Effect of losartan on renal microvasculature during chronic inhibition of nitric oxide visualized by micro-CT


Submitted 24 March 2003; accepted in final form 26 June 2003

NITRIC OXIDE (NO) is a physiologically important vasodilator, which plays a major role in the regulation of systemic and renal hemodynamics (16). Chronic inhibition of NO synthesis produces a general vasoconstriction that leads to an increased blood pressure and impairment of renal function including decreased renal blood flow (RBF) and glomerular filtration rate (GFR) without significant changes in urinary sodium and water excretion (3, 11, 30). Besides the functional changes, the inhibition of NO synthesis produces renal vascular damage including glomerulosclerosis, ischemia, necrosis, and increased interstitium (12, 24).

The mechanism underlying the effect of NO synthesis inhibition is not completely understood, but it is likely that the renin-angiotensin system is responsible for the renal and systemic alterations induced by the chronically reduced NO bioavailability. For instance, the simultaneous blockade of NO synthesis and ANG II AT1 receptor prevents the hypertension, renal functional and morphological consequences of the inhibition of NO synthesis alone (35). Ohishi et al. (20) found that the blockade of ANG II AT1 receptors attenuated the vasoconstriction elicited by the inhibition of NO synthesis in juxtamedullary afferent and efferent arterioles perfused in vitro. In vivo experiments (23) showed that NO modulated the ANG II response of cortical but not outer medullary blood flow. However, Ortiz et al. (21) demonstrated that in contrast to acute studies, chronic inhibition of NO synthesis caused a marked decrease in papillary flow, which was partially normalized by chronic blockade of AT1 receptors with losartan.

In the present study, we examined the effect of endogenous ANG II on AT1 receptors on renal vasculature during chronic inhibition of NO synthesis with N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME). Losartan was used to determine the role of AT1 receptors. To examine and measure the changes in renal vasculature, we used a three-dimensional X-ray microcomputed tomography (micro-CT) system (6, 15, 22). These changes were further evaluated by histological examination.

METHODS

Male Sprague-Dawley rats (250 g; Harlan, Indianapolis, IN) were housed in wire cages and maintained in a temperature-controlled room that was regulated on a 12:12-h light-dark cycle. Rats had free access to water and standard rat

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chow. The studies described here were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and approved by the Institutional Animal Care and Use Committee of the Mayo Clinic.

Three studies were conducted to determine the effects of simultaneous ANG II AT1 receptor blockade with losartan and NO synthesis blockade with L-NAME. The first study was a physiological study; the second was a histological study, and the third was a micro-CT study. In each study, three groups of rats were studied: control group receiving normal drinking water, ad libitum; L-NAME group receiving L-NAME (40 mg·kg⁻¹·day⁻¹) in the drinking water for 19 days; and L-NAME + losartan group receiving the same dose of L-NAME combined with losartan (40 mg·kg⁻¹·day⁻¹) for 19 days.

Physiological study. Metabolic and clearance studies were conducted to study the physiological effects of losartan on L-NAME-treated rats. Seven rats were included in each of the three groups. On day 18, 24-h urine samples were collected from metabolic cages for the measurement of urinary excretion variables. Urine and plasma were then analyzed and assayed for protein, creatinine, glucose, sodium, and potassium. Normal drinking water, ad libitum; L-NAME group receiving the same dose of L-NAME for 19 days.

To determine the volumes of the kidneys and their regions, resolution scans were carried out using a synchrotron X-ray source (4, 31). The scanners consist of an X-ray source, a detector, and a controlling computer. Scanning was performed by rotating the specimen in specific increments in the X-ray beam and acquiring an X-ray-transmitted image at each increment. An optical image of each X-ray at each angle of view was acquired on a clear cesium iodide crystal doped with thallium and projected with a lens to a cooled array-based camera with 24-μm square-detector pixels. The output images from the CCD were digitized to 16 bits and transferred to the controlling computer. A lens was positioned between the scintillator and the CCD camera to provide adequate magnification (i.e., CCD pixel was effectively 24 μm) and scans using the synchrotron X-ray source were made at ×5 (i.e., CCD pixel was effectively 5 μm). In this study, scans with the bench-top scanner were made at ×1 magnification and scans using the synchrotron source were made at ×5.

