Effect of Reduction of Nitric Oxide on Plasma and Kidney Tissue Angiotensin II Levels

Gabriela E. Garcia, Marvin R. Brown, Lucinda M. Wead, Sandra Braun, and Francis B. Gabbai

Nitric oxide synthase (NOS) blockade increases blood pressure (BP) and modifies glomerular and tubular function. Angiotensin II (AII) blockade restores glomerular and tubular function but does not lower BP. We measured plasma renin activity (PRA), plasma (AIIp), and kidney tissue (AIIk) AII with radioimmunoassay to investigate the dissociation between renal and systemic effects of NOS blockade. Two period clearance studies followed by plasma and renal tissue harvesting were performed in seven groups of rats. Groups 1 and 1A served as controls. Groups 2 and 2A received NaCl-NaHCO3 during the first period and N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA, 0.5 mg/kg/min) during the second period. Group 3 was similar to group 2 but renal perfusion pressure (RPP) was maintained constant by using an aortic snare. Groups 4 and 4A received N\textsuperscript{G}-nitro-L-arginine-methyl ester (L-NAME, 5 mg/100 mL of drinking water) for 2 weeks. NOS blockers decreased AIIp (group 1, 74 ± 7 pg/mL; group 2, 22 ± 1 pg/mL; group 3, 26 ± 1 pg/mL; group 4, 19 ± 3 pg/mL). The decrease in AIIp was a direct effect of L-NMMA independent of changes in perfusion pressure, as AIIp was similar in group 3 (normal RPP) and groups 2 and 4 (increased RPP). Measurements of PRA and AIIp demonstrated a similar reduction in PRA and AIIp in rats treated with NOS blocker. Although NOS blockers decreased AIIp, acute or chronic administration of NOS blockers did not modify AIIk (group 1, 1,192 ± 51; group 2, 1,354 ± 85; group 3, 1,348 ± 180; group 4, 1,276 ± 172 pg/kidney). Our findings demonstrate that NO blockers produce a dissociation between plasma and kidney AII levels. This dissociation can explain the beneficial effects of AII blockers on renal function and their lack of antihypertensive effects in anesthetized rats treated with NOS blockers. Am J Hypertens 1997;10:1103–1108 © 1997 American Journal of Hypertension, Ltd.

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in rats treated with NOS blockers, further supporting the presence of increased AII activity under these experimental conditions. However, the increase in AII activity in kidneys treated with NOS blockers is not clearly supported by studies analyzing the effect of NO on renin secretion. Indeed, evaluation of the role of NO on renin release has produced rather contradictory data, with various investigators demonstrating both increased and decreased renin release during NOS blockade. Moreover Tresham et al found that NO does not modify the renin release. Although the physiologic effects of NOS blockade on renal function clearly point to an increase in AII activity, the character of the interaction between NO and the renin angiotensin system is more difficult to interpret. We decided to investigate the interaction between NO and AII by evaluating the effects of acute and chronic NOS blockade on plasma renin activity and kidney tissue AII levels. Our results demonstrate that NOS blockade is very powerful in suppressing plasma renin activity and plasma AII acutely and chronically independent of changes in renal perfusion pressure (RPP). However, neither acute nor chronic NOS blockade modifies the intrarenal levels of AII, suggesting an important dissociation between intrarenal and circulating AII levels in the presence of NOS blockers.

METHODS

Experiments were performed in 37 male Munich-Wistar rats (200 to 270 g) obtained from Simonsen Laboratories (Gilroy, CA). Rats were anesthetized with Inactin (BYK, Konstanz, Germany) (10 mg/100 g body weight, intraperitoneally) and placed on a temperature-regulated table. Following placement of tracheostomy, the right jugular vein, left femoral artery, and the bladder were catheterized with PE-50 tubing. The left ureter was cannulated with PE-10 tubing. The femoral artery catheter was used for periodic blood sampling and monitoring the RPP with a transducer (model P23db; Statham Instruments, Gould Division Inc., Hato Rey, Puerto Rico) and recorded on a Statham (Statham Instruments) chart recorder. All rats received two infusions of NaCl-NaHCO$_3$ solutions throughout the experiment, one containing $[^3]$H inulin at rate of 0.8 mL/h and the other at a rate of 1.4 mL/h, which served as a vehicle for the NOS blocker infused during the second period. After 60 min of equilibration, urine was collected in preweighed containers under oil for two 20-min periods, and blood samples were taken from the femoral artery at the midpoint of each urine collection for $[^3]$H inulin concentration to compute left kidney glomerular filtration rate (GFR). After completing measurements for the first period, the NOS blocker or vehicle were started. After 30 min, urine from two 20-min clearance periods were again collected (experimental period). Seven groups of rats were studied. Groups 1 and 1A (n = 10) constituted the control group, in which NaCl-NaHCO$_3$ was infused during both periods. Group 2 and 2A (n = 10) received N$^6$-monomethyl-l-arginine (L-NMMA, Calbiochem, Corp., La Jolla, CA) 0.5 mg/kg/min during the second period. Group 3 (n = 6) was similar to group 2, but RPP was maintained constant (by clamping the suprarenal aorta) during L-NMMA infusion to investigate if the effect of L-NMMA on AII depended on increased RPP. Groups 4 and 4A (n = 11) received N$^6$-nitro-l-arginine-methyl ester (L-NAME, Bachem, Torrance, CA) 5 mg/100 mL of drinking water continuously for a period of 2 weeks prior to the acute study. In this group, studies were performed for only one period.

