NO mediates downregulation of RBF after a prolonged reduction of renal perfusion pressure in SHR

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NO mediates downregulation of RBF after a prolonged reduction of renal perfusion pressure in SHR. Am J Physiol Regul Integr Comp Physiol 285: R329–R338, 2003. First published April 24, 2003; 10.1152/ajpregu.00063.2003.—The aim of the study was to investigate mechanisms underlying the downregulation of renal blood flow (RBF) after a prolonged reduction in renal perfusion pressure (RPP) in adult spontaneously hypertensive rats (SHR). We tested the effect on the RBF response of clamping plasma ANG II in sevoflurane-anesthetized SHR. We also tested the effect of general cyclooxygenase (COX) inhibition and inhibition of the inducible COX-2. Furthermore, we assessed the effect of clamping the nitric oxide (NO) system. A prolonged period (15 min) of reduced RPP induced a downregulation of RBF. This was unchanged after clamping of plasma ANG II concentrations, general COX inhibition, and specific inhibition of COX-2. In contrast, clamping the NO system diminished the ability of SHR to downregulate RBF to a lower level. The downregulation of RBF was not associated with a resetting of the lower limit of autoregulation in the control group, in the ANG II-clamped group, or the NO clamped group. However, general COX inhibition and specific COX-2 inhibition enabled downward resetting of the lower limit of autoregulation. In conclusion, in SHR the renin-angiotensin system does not appear to play a major role in the downregulation of RBF after prolonged reduction of RPP. This response appears to be mediated partly by the NO system. We hypothesize that, in SHR, lack of downward resetting of the lower limit of autoregulation in response to a prolonged lowering of RPP could be the result of increased COX-2-mediated production of vasoconstrictory prostaglandins.

autoregulation; cyclooxygenase inhibition; nitric oxide inhibition; renal perfusion pressure; spontaneously hypertensive rats

CROSS TRANSPLANTATION STUDIES strongly suggest that a change in renal function is central in the pathogenesis of primary hypertension (4, 9). Although the underlying mechanisms still remain unresolved, experimental evidence suggests that abnormalities in renal hemodynamics are associated with the development and the maintenance of hypertension in the spontaneously hypertensive rat (SHR; see Refs. 2 and 7).

Different endocrine and paracrine substances are known to affect renal hemodynamics (23). The effect of the renin-angiotensin system (RAS) has been evaluated widely in normotensive rats (3, 10, 14, 26). When the arterial blood pressure is lowered for a prolonged period, renal blood flow (RBF) is downregulated, and RBF is now autoregulated around this new, lower value. This downregulation of RBF appears to be mediated solely by the renin-angiotensin system, since it is completely abolished by blocking the renin-angiotensin system (3, 14, 26). A similar downregulation also occurs in SHR in response to a prolonged lowering of the renal perfusion pressure (RPP), but, in contrast to the situation in normotensive rats, this downregulation of RBF does not appear to be mediated by the renin-angiotensin system (17). Presently, the mechanism underlying the downregulation of RBF in SHR is unknown. Adjustment of RBF in response to prolonged changes in arterial pressure is central to the optimal control of glomerular filtration rate (GFR), peritubular and medullary capillary flow, and, hence, the urinary excretion of sodium and water. Understanding the mechanisms that mediate these adjustments in the SHR may therefore be important in elucidating the renal mechanisms that ultimately lead to hypertension.

Metabolites from the cyclooxygenase (COX) enzymes may also influence RBF regulation. RBF and GFR are reduced in young SHR because of an increased renal vascular resistance (8). This increased resistance has been suggested to be the result of either an increased production or an increased sensitivity to vasoconstrictory prostaglandins [e.g., thromboxane A2 (TXA2); see Ref. 5]. Although RBF becomes normalized in adult SHR with established hypertension, an increased renal vascular reactivity toward vasoconstrictory prostaglandins seems to persist (2). It is generally accepted that vasoconstrictory prostaglandins only play a minor role in the regulation of adult SHR RBF.

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role under normal physiological conditions but that they may be of importance under pathophysiological conditions (23). Nitric oxide (NO) is of major importance in maintaining a low resistance in the renal vascular bed (23). The kidney contains all three isozymes of NO synthase (NOS; 1, 21). NOS-1 [neuronal NOS (nNOS)] is found in the macroca densa (29), and NOS-2 [inducible NOS (iNOS)] has been shown to be present both in vascular and tubular structures (21), whereas NOS-3 [endothelial NOS (eNOS)] predominantly is found in the vascular endothelium (30). Interestingly, in normotensive rats, NO plays a central role in the adjustments of RBF in response to variations in dietary salt intake (6). We therefore found it of interest to investigate whether NO might be involved in the downregulation of RBF in response to prolonged pressure reductions in the SHR.

