Endothelin A Receptor Blockade Alleviates Hypertension and Renal Lesions Associated with Chronic Nitric Oxide Synthase Inhibition

A. MARJAN G. VERHAGEN,* TON J. RABELINK,† BRANKO BRAAM,* TERRY J. OPGENORTH,† HERMANN-JOSEF GRÖNE,‡ HEIN A. KOOMANS,* and JAAP A. JOLES*†

*Department of Nephrology, University Hospital Utrecht, Utrecht, The Netherlands; †Abbott Laboratories, Abbott Park, Illinois; and ‡Department of Pathology, Philipps University, Marburg, Germany.

Abstract. Unopposed actions of vasoconstrictors, such as angiotensin, play an important role in the effects of chronic nitric oxide synthase (NOS) inhibition. In this study, it is hypothesized that endothelin (ET), another important vasoconstrictor, may also play a role in the development of hypertension and renal lesions during chronic NOS inhibition. The ETA receptor was blocked with A-127722 during chronic NOS inhibition with Nω-nitro-L-arginine (L-NNA), a potent NOS inhibitor without antimuscarinic action. Male Sprague Dawley rats were treated for 3 wk with L-NNA (40 mg/kg per d), L-NNA (40 mg/kg per d) + A-127722 (30 mg/kg per d), or remained untreated (control). In preliminary experiments, L-NNA (40 mg/kg per d) had been found to cause the maximum increase of systolic BP and a 35% decrease in renal NOS activity. Three weeks of L-NNA treatment resulted in a marked rise in systolic BP (240 ± 4 versus control 151 ± 7 mmHg; P < 0.01), proteinuria (209 ± 46 versus control 27 ± 3 mg/d; P < 0.01), and a fall in GFR (1.41 ± 0.16 versus control 2.23 ± 0.19 ml/min; P < 0.05). Renal morphology showed severe vascular injury, characterized by focal adhesion and infiltration of mononuclear cells into the intima and media of preglomerular arteries and arterioles. This was sometimes associated with necrosis of the media and partial or total obstruction of the lumen with thrombotic material. Ischemic glomeruli were also present. Tubulointerstitial damage was moderate and accompanied by an influx of monocytes and macrophages. A-127722 administered simultaneously with L-NNA completely prevented the increase in proteinuria (39 ± 8 mg/d) and glomerular ischemia. Vascular injury, tubulointerstitial damage, and the increase in systolic BP (191 ± 6 mmHg) were partially prevented. The protective effects of ETA receptor blockade suggest that ET has hemodynamic as well as nonhemodynamic effects in the cascade of events following chronic NOS inhibition. (J Am Soc Nephrol 9: 755-762, 1998)

Blockade of the endothelin (ET) system has proven beneficial in several models of chronic renal failure. Selective ETA and mixed ETA/ETB receptor blockade had antihypertensive and renoprotective effects in rats with 5/6 ablation (1,2) and in murine lupus nephritis (3). Mixed ETA/ETB receptor blockade was also effective in ameliorating proteinuria and glomerular lesions in a normotensive model of immune complex nephritis (4).

Hypertension and renal injury can also originate from chronic nitric oxide synthase (NOS) inhibition (5). Many patients with chronic renal failure may have an impaired nitric oxide (NO) system secondary to accumulation of asymmetrical dimethylarginine (6), low L-arginine levels (7), hyperhomocysteine (8,9), dyslipidemia (10), or hypertension (11). One of the consequences of impaired NO activity is that vasoconstrictors such as angiotensin II, ET, and thromboxane A2 become unopposed (12,13). This results in increased constrictor tone and hypertension. In addition, these vasoconstrictors may stimulate proliferation, extracellular matrix deposition, and leukocyte activation (14).