Three-dimensional volume images were reconstructed from the angular views using a modified Feldkamp’s filtered backprojection algorithm (9). The reconstructed images were comprised of cubic voxels, 6 or 21 μm on a side. Because of the large size of the three-dimensional volume arrays, the 21-μm voxels were resized to 42 μm for analysis. The opacity of each voxel was represented by a 16-bit gray scale value.

Analysis of images. Image analysis was carried out using the ANALYZE (32) software package. This package provides methods to compute, display, and analyze orthogonal and oblique sections from the reconstructed volume images. In addition, the software provides volume-rendering methods to envision the three-dimensional architecture of the renal vasculature. Glomerular diameters were determined by counting the number of sections through each individual glomerulus and multiplying the number of sections by section thickness. The number of glomeruli was determined by using the method of Sterio (34). With this method, the number of glomeruli was counted in small sample volumes systematically taken throughout the entire cortex.

To determine the volumes of the kidneys and their regions, a stereologic application was employed that randomly placed...
an array of points spaced orthogonally 2 mm apart throughout the entire scanned volume. The fraction of the volume array that was the tissue of interest was determined by counting the number of points within the boundary of the tissue and dividing by the total number of points (5). The tissue volume was then determined by multiplying the tissues of interest by the total scanned volume of the image. The volume fraction of vasculature in each of the renal tissues was determined following the method of Hillman et al. (14). The determinations were made from midtransverse slices that included the cortex, the outer stripe of the outer medulla, the inner stripe of the outer medulla, and the inner medulla. In addition, the volume fraction of tissue vasculature was also measured in glomeruli and in the nonglomerular tissue between glomeruli (interglomeruli). The interglomerular measurement provides an estimate of the volume fraction of capillaries in the cortical tissue that is not biased by opacity values of glomeruli and large blood vessels. To determine the volume fraction of tissue vascularization, the average opacity was measured from regions of interest (O(tissue)), and in the background matrix outside the kidney (O(bg)). The fraction of vasculature volume in each tissue was determined as

\[
\frac{O_{\text{tissue}} - O_{\text{bg}}}{O_{\text{artery}} - O_{\text{bg}}}
\]

This method assumes that the lead chromate is mixed homogeneously with the silicone rubber of the Microfil, that the vasculature is completely filled with Microfil, and that the Microfil has not “leaked” into the surrounding tissue.

**Statistical analysis.** The results are expressed as means ± SE, and the level of significance was considered to be \( P < 0.05 \) vs. control rats.

### RESULTS

**Physiological study.** MAP was significantly elevated (78%) and RBF and GFR decreased (65 and 42%, respectively) in the group treated with L-NAME alone compared with the control animals (Table 1). The simultaneous administration of losartan with L-NAME prevented the increase in MAP and the decrease in RBF. However, losartan did not significantly modify the increase in GFR induced by L-NAME. Sodium excretion was not significantly different among the three experimental groups (9.6 ± 2.37 in controls, 15.06 ± 4.64 in L-NAME, and 6.07 ± 1.89 \( \mu \text{eq} \cdot \text{min}^{-1} \cdot \text{g body wt}^{-1} \) in L-NAME + losartan). Both L-NAME-treated rats and L-NAME + losartan-treated rats had approximately a sixfold increase in PRA compared with control values. Treatment with L-NAME also increased the urinary protein excretion and the inclusion of losartan normalized it (Table 1). Whole body NO production measured as NOx was significantly lower in the L-NAME group than in the control animals, and losartan did not significantly alter those values.