The aim of the present study was to further characterize the response to a prolonged reduction in RPP in SHR and to investigate the extent to which the renin-angiotensin system, prostaglandins, or NO is involved in mediating the response. Our hypothesis was that the ability to downregulate RBF to a lower level after a prolonged period of reduced RPP involved either prostaglandins or NO rather than the renin-angiotensin system, as is the case in normotensive rats (26).

The results indicate that, in SHR with established hypertension, the downregulation of RBF is due, at least in part, to a reduction in the activity of the NO system. Neither the renin-angiotensin system nor prostaglandins were of importance in mediating the downregulation of RBF. Interestingly, it was found that blocking COX-2 allowed a downward resetting of the lower limit of autoregulation in response to a prolonged reduction of RPP.

METHODS

Animal Preparation

The experiments were performed in male SHR weighing 240–310 g obtained from Mollegård (Lille Skensved, Denmark). The experimental protocol was approved by the National Research Animal Committee. The rats had free access to food and water until immediately before the experiments and were fed ordinary rat chow (Altromin no. 1314; Petersen, Ringsted, Denmark) containing 87 mmol sodium/kg.

Anesthesia was induced with 8% sevoflurane delivered in 35% oxygen and 65% nitrogen. Polyethylene catheters were placed in the right jugular vein (PE-10) for infusion and in the left carotid artery (PE-50) for continuous measurement of the systemic blood pressure by a Statham P23-dB pressure transducer (Gould, Oxnard, CA). A tracheostomy was performed, and the rat was placed on a servo-controlled heating table to maintain body temperature at 37°C. The rat was connected to and ventilated by a small animal ventilator, tidal volume 1.7–2.1 ml depending on body weight, and a frequency of 60 breaths/min. The final sevoflurane concentration needed to maintain sufficient anesthesia was ~2%.

An intravenous bolus injection of 0.3 mg pancuronium bromide (Pavulon; Organon) in 0.4 ml of 0.9% saline was followed by continuous intravenous infusion of 0.6 mg/ml pancuronium bromide at 20 µl/min. Additional saline was given continuously at a rate of 20 µl/min.

The left kidney was exposed after a laparotomy, which was extended to the left flank. The left femoral artery was catheterized (PE-50) for measurements of the RPP. The left ureter was catheterized (PE-10 connected to PE-50) to ensure free urine flow. The left renal artery was stripped from any fat or fascias, and a precalibrated electromagnetic perivascular flow sensor (model 1401; Skalar Medical) was placed around it (lumen diameter 0.6–0.8 mm). The aorta was exposed, and a servo-controlled aortic clamp (RPC-2 controller; Electronic Workshop, McGill University) was placed above the bifurcation of the renal arteries. The controller of the clamp maintained a constant RPP by comparing the pressure signal from the femoral artery with a reference signal corresponding to a preset pressure.

Blood pressure and RBF were recorded on a stereo video recorder (Sony) through a frequency modulator (Reditech, Copenhagen, Denmark).

The kidney was superfused with heated saline (37°C) during the experiment.

Experimental Protocol

Control. INTACT RENIN-ANGIOTENSIN SYSTEM. Twelve rats were used. After completion of surgery, the rats were allowed to equilibrate for at least 30 min. A blood sample (~200 µl) for baseline renin measurements was taken just before the pressure reductions. RPP was reduced in steps of 10 mmHg from the spontaneous blood pressure down to 100 mmHg, with each step lasting 1 min. The pressure was kept at 100 mmHg for 15 min, after which the clamp was released. After 1–2 min at spontaneous blood pressure to allow stabilization of the perfusion pressure and the RBF, pressure reduction steps were repeated (Fig. 1). At the end of the experiment, a second blood sample was collected. EGTA (10 µl of 300 mM) was added to the blood samples to prevent coagulation. The blood samples were centrifuged at 7,000 rpm for 5 min, and the plasma was kept frozen for later renin measurements (see below).

The RBF at a blood pressure of 160 mmHg was set to 100%, and RBF was normalized to this flow. Average pressure and flow values were determined in the last 30 s of a pressure step.

ANG II clamp. CLAMPED RENIN-ANGIOTENSIN SYSTEM. Twelve rats were used. To clamp the level of ANG II, 12 µg/min (1.2 mg/ml dissolved in 0.9% saline) of the angiotensin-converting enzyme inhibitor captopril (ICN, Aurora, OH) were infused intravenously as soon as blood pressure and RBF were stable. When stable levels of blood pressure and RBF were obtained after ~10–15 min, infusion of ANG II (0.5 µg/ml dissolved in 0.9% saline; Sigma Chemicals) was started at 3 ng/min and increased until precapillary levels of blood pressure and RBF were obtained. The average infusion rate was 4.4 ± 0.4 ng/min. The rats were then allowed to equilibrate for at least 30 min. The protocol was otherwise similar to that of the experiments with an intact renin-angiotensin system, but no blood samples were taken. To prevent overhydration, saline was infused at a reduced rate of 10 µl/min.

