Angiotensin II and nitric oxide in neural control of intrarenal blood flow

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Rajapakse, Niwanthi W., Amanda K. Sampson, Gabriela A. Eppel, and Roger G. Evans. Angiotensin II and nitric oxide in neural control of intrarenal blood flow. Am J Physiol Regul Integr Comp Physiol 289: R745–R754, 2005. First published May 12, 2005; doi:10.1152/ajpregu.00477.2004.—We investigated the roles of the renin-angiotensin system and the significance of interactions between angiotensin II and nitric oxide, in responses of regional kidney perfusion to electrical renal nerve stimulation (RNS) in pentobarbital sodium-anesthetized rabbits. Under control conditions, RNS (0.5–8 Hz) reduced total renal blood flow (RBF; \(-89 \pm 3\% \) at 8 Hz) and cortical perfusion (CBF; \(-90 \pm 2\% \) at 8 Hz) more than medullary perfusion (MBF; \(-55 \pm 5\% \) at 8 Hz). Angiotensin II type 1 (AT1)-receptor antagonism (candesartan) blunted RNS-induced reductions in RBF (\(P = 0.03\)), CBF (\(P = 0.007\)), and MBF (\(P = 0.04\)), particularly at 4 and 8 Hz. Nitric oxide synthase inhibition with \(N^\omega\)-nitro-L-arginine (L-NNA) enhanced RBF (\(P = 0.003\)), CBF (\(P = 0.001\)), and MBF (\(P = 0.03\)) responses to RNS, particularly at frequencies of 2 Hz and less. After candesartan pretreatment, L-NNA significantly enhanced RNS-induced reductions in RBF (\(P = 0.04\) and CBF (\(P = 0.007\)) but not MBF (\(P = 0.66\)). Renal arterial infusion of angiotensin II (5 ng kg\(^{-1}\) min\(^{-1}\)) selectively enhanced responses of MBF to RNS in L-NNA-pretreated but not in vehicle-pretreated rabbits. In contrast, greater doses of angiotensin II (5 \(\sim\) 15 ng kg\(^{-1}\) min\(^{-1}\)) blunted responses of MBF to RNS in intact nitric oxide synthase. These results suggest that endogenous angiotensin II enhances, whereas nitric oxide blunts, neurally mediated vasoconstriction in the renal cortical and medullary circulations. In the renal medulla, but not the cortex, angiotensin II also appears to be able to blunt neurally mediated vasoconstriction.

renal medullary blood flow; renal cortical blood flow; sympathetic nervous system

RENAL MEDULLARY BLOOD FLOW plays a key role in the long-term regulation of blood pressure, mainly through its effects on tubular sodium and water handling (5–7, 29). Therefore, factors that regulate medullary blood flow should in turn have profound effects on the long-term set point of arterial pressure. There is good evidence that sympathetic drive can differentially regulate renal cortical and medullary blood flows. For example, electrical (15, 19, 25, 34) stimulation and reflex-mediated (23, 26) stimulation of the renal nerves have been shown to cause greater reductions in total renal blood flow (RBF) and cortical laser-Doppler flux (CLDF; an index of blood flow) than medullary laser-Doppler flux (MLDF). Furthermore, basal renal sympathetic drive appears to have little impact on medullary blood flow (41). This differential regulation of regional kidney blood flow by renal nerves is likely to represent an important regulatory mechanism in long-term blood pressure control, yet we are only beginning to understand the mechanisms underlying it (16, 17). One key factor seems to be nitric oxide (NO), since inhibition of nitric oxide synthase (NOS) (14, 40), but not cyclooxygenase (32), can enhance renal nerve stimulation (RNS)-induced vasoconstriction in the medullary circulation.

Another factor that might modulate responses of intrarenal blood flow to RNS is angiotensin II, especially because RNS can increase renin release from the juxtaglomerular apparatus and thus formation of angiotensin II in the kidney (22). However, at first sight, this would seem an unlikely candidate for a mechanism that blunts responses to RNS in the medulla, since angiotensin II type 1 (AT1)-receptor antagonism has been shown to inhibit RNS-induced reductions in RBF in anesthetized dogs (12, 36) and rats (8). Most evidence suggests that this is chiefly due to angiotensin II-induced facilitation of norepinephrine release from nerve terminals in response to RNS (35, 36) and through direct vasoconstrictor influences of angiotensin II, particularly on the efferent arteriole (38). Collectively, these data indicate that endogenous angiotensin II, by activation of AT1 receptors, facilitates neurally mediated renal vasoconstriction, at least when one considers total RBF.

However, angiotensin II has also been shown to have vasoconstrictor effects in the medullary circulation. Exogenous angiotensin II can increase medullary NO concentration (44) and medullary blood flow (10, 11, 33, 39), and these phenomena may be linked through the mechanism of "tubulovascular NO cross talk" (9). According to this concept, the epithelial cells of tubular elements within the outer medulla (mainly thick ascending limbs) are a major source of NO, including NO release stimulated by angiotensin II. This epithelium-derived NO may in turn regulate medullary blood flow through dilatation of outer medullary descending vasa recta (9). In anesthetized rabbits, the MLDF response to renal arterial boluses of angiotensin II is biphasic, consisting of an initial reduction followed by a later increase (10, 33). This increase in MLDF is blunted by NOS inhibition (33) or AT1-receptor antagonism (10), suggesting that angiotensin II-induced release of NO within the medullary circulation depends on activation of AT1 receptors. Intriguingly, we recently found that renal arterial infusion of angiotensin II, at a dose that did not affect basal MLDF, greatly blunted RNS-induced reductions in MLDF but not CLDF (19).

