Chronic Inhibition of Nitric Oxide Synthesis
A New Model of Arterial Hypertension
Miriam Oliveira Ribeiro, Edson Antunes, Gilberto de Nucci, Silvana Maria Lovisolo, and Roberto Zatz

Recent studies have indicated that acute inhibition of nitric oxide biosynthesis in the rat promotes arterial hypertension and renal vasoconstriction. We evaluated the renal and systemic effects of 4–6 weeks of nitric oxide blockade in Munich-Wistar rats receiving the nitric oxide inhibitor nitro-L-arginine orally. Age-matched untreated rats were used as controls. In an additional seven rats, nitric oxide blockade was carried out in conjunction with oral administration of the novel angiotensin II antagonist losartan potassium. Tail-cuff pressure rose progressively in nitro-L-arginine–treated rats, reaching 164±6 mm Hg at 4–6 weeks, compared with 108±3 mm Hg in controls. In rats concomitantly receiving losartan, tail-cuff pressure reached 125±6 mm Hg, still elevated compared with rats receiving losartan alone (98±3 mm Hg). Nitro-L-arginine–treated rats presented marked renal vasoconstriction and hypoperfusion, as well as a 30% fall in glomerular filtration rate and a 39% increase in filtration fraction. Treatment with Losartan normalized glomerular filtration rate, but not filtration fraction or renal vascular resistance. Plasma renin activity was elevated after nitro-L-arginine treatment. Renal histological examination revealed widespread arteriolar narrowing, focal arteriolar obliteration, and segmental fibrinoid necrosis in the glomeruli. In a separate group of rats, nitro-L-arginine administered for 1 week induced hypertension that was partially reversed by acute L-arginine, but not D-arginine or L-glycine, infusions. We conclude that chronic nitric oxide blockade may constitute a new model of severe arterial hypertension. Activation of the renin-angiotensin system may account, at least in part, for the vasoconstrictor activity after such inhibition. (Hypertension 1992;20:298–303)

**KEY WORDS** • endothelium-derived relaxing factor • kidney • hypertension, malignant • blood pressure • nitric oxide • rat studies

The endothelium releases a labile, diffusible vasorelaxing substance that has been termed endothelium-derived relaxing factor (EDRF).1 Recent observations have suggested that a major portion of the vascular effects of EDRF can be attributed to nitric oxide (NO).2 The vasorelaxing properties of EDRF/NO have been directly demonstrated by using in vitro preparations of conduit arteries.3 Moreover, acute in vivo administration of L-arginine analogues such as N prevail methyl L-arginine4 and N w-nitro-L-arginine-methyl ester (L-NAME),5 elicits marked arterial hypertension and renal vasoconstriction, presumably as a consequence of an abrupt inhibition of NO biosynthesis.4,6,7 These observations indicate that local release of NO in the microcirculation may occur on a continuous basis, thus modulating the effects of local and circulating vasoconstrictors and helping to finely regulate blood pressure and organ blood flow. In addition, micropuncture studies have suggested that angiotensin II (Ang II) may account for some of the renal microcirculatory alterations associated with acute NO inhibition.6

If NO exerts a tonic vasorelaxing effect on the microcirculation, its persistent inhibition might lead to the predominance of vasoconstrictor agents, resulting in arterial hypertension similar to that observed after chronic infusion of Ang II,8 norepinephrine,9 or thromboxane.10 Recently, Gardiner and coworkers11 demonstrated a marked blood pressure elevation in Brattleboro rats receiving NO inhibitors in the drinking water for up to 9 hours. However, the effects of longer NO inhibition on the renal and systemic microcirculations of healthy animals have not been systematically examined.

In the present study, we sought to determine the effect of chronic administration of L-NAME in the drinking water. By maintaining this regimen for 4–6 weeks, we observed profound alterations in systemic and renal hemodynamics, as well as in renal histology.

