. Denuded, endothelium was removed. ■ control plus intact, n = 8 (ACh) and 7 (SNP); ▲ treated plus intact, n = 9 (ACh) and 8 (SNP); ○, control plus denuded, n = 8 (ACh) and 7 (SNP); ●, treated plus denuded, n = 8 (ACh) and 8 (SNP). *P < 0.05, significant effect of previous treatment with enalapril when compared with responses in control SHR.
ANG II results in vasoconstriction when infused into isolated vasa recta bundles from the renal medulla of normotensive rats (28). As indicated previously, this result is not consistent with in vivo findings in normotensive rats (24). However, Zou and associates have proposed that a “counterregulatory mechanism” exists in vivo to protect the medulla from the effects of vasoconstrictors, such as norepinephrine (29), arginine-vasopressin (30), and ANG II (42). Because blood flow to the renal medulla is very low, further reductions in blood flow due to vasoconstrictor activity could result in a hypoxic state within the medulla. It is also possible that protection of the renal medulla from vasoconstriction may play a role in controlling arterial pressure. This concept was supported in recent studies using normotensive rats, in which infusion of low-dose L-NAME into the renal medulla caused the rats to become hypertensive with an intravenous dose of ANG II that would not normally produce hypertension (37). Similarly, a counterregulatory mechanism in the renal medulla that was functioning less than optimally could conceivably play a role in the development of hypertension in the SHR. In this case, arterial pressure would be expected to be increased to provide a normal level of MBF. This in fact is what was seen in the current study: at resting levels of arterial pressure, MBF was not significantly different between SHR and WKY rats. If there were a defect in the counterregulatory system in SHR, one would expect to see increased reaction to vasoconstrictors such as ANG II. In support of this, Kost and Jackson (18) showed that when compared with age-matched WKY rats infusion of ANG II resulted in a greater reduction in total RBF, a greater decrease in sodium and urine excretion, and a greater increase in renal vascular resistance for the SHR. The findings from the current study are consistent with these results and extend the observations by showing a marked difference between the MBF responses to ANG II in the SHR when compared with those in the WKY rats.

Recent evidence suggests a role for NO as a counter-regulatory system in the renal vasculature because NO has been shown to counteract the afferent and efferent arteriolar vasoconstrictor responses to ANG II in juxtedudillary nephrons of the rat (13). Moreover, NO counteracts the constrictor effect of ANG II on the renal medullary circulation in normotensive rats and further, ANG II actually stimulates NO production in the renal medulla (42). Interestingly, when the renal medullary NO system was slightly impaired by a low dose of L-NAME delivered to the interstitium of the medulla, the constrictor effect of ANG II was unmasked (42). This demonstrated that the NO system serves a protective role for the medullary circulation and that slight impairment of the NO system, which has little if any effect on resting blood flow, can have profound effects on medullary hemodynamics in the presence of vasoconstrictors.

Evidence exists that the renal NO system in the SHR is impaired. Ikenaga et al. (14) demonstrated that the NO synthase inhibitor N(G)-monomethyl-L-arginine,

### Table 3. Morphometric analysis of vessels from kidneys of 18- to 19-wk-old control SHR and SHR previously treated at 4 to 14 wks of age with enalapril (25 mg·kg⁻¹·day⁻¹)

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Lumen Area, μm² x 10⁴</th>
<th>Media Area, μm² x 10³</th>
<th>Media: Lumen Ratio</th>
<th>Medial SMC, Layers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interlobar</td>
<td>Control 12.91 ± 1.45</td>
<td>29.23 ± 1.75</td>
<td>2.64 ± 0.27</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Treated 13.80 ± 1.05</td>
<td>25.83 ± 1.15</td>
<td>2.00 ± 0.13*</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>Interlobular</td>
<td>Control 5.40 ± 0.74</td>
<td>17.28 ± 1.11</td>
<td>3.80 ± 0.37</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Treated 5.73 ± 0.56</td>
<td>14.57 ± 0.87*</td>
<td>2.89 ± 0.27*</td>
<td>3.0 ± 0.0</td>
</tr>
<tr>
<td>Afferent arteriole</td>
<td>Control 0.74 ± 0.10</td>
<td>3.51 ± 0.52</td>
<td>5.22 ± 0.47</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Treated 0.63 ± 0.07</td>
<td>2.84 ± 0.26</td>
<td>5.12 ± 0.45</td>
<td>1.9 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; data were obtained from 12 kidneys of control SHR and 13 kidneys of treated SHR. Number of vessels measured in each sample was 1–3 interlobar, 2–4 interlobular, and 2–4 afferent arteriole. SMC, smooth muscle cell. *P < 0.05, significant effect of previous treatment with enalapril.