Inhibition of COX. NONSELECTIVE COX INHIBITION. Eight rats were treated with the nonselective COX inhibitor indomethacin (Sigma). Indomethacin (13 mg dissolved in 7 ml of 0.9% saline and 3 ml of 1 M NaOH; solution adjusted to pH 7.4 and 260 mosmol/kg H2O) was given as a bolus of 5 mg/kg. The rat was then allowed to equilibrate for at least 30 min before a blood sample was drawn for later renin measurements. Otherwise, the protocol was as described for experiments with intact RAS.
specific COX-2 inhibition. Nine rats were treated with the specific COX-2 inhibitor NS-398 (Cayman Chemicals, Ann Arbor, MI). NS-398 (1 mg dissolved in 0.1 ml DMSO and 9.9 ml of 0.9% saline) was infused at 2 μg/min. The rats were then allowed to equilibrate for at least 30 min. Otherwise, the protocol was as above except no blood samples were drawn.

NO clamp. N<sup>ω</sup>-nitro-L-arginine methyl ester-sodium nitroprusside. Eight rats were treated with N<sup>ω</sup>-nitro-L-arginine methyl ester (L-NAME). L-NAME (50 mg dissolved in 20 ml of 0.9% saline) was infused at 10 mg·kg<sup>−1</sup>·h<sup>−1</sup>. When stable levels of blood pressure and RBF were obtained after ~5–10 min, an infusion of sodium nitroprusside (SNP, Nitropress; Abbott Laboratories) was begun at a rate of 1–2 μg/min (1.5 ± 0.1 μg/min) to return RPP and RBF to control levels. The rats were then allowed to equilibrate for at least 30 min. Otherwise, the protocol was as above.

L-NAME/SNP. In eight additional rats, the above protocol was modified so that L-NAME and SNP were given simultaneously. This was necessary, since it proved difficult to restore RBF to the control level when there was a delay between the administration of L-NAME and SNP. The rate of SNP needed to restore RPP and RBF was significantly lower in these rats (0.8 ± 0.1 μg/min). The rats were then allowed to equilibrate for at least 30 min before a blood sample was taken for later renin measurements. Otherwise, the protocol was as above.

Renin assay. Plasma renin concentration (PRC) was measured using the protocol of Lykkegaard and Poulsen (19). Aliquots of plasma were diluted 20- to 80-fold with Tris buffer containing human albumin, and 5–μl portions of these samples were incubated for 24 h at 37°C with 20 ml of a reaction mixture that contained purified rat renin substrate (~1,200 ng ANG I equivalents/ml). This incubation was followed by RIA of generated ANG I. PRC was measured in reference to renin standards obtained from the National Institute for Biological Standards and Control (Potters Bar, Herts, United Kingdom).

Data Analysis and Statistics

The autoregulatory efficiency was quantified by the autoregulatory index (ARI), which was calculated as the ratio of the normalized decreases in RBF and RPP (%change in RBF/%change in RPP) in the pressure range from 140 to 160 mmHg. If there is perfect autoregulation ARI is zero, whereas if there is no autoregulation ARI is one.

The lower limit of autoregulation was determined by two independent methods. In the first case, the lower limit was determined as the inflection point of the autoregulation curve. For each autoregulation curve, the inflection point was found as the intersection of two straight lines fitted by eye to the seven points on the curve. The fits were made in a blinded design, in that the person was unaware of which group the experiment belonged to. If the autoregulation curve had no apparent inflection point, it was classified as continually decreasing if the slope was statistically different from zero and if the RBF decreased to a value <95% at 100 mmHg (ARI >0.1). In this case, the lower limit of autoregulation was set at 160 mmHg, i.e., no autoregulation. Otherwise, it was classified as horizontal, and the lower limit of autoregulation was set at the lowest possible value (100 mmHg), i.e., perfect autoregulation.

In the second case, the lower limit was determined from the averaged autoregulation curve using an ANOVA for repeated measures. The first RPP where RBF was significantly lower (least significant difference test) different from the flow at 160 mmHg was taken as the lower limit of autoregulation.

Inflection points and RBF values after pressure reduction were compared between groups by one-way ANOVA and least significant difference test. PRCs before and after the experiment were compared using Student’s t-test for paired measurements.