Collectively, these data have generated the hypothesis that angiotensin II modulates responses of the renal vasculature to RNS in a regionally specific manner. Thus, in vascular elements regulating medullary blood flow [juxtamedullary arterioles and particularly descending vasa recta (17)], angiotensin II might increase NO levels, which could in turn blunt neurally mediated vasoconstriction. However, the effects of AT1-receptor blockade on responses of medullary perfusion to RNS have not previously been examined.

In the present study, we tested the hypothesis that endogenous angiotensin II, via activation of AT1 receptors, contrib-
utes to the differential impact of RNS on cortical and medullary blood flow. We also tested whether prior AT<sub>1</sub>-receptor antagonism can alter the impact of inhibition of NOS on responses to RNS and whether prior blockade of NOS can alter the impact of exogenous angiotensin II on responses to RNS, either of which could indicate an interaction between angiotensin II and NO in neural regulation of intrarenal blood flow.

METHODS

Animals

Fifty New Zealand White rabbits (mean weight of 2.72 ± 0.04 kg) were used across three experimental protocols. Rabbits were meal fed and provided with water ad libitum (18). All experiments were conducted in accordance with the American Physiological Society (1) and approved in advance by the Animal Ethics Committee of the Department of Physiology, Monash University.

Surgical Preparations

The experimental preparation that we used has been described in detail previously (15, 19). Briefly, rabbits were anesthetized with pentobarbital sodium (90–150 mg plus 30–50 mg/h; Nembutal; Rhone Merieux, Pinkenba, Queensland, Australia) and artificially ventilated. The left kidney was exposed and placed in a stable cup. The (left) renal nerves were placed across a stimulating electrode and sectioned proximally. We measured RBF using a transit-time ultrasound flow probe (type 2SB, Transonic Systems, Ithaca, NY) placed around the renal artery. A needle-type laser-Doppler flow probe (26 gauge, DPA2, Moor Instruments, Millwey, Devon, UK) was advanced ~9 mm into the kidney to measure MLDF. A standard plastic laser-Doppler flow probe was placed on the dorsal surface of the kidney to measure CLDF (DP2b, Moor Instruments). For protocols 2 and 3, a catheter was placed in a side branch of the renal artery (suprarenalombar artery) for administration of angiotensin II (33). Arterial pressure was measured via an ear artery catheter (26). After completion of all surgical procedures, the infusion of Hartmann’s solution was replaced with a solution containing four parts Hartmann’s solution and one part 10% vol/vol polygeline (Haemaccel, Rhone Merieux, Pinkenba, Queensland, Australia) and artificially maintained at 37°C by a heating pad. We also tested whether endogenous NO modulates responses of regional kidney perfusion to RNS, in the presence of an intact renin-angiotensin system. Group 3 received candesartan (10 µg/kg + 10 µg·kg⁻¹·h⁻¹ iv; Astra Zeneca) after the first stimulation sequence and L-NNA after the second stimulation sequence. This tested whether endogenous angiotensin II (acting via AT<sub>1</sub>-receptors) modulates responses of intrarenal perfusion to RNS and also whether endogenous NO modulates responses of regional kidney blood flow to RNS after blockade of AT<sub>1</sub>-receptors. Ten minutes after the third stimulation sequence was completed, rabbits in all three groups received three intravenous bolus doses of angiotensin II (10, 100, and 1,000 ng/kg) in ascending order. A 5- to 10-min recovery period was allowed between each dose of angiotensin II.

Protocol 2: effects of exogenous angiotensin II with and without NOS blockade. Rabbits were randomized to two groups, each being subjected to two sequences of RNS. Group 1 (n = 6) received the vehicle for L-NNA, starting 20 min before the first stimulation sequence. Fifteen minutes before the start of the second stimulation sequence, a renal arterial infusion of angiotensin II (5 ng·kg⁻¹·min⁻¹) commenced, which continued until the end of the experiment. Group 2 (n = 6) received L-NNA starting 20 min before the beginning of the first stimulation sequence (20 mg/kg + 5 mg·kg⁻¹·min⁻¹ iv) and then renal arterial angiotensin II (as for group 1) starting 15 min before the second stimulation sequence began.

Protocol 3: effects of high-dose exogenous angiotensin II. Rabbits were randomized to two groups, each being subjected to two sequences of RNS. Group 1 (n = 9) received a renal arterial infusion of 154 mM NaCl, starting 15 min before the second stimulation sequence. Group 2 (n = 9) received a renal arterial infusion of angiotensin II (5–15 ng·kg⁻¹·min⁻¹) titrated in each rabbit to reduce basal RBF by ~40%, starting 15 min before the second stimulation sequence began.

Electrical Nerve Stimulation

Sequences of RNS, at supramaximal voltage (3–5 V), were generated by computer (25). Each stimulation sequence consisted of five 3-min stimulus trains (0.5, 1, 2, 4, and 8 Hz; 2-ms pulse duration). These frequencies were applied in random order. Recovery periods of 8–10 min were allowed between each stimulus train.