Plasma Renin Activity and Angiotensin II Measurement

At the end of the experimental period, plasma and kidney tissue were obtained in groups 1 to 4 for AII measurement by radioimmunoassay (RIA) as previously described. One milliliter of blood was collected in a syringe containing 20 μL of EDTA (0.16 mol/L) and 10 μL of converting enzyme inhibitor (0.1 mmol/L). Samples were spun at 4°C and plasma stored at −70°C until processed. Plasma was extracted using a Bondelut C$_{18}$ column (Analytichem International, Harbor City, CA) previously washed with methanol and triethylamine-formic acid. The column was rinsed with triethylamine formic acid buffer and AII was eluted off with acetonitrile triethylamine formic acid 70:30, lyophilized on a Speed Vac overnight (model RH200-12, Savant Instruments Inc., Farmingdale, NY), and kept at −20°C until assayed. This extraction procedure yielded 92.5% ± 1% recovery of AII.

Renal Tissue Processing

Kidneys were perfused free of blood with 50 mL of a solution containing 4.9 mM 8-hydroxyquinoline hemisulfate, 2.6 mM EDTA, and 3% BSA administered through an aortic catheter. Bloodless kidneys were excised, placed in a plastic container, flash-frozen in liquid nitrogen, and stored at −70°C until further processing. Individual whole kidneys were homogenized (Polytron, Brinkmann Instruments Inc., Westbury, NY; 10 min at setting 6) in 2 mL of RIA-BSA 0.25% buffer, added to 9 mL of homogenizing medium (1 N glacial acetic acid, 0.02 N hydrochloric acid, and 0.1% 2 mercaptoethanol), and heated to 90°C for 10 min, then centrifuged at 30,000 g for 20 min. The first supernatant (S$_1$) was removed and pellet resuspended in 4.5 mL of homogenizing medium and centrifuged at 30,000 g for 20 min. The second supernatant was combined with S$_1$ and lyophilized overnight using a Speed Vac centrifuge. The resulting lyophilizate was resuspended in 2 mL of RIA-BSA 0.25% buffer and 500 μL of this extracted...
using a Bondelut C18 column as described above for plasma. This combined procedure yielded 57% ± 2% recovery of AII.

Plasma renin activity and plasma AII were measured in groups 1A, 2A, and 4A to evaluate the effects of NOS inhibition on both hormones. For plasma renin activity measurement, 1.0 mL of blood was collected in a tube containing 20 μL of EDTA alone. Samples were spun at 4°C and stored at −70°C until processed. Plasma renin activity was measured using a commercial kit (New England Nuclear, Boston, MA).

Radioimmunoassay for AII Resuspended lyophilized material, 200 μL, was added to 100 μL of specific AII-antibody (Ab# 127 kindly provided by Dr. Wylie Vale, Salk Institute, La Jolla, CA) diluted 1/62,500. After a 2-day incubation at 4°C, 100 μL of 125I-AII (6,000 counts/min) (New England Nuclear) were added to each tube and incubated again at 4°C overnight. Normal rabbit serum, 100 μL (diluted 1/200) and goat anti-rabbit IgG, 100 μL (diluted 1/40) were added to each tube, followed by 500 μL of 10% polyethylene glycol in RIA buffer. Tubes were allowed to sit in the cold for 1 h and then spun for 30 min. The supernatant was then decanted and the pellet was counted in a γ-counter (Searle [Chicago, IL] model 1185, 80% efficiency). All concentrations were calculated using a computer aided logit/log transformation of the standard curve. Cross-reactivity of this antibody for AII with AI is 0.33% and with AIII is 68%. Minimum detection level of the RIA is 1 pg/tube. All samples were run in the same assay.