Results are presented as means ± SE of original or normalized data. A P value <0.05 was considered statistically significant.
RESULTS

Mean arterial pressure and initial RBF values are shown in Table 1. The rats used in the ANG II clamp experiments were smaller, and their RBF was significantly lower ($P < 0.01$) compared with the control rats. RBF was also significantly lower ($P < 0.05$) in rats used for the first NO clamp for no obvious reason. Otherwise, the rats were of comparable physiological status.

Control

Figure 1 shows the results of a typical experiment. After a prolonged (15-min) reduction of the RPP to 100 mmHg, release of the aortic clamp did not result in full restoration of the RBF despite a normalization of the RPP. During the 15 min of reduced RPP there was no decline in RBF (Fig. 1); this was a consistent observation in all experiments. Upon release of the aortic clamp, RBF returned from 62 ± 6 to 85 ± 3% of the initial value ($P < 0.01$; Fig. 2). The RPP increased to 177 ± 4 mmHg after release of the aortic clamp compared with an initial value of 165 ± 3 mmHg ($P < 0.05$).

The PRC increased significantly after 15 min of reduced perfusion pressure from 67 ± 25 × 10⁻⁵ Goldblatt units (GU)/ml to 182 ± 50 × 10⁻⁵ GU/ml (Table 2).

![Graph](image)

**Fig. 2. End points.** Values that RBF increased to after the aortic clamp was released after 15 min of reduced RPP. Values are means ± SE. # $P < 0.05$ vs. control.

Table 2. *Physiological status of the SHR rats*

<table>
<thead>
<tr>
<th></th>
<th>Body Wt, g n</th>
<th>MAP, mmHg</th>
<th>RBF, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>263 ± 2 12</td>
<td>165 ± 3</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
<td>ANG II clamp</td>
<td>248 ± 4 12</td>
<td>177 ± 4</td>
<td>5.9 ± 0.3</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>262 ± 2 9</td>
<td>165 ± 3</td>
<td>6.3 ± 0.5</td>
</tr>
<tr>
<td>NS-398</td>
<td>275 ± 6 8</td>
<td>165 ± 3</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>L-NAME-SNP</td>
<td>262 ± 2 8</td>
<td>165 ± 3</td>
<td>6.0 ± 0.4</td>
</tr>
<tr>
<td>L-NAME/SNP</td>
<td>262 ± 2 9</td>
<td>165 ± 3</td>
<td>6.0 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. # $P < 0.05$.

Figure 3 shows the autoregulation of RBF in control SHR before and after RPP was kept at 100 mmHg for 15 min. Values are normalized to the RBF value at 160 mmHg. Autoregulation was still present, albeit around a lower value of RBF, after the prolonged lowering of the RPP. There was a tendency for the ARI to increase after the prolonged pressure reduction, but the difference did not reach statistical significance ($P < 0.08$; Table 3). There was no resetting of the lower limit of autoregulation after the pressure reduction (Table 4). This was the case regardless of whether the lower limit was determined from the inflection points of the individual autoregulatory curves or from the averaged RBF curves using an ANOVA-based approach (Table 4).

At all perfusion pressures in the second set of pressure reductions, RBF was significantly lower compared with the corresponding value in the first set of pressure reductions. At 100 mmHg, RBF reached a value of only 49 ± 6% of the initial value ($P < 0.05$ compared with the corresponding value obtained before the prolonged pressure reduction (62 ± 6%); Table 5).

**ANG II clamp**

After inhibition of angiotensin-converting enzyme and subsequent infusion of 4.4 ± 0.4 ng/min ANG II, there were no changes in RPP and RBF as these parameters returned to the levels observed before infusion of the angiotensin-converting enzyme inhibitor (Table 1). The response of RBF to the prolonged reduction of RPP was unaffected by clamping of the plasma ANG II concentration (Fig. 4). RBF was depressed to the same extent (72 ± 6%) during the prolonged pres-
pressure reduction, and upon release of the aortic clamp RBF returned to 88 ± 4% of the initial value (P < 0.01, Fig. 2). These values were not significantly different from those obtained in the control rats. The RPP did not change significantly after release of the aortic clamp [165 ± 3 vs. 163 ± 3 mmHg; not significant (NS)].

When compared with the untreated SHR, clamping the plasma ANG II levels by itself did not affect the autoregulatory behavior to any significant extent (Fig. 4 and Tables 3 and 4). The response to the prolonged pressure reduction also remained nearly unchanged compared with the unclamped condition. Thus there was no significant change in ARI after the prolonged pressure reduction (Table 3), and, like in the control situation before the clamp, the pressure reduction did not elicit a resetting of the lower limit of autoregulation (Table 4).