Recording of Hemodynamic Variables

Signals were processed and acquired digitally as previously described (14, 15), to provide 2-s averages of mean arterial pressure (MAP; mmHg), heart rate (HR; determined from the arterial pressure pulse; beats/min), RBF (ml/min), CLDF (perfusion units), and MLDF (perfusion units). The values of CLDF (6 ± 1 U) and MLDF (12 ± 1 U) during the 60 s immediately after the rabbit was humanely killed by overdose with pentobarbital sodium (300 mg) were subtracted from the values obtained during the experiment, before data analysis was performed.

Statistical Methods

Data are expressed as means ± SE. All statistical tests were performed using the software package SYSTAT (version 9, SPSS, Chicago, IL). Two-sided P ≤ 0.05 was considered statistically significant. Baseline levels of each variable were averaged over 30-s control periods immediately before each stimulus train, across all five frequencies in each RNS sequence. Responses to RNS were determined by comparing the levels of each variable, during the last 30 s of each stimulus train, with the control values during the 30 s immediately before stimulation. We used ANOVA to test, within each protocol, whether baseline variables and responses to RNS differed 1) between the experimental groups during the control period (P<sub>group</sub>) and 2) during stimulation sequence 2 compared with stimulation sequence 1 (and [for protocol 1] during stimulation sequence 3 compared with stimulation sequence 2 P<sub>realign</sub>). We also tested whether responses of MLDF to RNS differed from those of CLDF (P<sub>Region</sub>). Thus our main biological hypotheses were tested in a “within-animal” fashion.
Table 1. Mean baseline levels of systemic and renal hemodynamic variables in protocol 1

<table>
<thead>
<tr>
<th>Group 1</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>RBF, ml/min</th>
<th>CLDF, U</th>
<th>MLDF, U</th>
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<tr>
<td>Control</td>
<td>71 ± 3</td>
<td>247 ± 11</td>
<td>31 ± 2</td>
<td>285 ± 17</td>
<td>76 ± 13</td>
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<tr>
<td>Vehicle 1</td>
<td>67 ± 3</td>
<td>243 ± 11</td>
<td>36 ± 1</td>
<td>272 ± 8</td>
<td>70 ± 15</td>
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<tr>
<td>Vehicle 2</td>
<td>69 ± 4</td>
<td>240 ± 11</td>
<td>37 ± 1</td>
<td>261 ± 9</td>
<td>79 ± 23</td>
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<table>
<thead>
<tr>
<th>Group 2</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>RBF, ml/min</th>
<th>CLDF, U</th>
<th>MLDF, U</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>76 ± 4</td>
<td>230 ± 9</td>
<td>23 ± 4</td>
<td>269 ± 21</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>Vehicle 1</td>
<td>75 ± 5</td>
<td>230 ± 9</td>
<td>23 ± 1</td>
<td>269 ± 9</td>
<td>51 ± 1*</td>
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<tr>
<td>t-L-NNA</td>
<td>95 ± 5†</td>
<td>195 ± 9†</td>
<td>17 ± 1*</td>
<td>218 ± 10*</td>
<td>36 ± 2*</td>
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<th>Group 3</th>
<th>MAP, mmHg</th>
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<th>RBF, ml/min</th>
<th>CLDF, U</th>
<th>MLDF, U</th>
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<tr>
<td>Control</td>
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<td>244 ± 7</td>
<td>24 ± 3</td>
<td>273 ± 11</td>
<td>61 ± 14</td>
</tr>
<tr>
<td>Candesartan</td>
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<td>245 ± 7</td>
<td>24 ± 2</td>
<td>313 ± 18</td>
<td>58 ± 11</td>
</tr>
<tr>
<td>t-L-NNA</td>
<td>72 ± 5†</td>
<td>220 ± 10†</td>
<td>21 ± 3†</td>
<td>256 ± 26†</td>
<td>34 ± 6†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of animals. t-L-NNA, Nω-nitro-l-arginine; MAP, mean arterial pressure; HR, heart rate; RBF, renal blood flow; CLDF, cortical laser-Doppler flux; MLDF, medullary laser-Doppler flux. See text for explanation of groups and protocols. *P < 0.05 and †P < 0.01 indicate the outcomes of ANOVA, which tested whether baseline levels observed during stimulation sequence 2 differed from those observed during stimulation sequence 1 and whether baseline levels observed during stimulation sequence 3 differed from those observed during stimulation sequence 2 within each group of rabbits.

For analysis of responses to exogenous angiotensin II in protocol 1, levels of hemodynamic variables during the final 30 s of the control periods and periods of maximum reductions and/or increases after the angiotensin II boluses were subjected to repeated-measures ANOVA. We tested whether angiotensin II had dose-dependent effects in the vehicle-control group (P_{treat}) and whether each of the treatments affected these responses (P_{group}). P values from within-subject factors in repeated-measures analyses were conservatively adjusted using the Greenhouse-Geisser correction (27).