In the present study, we explored the role of ET-1 in the development of hypertension and renal lesions during chronic NOS blockade. ET-1 and ET receptors are widely expressed in the kidney (15,16). Support for participation of ET-1 in the development of the systemic and renal changes induced by chronic blockade of NOS with Nω-nitro-L-arginine methyl ester (L-NAME), are the increase in ET-1 gene expression observed in conduit arteries (aorta and mesenteric arteries) of spontaneously hypertensive rats (17) and the increase of urinary ET-1 excretion observed in normal rats (18). However, L-NAME treatment of spontaneously hypertensive rats was associated with decreased expression of ET-1 by resistance arteries, in which hypertrophy of smooth muscle was nearly absent (17,19). Moreover, in nonhypertensive rats, no important role for ET-1 in the pathogenesis of L-NAME-induced hypertension

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Correspondence to Dr. Jaap A. Joles, Department of Nephrology and Hypertension (Room F03.226), University Hospital Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands.
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could be identified; vascular expression of ET-1 was not changed during L-NNAME, and ETA receptor blockade with A-127722 did not affect BP or vascular lesions (20).

Variation in the response to L-NNAME may be due to its divergent effects: proliferation and upregulation of ET-1 in conduit arteries and downregulation of ET-1 in resistance arteries, possibly in association with antiproliferative effects. The inhibitory effect of L-NNAME on hypertension may be due to antimuscarinic properties (21). Muscarinic receptor-mediated stimulation of DNA synthesis and growth factor-like activity have been described (22,23). Therefore, we chose Nω-nitro-L-arginine (L-NNA) (24), a NOS inhibitor (25) that is tenfold more potent than L-NAME (25-27) and does not have antimuscarinic properties (21), to evaluate the effects of chronic administration of the potent ETA receptor antagonist A-127722 (28,29) on BP, proteinuria, and renal vascular, glomerular, and tubulointerstitial lesions induced by NO deficiency.

Materials and Methods

Animals

Male Sprague Dawley rats (4 to 5 wk old; 125 to 150 g; Harlan-Olac, Blackthorn, United Kingdom) received a standard diet (RMH-TM; Hope Farms, Woerden, The Netherlands) and had free access to tap water. They were exposed to a 12-h light/dark cycle, an ambient temperature of 22°C, and humidity of 60%. Sentinel animals, which were monitored regularly for infection by nematodes and pathogenic bacteria, as well as for antibodies to a large number of rodent viral pathogens (International Council for Laboratory Animal Science, Nijmegen, The Netherlands), consistently tested negative for infection throughout the experiment. The protocol was approved by the Utrecht University Board for studies in experimental animals.

Preliminary Studies

In a preliminary study, dose dependency of the effects of L-NNAME (Sigma Chemical Co., St. Louis, MO) on systolic BP was measured. L-NNAME was mixed through finely ground chow at concentrations of 0, 3, 10, 30, 100, 500, and 5000 mg/kg. This resulted in intakes of approximately 0, 0.24, 0.8, 2.4, 8, 40, and 400 mg/kg per d. Each concentration was administered to three rats, and the systolic BP was measured after 3 wk. Subsequently, the rats in the control group and those in the 40 mg/kg per d L-NNAME group were anesthetized with 60 mg/kg sodium pentobarbital intraperitoneally, and one kidney was removed and directly homogenized in an ice-cooled homogenization buffer (vol/vol: 1:5) consisting of: 50 mM Tris buffer, 320 mM sucrose, 1 mM ethylenediaminetetraacetic acid, 1 mM dithiothreitol, 2 mM aprotinin, and 100 mM phenylmethylsulfonil fluoride, frozen in liquid nitrogen and stored at −80°C for determination of NOS activity. NOS activity was determined as the formation of L-3H-citrulline from L-3H-arginine. Homogenate (50 µL) was incubated in a tube, the NADPH was substituted by 0.1 M L-NAME to determine nonspecific activity. The reaction was stopped by placing the tubes on ice and adding 20 mM ice-cold Hepes buffer, followed by separation of arginine and citrulline on Dowex 50X8-200 and detection of 3H-citrulline by scintillation counting. Measurements were performed in triplicate, and the results are presented per milligram of protein.