Other Analytic Methods [3H]Inulin activity in plasma and urine was monitored on a model B4530 Tri/Carb Packard liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, IL). GFR was determined as previously described.18

Statistics Paired Student’s t test was used for comparison of the first and experimental periods. Tukey multiple comparison analysis was used for comparison among groups.19 The level of statistical significance was defined at a value of P < .05. All results are expressed as mean ± SEM.

RESULTS
Renal perfusion pressure, GFR, and urine flow rate during control and experimental periods are presented in Table 1. No significant differences were found among the three groups during the first period except for lower urine flow rate in group 3. L-NMMA (group 2) increased renal perfusion pressure (102 ± 3 to 120 ± 4 mm Hg P < .05), urine rate flow (2.4 ± 0.2 to 3.9 ± 0.6 μL/min, P < .05) but did not modify GFR as previously demonstrated.4,8 Placing an aortic clamp during L-NMMA administration in group 3 maintained renal perfusion pressure constant during both periods. In the absence of changes in renal perfusion pressure, a significant decrease in GFR was observed when compared with control values (1.2 ± 0.1 to 0.9 ± 0.1 μL/min, P < .05) but not when compared with values for group 1 or 2. In spite of a decrease in GFR, urine flow rate increased revealing an important tubular effect of L-NMMA independent of RPP. Chronic administration of L-NAME (group 4) produced a significant increase in renal perfusion pressure when compared with that of control rats and rats acutely treated with L-NMMA. The increase in renal perfusion pressure was associated with a significant decrease in GFR (0.8 ± 0.1 v. 1.3 ± 0.1 mL/min in the

<table>
<thead>
<tr>
<th>Group</th>
<th>RPP (mm HG)</th>
<th>GFR (mL/min)</th>
<th>UV (μL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>110 ± 4</td>
<td>1.3 ± 0.1</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>102 ± 3</td>
<td>1.3 ± 0.2</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>L-NMMA + clamp</td>
<td>107 ± 1</td>
<td>1.2 ± 0.1</td>
<td>1.8 ± 0.2†</td>
</tr>
<tr>
<td>L-NAME</td>
<td>142 ± 4</td>
<td>0.8 ± 0.1†</td>
<td>2.5 ± 0.3</td>
</tr>
</tbody>
</table>

* P < .05 control v experimental (paired t test); † P < .05 v group 1 (Tukey test); ‡ P < .05 other groups (Tukey test). L-NMMA: N^G-monomethyl-L-arginine, L-NAME: N^G-nitro-L-arginine-methyl ester.

TABLE 2. PLASMA (AIIp) AND KIDNEY TISSUE (AI|p) ANGIOTENSIN II LEVELS IN THE FOUR DIFFERENT GROUPS

<table>
<thead>
<tr>
<th>Group</th>
<th>AIIp (pg/mL)</th>
<th>AIIk (pg/kidney)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>74 ± 7</td>
<td>1192 ± 51</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>22 ± 1*</td>
<td>1354 ± 85</td>
</tr>
<tr>
<td>L-NMMA + clamp</td>
<td>26 ± 1*</td>
<td>1348 ± 180</td>
</tr>
<tr>
<td>L-NAME</td>
<td>19 ± 3*</td>
<td>1276 ± 172</td>
</tr>
</tbody>
</table>

* P < .05 v group 1 (Tukey test).
control group, \( P < .05 \). No significant changes were observed for urine flow rate.

Table 2 depicts the values of plasma and kidney AII. Acute and chronic NOS blockade (groups 2 and 4) produced a significant decrease in plasma AII from \( 74 \pm 7 \) pg/mL in the control group to \( 22 \pm 1 \) pg/mL in group 2, and to \( 19 \pm 3 \) pg/mL in group 4. Interestingly, controlling renal perfusion pressure did not modify the effect of NOS blockade on plasma AII, which was \( 26 \pm 1 \) pg/mL in group 3, a value not different from groups 2 and 4. In spite of significant reduction in plasma AII during NOS blockade, no changes were found in kidney AII during acute or chronic administration of NOS blockers. To further define the mechanism for the reduction in plasma AII during NOS inhibition, we measured plasma renin activity and plasma AII in a subset of rats from groups 1, 2, and 4 (groups 1A, 2A, and 4A). As shown in Figure 1, NOS inhibition reduced plasma AII to \( 53\% \pm 7\% \) of the control value and plasma renin activity to \( 42\% \pm 14\% \) of the control value in group 2A. Chronic administration of L-NNAME in group 4A reduced plasma AII levels to \( 41\% \pm 4\% \) of control, and plasma renin activity to \( 40\% \pm 11\% \) of control. These results demonstrate that the decrease in plasma AII during NOS blockade is a reflection of the changes in plasma renin activity induced by NO suppression.