RESULTS

Protocol 1: Effects of AT₁ Receptor and NOS Blockade

Baseline hemodynamics. During the first (control) stimulation sequence, baseline levels of MAP, HR, RBF, CLDF, and MLDF, when averaged across all 20 rabbits, were 72 ± 2 mmHg, 245 ± 4 beats/min, 27 ± 2 ml/min, 264 ± 10 U, and 67 ± 5 U, respectively. These variables did not vary according to the treatment that was to follow (P_{group} = 0.1). In vehicle-treated rabbits (group 1), baseline levels of all measured variables remained stable across the course of the experiment (Table 1). In the rabbits of group 2, vehicle treatment was accompanied by little change in MAP, HR, RBF, and CLDF, but there was a significant reduction in MLDF (−14 ± 4% ;P_{treat} = 0.02). Subsequent t-L-NNA treatment increased MAP (30 ± 9%) and reduced HR (−14 ± 4%), RBF (−22 ± 4%), CLDF (−13 ± 2%), and MLDF (−25 ± 5%) (Table 1). In the rabbits of group 3, candesartan alone increased RBF (43 ± 12%) and CLDF (16 ± 3%) but had little effect on MLDF or HR. Candesartan also tended to reduce MAP (−7 ± 3%), but this did not reach statistical significance. Subsequent administration of t-L-NNA reduced HR (−10 ± 2%), RBF (−35 ± 8%), CLDF (−19 ± 5%), and MLDF (−36 ± 6%) but did not significantly alter MAP (11 ± 5% increase) (Table 1).

Responses to RNS under control conditions. During the initial stimulation sequence, responses of RBF (P_{group} = 0.08), CLDF (P_{group} = 0.19), and MLDF (P_{group} = 0.07) to RNS did not differ significantly between the three treatment groups when analyzed across all five frequencies. However, responses at 2 Hz did appear to differ between the groups in that MLDF was consistently reduced (by −34 ± 5%) in group 3 but not in group 1 (+10 ± 7% change) or group 2 (−4 ± 1% change). When averaged across all 20 rabbits, RNS caused frequency-dependent reductions in RBF (−89 ± 3% at 8 Hz; P_{frequency} < 0.001), CLDF (−90 ± 2% at 8 Hz; P_{frequency} < 0.001), and MLDF (−55 ± 5% at 8 Hz; P_{frequency} < 0.001). RNS induced significantly greater reductions in CLDF than in MLDF (P_{region} < 0.001). As we have found previously (14, 15, 32), RNS caused a slight but significant increase in MAP (7 ± 1% at 8 Hz) but had little effect on HR (data not shown).

Effects of vehicle, t-L-NNA, and candesartan on responses to RNS. In the rabbits of group 1, responses of RBF, CLDF, and MLDF to RNS remained remarkably stable across the three stimulation sequences (P_{treat} = 0.18) (Fig. 1). In the
rabbits of group 2, responses of RBF, CLDF, and MLDF to RNS were similar during stimulation sequences 1 and 2 (after vehicle treatment) ($P_{\text{Treat}} \geq 0.24$; Fig. 2). In contrast, RNS-induced reductions in RBF ($P_{\text{Treat}} = 0.003$), CLDF ($P_{\text{Treat}} = 0.001$), and MLDF ($P_{\text{Treat}} = 0.03$) were enhanced after L-NNA treatment (Fig. 2). For example, 2-Hz RNS reduced RBF, CLDF, and MLDF by 2%, 5%, and 6%, respectively, before NOS blockade and by −37 ± 5%, −39 ± 5%, and −23 ± 4%, respectively, after NOS blockade. Similarly, 0.5-Hz RNS altered RBF, CLDF, and MLDF by −6 ± 2%, −5 ± 1%, and +1 ± 3%, respectively, before NOS blockade and by −10 ± 3%, −8 ± 2%, and −8 ± 2%, respectively, after NOS blockade. However, responses of MLDF to RNS frequencies > 2 Hz were not enhanced after L-NNA. In rabbits from group 3, RNS-induced reductions in RBF ($P_{\text{Treat}} = 0.03$), CLDF ($P_{\text{Treat}} = 0.007$), and MLDF ($P_{\text{Treat}} = 0.04$) were blunted after candesartan treatment (Fig. 3). For example, 4-Hz RNS reduced RBF, CLDF, and MLDF by −81 ± 5%, −85 ± 5%, and −68 ± 4%, respectively, under control conditions but only by −71 ± 7%, −71 ± 7%, and −45 ± 5%, respectively, after candesartan treatment. However, candesartan appeared to have less effect on responses to RNS at ≤ 2 Hz. Subsequent administration of L-NNA significantly enhanced RNS-induced reductions in RBF ($P_{\text{Treat}} = 0.04$) and CLDF ($P_{\text{Treat}} = 0.007$), with the effect being most prominent at 4 Hz. In contrast, in these rabbits, L-NNA had little effect on responses of MLDF to RNS at any frequency ($P_{\text{Treat}} = 0.66$). The differences in MLDF responses to RNS during stimulation sequences 2 (during vehicle infusion) and 3 (during L-NNA infusion) in rabbits of group 2 differed significantly from those during stimulation sequences 2 (during candesartan infusion) and 3 (during L-NNA infusion) in rabbits of group 3 ($P_{\text{Group}} = 0.035$). This

![Fig. 2. Effects of L-arginine (L-NNA; 20 mg/kg + 5 mg·kg⁻¹·h⁻¹) on renal hemodynamic responses to renal nerve stimulation in protocol 1. Symbols and error bars indicate means ± SE of data from rabbits in group 2 ($n = 8$). $P$ values indicate the outcomes of ANOVA, which tested whether renal hemodynamic responses during stimulation sequence 2 (●, performed after vehicle treatment was started) differed from those observed during the initial stimulation sequence (○) and whether renal hemodynamic responses during stimulation sequence 3 (▲, performed after L-NNA treatment was started) differed from those observed during stimulation sequence 2. See text for explanation of groups and protocols.](http://ajpregu.physiology.org/)