**Morphometric analysis of renal vessels.** As shown in Table 3, 10-wk treatment with enalapril resulted in a persistent reduction of the ratio of media area to lumen area in interlobar and interlobular arteries. No significant differences were found for the number of medial smooth muscle cell layers in these vessels from control and treated SHR. There were no significant differences between parameters for afferent arterioles in treated and control SHR.

### DISCUSSION

**Effect of ANG II on MBF in SHR and WKY Rats**

The importance of MBF in the control of arterial pressure has been demonstrated by the selective, chronic infusion of L-NAME (26) or captopril (21) into the renal medulla of rats, resulting in a decrease or increase in MBF, respectively, and an increase or decrease in arterial pressure, respectively. We have shown previously that 3-day losartan treatment of SHR resulted in a decrease in arterial pressure and an increase in the slope of the relationship between RAP and MBF (8), suggesting that the renal medullary circulation of the SHR is sensitive to endogenous levels of ANG II. Contrary to this, the renal medullary circulation of normotensive rats is relatively insensitive to the vasoactive effects of endogenous levels of ANG II (24). The results of the current study are consistent with these findings in that MBF in WKY rats was unaffected by the ANG II doses used, whereas MBF in the SHR was decreased significantly in a dose-dependent fashion. Thus the medullary circulation of the SHR appears to be more sensitive to ANG II when compared with that of WKY rats, and this effect may contribute to the hypertension in SHR. Interestingly, a similar sensitivity to ANG II has been demonstrated recently for the medullary circulation of Dahl salt-sensitive rats (38).

The effect of ANG II on the renal medullary circulation is complex. For example, it has been shown that
while having profound effects on pressure natriuresis in the normotensive WKY rats, did not significantly affect pressure natriuresis in the SHR, suggesting that the NO system is impaired or functioning differently in the SHR. Furthermore, they demonstrated that intravenous infusion of L-arginine, the substrate for NO synthase, increased sodium excretion in the SHR but not in WKY rats. In another study, similar observations were made, noting that infusion of L-arginine but not D-arginine improved pressure natriuresis in the SHR (19) and that L-arginine resulted in enhanced increases in MBF in response to increases in arterial pressure, i.e., the pressure-dependence of MBF, which has been shown to be impaired in the SHR (7), was restored by L-arginine.

In the current study, it was found that MBF was insensitive to ANG II in WKY rats receiving intramedullary saline or L-arginine. Because it has been demonstrated previously that L-arginine delivered intravenously resulted in an increase in medullary NO production (41), our data suggest that further NO production had no effect on MBF in the WKY rat. In contrast, when low-dose L-NAME was infused into the renal medulla of WKY rats, the sensitivity of the medullary circulation to ANG II was unmasked, supporting the concept advanced by Zou et al. (42), that the NO system is involved in countering the vasoconstrictor effect of ANG II on the medullary circulation.

The infusion of a low dose of L-NAME into the renal medulla of the SHR further increased the sensitivity of MBF to ANG II. If the NO system in the SHR is already impaired, resulting in heightened sensitivity to ANG II, it stands to reason that further impairment of the NO system would lead to even further enhanced sensitivity to ANG II. Unlike that in WKY rats, the infusion of L-arginine into the renal medulla of the SHR virtually eliminated the sensitivity of MBF to ANG II. It is possible that intramedullary L-arginine infusion enhanced the response of the NO system to ANG II, thus increasing the amount of NO produced, leading to a decrease in the sensitivity of MBF to ANG II.

Effect of Long-Term Enalapril in SHR

Numerous studies have demonstrated that long-term ACE inhibitor treatment can prevent the full development of hypertension in the SHR and that an antihypertensive effect persists long after cessation of treatment (6, 7, 23). We have previously demonstrated that long-term ACE inhibitor treatment improves the pressure-dependence of the relationship between RAP and MBF in SHR (7). The underlying reason for this persistent improvement in medullary hemodynamics, however, is not known. Numerous studies have shown that long-term ACE inhibitor treatment can prevent structural changes in the vasculature that normally occur as the SHR ages (23). Our morphometric analysis of renal vessels in this study supports the results obtained in other vascular beds and shows that persistent effects on vascular structure are produced by long-term enalapril treatment in SHR. Prevention of vascular hypertrophy would result in a lower structurally based renal vascular resistance, which in turn could result in an increased MBF at a given level of arterial pressure and also reduce vascular responsiveness to vasoconstrictors. Vascular hypertrophy alone, however, does not fully explain the blunted relationship between RAP and MBF observed in the SHR. Evidence also suggests an enhanced vascular tone in the SHR compared with the WKY rat (10). As described previously, this may be due in part to an impaired counter-regulatory system in the SHR.