**Histological study.** The histological study showed evidence of some segmental glomerular sclerosis in focal regions of the L-NAME kidneys. This sclerosis was essentially absent in the control kidneys and in the L-NAME + losartan kidneys. In contrast to the kidneys of the control group, the kidneys from both the L-NAME and L-NAME + losartan groups had collapsed and shrunken glomeruli. The associated nephrons were atrophied with collapsed lumens and dense, separated cells (Fig. 1). The atrophied nephrons were more frequent in the kidneys of the L-NAME group than in the kidneys of L-NAME + losartan group. Occasionally, casts were observed in the tubules of the kidneys from the L-NAME and the L-NAME + losartan groups (Fig. 2). Some of the casts were homogeneous, whereas others contained cell debris. The tubular cells surrounding the casts were flattened and contained dense granules. In some cases, the cells appeared atrophic. In both groups, the atrophied nephrons were surrounded by nephrons and glomeruli that were similar to those of the control kidneys, which suggests that the atrophy was not a fixation artifact. Also, many of the tubules of the juxtamedullary region and outer stripe of the outer medulla of the L-NAME and some of the tubules in the L-NAME + losartan kidneys were collapsed and contained sloughed cells and cellular debris (Fig. 2).

**Micro-CT study.** There were slight but significant differences among the three groups in the fraction of cortical and medullary tissue volumes (Table 2). However, there were apparent differences in the amount of microvasculature among the three groups. In general, the images of L-NAME kidneys were less opaque than the control kidneys, whereas the opacity of the L-NAME + losartan kidneys appeared to be similar to the control kidneys (Fig. 3). High-resolution images obtained with the synchrotron source showed that the differences in opacity were related to the distribution of microvessels (Fig. 4). Furthermore, the veins contained Microfil, indicating that the differences were not a consequence of incomplete

### Table 1. Physiological values from control (n = 7), L-NAME (n = 7), and L-NAME + losartan (n = 7)-treated rats

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>RBF, ml·min⁻¹·g⁻¹</th>
<th>GFR, ml·min⁻¹·g⁻¹</th>
<th>Uₚrot, mg·24 h⁻¹</th>
<th>NOₓ, μmol·24 h⁻¹·kg⁻¹</th>
<th>PRA, ng·L⁻¹·ml⁻¹·h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>102 ± 7</td>
<td>11.6 ± 1.0</td>
<td>1.32 ± 0.10</td>
<td>5.81 ± 0.79</td>
<td>7.31 ± 0.54</td>
<td>2.68 ± 0.41</td>
</tr>
<tr>
<td>L-NAME</td>
<td>182 ± 14*</td>
<td>4.1 ± 0.7*</td>
<td>0.76 ± 0.15*</td>
<td>10.96 ± 1.4*</td>
<td>1.33 ± 0.21*</td>
<td>14.52 ± 3.58*</td>
</tr>
<tr>
<td>N + L</td>
<td>104 ± 5</td>
<td>9.0 ± 1.0</td>
<td>0.83 ± 0.12*</td>
<td>7.65 ± 0.55*</td>
<td>2.70 ± 0.65*</td>
<td>26.37 ± 6.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. L-NAME, N⁵-nitro-L-arginine methyl ester; N + L, L-NAME + losartan; MAP, mean arterial pressure; RBF, renal blood flow; GFR, glomerular filtration rate; Uₚrot, excretion of protein; NOₓ, excretion of nitrates/nitrites; PRA, plasma renin activity. *P < 0.05 vs. control rats.

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*AJP-Renal Physiol • VOL 285 • NOVEMBER 2003 • www.ajprenal.org*
vascular filling. Compared with kidneys from the control group, the kidneys of the L-NAME group had a significant reduction in the percentage of vasculature (vascular fraction), which was progressively accentuated from the renal cortex toward the renal papilla (Table 2). There was a 37% reduction of vascular fraction in the renal cortex, whereas in the outer and inner stripe of the outer medulla the reduction was 44% and in the inner medulla it reached 50%. In the group that received losartan along with L-NAME, the vascular fraction in the cortex and outer stripe of the outer medulla was similar to that of the control group, but that of the inner stripe of the outer medulla and inner medulla was less than that of the control group. In these two regions, the reduction was 21 and 29% less than the control values, respectively.