![Fig. 3. Effects of candesartan (10 μg/kg + 10 μg·kg⁻¹·h⁻¹) and subsequently L-NNA (20 mg/kg + 5 mg·kg⁻¹·h⁻¹) on renal hemodynamic responses to renal nerve stimulation in protocol 1. Symbols and error bars indicate means ± SE of data from rabbits in group 3 ($n = 6$). $P$ values indicate the outcomes of ANOVA, which tested whether responses during stimulation sequence 2 (●, performed after candesartan treatment was started) differed from those observed during the initial stimulation sequence (○) and whether responses during stimulation sequence 3 (▲, performed after L-NNA treatment was started) differed from those observed during stimulation sequence 2. See text for explanation of groups and protocols.](http://ajpregu.physiology.org/)
was not the case for RBF ($P_{\text{Group}} = 0.19$) or CLDF ($P_{\text{Group}} = 0.27$) (Fig. 3). RNS-induced reductions in CLDF were always greater than those of MLDF, regardless of the treatment (vehicle, L-NNA, candesartan, or candesartan + L-NNA) administered ($P_{\text{Region}} < 0.001$).

Responses to bolus doses of angiotensin II. In vehicle-treated rabbits (group 1), intravenous angiotensin II caused dose-dependent reductions in RBF and CLDF ($P_{\text{Dose}} \leq 0.01$). The response of MLDF to bolus angiotensin II was biphasic, consisting of an initial reduction (during the first 20 s of the response) followed by a later increase (during the period 20–70 s after the bolus). Also, angiotensin II dose dependently increased MAP (by $21 \pm 3$ mmHg at 1,000 ng/kg; $P_{\text{Dose}} = 0.003$) but had little effect on HR (data not shown). Compared with vehicle-treated rabbits, in rabbits treated with L-NNA alone, in rabbits treated with L-NNA and angiotensin II (group 2) angiotensin II produced greater reductions in CLDF ($P_{\text{Group}} = 0.03$) and tended to produce greater reductions in RBF ($P_{\text{Group}} = 0.07$) and MLDF ($P_{\text{Group}} = 0.09$). Compared with rabbits treated with L-NNA alone, in rabbits treated with candesartan plus L-NNA (group 3), angiotensin II-induced reductions in RBF ($P_{\text{Group}} \leq 0.004$), CLDF ($P_{\text{Group}} = 0.001$), and MLDF ($P_{\text{Group}} = 0.002$) and also angiotensin II-induced increases in MAP ($P_{\text{Group}} < 0.001$) were significantly reduced. Angiotensin II-induced increases in MLDF were virtually abolished by combined candesartan and L-NNA treatment (Fig. 4).

Protocol 2: Effects of Exogenous Angiotensin II With and Without NOS Blockade

Baseline hemodynamics. During control periods in stimulation sequence 1, MAP was 46% greater and CLDF was 23% less in L-NNA-pretreated rabbits than in vehicle-pretreated rabbits (Table 2). Levels of RBF and MLDF did not differ significantly between the two groups of rabbits. Renal arterial infusion of angiotensin II (5 ng·kg⁻¹·min⁻¹) reduced CLDF similarly in both groups of rabbits (by ~20%) but did not significantly alter RBF, MLDF, or MAP.

Responses to RNS. In vehicle-pretreated rabbits, responses of CLDF to RNS were slightly attenuated during renal arterial infusion of angiotensin II, although this did not reach statistical significance ($P = 0.06$). Responses of RBF and MLDF to RNS in this group of rabbits were not significantly altered by angiotensin II infusion. In contrast, in rabbits pretreated with L-NNA, responses of MLDF (but not RBF or CLDF) were significantly enhanced during infusion of angiotensin II compared with control responses. For example, 1-Hz RNS reduced MLDF by $-7 \pm 5\%$ during stimulation sequence 1 in L-NNA-pretreated rabbits and by $-31 \pm 10\%$ during stimulation sequence 2, in which angiotensin II was infused into the renal artery (Fig. 5).

Protocol 3: Effects of High-Dose Exogenous Angiotensin II

Baseline hemodynamics. These were indistinguishable in the two groups of rabbits during control periods in stimulation sequence 1 (Table 3). Renal arterial infusion of angiotensin II (5–15 ng·kg⁻¹·min⁻¹) reduced RBF (by $-38 \pm 3\%$) and CLDF (by $-33 \pm 4\%$) but did not significantly alter MAP or MLDF. In contrast, baseline hemodynamic variables changed little in response to renal arterial infusion of the vehicle for angiotensin II.