**Methods**

Sixty-eight adult male Munich-Wistar rats (203–291 g) were obtained from an established colony at the
Renal plasma flow (RPF) was calculated as

\[ \text{RPF} = \frac{\text{GFR}}{\text{FF}} \]

where GFR is glomerular filtration rate. Renal total vascular resistance (RVR) was estimated by the expression

\[ \text{RVR} = \frac{\text{MAP} \cdot (1 - \text{Ht})}{\text{RPF}} \]

where MAP is mean arterial pressure.

In three rats, L-NAME was given as described for 4 weeks and then discontinued. Tail-cuff pressure (TCP) was then determined weekly for the following 2 weeks.

**Effect of Angiotensin II Inhibition on L-NAME-Treated Rats**

In a second set of rats \( (n=7) \), L-NAME was administered as described above. To evaluate the effect of Ang II in a state of chronic NO inhibition, the rats also received a novel, orally active, nonpeptidic Ang II receptor antagonist, losartan potassium \((\text{DuP 753, Du-Pont Merck Pharmaceutical Co., Wilmington, Del.})\). 14 30 mg • 100 ml\(^{-1}\), in the drinking water, corresponding to an average daily intake of approximately 30 mg • kg\(^{-1}\). The rats were followed for 4–6 weeks and TCP was determined weekly. At the end of this period the rats were subjected to renal functional and morphological studies as described above. Effective Ang II blockade was confirmed by the absence of pressor response to acute intravenous injections of Ang II (50 ng), which have previously been shown to promote sharp blood pressure elevation in untreated rats. To evaluate the effect of Ang II inhibition alone, an additional group of rats \( (n=13) \) not receiving L-NAME, received losartan as described above for 4 weeks. Weekly TCP determinations were also performed in this group. After 4 weeks, five of these rats were anesthetized and prepared for renal functional studies as described above.

**Effects of t-Arginine, d-Arginine, and L-Glycine in L-NAME-Treated Rats**

Eight rats received L-NAME as described above for 1 week. The rats were then anesthetized with thiobutabarbital (Inactin) \((100 \text{ mg} \cdot \text{kg}^{-1} \text{i.p.})\), and the left femoral artery was cannulated for blood pressure monitoring as described above. The right jugular vein was catheterized for fluid infusion. After a 30-minute stabilization period, baseline arterial pressure was recorded. The rats then received a bolus injection of 200 mg • kg\(^{-1}\) t-arginine \((\text{Sigma})\) dissolved in 0.5 ml saline solution, followed by an 80 mg • ml\(^{-1}\) • min\(^{-1}\) infusion for 10 minutes. A second blood pressure determination was then performed. In a second group of L-NAME-treated rats \( (n=5) \), t-arginine was replaced by d-arginine. To ascertain whether amino acids other than L-arginine might influence blood pressure in the setting of chronic NO blockade, five rats that had received L-NAME for 1 week were prepared as described above and were administered a bolus injection of L-glycine, 85 mg • kg\(^{-1}\) in 0.5 ml saline, followed by a 35 mg • kg\(^{-1}\) • min\(^{-1}\) infusion for 10 minutes. This dosage was equimolar to that used for the L-arginine infusions.

To evaluate the effect of L-arginine per se, eight control rats were subjected to the same protocol as described above. To exclude a possible influence of blood pressure levels on the hemodynamic response to L-arginine, an additional group of control rats \( (n=5) \) received continuous intravenous infusions of nonrepi-
neprin (3 µg·min⁻¹·kg⁻¹) before L-arginine infusion. In preliminary experiments, this dose of norepinephrine was shown to elevate blood pressure to levels comparable to those observed in rats treated with L-NAME for 1 week.

**Morphological Studies**

In 11 L-NAME-treated rats, including those undergoing renal functional studies, the left kidney was removed, weighed, and fixed in Duboscq-Brazil solution, and the right kidney was perfusion-fixed in situ with 1.25% glutaraldehyde in phosphate buffer. After fixation, two microradial slices of the right kidney, 2–3 mm thick, were embedded in paraffin after conventional processing, and 3-µm-thick sections were stained by hematoxylin-eosin or by the periodic acid-Schiff reaction for light microscopy. Sections obtained in the same manner from the left kidney were stained with silver methenamine, phosphotungstic hematoxylin, or Masson trichromic, for specific staining of extracellular matrix, fibrin, and collagen, respectively.