The reduced vasculature in the cortex of the kidneys from the L-NAME group was accompanied by a 20% decrease in the number of opacified glomeruli (Table 3) compared with the kidneys of the control group. Along with the decrease in the number of opacified glomeruli, there was also a decrease in the diameter of cortical and juxtamedullary glomeruli. These decreases were not evident in the group that received losartan and L-NAME.

The glomerular and interglomerular vascular fraction in the kidneys of the L-NAME group were less than those of the control group. In the kidneys of the group that received losartan and L-NAME, the interglomeru-
lar vascular fraction was similar to the control kidneys but the glomerular vascular fraction was slightly less than the control kidneys (Table 3).

DISCUSSION

The purpose of this study was to examine the changes in intrarenal microvasculature in chronic NO-deficient hypertension and to evaluate the role of ANG II in these alterations. Overall, our results were consistent with a substantial amount of information concerning the action of NO and ANG II in mediating changes in renal function and structure. However, previous information has not been available in regards to microvessel density in renal tissue during the hypertension. To measure the changes in microvasculature, we used micro-CT, which provides a means to obtain quantitative volumetric information (6, 15, 22). NO is an important physiological antagonist of vasoconstrictors such as ANG II. By inhibiting the synthesis of NO with L-NAME, the effect of endogenous ANG II was revealed. In the absence of NO, there is an increase in total peripheral resistance, which is attenuated by the AT1 receptor blocker losartan (13, 33). Verhagen et al. (35) demonstrated that chronic inhibition of NO synthesis did not raise endogenous ANG II values but did cause detrimental effects on the kidney. Blockade of ANG II AT1 receptors reduced the detrimental effects. Other vasodilators, such as converting enzyme inhibitors, and calcium channel blockers also reduce the detrimental effects of NO-deficient hypertension (18, 26).

In the present study, the administration of L-NAME reduced 24-h NOx confirming the systemic inhibition of NO synthesis. When losartan was administered with L-NAME, the 24-h NOx remained at similar low levels. This dose of L-NAME elevated blood pressure, which is in agreement with other studies that showed that systemic NO blockade at different doses causes general vasoconstriction and hypertension (28, 30). In addition, there was a decrease in RBF and GFR as well as an increase in proteinuria, which accompanied the hypertension that has been previously reported (11, 25). Likewise, L-NAME treatment produced an elevation of PRA with respect to control values, indicating an activation of the renin-angiotensin system. Under these conditions, the significant fall on RBF may have been

Table 2. Tissue and vascular composition of kidneys from control (n = 6), L-NAME (n = 8), and L-NAME + losartan (n = 6)-treated rats

<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
<th>OSOM</th>
<th>ISOM</th>
<th>Papilla</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue volume, μl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>851 ± 68</td>
<td>243 ± 28</td>
<td>202 ± 17</td>
<td>41 ± 4</td>
</tr>
<tr>
<td>L-NAME</td>
<td>951 ± 44</td>
<td>315 ± 5*</td>
<td>233 ± 16</td>
<td>64 ± 3*</td>
</tr>
<tr>
<td>N + L</td>
<td>854 ± 40</td>
<td>310 ± 24</td>
<td>193 ± 7</td>
<td>60 ± 5*</td>
</tr>
<tr>
<td>Percentage of kidney volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>63.6 ± 0.7</td>
<td>18.0 ± 1.1</td>
<td>15.2 ± 1.1</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>L-NAME</td>
<td>60.8 ± 0.5*</td>
<td>20.3 ± 0.7</td>
<td>14.8 ± 0.5</td>
<td>4.1 ± 0.2*</td>
</tr>
<tr>
<td>N + L</td>
<td>60.3 ± 0.6*</td>
<td>21.8 ± 0.6*</td>
<td>13.7 ± 0.5</td>
<td>4.2 ± 0.2*</td>
</tr>
<tr>
<td>Vascular volume fraction of tissue, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>19.72 ± 1.61</td>
<td>18.24 ± 1.69</td>
<td>28.97 ± 2.61</td>
<td>24.81 ± 3.13</td>
</tr>
<tr>
<td>L-NAME</td>
<td>12.73 ± 0.45*</td>
<td>10.67 ± 0.16*</td>
<td>15.83 ± 0.61*</td>
<td>12.48 ± 0.31*</td>
</tr>
<tr>
<td>N + L</td>
<td>21.26 ± 0.93</td>
<td>18.03 ± 0.83</td>
<td>22.16 ± 0.69*</td>
<td>17.6 ± 1.04*</td>
</tr>
</tbody>
</table>