When RPP was reduced to 100 mmHg after the prolonged pressure reduction, RBF was 64 ± 6% of the initial value, which was significantly higher than the 49 ± 6% found in the control SHR rats (P < 0.05, Table 5).

**Inhibition of COX.** Inhibition of COX with the non-specific COX inhibitor indomethacin did not change either RPP or RBF significantly (Table 1). Indomethacin slightly attenuated the decrease in RBF during the reduction in RPP compared with the control condition (80 ± 3 vs. 62 ± 6%, P < 0.05, Table 5). However, when the clamp was released, RBF increased to nearly the same value as in the controls (84 ± 3 vs. 85 ± 3%, NS; Fig. 2).

Figure 5 shows the RBF autoregulation before and after reduction of the RPP to 100 mmHg for 15 min in the indomethacin-treated SHR. The autoregulatory efficiency, as expressed by the ARI, was unchanged after the pressure reduction period (Table 3). By itself, indomethacin had no effect on the lower limit of autoregulation or the ARI (Tables 3 and 4). However, in contrast to the control rats, the indomethacin-treated SHR showed a significant reduction of the lower limit of autoregulation after the 15-min reduction of RPP (Table 4).

Indomethacin attenuated the decrease in RBF when RPP was reduced below the lower limit of autoregulation, i.e., to a RPP of 100 mmHg. Before the prolonged pressure reduction period, RBF was reduced to 80 ± 3% at a RPP of 100 mmHg, and after the RPP reduction period it was 72 ± 5% of the initial value. Both values were significantly larger than the corresponding values found in the control experiments (Table 5).

In the indomethacin-treated rats, plasma renin increased from 68 ± 14 × 10⁻⁵ GU/ml to 221 ± 58 × 10⁻⁵ GU/ml after the pressure reduction period (Table 2).

Inhibition of the inducible COX-2 enzyme with NS-398 had effects very similar to those seen with indomethacin. It had no significant effects on either RPP or RBF (Table 1), and after the prolonged reduction in RPP the RBF returned to a value of 84 ± 3% (Fig. 2), a value that was identical to that found in the indomethacin-treated rats and not significantly different from the value found in the control rats.

Like in the indomethacin-treated SHR, the ARI was unchanged after the pressure reduction period (Fig. 6 and Table 3), and, like in the indomethacin-treated SHR, the NS-398-treated rats showed a significant resetting of the lower limit of autoregulation after the prolonged pressure reduction period (Table 4). However, NS-398 appeared to be slightly less efficient when it came to preventing the decrease in RBF when RPP was decreased below the lower limit of autoregulation (100 mmHg; Table 5). This was the case both before and after the prolonged pressure reduction period. The values found in the NS-398-treated SHR fell between the values found in the control and the indomethacin-treated rats and did not differ significantly from either set (Table 5).

**NO clamp.** When the SHR rats were given l-NAME followed by SNP, RPP was brought back to the initial value, whereas RBF tended to remain decreased (P = 0.06; Table 1). Administration of SNP together with l-NAME prevented the transient vasoconstriction, and

### Table 3. ARI calculated in the pressure range from 140 to 160 mmHg

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ANG II Clamp</th>
<th>Indomethacin</th>
<th>NS-398</th>
<th>l-NAME-SNP</th>
<th>l-NAME/NO clamp</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before</strong></td>
<td>0.41 ± 0.14</td>
<td>0.42 ± 0.11</td>
<td>0.28 ± 0.11</td>
<td>0.22 ± 0.11</td>
<td>0.09 ± 0.07</td>
<td>0.23 ± 0.08</td>
</tr>
<tr>
<td><strong>After</strong></td>
<td>0.73 ± 0.15</td>
<td>0.50 ± 0.12</td>
<td>0.13 ± 0.09</td>
<td>0.15 ± 0.08</td>
<td>0.14 ± 0.08</td>
<td>0.21 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE. ARI, autoregulatory index.
consequently it was possible to avoid the decrease in RBF (Table 1).

Except for the above-mentioned effect on the RBF baseline value, the effects of the two protocols on the RBF responses to the pressure reductions were similar. Clamping NO significantly reduced the decrease in RBF during the pressure reduction period compared with the control rats (88 ± 3 and 87 ± 3% vs. 62 ± 6%; Table 5). Furthermore, when the clamp was released, RBF returned to a value of 93 ± 2% (NS) and 95 ± 3% (P < 0.05) of the initial value (see Fig. 2). Thus there was a nearly complete restoration of RBF after the termination of the prolonged pressure reduction period in the L-NAME- and SNP-treated rats.