Experimental Protocol

A dose of 40 mg/kg per d L-NNA was chosen for the experiments. Three groups of rats were studied. The control group (n = 8) received normal chow and water. The second group (n = 9) received L-NNA mixed through finely ground chow (0.5 g/kg chow). The third group (n = 8) received L-NNA mixed through finely ground chow (0.5 g/kg chow) in combination with the ETA receptor antagonist A-127722 (Abbott Laboratories, Abbott Park, IL) dissolved in its drinking water (375 mL/L). This resulted in an intake of approximately 40 mg/kg per d L-NNA and 30 mg/kg per d A-127722.

BP, Body Weight, and Renal Function

Rats were kept on their diet for 3 wk. Systolic BP was measured weekly in the awake rats, starting 1 wk before the start of the diet (week 0) by the tail-cuff method (ITTC, San Diego, CA). Urine was collected weekly starting at week 0 for determination of urinary protein and creatinine excretion. The rats were weighed and placed in metabolism cages for 24 h, with free access to food and water. At the end of the experiment, the animals were anesthetized with 60 mg/kg sodium pentobarbital intraperitoneally, and the femoral artery was cannulated for direct measurement of mean arterial pressure and to collect blood for determination of plasma creatinine concentration. Urinary protein levels were determined by the Bradford method. Plasma and urinary creatinine levels were determined colorimetrically (Sigma Chemical Co.). The creatinine clearance, calculated by the standard formula, was used as an estimate of GFR.

Morphologic Studies

At the end of the experiment, the kidneys were removed, weighed, and then processed for histology and immunohistochemistry. Kidneys were cut into slices and either immersion-fixed in phosphate-buffered saline (PBS) formaldehyde (4%, pH 7.35) (PBS: 99 mM NaH2PO4 × H2O, 108 mM Na2HPO4 × 2 H2O and 248 mM NaCl) and embedded in paraffin or immersion-fixed in methacarn (60% methanol, 30% chloroform, 10% acetic acid) for 24 h, stored in 70% ethanol, and subsequently embedded in paraffin.

Light microscopy was done on 3-µm sections of the formaldehyde-fixed tissue stained by periodic acid-Schiff. The sections were numbered. The pathologist (Dr. Gröne) was blinded to the experimental groups.

Preglomerular vessels with endothelial damage, thrombus, necrosis, and vasculitis were assessed as showing either a mild, moderate, or severe degree of injury and evaluated in both cortex and outer medulla. The degree-specific vascular injury index was defined as the number of vessels with the respective degree of injury encountered in a whole kidney section divided by the total number of vessels in a whole kidney section. The total vascular injury index was taken as the sum of all vessels with all degrees of vascular injury, whereby the number of vessels with degree 1 was multiplied by 1, that with degree 2 by 2, and that with degree 3 by 3, divided by the total number of vessels in a whole kidney section, and multiplied by 100.

One hundred glomeruli were evaluated. Protein droplets in glomerular visceral epithelial cells were assessed by calculating the percentage of affected glomeruli. The glomerular ischemia index was taken as the sum of all glomeruli with mild (1), moderate (2), or severe (3) ischemia, whereby the number of glomeruli with degree 1 was multiplied by 1, that with degree 2 by 2, and that with degree 3 by 3.

Tubulointerstitial damage was qualitatively assessed in cortex and
outer medulla as mild, moderate, or severe, depending on the presence of infiltrated mononuclear cells and tubular damage in 20 microscopic fields with ×100 magnification, and an average score per rat was calculated.

Immunohistochemistry was carried out on 5-μm sections of the methacarn-fixed tissue, using the alkaline phosphatase anti-alkaline phosphatase technique (Dako, Hamburg, Germany). Tissue sections were deparaffinized, rehydrated, and incubated with the mouse monoclonal antibody ED-1 (Seropec/Camon, Wiesbaden, Germany) to demonstrate monocytes/macrophages. After application of ED-1 (dilution 1:400) to the slides for 2 h at 22°C, a rabbit anti-mouse antibody was used at a dilution of 1:40 at 22°C for 1 h. Alkaline phosphatase mouse monoclonal antibody (diluted 1:400) was then incubated at 22°C for 1 h. All dilutions were done in PBS (pH 7.6). For staining, slides were exposed to a solution of sodium nitrite (28 mM), new fuchsin (basic fuchsin, 21 mM), naphthol-AS-BI-phosphate (0.5 mM), dimethylformamide (64 mM), and levamisole (5 mM) in 50 mM Tris/HCl buffer (pH 8.4, containing 146 mM NaCl) for 15 min.