**DISCUSSION**

It has been established that blockade of NOS produces two effects: a significant and consistent elevation in blood pressure, and significant alterations in renal function.\textsuperscript{4–8,20,21} Blockers of NOS produce hypertension, which could depend upon a reduction in the direct effects of NO generated in endothelial cells acting upon vascular smooth muscle cells, but could also be partially mediated by NO effects on the renin angiotensin system. The second alternative mechanism is of particular interest because it has been observed that NO can modify renin secretion. Different investigators have demonstrated increased renin release, both in vivo and in vitro, with administration of NOS blockers.\textsuperscript{9–11,22} However, other investigators have concluded that blockade of NOS exhibits the opposite effect: a decrease in renin release.\textsuperscript{12–15} The decrease in renin release is independent of a rise in RPP.\textsuperscript{23,24} The results of the present study demonstrate that NOS blockers decrease plasma renin activity and plasma AII after acute or chronic administration. When we controlled RPP using an aortic clamp, our results were in agreement with those of Johnson et al.\textsuperscript{24} demonstrating that the decrease in renin and AII observed is independent of the elevation of RPP and could be the direct effect of NOS blockade on the juxtaglomerular apparatus. NOS is highly expressed in kidney macula densa cells.\textsuperscript{25} This finding provides a possible role of NO during signal transfer in the juxtaglomerular apparatus. Because blockade of NOS decreases plasma AII, these results support the absence of an antihypertensive effect of both converting enzyme inhibitors and AII receptor blockers in rats treated with NOS blockers.

Some studies have found that angiotensin blockade decreases or reverses hypertension during long-term NOS inhibition (\( \geq 3 \) weeks).\textsuperscript{12,32,33} Under such conditions, hypertension was associated with mild degree of renal failure and marked renal histological changes such as focal glomerular collapse, arteriolar luminal obliteration, and partial fibrinoid necrosis. These data suggest that other mechanism(s) may be involved in the induction of hypertension during the chronic inhibition of NOS. When sustained vasoconstriction compromises adequate kidney perfusion, increased plasma renin activity can be expected, as demonstrated by Ribeiro et al in a model of chronic inhibition of NOS.\textsuperscript{12}

In spite of the fact that blockade of NOS suppresses plasma AII, we have found that the same blockade induces changes in glomerular and tubular function that are similar to those with AII administration.\textsuperscript{8} Previous studies from our group have shown that blockade of NOS decreases nephron plasma flow and the glomerular ultrafiltration coefficient, whereas renal vascular resistance and the transcapillary hydrostatic pressure gradient are increased.\textsuperscript{7,8} These effects are strikingly similar to those results previously described by Blantz et al several years ago with AII infusion.\textsuperscript{29} These effects after NOS blockade on glomerular function are prevented by administration of AII receptor blockers.\textsuperscript{8} Studies from our
laboratory have also demonstrated an effect on the proximal tubule during blockade of NOS, an effect that is prevented by the administration of AII antagonists. In spite of the physiologic evidence provided suggesting increased AII activity, measurements of intrarenal AII demonstrated no absolute difference between control and L-NMMA treated animals. Our results demonstrate that NO modulates the intrarenal effects of AII. The specific mechanism mediating this regulation is not actually known, but these results may explain the apparent capacities of converting enzyme inhibitors and AII receptor blockers to prevent the glomerular and tubular alterations that result from blockade of NOS. Our results then suggest that these glomerular and tubular effects may not be the consequence of absolute increases in AII activity within the kidney but rather the consequence of a reduction in NO content, a natural antagonist of the glomerular and tubular actions of AII.

The dissociation between plasma and kidney AII was rather unexpected. Previous studies by Fox and coworkers and observations from our laboratory (unpublished observations) have shown that kidney AII levels correlate in general with changes in plasma AII concentration. For example, low salt diet increases both plasma and kidney AII, whereas high salt diets decrease both plasma AII and kidney AII. Furthermore, administration of converting enzyme inhibitors tend to decrease both plasma AII and kidney AII content. Previous evidence of a dissociation between renin and kidney AII have only been provided in the two kidney, one clip Goldblatt model, where kidney AII remains high in the presence of suppressed renin values in the unclipped kidney.

In conclusion, our results demonstrate that blockade of NOS leads to a significant and major reduction in plasma renin activity and in plasma AII, an event that is independent of changes in renal perfusion pressure. These results also suggest that, in the anesthetized rat, the renal effects of blockade of NOS are not due to an absolute elevation in kidney AII, but rather to the absence of the effect of the physiologic antagonist of AII, NO, allowing an increased expression of AII activity in the kidney.

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