Studies have suggested that endothelial function is impaired in hypertension (20, 39). Furthermore, there is considerable evidence that long-term ACE inhibitor treatment improves endothelial function or prevents endothelial dysfunction in the young SHR (2, 3, 16). In our current study, the MBF response to ANG II normally seen in control SHR was abolished totally in the 10-wk ACE inhibitor-treated SHR. This persistent effect of enalapril was not seen on total RBF (primarily cortical flow), because reductions in RBF in response to ANG II were similar in control and treated SHR. In series 1 of the current study, we showed that intramedullary L-arginine administered to control SHR also abolished the MBF response to the ANG II doses used. It is possible that long-term ACE inhibitor treatment enhanced the response of the medullary NO system to ANG II, resulting in a decreased vascular sensitivity to ANG II.

If the NO system is responsible for the reduced sensitivity to ANG II in the ACE inhibitor-treated SHR, it would be expected that intramedullary L-NAME would result in a greater change in MBF responsiveness for the ACE inhibitor-treated SHR compared with that in control SHR, i.e., NO-induced vasodilation is playing an important role in offsetting ANG II vasoconstriction in treated rats. Consistent with this suggestion, intrarenal medullary L-NAME increased the MBF response to 50 ng·kg⁻¹·min⁻¹ ANG II from −22 to −38% in control SHR and from a response not significantly different from zero to −37% in treated SHR. A similar difference was seen when comparing the effect of intrarenal medullary L-NAME on the MBF response to ANG II in SHR and WKY rats in series 1. Overall, these results support the conclusion that long-term enalapril treatment in SHR resulted in a persistent enhancement of the counter-regulatory role of the NO system in modulating the vasoconstrictor action of ANG II in the renal medulla.

Results from the aortic ring study are also consistent with a normalization of function of the NO system, inasmuch as long-term enalapril treatment greatly enhanced endothelium-dependent relaxation to ACh. These results agree with those of others either when SHR are on treatment (2, 15) or off treatment (16, 31). If the NO system is affected similarly in the renal medulla by enalapril treatment, then this could contribute to the reduced medullary response to ANG II which was observed. In agreement with Keaton et al. (16), aortic ring responses to phenylephrine or to SNP
were not significantly different between control and treated SHR, suggesting that the difference in response to ACh between the control and 10-wk treated SHR was linked to the endothelium, not to the vascular smooth muscle. Furthermore, denuding of the rings abolished differences in the relaxation responses to ACh between the control and 10-wk-treated SHR. Thus we have demonstrated that long-term ACE inhibitor treatment has a persistent effect on endothelium-dependent vasodilation in the same rats that show reduced renal medullary responses to ANG II. These results are consistent with an improvement in the NO system in enalapril-treated SHR.

In summary, this study has demonstrated differences between the SHR and WKY rats in the sensitivity of the renal medullary circulation to ANG II, which may be due in part to an altered NO system. This study has also provided evidence that is consistent with an improvement of the renal medullary NO system in the SHR following long-term ACE inhibitor treatment, which may explain the restoration of the pressure-dependence between RAP and MBF, enhanced pressure natriuresis, and persistently lower arterial pressure in treated SHR.

Perspectives

Results from this study demonstrate that the SHR is less able to modulate the vasoconstrictor effect of ANG II on renal medullary hemodynamics when compared with WKY rats. Furthermore, our data also suggest that this reduced counterregulatory ability was due, at least in part, to an impairment in the NO system, which is consistent with previously demonstrated endothelial dysfunction in the SHR. Overall, because the renal medulla has been shown to have an important role in the long-term regulation of arterial pressure (5), the results of our studies provide a possible cause-and-effect link between endothelial dysfunction and hypertension in SHR. Failure to adequately modulate renal MBF in response to changes in arterial pressure and to counterregulate vasoconstrictor tone in the renal medulla may contribute to the development and maintenance of hypertension in SHR. This overall hypothesis is supported by data in this study and previous studies showing that persistent antihypertensive effects of ACE inhibition in SHR are accompanied by 1) a restoration of endothelium-dependent vasodilation (15, 16), 2) improved renal medullary hemodynamics (7), and 3) normalization of a counterregulatory vasoconstrictor mechanism in the renal medulla. The mechanism for these long-lasting effects of ACE inhibition is not known.

The underlying reason for the apparent dysfunctional NO system in the renal medulla of SHR is not known. Although the ability to synthesize NO in the renal medulla of SHR is not compromised (27), it is possible that elevated levels of superoxide in the endothelium of SHR reduce the bioavailability of NO (11). This in turn would lead to impaired modulation of medullary hemodynamics in response to changes in arterial pressure and to vasoconstrictors. In support of this proposal, it is of interest that superoxide scavengers have been shown to reduce arterial pressure in the SHR, while having no significant effect in normotensive rats (36).

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