The number of ED-1-antigen-positive monocytes/macrophages was determined in 100 glomeruli, all vessels, and tubulointerstitium. In vessels, the number of ED-1-antigen-positive monocytes/macrophages that was attached to the endothelium or that had infiltrated into the intima or media was determined. The number of ED-1-antigen-positive monocytes/macrophages infiltrated into the tubulointerstitium was counted in 20 microscopic fields with ×400 magnification, and an average score per rat was calculated.

Statistical Analyses

Results are expressed as mean ± SEM for quantitative data and as median and range for semiquantitative data. Quantitative data were tested by two-way ANOVA for repeated measures and semiquantitative data by the Kruskal-Wallis ANOVA. If the variance ratio (F) reached statistical significance (P < 0.05), quantitative data were further analyzed with the Student-Newman-Keuls test for multiple comparisons, and semiquantitative data with Dunn’s test for multiple comparisons.

Results

l-NNA: Effect on NOS Activity and Dose-Dependent Effect on BP

After 3 wk of oral administration of l-NNA, the maximum increase in systolic BP was found at a dose of 40 mg/kg per d (P < 0.001) (Figure 1). In rats treated with l-NNA at 40 mg/kg per d, renal NOS activity was reduced to 2.45 ± 0.09 pmol of 3H-citrulline/min per mg of protein compared with 3.79 ± 0.04 pmol of 3H-citrulline/min per mg of protein in controls (P < 0.001).

BP, Body Weight, and Renal Function

l-NNA treatment (40 mg/kg per d) during 3 wk resulted in a 10% decrease in body weight (250 ± 7 g versus 279 ± 5 g in control, P < 0.05), a decrease in GFR (1.41 ± 0.16 ml/min versus 2.23 ± 0.19 ml/min in control, P < 0.05), and a marked rise in systolic BP (240 ± 4 mmHg versus 151 ± 7 mmHg in control, P < 0.01) (Figure 2) and proteinuria (209 ± 46 mg/d versus 27 ± 3 mg/d in control, P < 0.01) (Figure 3). Intravenous measured mean BP showed a similar increase (195 ± 9 mmHg versus 120 ± 7 mmHg in control, P < 0.01). Systolic BP was already increased by l-NNA after 1 wk and proteinuria after 2 wk. One rat died 1 d before the end of l-NNA treatment after 2 to 3 d of decreasedmotility and weight loss. Most remaining rats showed clear signs of illness, such as weight loss, ruffled hair, decreased motility, and uncleaned nostrils and eyes after 3 wk.

A-127722 administered with l-NNA prevented the decreases in body weight (269 ± 8 g) and GFR (1.98 ± 0.16 ml/min), and the increase in proteinuria (39 ± 8 mg/24 h). These values were not statistically different from those found in the control group. Systolic BP was intermediate between the l-NNA and control group (194 ± 7 mmHg), and in the second and third week of combined treatment it was significantly lower than in the rats treated with l-NNA alone (P < 0.05). Mean arterial BP was similarly reduced (135 ± 10 mmHg). None of the rats in this group or in the control group showed signs of illness.

Figure 1. Systolic BP in rats treated for 3 wk with (l-NNA). l-NNA was mixed through finely ground chow at concentrations of 0, 3, 10, 30, 100, 500, and 5000 mg/kg. This resulted in intakes of approximately 0, 0.24, 0.8, 2.4, 8, 40, and 400 mg/kg per d. Each concentration was administered to three rats. *P < 0.05 versus control; **P < 0.001 versus control.

Figure 2. Systolic BP in rats treated for 3 wk with l-NNA (●). l-NNA plus A-127722 (▲), or control rats (○). *P < 0.05 versus control; **P < 0.05 versus l-NNA.
Figure 3. Proteinuria in rats treated for 3 wk with L-NNA (●), L-NNA plus A-127722 (▲), or control rats (○). *P < 0.05 versus control; **P < 0.05 versus L-NNA.