**Analytical Procedures**

PRA was estimated by measuring the generation of angiotensin I with a commercially available radioimmunoassay kit (Baxter Healthcare Corp., Cambridge, Mass.) adapted for small samples. Plasma and urine activities of ¹⁴C were measured in a scintillation counter (Beckman Instruments).

**Statistics**

Statistical differences between groups were evaluated by one-way analysis of variance and pairwise comparisons according to the Bonferroni method. In addition, Student's paired t test was used where appropriate, and a value of p < 0.05 was considered significant.

**Results**

L-NAME–treated rats appeared generally healthy but gained weight at a slightly lower rate than untreated rats (Table 1). Tail-cuff pressure rose progressively in L-NAME rats, reaching 168 ± 7 mm Hg by day 30 of treatment, compared with 108 ± 5 mm Hg in untreated controls (p < 0.05). In rats treated concomitantly with losartan, TCP reached only 125 ± 6 mm Hg. However, this value was still elevated compared with those encountered in rats treated with losartan alone (100 ± 2 mm Hg, p < 0.05) (Figure 1). After 15 days of L-NAME administration, PRA was identical in control and treated rats. PRA was numerically elevated in rats treated with L-NAME for 4–6 weeks (16.8 ± 4.5 versus 4.5 ± 0.4 ng angiotensin I [Ang I]·ml⁻¹·hr⁻¹ in controls, 0.05 < p < 0.1). PRA varied widely in this group, ranging from 1.1 to 67.0 ng Ang I·ml⁻¹·hr⁻¹ (Figure 2). A positive numerical not statistically significant correlation was found between TCP and PRA (r = 0.30, p > 0.1). As expected, Ang II inhibition was associated with a much more pronounced increase in TCP in rats treated with L-NAME and losartan (71.9 ± 7.4 ng Ang I·ml⁻¹·hr⁻¹, p < 0.05 versus control and versus L-NAME).

Figure 3 depicts the blood pressure effect of acute administration of l-arginine, d-arginine, and L-glycine in control and L-NAME–treated rats. As expected, administration of d-arginine had no discernible effect on blood pressure in L-NAME–treated rats. Equally ineffective was the administration of an equimolar dose of L-glycine. In contrast, acute administration of L-arginine promoted a 26-mm Hg lowering of mean arterial pressure in rats receiving L-NAME for 1 week. L-Arginine had no hemodynamic effect in rats not receiving L-NAME. This divergent effect of L-arginine was unrelated to the observed differences in baseline arterial pressure since L-arginine was hemodynamically ineffective in rats not treated with L-NAME even after blood pressure had been raised by prior norepinephrine infusions (Figure 3).

Withdrawal of L-NAME after 4 weeks of treatment was associated with a gradual lowering of TCP from...
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FIGURE 2. Plot shows plasma renin activity (PRA) in rats after 2 and 4–6 weeks of N\textsuperscript{G}-nitro-L-arginine-methyl ester administration (△) and in controls (●). Al, angiotensin I.

163±14 mm Hg to 147±13 mm Hg 1 week and to 121±2 mm Hg 2 weeks after L-NAME discontinuation.

Table 1 summarizes the findings obtained in renal functional studies performed after 4–6 weeks of L-NAME administration. No significant differences in kidney weight were seen among the groups. Mean arterial pressure in the experimental groups, measured at the femoral artery while the rats were under anesthesia, was markedly elevated as compared with control animals. Treatment with losartan largely attenuated the L-NAME-induced hypertension, although mean arterial pressure remained significantly higher than in losartan-treated control rats.

In rats receiving L-NAME, GFR and RPF were reduced to 70% and to less than 50%, respectively, compared with control animals. Accordingly, FF was markedly elevated to 0.43±0.04 compared with 0.31±0.01 in control animals. These renal hemodynamic abnormalities were associated with striking vasoconstriction, in which RVR reached 41.6±4.7 mm Hg · ml\textsuperscript{-1} · min in L-NAME-treated rats, as opposed to 12.6±1.3 mm Hg · ml\textsuperscript{-1} · min in the control group. In rats treated with L-NAME and losartan, GFR was identical to that observed in losartan-treated controls. However, losartan treatment promoted no lowering of FF. Accordingly, RPF remained numerically lower, whereas RVR was numerically increased in these animals, as compared with losartan-treated controls.