Responses to RNS. Responses of RBF and CLDF to RNS during stimulation sequence 1 were not significantly different in the two groups of rabbits ($P_{\text{Group}} \geq 0.18$). However, responses of MLDF to RNS were consistently greater in group 2 than in group 1 ($P_{\text{Group}} = 0.008$). In group 1, responses of RBF, CLDF, and MLDF to RNS were similar during stimulation sequence 1 (control) compared with stimulation sequence 2 (during renal arterial infusion of a saline vehicle). In group 2, renal arterial infusion of angiotensin II had little impact on responses of RBF or CLDF to RNS, but responses of MLDF were attenuated relative to the control responses. For example,

Fig. 4. Responses to intravenous bolus doses of angiotensin II in protocol 1. Responses in rabbits treated with vehicle only (○; group 1, $n = 6$), L-NNA (●; group 2, $n = 8$; 20 mg/kg + 5 mg·kg⁻¹·h⁻¹), or candesartan (cand; 10 μg/kg + 10 μg·kg⁻¹·h⁻¹) plus L-NNA (▲; group 3, $n = 6$) are shown. $P$ values indicate the outcomes of ANOVA, which tested whether responses to angiotensin II differed in rabbits treated with L-NNA compared with vehicle-treated rabbits and in rabbits treated with candesartan plus L-NNA compared with those treated with L-NNA only. MAP, mean arterial pressure. See text for explanation of groups and protocols.
2-Hz RNS reduced MLDF by \(-44 \pm 9\%\) during stimulation sequence 1, but only by \(-29 \pm 8\%\) during stimulation sequence 2, during which angiotensin II was infused into the renal artery (Fig. 6).

**DISCUSSION**

The aim of our study was to investigate the roles of endogenous angiotensin II acting at AT1 receptors and the significance of interactions between NO and angiotensin II, in the differential regulation of regional kidney blood flow by RNS.

As shown previously by ourselves (14, 15, 19, 20, 25) and others (34), RNS reduced CLDF more than MLDF. Consistent with our group’s previous findings (14, 32), NOS blockade enhanced neurally mediated renal vasoconstriction, particularly at low frequencies within the medulla. Thus endogenous NO appears to contribute to the mechanisms underlying the relative insensitivity of the medullary circulation to low-frequency RNS. Our present data also demonstrate that AT1-receptor antagonism with candesartan can blunt RNS-induced reductions in RBF and CLDF. This confirms and extends previous studies of the effects of AT1-receptor antagonists on responses of global RBF to RNS (8, 12, 36, 42) and suggests that endogenous angiotensin II, acting at AT1 receptors, aug-

### Table 2. Mean baseline levels of systemic and renal hemodynamic variables in protocol 2

<table>
<thead>
<tr>
<th></th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
<th>RBF (ml/min)</th>
<th>CLDF (U)</th>
<th>MLDF (U)</th>
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<tbody>
<tr>
<td><strong>Control</strong></td>
<td>70 ± 3</td>
<td>244 ± 15</td>
<td>23 ± 2</td>
<td>318 ± 28</td>
<td>37 ± 8</td>
</tr>
<tr>
<td><strong>Angiotensin II</strong></td>
<td>72 ± 4</td>
<td>229 ± 18</td>
<td>21 ± 3</td>
<td>256 ± 7</td>
<td>35 ± 6</td>
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**Effects of renal arterial angiotensin II under control conditions**

(group 1, n = 6)

<table>
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<tr>
<th></th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
<th>RBF (ml/min)</th>
<th>CLDF (U)</th>
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<tr>
<td>L-NNA</td>
<td>102 ± 4</td>
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<tr>
<td>Angiotensin II</td>
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<td>(P_{\text{Group}})</td>
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<td>(P_{\text{Treat}})</td>
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<td>(P_{\text{Group}\times\text{Treat}})</td>
<td>0.10</td>
<td>0.02</td>
<td>0.46</td>
<td>0.57</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of animals. See text for explanation of groups and protocols. \(P\) values are the outcomes of ANOVA, which tested whether baseline levels differed in the 2 groups of rabbits (\(P_{\text{Group}}\)) and whether renal arterial infusion of angiotensin II (5 ng·kg\(^{-1}\)·min\(^{-1}\)) affected the levels of these variables in a manner independent (\(P_{\text{Treat}}\)) or dependent (\(P_{\text{Group}\times\text{Treat}}\)) on whether the rabbits were pretreated with L-NNA or its vehicle.

Fig. 5. Effects of renal arterial infusion of angiotensin II (5 ng·kg\(^{-1}\)·min\(^{-1}\)) on renal hemodynamic responses to renal nerve stimulation in rabbits pretreated with L-NNA (20 mg/kg + 5 mg·kg\(^{-1}\)·min\(^{-1}\); n = 6) or its vehicle (n = 6). Symbols and error bars indicate means ± SE. \(P\) values indicate the outcomes of ANOVA, which tested whether responses during stimulation sequence 2 (○) performed after angiotensin II infusion was started) differed from those observed during the initial stimulation sequence (○).
ments RNS-induced vasoconstriction in vascular elements regulating cortical blood flow.

Our novel findings include the observation that the AT1-receptor antagonist candesartan blunted responses of MLDF to RNS. To our knowledge, this is the first demonstration that endogenous angiotensin II can modulate neural control of medullary blood flow. Our data indicate that the dominant role of endogenous angiotensin II, with regard to neural control of medullary blood flow, is to enhance vasoconstrictor responses to RNS within the medullary circulation. This may be mediated through the direct vasoconstrictor action of angiotensin II (22, 38) and/or through facilitation of norepinephrine release from adrenergic nerve terminals (12, 35, 36).