Values are means ± SE. OSOM, outer stripe outer medulla. ISOM, inner stripe outer medulla. *P < 0.05 vs. control rats.

Fig. 3. Midcoronal microcomputed tomography (micro-CT) 40-μm sections of kidneys from control (A), L-NAME (B), and L-NAME + losartan (C)-treated rats. Opacity of cortex, outer stripe of outer medulla (OSOM), inner stripe of outer medulla (ISOM), and inner medulla (IM) in each kidney indicate fraction of vasculature filled with Microfil. The contrast and grayscale range are the same in each section. The images are from the benchtop micro-CT scanner, and the displayed voxel size is 40 μm.
an important contributing factor on renin release. As expected, blockage of ANG II AT1 receptors with losartan during the inhibition of NO synthesis prevented the hypertension and most of the alterations in renal hemodynamics associated with L-NAME administration, suggesting that ANG II is an important factor mediating these alterations through the activation of AT1 receptors (11, 25). However, GFR remained lower than control values, indicating that GFR was more dependent on NO than ANG II. Erley et al. (8) also observed that losartan did not normalize GFR in L-NAME-treated rats and suggested that NO plays a major role in the regulation of GFR, which values could not be normalized in the absence of NO. Interestingly, in other studies (11), we demonstrated normalization in GFR after losartan treatment. However, the dose of losartan, the length of the treatment, and the strain of rats used for these experiments were different than those used in the present study. Furthermore, other factors that might be involved in hypertension induced by NO blockade could also influence GFR. For instance, the sympathetic nervous system has an effect on GFR and it is involved in the hypertension induced by NO blockade (27). In addition, other factors such as prostanooids and endothelin may influence GFR, renal hemodynamics, and excretory function during the chronic inhibition of NO (10).

The results of the present study demonstrate the powerful effect of endogenous ANG II on the preglomerular vasculature in the absence of NO synthesis. In the micro-CT images of kidneys from L-NAME-treated rats, there was a decrease in the vascular volume fraction, number, and diameter of the opacified glomeruli, which corresponded to the reduced GFR. Furthermore, as indicated by the interglomerular tissue opacity, the postglomerular, peritubular circulation was also reduced. It is unlikely that the reductions were a result of edema because the differences in total and relative amount of cortical and medullary tissues in the three groups were small. When the angiotensin AT1 receptors were blocked with losartan during the blockade of NO synthesis, the number and diameters of glomeruli were similar to control values and the cortical postglomerular circulation was preserved. However, the vascular fraction of the glomeruli was slightly lower than control values and corresponded to the low GFR values previously discussed.