Administration of L-NAME was associated with widespread renal morphological alterations. Marked thickening of the wall of small arteries and arterioles was systematically observed, frequently in association with collapse of the glomerular tuft (Figure 4). Extreme thickening of the arteriolar wall and nearly complete luminal obliteration were encountered in three rats (27%). In addition, partial fibrinoid necrosis of the arteriolar wall was observed in these animals. These lesions stained positively with phosphotungstic hematoxylin. Extensive glomerular segmental areas of hyalinization, sharply delimited from adjacent healthy tissue, were observed in seven rats (64%). These lesions were also positive for phosphotungstic hematoxylin, but no staining was obtained by silver methenamine, suggesting the presence of segmental fibrinoid necrosis of the glomerular tuft. Collagen-like material, staining in blue with Masson trichromic, was frequently seen around the outermost portions of these areas. Lysis of the central portions of these lesions was also observed, with occasional filling of this space with red blood cells, suggesting the formation of microaneurysms. Glomerular segmental sclerotic lesions were infrequently observed. Focal areas of tubular atrophy and interstitial inflammatory infiltration were occasionally seen in these animals. In rats receiving L-NAME and losartan simultaneously and in losartan-treated controls, the morphological appearance of arterioles, interstitium, and glomeruli was entirely inconspicuous.
Discusson

Previous evidence obtained after acute inhibition of NO biosynthesis has suggested that NO exerts a basal relaxing effect on renal and systemic microvessels, thus modulating the effects of local and systemic vasoconstrictors.\(^6,7\) This concept is supported by the present finding that prolonged inhibition of NO synthesis by administration of an \(L\)-arginine analogue resulted in severe and persistent elevation of arterial pressure, characterizing a new model of severe arterial hypertension. A major role of basal NO biosynthesis in the regulation of arterial pressure is also suggested by the finding that acute intravenous administration of excess \(L\)-arginine after 7 days of L-NAME was associated with marked lowering of blood pressure. That this effect of \(L\)-arginine is likely to be specifically related to NO synthesis is suggested by the negligible hemodynamic effects induced by \(L\)-arginine in untreated controls, irrespective of their baseline arterial pressure levels, and the equally trivial blood pressure effect of the enantiomer \(D\)-arginine and the structurally unrelated amino acid \(L\)-glycine in rats with L-NAME-induced hypertension. However, since hypertension was not entirely reversed by \(L\)-arginine infusion, the possibility remains that persistent NO inhibition may have promoted additional humoral, structural, or both, alterations that also contributed to raise blood pressure in this model.

In rats given L-NAME orally for 2 months, Baylis et al\(^8\) recently reported preliminary data quite similar to those described in the present study. These investigators reported that daily oral administration of L-NAME was associated with arterial hypertension and diminished single nephron GFR. In addition, they reported an elevation in glomerular hydraulic pressure as well as a decrease in glomerular ultrafiltration coefficient, in keeping with the glomerular hemodynamic findings previously described in association with acute NO blockade.\(^6\) Besides underlining the reproducibility of this new model of arterial hypertension, the results obtained by Baylis et al\(^9\) constitute independent evidence that NO plays a crucial role in the long-term regulation of systemic blood pressure in the rat. That a similar mechanism might act in other species as well is suggested by preliminary data obtained by Salazar et al.,\(^10\) who showed that daily intravenous administration of L-NAME to the dog also leads to marked arterial hypertension.