Figures 7 and 8 show the corresponding autoregulatory responses. In both groups, the autoregulatory efficiency, as expressed by the ARI, was unchanged after the pressure reduction period (Table 3). Clamping the NO system by itself resulted in a significant resetting of the lower limit after the prolonged changes in RPP (Table 4). When RPP was reduced to 100 mmHg, the RBF remained at a level significantly greater than seen in the untreated control rats. Thus, at a RPP of 100 mmHg, RBF was reduced to 83 ± 3 and 87 ± 3%, respectively, during the first period, and to 83 ± 5 and 81 ± 4%, respectively, in the second autoregulatory period. All values are significantly above the values obtained in the corresponding periods in the control rats (Table 5).

### DISCUSSION

The purpose of the present experiments was to examine the response of RBF to a prolonged reduction in RPP in adult SHR at a time when hypertension is manifest. After a period of prolonged reduction in the RPP to 100 mmHg, SHR downregulates RBF to a lower level, but this downregulation does not seem to be dependent on an intact and adjustable renin-angiotensin system (26). However, when NO levels were clamped by the simultaneous infusion of L-NAME and SNP, the ability to downregulate RBF to a lower level after prolonged reduction in perfusion pressure was attenuated significantly. This suggests that the NO system plays an important role in RBF regulation in SHR in response to prolonged changes in RPP.

In the SHR the ANG II clamp did not change the RBF response to a prolonged change in RPP. This is in

### Table 4. Lower limit of autoregulation as determined by either the inflection point or the ANOVA-based method (see text for details) during the first and second set of pressure reductions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>First Pressure Reduction</th>
<th>Second Pressure Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ANOVA based</td>
<td>Infection point</td>
</tr>
<tr>
<td>Control</td>
<td>130</td>
<td>140 ± 2</td>
</tr>
<tr>
<td>Ang II clamp</td>
<td>130</td>
<td>142 ± 4</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>130</td>
<td>141 ± 2</td>
</tr>
<tr>
<td>NS-398</td>
<td>130</td>
<td>137 ± 5</td>
</tr>
<tr>
<td>L-NAME-SNP</td>
<td>110</td>
<td>126 ± 6†</td>
</tr>
<tr>
<td>L-NAME/SNP</td>
<td>130</td>
<td>122 ± 6‡</td>
</tr>
</tbody>
</table>

Values for inflection point are means ± SE. Units are mmHg. Lower limits found by ANOVA. *P < 0.05 vs. before value. †P < 0.05 and ‡P < 0.01 vs. corresponding control value.

### Table 5. End points of RBF after reduction of the RPP to 100 mmHg during the first and second set of pressure reductions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RBF End Point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First set</td>
</tr>
<tr>
<td>Control</td>
<td>62 ± 6</td>
</tr>
<tr>
<td>Ang II clamp</td>
<td>72 ± 6</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>80 ± 3*‡</td>
</tr>
<tr>
<td>NS-398</td>
<td>72 ± 9</td>
</tr>
<tr>
<td>L-NAME-SNP</td>
<td>88 ± 3*†</td>
</tr>
<tr>
<td>L-NAME/SNP</td>
<td>87 ± 3*†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Units are %. RPP, renal perfusion pressure. Pooling the result (RBF after 15 min of RPP reduction) of the L-NAME groups showed that only in this group did RBF increase to a significantly higher level than the rest of the groups, whereas the cyclooxygenase inhibition groups were not significantly different from the control and the Ang II-clamped groups. *P < 0.05, †P < 0.01 vs. value in control rats.
striking contrast to the effects observed in normotensive Sprague-Dawley rats (3, 14, 26). As we and others have shown previously, lowering the RPP for a 15-min period induces a downregulation of RBF, and this effect is completely blocked by clamping the renin-angiotensin system (3, 14, 26). Another striking difference between hyper- and normotensive rats was the lack of a gradual decrease in RBF during the 15 min of reduced perfusion pressure in SHR. In the normotensive rats, this gradual decrease in RBF occurred in parallel with the activation of RAS, and it was blocked by the ANG II clamp (14, 26). As is evident from Table 2, lowering the RPP in SHR led, as expected, to a significant increase in PRC, but the only discernible effect of clamping plasma ANG II was a blunting of the decrease in RBF when RPP was reduced below the autoregulatory range. In the ANG II-clamped SHR, RBF only decreased to 72 ± 9% of the initial value (Table 5) when RPP was reduced to 100 mmHg (Fig. 4) during the first set of pressure reductions, and to 64 ± 6% during the second set of pressure reductions. In comparison, in the control SHR, the RBF decreased to 62 ± 5% (NS) and 49 ± 6% (P < 0.05), respectively, of the initial value (Fig. 3 and Table 5). This effect is most likely because of the lack of an increase in plasma ANG II and hence renal vasoconstriction during the pressure reduction in the clamped rats.