The decreased number, diameter, and vascular fraction of glomeruli in the kidneys of L-NAME-treated rats were related to the glomerular sclerosis and atrophy observed in the histological study, and the capillaries of those glomeruli were not sufficiently opacified in the micro-CT images to be seen or counted. Pereira and Mandarim-de-Lacerda (24) observed glomerular

Table 3. Glomerular data of kidneys from control (n = 6), L-NAME (n = 8)-, and L-NAME + losartan (n = 6)-treated rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>L-NAME</th>
<th>N + L</th>
</tr>
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<tbody>
<tr>
<td><strong>Number of glomeruli</strong></td>
<td>36,267 ± 3,572</td>
<td>28,824 ± 839*</td>
<td>34,095 ± 1,537</td>
</tr>
<tr>
<td><strong>Glomerular diameter, μm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer cortex</td>
<td>160 ± 6</td>
<td>146 ± 5*</td>
<td>160 ± 4</td>
</tr>
<tr>
<td>Juxtamedullary cortex</td>
<td>156 ± 11</td>
<td>141 ± 5*</td>
<td>163 ± 4</td>
</tr>
<tr>
<td><strong>Glomerular vascular fraction, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer cortex</td>
<td>41 ± 2</td>
<td>24 ± 1*</td>
<td>36 ± 1</td>
</tr>
<tr>
<td>Juxtamedullary cortex</td>
<td>34 ± 1</td>
<td>21 ± 1*</td>
<td>33 ± 1</td>
</tr>
<tr>
<td><strong>Interglomerular vascular fraction, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer cortex</td>
<td>18 ± 1</td>
<td>11 ± 1*</td>
<td>20 ± 1*</td>
</tr>
<tr>
<td>Juxtamedullary cortex</td>
<td>16 ± 1</td>
<td>10 ± 1*</td>
<td>19 ± 1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. control rats.
glomerular atrophy in chronic L-NAME treatment. The erda (24) also noted tubular atrophy that accompanied tubular atrophy in both the group treated with pronounced after 28 days.

In addition, the kidneys used in our study were from rats treated with L-NAME for 19 days, whereas the rats in Pereira and Mandarim-de-Lacerda’s study (24) were treated for 40 days. It is possible that by 40 days, the nonatrophic, remnant glomeruli and nephrons had undergone further hypertrophy and expansion of the mesangial matrix than at the 19 days of treatment of the present study. In fact, Qiu et al. (28) observed that at 21 days of L-NAME treatment, the glomerular lesions were mild but were more pronounced after 28 days.

In addition to glomerular atrophy, we also observed tubular atrophy in both the group treated with L-NAME and the group treated with L-NAME and losartan. These results imply that the atrophy was mediated more by the lack of NO availability than by ANG II AT1 receptors. Pereira and Mandarim-de-Lacerda (24) also noted tubular atrophy that accompanied glomerular atrophy in chronic L-NAME treatment. The atrophy observed in the present study may have been partially a result of the decreased glomerular function. However, in the medulla, the atrophy may also have been a result of ischemic injury from hypoperfusion.

The proteinuria observed in rats receiving L-NAME most likely resulted from functional disruption of the glomerular wall as described by Arcos et al. (1). The tubular casts observed in the histology part of our study were most likely reflective of the proteinuria. In addition, cell debris from atrophic tubules may have contributed to the proteinuria. The proteinuria and renal casts were reduced in rats that received both L-NAME and losartan, suggesting that a major determinant was ANG II via AT1 receptors in the absence of NO. This change was unlikely due to elevated glomerular pressure because the administration of L-NAME caused an increase in afferent vascular resistance and concomitant decreases in GFR and RBF. Therefore, the results of the present study imply that altered glomerular permeability to protein was the primary factor and that the proteinuria was directly mediated by the effect of ANG II AT1 receptors.