Morphologic Studies
The most striking effect of 3 wk with L-NNA treatment in rats was vascular injury characterized by focal adhesion and infiltration of mononuclear cells into the intima and media of preglomerular arteries and arterioles (Figure 4). This was sometimes associated with a partial or total obstruction of the lumen with thrombotic material, and/or necrosis of the media (Table 1). Mild injury was observed more frequently than moderate or severe injury. The number of monocytes and macrophages attached to the endothelium and infiltrated into the intima and media of blood vessels was increased (Figure 5), significantly so in the arteries (Table 2).

Figure 4. Intrarenal artery in a rat treated with L-NNA with pronounced infiltration of the intima and media by mononuclear cells. Some vascular smooth muscle cells in the periphery of the media are necrotic, and the periadventitial tissue is infiltrated by mononuclear cells. Magnification, ×400.

Glomerular damage was characterized by an increase in the number of glomeruli with protein droplets in visceral epithelial cells and the occurrence of glomeruli with ischemic retraction and solidification of the capillary convolute (Figure 4, Table 1). However, only 7 ± 2% of the glomeruli showed such ischemic changes. A few glomeruli also showed necrosis, adhesion to Bowman’s capsule, and extracapillary proliferation of cells (probably parietal epithelia). The number of monocytes and macrophages in the glomeruli was not significantly increased (Table 2). L-NNA induced a moderate tubulointerstitial damage (Figure 6, Table 1) and an increase in the number of monocytes and macrophages in the tubulointerstitium (Table 2).

A-127722 administered with L-NNA markedly diminished vascular injury (Figure 7) in seven of the eight rats in this group (P < 0.05) (Table 1) and prevented glomerular ischemia in all rats (P < 0.01). This can be seen by comparison of the median values in relation to the indicated range. There was no significant effect on the number of glomeruli with protein droplets. Tubulointerstitial damage was intermediate between the control and L-NNA group (Table 1). The number of monocytes and macrophages in the blood vessels, glomeruli, and tubulointerstitium was intermediate between the control and L-NNA group (Table 2).

Discussion
Treatment of rats for 3 wk with L-NNA (40 mg/kg per d) decreased renal NOS activity and resulted in severe hypertension and proteinuria. Changes in renal morphology after 6 to 8 wk of treatment with the NOS inhibitor L-NAME (5,18,30-34) and after 3 wk of L-NNA in the present study were similar, with prominent vascular lesions including thrombosis and necrosis in arterial media, glomerular collapse, and tubulointerstitial damage characterized by tubular atrophy and vacuolization and interstitial inflammatory infiltration. In contrast to the L-NAME-model, there are a paucity of chronic changes such as focal segmental glomerulosclerosis, fibrinoid necrosis in glomeruli, vascular and interstitial fibrosis, and “onion skin”-like vascular lesions in the L-NNA model. These differences are unexplained, but may be due to the fact that L-NNA is more potent than L-NAME (25-27) and has no additional antimuscarinic effect (21) that interferes with its NOS-blocking action. As a consequence, L-NNA induces NOS inhibition that is so rapid and severe that the development of chronic lesions appears to be obstructed.

Blockade of ET_A receptors could, for the most part, but not completely, prevent renal injury and hypertension after chronic NOS inhibition. Sventek et al. (20) showed that after oral treatment with A-127722 at the same dose, the response to a bolus intravenous injection of a large dose of ET-1 was inhibited to a degree similar to that found at doses that effectively lowered BP in ET-dependent hypertensive models such as deoxycorticosterone acetate-salt hypertensive rats (19). Therefore, this dosage was presumed to effectively inhibit endogenously generated ET-1, of which the concentration is probably much lower (20). We found that blockade of ET_A receptors could completely prevent the increase in proteinuria, decrease
Table 1. Renal vascular, glomerular, and tubulointerstitial damage in control, L-NNA-, and L-NNA plus A-127722-treated rats