After prior AT1-receptor blockade in protocol 1, we were unable to detect an effect of L-NNA treatment on MLDF responses to RNS. Nevertheless, consistent with previous observations by Egi et al. (12) in anesthetized dogs, effects of L-NNA on responses of total RBF (and CLDF) remained intact. One possible interpretation of this observation is that, in the renal medullary but not in the cortical circulation, the ability of endogenous NO to blunt neurally mediated vasoconstriction depends on tonic activation of AT1 receptors. However, this conclusion must be tempered by the fact that responses of MLDF to RNS appeared to differ under control conditions in the two groups of rabbits treated with L-NNA in protocol 1. The apparent inability of NOS blockade to affect responses of MLDF to RNS after AT1-receptor blockade might simply reflect the apparent greater sensitivity of MLDF to RNS in this group of rabbits under control conditions (see RESULTS). Nev-

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**Table 3. Mean baseline levels of systemic and renal hemodynamic variables in protocol 3**

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>RBF, ml/min</th>
<th>CLDF, U</th>
<th>MLDF, U</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effects of renal arterial infusion of a saline vehicle (group 1, n = 9)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>70±2</td>
<td>235±7</td>
<td>20±2</td>
<td>253±13</td>
<td>36±7</td>
</tr>
<tr>
<td>Vehicle</td>
<td>70±3</td>
<td>232±8</td>
<td>18±2</td>
<td>236±12</td>
<td>37±10</td>
</tr>
<tr>
<td><strong>Effects of renal arterial infusion of angiotensin II (5–15 ng·kg⁻¹·min⁻¹) (group 2, n = 9)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>70±3</td>
<td>249±8</td>
<td>19±1</td>
<td>263±17</td>
<td>48±8</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>71±3</td>
<td>222±9</td>
<td>12±1</td>
<td>172±11</td>
<td>51±11</td>
</tr>
<tr>
<td>P_{Group}</td>
<td>0.94</td>
<td>0.88</td>
<td>0.14</td>
<td>0.102</td>
<td>0.29</td>
</tr>
<tr>
<td>P_{Treat}</td>
<td>0.90</td>
<td>0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.71</td>
</tr>
<tr>
<td>P_{Group*Treat}</td>
<td>0.87</td>
<td>0.02</td>
<td>0.003</td>
<td>0.003</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of animals. See text for explanation of groups and protocols. P values are the outcomes of ANOVA, which tested whether baseline levels differed in the 2 groups of rabbits (P_{Group}) and whether the levels of these variables changed, between the first and second stimulation sequence, in a manner independent (P_{Treat}) or dependent (P_{Group*Treat}) on whether they received angiotensin II or its saline vehicle.

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**Fig. 6. Effects of renal arterial infusion of angiotensin II (5–15 ng·kg⁻¹·min⁻¹; n = 9) or its saline vehicle (n = 9) on renal hemodynamic responses to renal nerve stimulation. The dose of angiotensin II was titrated to reduce RBF by ~40% (see Table 3). Symbols and error bars indicate means ± SE. P values indicate the outcomes of ANOVA, which tested whether responses during stimulation sequence 2 (●; performed after infusion of angiotensin II or its vehicle was started) differed from those observed during the initial stimulation sequence (○).**
etertheless, our observations in *protocol 1* prompted us to further examine the notion that, within the medullary circulation, angiotensin II may have actions to both enhance and blunt neurally mediated vasoconstriction.

To further explore the possible interactions between angiotensin II and NO in neural control of regional kidney perfusion, we examined the effects of renal arterial infusion of angiotensin II on responses to RNS in rabbits pretreated with 1-NNA or its vehicle. In *protocol 2*, we used a relatively low dose of angiotensin II (5 ng·kg⁻¹·min⁻¹), which reduced baseline CLDF by ~20% in both 1-NNA-pretreated and vehicle-pretreated rabbits but did not significantly alter RBF or MLDF. The angiotensin II infusion did not significantly alter responses of RBF or CLDF to RNS, but the responses of MLDF were significantly enhanced in 1-NNA-pretreated rabbits but not in vehicle-pretreated rabbits. Thus exogenous angiotensin II appears to be able to selectively enhance responses of MLDF to RNS under conditions of NOS blockade but not when NOS is intact. These data are consistent with the view that angiotensin II can enhance neurally mediated vasoconstriction in the medullary circulation. However, at least in the case of exogenous angiotensin II, this effect may normally be counterbalanced by the actions of NO. This latter conclusion is consistent with observations that angiotensin II can increase levels of NO in the medulla (43) and NO can blunt adrenergic vasoconstriction within the medullary circulation (14, 33, 43).

In a previous study, we found that renal arterial infusion of angiotensin II, at a dose that reduced basal RBF by ~30%, blunted responses of MLDF (but not RBF or CLDF) to RNS (19). The fact that we did not observe this phenomenon in *protocol 2* may reflect the relatively low dose of angiotensin II that we administered, which might not have been sufficient to recruit medullary vasodilator mechanisms. Therefore, in *protocol 3*, we titrated the dose of angiotensin II so that it reduced basal RBF by ~40% (5−15 ng·kg⁻¹·min⁻¹) in each rabbit. Under these conditions, we reproduced our original finding (19), in that responses to RNS of MLDF, but not of RBF or CLDF, were significantly blunted during renal arterial infusion of angiotensin II but not its saline vehicle. Thus exogenous angiotensin II appears to be able to selectively blunt neurally mediated vasoconstriction in the medullary circulation. It remains to be determined whether this effect is mediated by NO. Angiotensin II-induced NO release in the kidney has been demonstrated to result from activation of AT₁ receptors (37) and AT₂ receptors (4) and from both endothelial (37) and epithelial (9) sources. The epithelial cells of the thick ascending limb of Henle’s loop may be a key site where angiotensin II acts to increase NO levels within the outer medulla, which would presumably dilate outer medullary descending vasa recta to increase medullary blood flow (9).