Recent results from Navar and coworkers (23) suggest that systemic administration of an angiotensin-converting enzyme inhibitor may fail to inhibit the intrarenal formation of ANG II. It could therefore be argued that the failure of the ANG II clamp to affect the downregulation of RBF was because of a failure of captopril to block the relevant ANG II production. Although this possibility cannot be excluded, we find it less likely for several reasons. First, acute administration of captopril results in a decrease in renal hemodynamic resistance, showing that at least part of the hemodynamically relevant ANG II production is blocked. Second, in normotensive rats a similar protocol results in a complete blockade of both the resetting of autoregulation and the downregulation of RBF, indicating that at least part of the relevant ANG II production can be blocked by systemic captopril administration.

The only one of the present experimental maneuvers that had a marked effect on the downregulation of RBF after a prolonged lowering of the RPP was the NO clamp. This suggests that in SHR the NO system plays an important role in RBF regulation in response to changes in arterial pressure. Clamping of the NO system nearly abolished the downregulation of RBF, implying that a decreased NO synthesis could be a central regulator of renal blood flow.
mechanism underlying the vasoconstriction after the prolonged lowering of RPP seen in control SHR. The present data do not allow a conclusion as to the site of action. The kidney contains NOS-1 (nNOS), which is present in the macula densa; NOS-2, which is found in both vascular and tubular segments (21); and NOS-3 (eNOS), which is found predominantly in the vascular endothelium (29). In normotensive rats, acute inhibition of NOS-1 in the macula densa using the specific inhibitor 7-nitroindazole increases both the maximum response and the sensitivity of the tubuloglomerular feedback (TGF) mechanism (25, 32). In contrast, in SHR a similar blockade was without effect on TGF (32). Likewise, acute inhibition of NOS-2 using amino-guanidine had no effect on either RBF (20) or TGF (28). Therefore, it appears more likely that the present effect is elicited by changes in the activity of eNOS (NOS-3). The observation that general NOS inhibition with Nω-nitro-L-arginine (l-NNA) had no effect on the TGF response in SHR (27) suggests that the effect is on the vessels rather than on the TGF mechanism. Because RPP is reduced while RBF is relatively well maintained, shear stress will decrease, and this will be expected to decrease endothelial NO production. If normalization of the NO production is delayed after restoration of the RPP, there will be a period where NO production is lower compared with the control period, thus causing vasoconstriction. Although this could explain an NO-mediated downregulation of RBF in control rats, this issue clearly warrants further experiments for a full clarification of the underlying mechanism.

In normotensive rats, a prolonged decrease in RPP not only causes a downregulation of RBF but also a resetting (decrease) of the lower limit of autoregulation (3, 22, 26). This appears not to be the case in SHR. As the results of the present study show, a prolonged reduction of RPP does induce a downregulation of RBF but not a downward resetting of the lower limit of autoregulation. This is in agreement with a previous study by Iversen and coworkers (17). In 12-wk-old SHR, they found that a reduction in RPP to 90 mmHg for 10 min induced a downregulation of RBF but no resetting of the lower limit. Interestingly, they also found that a 10-min decrease in RPP to 122 mmHg, which is above the lower limit of autoregulation, induced a downward resetting of the lower limit of autoregulation from 120 ± 4 to 106 ± 2 mmHg but not a regulation of RFB to a lower level (17). The reduction to 90 mmHg and the present reduction to 100 mmHg are both below the lower limit of autoregulation in SHR, and both seem to induce a downregulation of RBF but no resetting of the lower limit of autoregulation as opposed to a reduction to a level above the lower limit.

In agreement with previous studies (18, 23), neither indomethacin nor NS-398 per se influenced the lower limit of autoregulation during control conditions. However, after the administration of either indomethacin or NS-398, the prolonged reduction in RPP now caused a downward resetting of the lower limit (Table 4). This effect appears to be mediated by the COX-2 enzyme, since there was no difference in the effect between the nonselective COX inhibitor indomethacin and the COX-2 inhibitor NS-398. COX-2 is constitutively expressed in the macula densa (13), where it has been suggested to play a role in the modulation of both the TGF response and renin release (16). It is still unclear which prostaglandins are produced in the macula densa. Recent studies by Ichihara and coworkers (15) in the isolated, blood-perfused juxtaglomerular nephron preparation from normotensive rats have suggested that COX-2 may lead to the production of vaso-dilatory prostaglandins during increases in RPP (15). However, based on the present results, we hypothesize that the lack of resetting of the lower limit in control SHR could be the result of an increased production of vasoconstrictory prostaglandins during lowering of the RPP. Alternatively, it could be the result of an increased sensitivity toward vasoconstrictory prostaglandins, since there is evidence to suggest an increased number of thromboxane A2 receptors in the renal vascular bed in SHR (2). This hypothesis is supported by the observation that, when RPP was lowered below the lower limit of autoregulation, the decrease in RBF was blunted in the treated compared with the untreated rats (72 ± 9 and 80 ± 3% vs. 62 ± 6% of control at 100 mmHg). This suggests that both compounds inhibited the formation of a vasoconstrictory prostaglandin (e.g., thromboxane A2), and this inhibition could unmask a downward resetting of the lower limit of autoregulation after prolonged reductions of RPP.