Predominance of vasoconstrictor over vasodilator activity in the microcirculation, directly raising peripheral resistance, is likely to account for a large fraction of the arterial hypertension observed in this model, although the participation of pathogenetic factors such as extracellular fluid accumulation\(^11,12\) cannot be excluded. Consistent with this hypothesis is the previous observation that acute oral or intravenous administration of \(L\)-arginine analogues in the rat markedly raises renal,\(^6,7,13\) splanchnic,\(^14\) and mesenteric\(^15\) vascular resistances, as well as the finding in this study of a threefold increase in RVR in rats receiving L-NAME for 4–6 weeks. Additional peripheral vasoconstriction may have resulted from sympathetic hyperactivity directly associated with NO inhibition, since NO has been recently suggested to play a role as an inhibitory autonomic neurotransmitter.\(^16\) Widespread arterial vasoconstriction, particularly in the renal territory, may also have resulted from autoregulatory phenomena directly elicited by the rise in arterial pressure. However, renal vasoconstriction observed in other models such as deoxycorticosterone acetate (DOCA)–salt hypertension\(^17\) or two-kidney, one clip Goldblatt hypertension\(^18\) is only modest compared with that observed in the present study, suggesting that more direct vascular effects of NO were chiefly responsible for the observed increase in RVR. Additional vasoconstriction may have resulted from the widespread morphological alterations encountered in the renal microcirculation. However, this appears to be an unlikely possibility, given that withdrawal of L-NAME was associated with near normalization of TCP after 2 weeks.

The exact nature of the tonic vasoconstrictor activity disclosed in this study by NO inhibition remains to be elucidated. The present data suggest that the renin-angiotensin system may have played a prominent role in the development of hypertension in this model. Effective inhibition of the renin-angiotensin system by administration of a specific Ang II antagonist largely attenuated the development of arterial hypertension and prevented renal functional and morphological deterioration in L-NAME–treated rats. In addition, hyperactivity of the renin-angiotensin system, perhaps directly related to NO inhibition,\(^19\) is likely to have contributed to aggravate arterial hypertension at more advanced stages of this model, since PRA was markedly elevated in several rats that had received L-NAME for over 1 month. However, the administration of losartan to L-NAME rats failed to completely prevent arterial hypertension. In addition, although GFR was normalized, FF was not reduced by Ang II inhibition, and some degree of renal hypoperfusion persisted in these rats. These findings suggest that other circulating, locally released, or both, vasoconstrictor agents must also have contributed to raise blood pressure and promoted renal circulatory abnormalities in the setting of persistent NO inhibition.

Arterial hypertension was associated in our model with marked renal histological changes. Some of these, such as arteriolar wall thickening, are characteristic of prolonged arterial hypertension.\(^20\) Focal glomerular collapse, also described in association with long-standing hypertension,\(^21\) may provide a morphological basis for the increased PRA frequently observed in the present study.\(^22\) In addition, a substantial fraction of the animals exhibited microvascular lesions characteristic of malignant hypertension, such as extreme arteriolar hypertrophy with luminal obliteration.\(^9,23\) Of additional interest are the segmental glomerular lesions frequently observed in this study. These lesions are similar to the microaneurysms previously described in association with DOCA-salt hypertension.\(^23\) Their histochmical characteristics suggest the presence of a fibrinlike material consistent with the development of malignant hypertension.\(^24,25\) The pathogenesis of these lesions is unknown, although glomerular hypertension, demonstrated in DOCA-salt rats\(^26\) and after acute and chronic\(^27\) NO inhibition, may represent a contributing factor to such glomerular injury. Direct detrimental effects of L-NAME in the kidney are unlikely since losartan prevented development of microangiopathy.
In summary, the present study describes a new model of persistent arterial hypertension induced by chronic inhibition of NO biosynthesis. This hypertension can be partially reversed by acute administration of l-arginine and may involve an important participation of the renin-angiotensin system. At more advanced phases, the model is characterized by a depressed renal function and renal hypertensive microangiopathy, with a fraction of the animals eventually exhibiting functional and morphological characteristics of malignant hypertension. Further investigation is required to unravel the detailed mechanisms underlying the development and aggravation of arterial hypertension in this new model.

Acknowledgments

Losartan potassium was a donation of The Du Pont Merck Pharmaceutical Co., Wilmington, Del. We are indebted to Regina Maria Padilha and Marinete Miristini dos Santos for expert technical assistance.

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Hypertension. 1992;20:298-303
doi: 10.1161/01.HYP.20.3.298

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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