The apparent discrepancy between our observation in *protocol 1* (that candesartan blunted responses of RBF, CLDF, and MLDF to RNS) and our observations in *protocols 2 and 3* (that exogenous angiotensin II did not affect responses of RBF and CLDF to RNS and either had no effect, blunted, or enhanced (after NOS blockade) responses of MLDF) deserves comment. One possible explanation relates to differences in bioavailability between exogenous angiotensin II, which is rapidly degraded in the kidney (half-life ~16 s) (2), and that of the more stable candesartan (half-life ~9 h) (3). Thus intravenously administered candesartan would likely reach similar concentrations throughout the kidney, including vascular smooth muscle and its associated sympathetic innervation. In contrast, angiotensin II infused into the renal artery would reach relatively high concentrations within the vascular and tubular lumen and so be able to stimulate NO release from endothelial and/or epithelial cells (9). However, the vascular endothelium and the interstitium would act as barriers for diffusion of exogenous angiotensin II from sites within the vascular and tubular lumen to vascular smooth muscle (and its associated sympathetic innervation).

RNS was associated with small increases in MAP (5 ± 1 mmHg at 8 Hz) but no change in HR. Le Fevre et al. (24) recently showed that pressor responses to prolonged (up to 180 min) stimulation of the renal nerves at 1 Hz in rabbits are abolished by angiotensin-converting enzyme inhibition, suggesting that it is mediated by angiotensin II. In contrast, we observed a small increase in MAP in response to RNS even after candesartan treatment (7 ± 3 mmHg at 8 Hz; data not shown). Thus the pressor response to these short trains of RNS likely reflects the direct impact of increased renal vascular resistance on total peripheral resistance. The lack of effect of RNS on HR in our study probably reflects the use of pentobarbital anesthesia, which greatly depresses the baroreceptor reflex (30).

There are some limitations of this study. First, there is evidence that NO can inhibit release of norepinephrine from prejunctional sites in response to RNS (28). Thus enhanced responses of RBF, CLDF, and MLDF to RNS in rabbits pretreated with l-NNA could be due to facilitated release of norepinephrine, to a postjunctional effect to enhance responses to norepinephrine, or to both. Our present results do not allow us to identify the precise mechanisms by which NO, or angiotensin II, modulates renal hemodynamic responses to RNS. Second, l-NNA reduced baseline levels of RBF, CLDF, and MLDF, and candesartan increased (and exogenous angiotensin II reduced) baseline levels of RBF and CLDF. Therefore, the effects of candesartan, l-NNA, and exogenous angiotensin II on responses of intrarenal blood flow to RNS could be secondary to the effects of these treatments on baseline levels of systemic and renal hemodynamics. However, this seems unlikely because our previous findings have indicated that changes in renal vascular tone per se (19) and changes in arterial pressure similar to those produced by l-NNA and candesartan in the present study (14) have little impact on responses of regional kidney blood flow to RNS. Moreover, even large increases in renal perfusion pressure, produced using an extracorporeal circuit preparation in anesthetized rabbits, have little impact on the responses of MLDF to RNS (21). Nevertheless, our present conclusions might be different if they were based on the effects of RNS on absolute rather than percent changes in RBF, CLDF, and MLDF. Our view is that percent changes, rather than absolute changes, reflect the functional state of the vasculature, since Poiseuille’s relationship predicts that the relative response of flow to a given (relative) change in vascular caliber is independent of the resting caliber of the vessel (see Ref. 21). Another important limitation of our study relates to the use of electrical stimulation of the renal nerves. This technique provides information regarding the potential actions of sympathetic activation on renal hemodynamics but not necessarily regarding the physiological control of renal hemodynamics under basal conditions.

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In protocol 1, intravenous angiotensin II dose-dependently increased MAP and reduced RBF and CLDF, while having a biphasic effect (reduction followed by increase) on MLDF. These renal hemodynamic effects were remarkably similar to those that we have previously observed in response to renal arterial boluses of angiotensin II (10, 31, 33); therefore, they likely reflect direct actions of angiotensin II within the kidney, rather than changes secondary to its pressor effect. Candesartan greatly blunted all of the effects of angiotensin II, indicating that the dose that we used provided significant blockade of AT1 receptors.

In conclusion, our present results suggest that endogenous angiotensin II enhances RNS-induced vasoconstriction in vascular elements regulating both cortical and medullary blood flow. This observation does not support the hypothesis that the relative insensitivity of medullary blood flow to renal nerve activation is due to differential effects of endogenous angiotensin II in the cortical and medullary circulations. Our data confirm that endogenous NO buffers neurally mediated vasoconstriction in the renal cortical and medullary circulations. The fact that we could not detect an effect of NOS blockade on responses of MLDF to RNS in rabbits pretreated with candesartan might indicate rather complex interactions between endogenous angiotensin II and NO in the medullary circulation. However, we cannot exclude the possibility that this observation was an artifact secondary to possible between-group differences in control responses of MLDF to RNS in protocol 1. Nevertheless, our observations of the effects of renal arterial infusions of angiotensin II in protocols 2 and 3 are consistent with the idea that angiotensin II can produce competing effects to both enhance and blunt neurally mediated reductions in medullary blood flow. Nevertheless, the dominant action of endogenous angiotensin II is to enhance RNS-induced vasoconstriction in the medullary circulation.

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