COX-2-derived metabolites stimulate renin release during reductions in RPP (11, 12). However, it does not appear likely that this mechanism played a major role under the present circumstances. The increase in the PRC after RPP lowering was unchanged after indomethacin treatment, and clamping of the renin-angio-

![Fig. 8. Effect on RBF autoregulation when plasma NO is clamped. The response of RBF to stepwise reduction of RPP before (solid line, •) and after (dashed line, ●) RPP had been reduced to 100 mmHg for 15 min (n = 9). Responses are shown as normalized to the RBF value at 160 mmHg before RPP was reduced. Significant changes in RBF are found by ANOVA for repeated measurements. Values are means ± SE, *P < 0.05 vs. before value at 160 mmHg, †P < 0.05 vs. before value at 160 mmHg, ‡P < 0.05 vs. after value at 160 mmHg. #P < 0.01 vs. after value at 160 mmHg. $P < 0.05 vs. corresponding before value. ∆P < 0.01 vs. corresponding before value.](http://ajpregu.physiology.org/10.1152/ajpregu.00333.2003)
tensin system did not by itself cause a resetting of the lower limit of autoregulation. It therefore seems unlikely that the effect of the COX inhibitors was secondary to an effect on the renin-angiotensin system.

Recently, Iversen et al. (17) showed that neither sympathetic nerves, the renin-angiotensin system, nor a macula densa-mediated mechanism could account for the downregulation of RBF that follows a 10-min reduction of RPP in SHR. They suggested that the downregulation was the result of mechanical adjustments of the vessel wall. The present study confirms that the renin-angiotensin system is not involved, but suggests that, rather than mechanical factors, the downregulation of RBF may be the result of a decreased activity of the NO system.

Clamping the NO system by itself affected RBF autoregulation. Not at any measured RPP did RBF decrease below 80% of the initial value (Figs. 7 and 8). Also, clamping the NO system caused a resetting of the lower limit of autoregulation to a lower level, but there was no significant effect on the ARI (Table 3). In 2K1C hypertensive rats, Turkstra et al. (31) also found that the lower limit of autoregulation was reset to a lower value of 72 ± 5 mmHg compared with 85 ± 3 mmHg before treatment with L-NNA. They suggested that the effect was because of lack of a shear stress-mediated reduction in NO release during the lowering of the RPP.

The downward resetting of the lower limit of autoregulation after the prolonged reduction in RPP in the COX-inhibited SHR together with the above effects of NO blockade suggest that the lower limit of autoregulation does not represent a state of maximal dilation of the renal preglomerular vessels but rather that it is because of a dynamic balance between vasoconstrictory and vasodilatory stimuli and that a shift in this balance resets the lower limit of autoregulation.

Adjustment of RBF in response to prolonged changes in arterial pressure is central to the optimal control of GFR, peritubular and medullary capillary flow, and hence, the urinary excretion of sodium and water. The results of the present study suggest that the mechanisms that mediate this response are qualitatively different between normotensive rats and SHR.

Whereas the renin-angiotensin system seems to play a central role in the normotensive rats, the NO system appears to be the important mediator in the SHR. The lack of a downward resetting of the lower limit of autoregulation after prolonged reduction in RPP and the lack of reduction in RBF when RPP is maintained at a reduced value suggest that the response in SHR to prolonged changes in RPP is suboptimal. The present study was performed in SHR with established hypertension, and consequently it is impossible to decide whether the difference is primary or secondary to the high blood pressure. Clearly, more research is needed to decide this important question.

In conclusion, in SHR, the renin-angiotensin system does not appear to play a major role in the downregulation of RBF after a prolonged (15-min) reduction of RPP. Instead, this response appears to be mediated, at least in part, by the NO system. Furthermore, we hypothesize that the lack of resetting of the lower limit of autoregulation in response to a pronounced lowering of RPP reported by us and others could be because of an increased, COX-2-mediated production of vasoconstrictory prostaglandins.

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