<table>
<thead>
<tr>
<th>Category</th>
<th>Control (n = 8)</th>
<th>L-NNA (n = 9)</th>
<th>L-NNA plus A-127722 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vascular injury score (1 to 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mild</td>
<td>0.0</td>
<td>25.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.75&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>(0.0 to 1.6)</td>
<td>(5.8 to 42.2)</td>
<td>(0.0 to 46.0)</td>
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</tr>
<tr>
<td>moderate</td>
<td>0.0</td>
<td>4.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
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<td>(0.0 to 0.0)</td>
<td>(0.6 to 9.5)</td>
<td>(0.0 to 11.0)</td>
<td></td>
</tr>
<tr>
<td>severe</td>
<td>0.0</td>
<td>4.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.85</td>
</tr>
<tr>
<td>(0.0 to 0.0)</td>
<td>(0.0 to 27.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total vascular injury index</td>
<td>0.00</td>
<td>39.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>(0.00 to 0.02)</td>
<td>(25 to 129)</td>
<td>(3 to 108)</td>
<td></td>
</tr>
<tr>
<td>Glomeruli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% with protein droplets</td>
<td>1</td>
<td>8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6</td>
</tr>
<tr>
<td>(0 to 4)</td>
<td>(2 to 22)</td>
<td>(1 to 15)</td>
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<tr>
<td>glomerular ischemia index</td>
<td>0</td>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>(0 to 0)</td>
<td>(3 to 23)</td>
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<tr>
<td>Tubulointerstitial damage</td>
<td>0.03</td>
<td>1.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.65&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>(0.00 to 0.10)</td>
<td>(1.00 to 2.10)</td>
<td>(0.30 to 0.90)</td>
<td></td>
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</table>

<sup>a</sup> Values given are median and range. L-NNA, N<sup>ω</sup>-nitro-L-arginine.

<sup>b</sup> P < 0.01 versus control.

<sup>c</sup> P < 0.05 versus L-NNA.

<sup>d</sup> P < 0.01 versus L-NNA.

Figure 5. Immunohistochemical detection of monocytes and macrophages with monoclonal ED-1 antibody staining in a rat treated with L-NNA. ED-1-positive cells can be observed in arterioles and tubulointerstitium. Magnification, X400.

in glomerular filtration, and the occurrence of ischemic glomeruli. Only mild vascular injury was observed in all but one of the rats treated with A-127722. One explanation could be that ETA receptor blockade reduces the imbalance between vasoconstrictors and vasodilators that appears after NOS inhibition. However, the complete absence of proteinuria, as well as functional and morphologic glomerular damage in the presence of only partial restoration of BP, suggests that part of the protective effect of ETA receptor blockade was independent of hemodynamics, possibly because some of the effects of ET are nonhemodynamic. Indeed, aging transgenic mice with a high expression of human ET-1 in the kidney, brain, and lung develop glomerulosclerosis as well as interstitial fibrosis, but no hypertension (35). In addition, ET stimulates the production of superoxide radicals by macrophages (36). Chronic NOS inhibition also causes a cascade of effects leading to platelet aggregation, as well as adhesion and emigration of leukocytes from the bloodstream. This cascade is characterized by enhanced production of superoxide radicals that results in upregulation of cytokines and adhesion molecules (37,38). Thus, the effects of NOS inhibition and ET on the production of superoxide radicals coincide. The resulting vascular obliteration may induce glomerular ischemia. It should be emphasized that chronic NOS inhibition will probably generate secondary effects due to the severe vascular damage, both in the kidney and elsewhere, which may have resulted in upregulation of ET-1 production (3). Part of the protection afforded by A-127722 may well have been due to amelioration of these secondary effects.