The micro-CT data revealed that after chronic NO inhibition, there was a decrease in the fraction of cortical and medullary vasculature filled with Microfil. In the L-NAME kidneys with ANG II AT1 receptor blockade, although the cortical and outer medullary vascular fraction was similar to that of the control kidneys, the inner medullary vascular fraction was lower than that of the control kidneys. These results are in agreement with the fact that the cortical and outer medullary circulation is mainly dependent on ANG II AT1 receptors and that the inner medullary (papillary) circulation is highly dependent on NO availability. Previous studies (21, 23) showed that when NO was inhibited, the effects of ANG II on papillary circulation were small and appeared not to be an important controller of papillary blood flow. Furthermore, Zou and Cowley (38) showed that ANG II binding sites were absent in the inner medulla and were located primarily in the interstitial cells of the outer medulla located between the tubules and vasa recta. These cells have contractile properties and they may influence medullary blood flow, because they are able to secrete prostaglandins. The structural and hormonal differences between kidney regions could explain the differential responses in the outer and inner medullary circulation to chronic NO inhibition and ANG II receptor blockade.

During initial phases of NO inhibition, it is probable that the reductions in intrarenal blood flow are a result of vasoconstriction (21). However, during chronic NO inhibition, it is probable that the initial vasoconstriction progresses to rarefaction of the microvasculature as a consequence of pathological changes related to hypoperfusion. In the micro-CT images of the present study, it is unlikely that the microvessels were constricted so that the microvasculature was incompletely filled with Microfil for several reasons: before infusion with Microfil, the vasculature was flushed with calcium-free saline to provide vasorelaxation; the opacity values were above background levels; the micro-CT images showed that the venous circulation was filled with Microfil; and the high-resolution synchrotron micro-CT images showed Microfil in the microvessels down to ~10-μm diameter. In other studies, Qiu et al. (28) showed that administration of l-arginine in acute phases of NO inhibition reversed the hypertensive effect, but following chronic NO inhibition the administration of l-arginine did not reverse the hypertension. This observation implies mechanisms other than vasoconstriction were responsible for the chronic hypertension. In chronic NO inhibition, vascular pathology such as wall thickening and lipid deposition has been described previously (37). In rats receiving renal ischemic injury, there is a permanent reduction in the spatial density of peritubular capillaries and eventual interstitial fibrosis (2, 36).

A decrease in vascular filling, as a result of vascular rarefaction, would contribute to the increase in vascular resistance and hypoperfusion seen in the physiological part of this study as well as in previous studies published by us (21). The decreases in vascular volume fraction were greater in the medulla than in the cortex. This is in agreement with cortical and medullary blood flow measurements made in previous studies (21, 23). In the present study, the increase in blood pressure was not accompanied by a concomitant increase in sodium excretion. This could have been due to an
exaggerated decrease in medullary circulation, which is known to regulate sodium excretion (19). Furthermore, higher amounts of NO have been observed in the renal medulla with respect to the cortex (38). Consequently, it is likely that blockade of NO synthesis would produce a more pronounced effect on the medullary circulation.

In conclusion, hypertension after chronic NO synthesis inhibition is accompanied by alterations in renal function and anatomy. In this study, we demonstrated using micro-CT that chronic NO inhibition produces a generalized reduction in renal vascular volume fraction that may contribute to the reduction in whole kidney blood flow and excretory function. Because simultaneous AT1 receptor blockade maintains vascular volume fraction at control values, it is likely that altered intrarenal blood flow may be mediated by ANG II through AT1 receptors. Furthermore, the blockade of AT1 receptors counteracted most of the anatomic and functional alterations induced by L-NAME, indicating the major role of ANG II in the hypertension induced by inhibition of NO synthesis.

We thank K. Zodrow for the preparation of this manuscript and J. Patterson for the preparation of the figures. We thank P. E. Beighley, S. M. Jorgensen, D. A. Reyes, and M. S. Chmelik for technical assistance.

DISCLOSURES

This work was supported in part by National Institutes of Health Grants HL-16496 and EB-00305, Mayo Foundation, and the National Synchrotron Light Source, Brookhaven National Laboratory, which is supported by the U.S. Department of Energy, Division of Materials Sciences and Division of Chemical Sciences under contract no. DE-AC02–98CH10886. M. C. Ortiz was supported by a postdoctoral fellowship grant from the American Heart Association (#0029000129) and by the Spanish M. E. C. (PP-0029900129).

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