The tubulointerstitial injury may have been due to both vascular injury and glomerular ischemia. Upregulation of cytokines and adhesion molecules will stimulate emigration of mononuclear cells from the bloodstream into the interstitium
Table 2. Renal vascular, glomerular, and tubulointerstitial monocytes plus macrophages in control, l-NNA-, and l-NNA plus A-127722-treated rats\(^a\)

<table>
<thead>
<tr>
<th>Category</th>
<th>Control ((n = 8))</th>
<th>l-NNA ((n = 9))</th>
<th>l-NNA plus A-127722 ((n = 8))</th>
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<tr>
<td>Vessels</td>
<td></td>
<td></td>
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<tr>
<td>artery</td>
<td>0.24</td>
<td>0.96(^b)</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>(0.00 to 0.44)</td>
<td>(0.42 to 1.79)</td>
<td>(0.00 to 10.9)</td>
</tr>
<tr>
<td>arteriole</td>
<td>0.05</td>
<td>0.29</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>(0.02 to 0.09)</td>
<td>(0.00 to 2.25)</td>
<td>(0.03 to 1.03)</td>
</tr>
<tr>
<td>Glomeruli</td>
<td>0.91</td>
<td>1.22</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>(0.61 to 1.44)</td>
<td>(1.11 to 1.92)</td>
<td>(0.54 to 1.69)</td>
</tr>
<tr>
<td>Tubulointerstitium</td>
<td>3.60</td>
<td>8.28(^c)</td>
<td>4.84</td>
</tr>
<tr>
<td></td>
<td>(2.69 to 4.13)</td>
<td>(7.29 to 9.32)</td>
<td>(3.14 to 9.56)</td>
</tr>
</tbody>
</table>

*Values given are median and range.

\(^b\) \(P < 0.05\) *versus* control.

\(^c\) \(P < 0.01\) *versus* control.

Vascular obliteration and glomerular ischemia may cause tubular atrophy. Another possibility is that tubulointerstitial injury is a direct effect of inhibition of tubular NO synthesis. Renal tubules produce a large amount of NO under normal circumstances (40,41). NO may prevent tubulitis by the ability of NO to downregulate leukocyte proliferation and macrophage activation (42). However, during hypoxia/reoxygenation injury, NO may react with superoxide radicals to form peroxynitrite (43), which could contribute to injury. Indeed, in isolated proximal tubules, hypoxia was accompanied by an increase in calcium-independent NOS activity and NO\(_2/\text{NO}_3\) production, effects that were blocked by NOS inhibition (44). However, in our model it is unlikely that such an effect of tubular ischemia on NO production played a major role in the generation of tubular injury, because then the effects of NOS inhibition would have been self-limiting. The observed tubulointerstitial injury may also, at least in part, be related to proteinuria. Excessive protein reabsorption induces functional alterations of tubular cells and overexpression of inflammatory and vasoactive molecules, such as monocyte chemoattractant protein-1 and osteopontin, which may attract inflammatory cells into the renal interstitium (45). ETA receptor blockade could completely prevent proteinuria; however, it had no effect on the percentage of glomeruli with protein droplets, and mild tubulointerstitial damage was observed in the rats treated with A-127722. This suggests that there was still glomerular protein...
loss and, consequently, tubular protein reabsorption that may have contributed to the remaining tubulointerstitial injury.

In the single L-NAME study using A-127722 (20), effects on the kidney were not evaluated. Other ET receptor blockers are less potent than A-127722 (29), and their protective action has not been very promising in this model. ET\(_A\) receptor blockade with FR139317 (31) and ET\(_A/ET\_B\) blockade with bosentan (34) did not affect hypertension, renal and systemic vasoconstriction, glomerular collapse, and interstitial expansion. Administration of bosentan did blunt the development of sudanophilic lesions in branches of arcuate arteries and in interlobular arteries, but failed to affect arterial hypertension or the degree of glomerular injury (33). The findings of the present study show that ET\(_A\)-receptor blockade with a potent antagonist at an adequate dose does provide some protection to both the hemodynamic and nonhemodynamic effects of NOS inhibition.

In conclusion, the activity of ET seems to be necessary for full expression of the renal injury and hypertension induced by chronic NOS inhibition with L-NAME. Our findings suggest further exploration of ET\(_A\) receptor blockade as a strategy to alleviate renal injury in patients with chronic renal failure, who are assumed to have impaired NO activity.

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