Chronic Nitric Oxide Synthase Inhibition Aggravates Glomerular Injury in Rats With Subtotal Nephrectomy

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ABSTRACT

Besides its glomerular hemodynamic effects, nitric oxide (NO) inhibits platelet aggregation and mesangial cell proliferation, two mechanisms possibly involved in the pathogenesis of glomerulosclerosis (GS). Chronic NO synthase inhibition in the rat leads to marked arterial hypertension and promotes glomerular and interstitial injury, but only mild GS. In this study, NO synthase blockade by nitro-L-arginine methyl ester (L-NAME) was associated with 5/6 nephrectomy, a well-known model of GS. Sixty-eight adult male Munich-Wistar rats were distributed among four groups: SHAM (no renal ablation or drug treatment), NX (5/6 nephrectomy), NX+NAME (5/6 nephrectomy and chronic treatment with L-NAME, 5 mg/dl in drinking water) and NX+NAME+L (as in group NX+NAME but also receiving the angiotensin II receptor inhibitor Losartan potassium (L), 25 mg/dl in drinking water). One week after ablation, rats of Group NX showed moderate glomerular hypertension and hypertrophy. Although glomerular enlargement was also modest in Group NX+NAME, glomerular hypertension was particularly severe in this group. Both alterations were absent in Group NX+NAME+L. Only incipient glomerular and interstitial injury occurred at this phase. Three weeks after ablation, renal structural injury was still modest in Group NX. By contrast, Group NX+NAME exhibited marked GS, glomerular ischemic injury, interstitial expansion, and creatinine retention. Renal injury was largely prevented in Group NX+NAME+L. Tuft enlargement occurred in all groups but was most prominent in Group NX. NO synthase inhibition aggravates parenchymal injury and functional impairment in the remnant kidney by mechanisms that may involve glomerular hypertension and renin-angiotensin activation but that appear to be unrelated to glomerular enlargement.

Key Words: Progressive glomerulopathy, renal ablation, nitric oxide, glomerular hypertrophy, glomerular hypertension

The mechanisms leading to the development of progressive glomerulosclerosis (GS) in humans and in several experimental models of renal disease are far from established. The pathogenesis of GS may include glomerular hypertension (1-3), glomerular hypertrophy (4,5), abnormal proliferation of glomerular cells (6), intracapillary thrombosis (3,7), and macrophage activation (8), among others.

Given the multiple effects of nitric oxide (NO) on hemodynamic and nonhemodynamic biologic phenomena (9-14), inhibition of its biosynthesis might facilitate GS by at least three mechanisms: (1) intracapillary hypertension; (2) enhanced cell proliferation; and (3) enhanced platelet activity. We and others have recently shown that the chronic inhibition of NO promotes arterial hypertension and glomerular ischemic injury in the rat (15,16). However, we found only slight GS in rats undergoing NO synthase blockade for up to 45 days (16), suggesting that glomerular hypertension, enhanced platelet activity, and/or increased mitogenic capability may require additional glomerular derangement to promote GS of appreciable severity within this period of observation. We provided additional support for this notion by showing that dietary salt overload exacerbates GS in rats with chronic NO synthase blockade (11).

In this study, we combined chronic NO synthase inhibition and 5/6 renal ablation (NX), a well-known model of progressive GS (1). By doing so, we were able to demonstrate that interaction between NX and NO synthase inhibition leads to the development of much more severe GS than would be obtained with either of these procedures alone.

METHODS

Ablation Procedure and Experimental Groups

Sixty-eight adult male Munich-Wistar rats weighing 245 to 275 g, obtained from an established colony at the University of São Paulo, were used in this study. All rats received food (22% protein) and tap water ad libitum. Fifty-two rats were subjected to NX before study. For this purpose, a ventral
laponotomy was performed under aseptic conditions after anesthesia with sodium nembutal, 50 mg/kg ip. The right kidney was then removed, while two-thirds of the left kidney underwent acute infarction by ligation of two first-order branches of the main renal artery (1). Recovery from anesthesia and from the surgical procedure was complete within 24 h. Rats were distributed among the following four experimental groups.

(1) SHAM (N = 16). In this group, rats underwent a ventral laparotomy under anesthesia as described. However, only handling of the renal pedicle without the removal of renal mass was performed. (2) NX (N = 16). Rats in this group were subjected to 5/6 renal ablation as described. (3) NX + NAME (N = 20). Rats were handled as in Group NX but also received the NO synthase inhibitor nitro-l-arginine methyl ester (l-NAME), dissolved in the drinking water at 5 mg/dL, corresponding to an individual daily ingestion of approximately 6 mg/kg. (4) NX + NAME + L (N = 16). Rats were treated as in Group NX + NAME but also received the angiotensin II inhibitor Losartan potassium (L), formerly DuP753, 25 mg/dL in the drinking water, corresponding to a daily ingestion of approximately 35 mg/kg. l-NAME and losartan treatments were started on the day after the ablation procedure and were maintained until euthanasia. Individual values for food intake were similar among groups.

Short-Term Studies

To examine renal functional and structural parameters, eight rats of each group were subjected to micropuncture experiments after 1 wk of treatment. On the morning of the experiment, 400-μL blood samples were obtained from a tail vein for plasma renin activity (PRA) determination in Groups SHAM, NX, and NX + NAME. Rats from these groups and from Group NX + NAME + L were then anesthetized with an ip injection of fentanyl (100 mg/kg body wt) and placed on a heated table. Rectal temperature was maintained at 37 ± 0.5°C. The left femoral artery was catheterized with PE-50 polyethylene tubing for the determination of baseline arterial hematocrit and subsequent periodic blood sampling, as well as for continuous monitoring of mean arterial pressure with a P23Db Statham pressure transducer connected to a chart recorder (Model AM8200; Anamed, São Paulo, Brazil). After tracheotomy, the jugular veins were cannulated with PE-50 polyethylene tubing for the infusion of homologous plasma and inulin. Saline solution containing 14C-labeled inulin (2 μCi/mL) was infused at the rate of 1.5 mL/h throughout the experiment. A continuous infusion of homologous plasma was maintained throughout the experiment to replace surgical fluid losses (17). An amount of plasma equivalent to 1% body weight was given for 45 min, followed by an infusion of 0.5 to 1.0 mL/h until the end of the experiment. The left kidney was exposed, freed from the adrenal gland and perirenal fat, and immobilized with a Lucite holder. Isotonic saline was slowly and continuously dripped on the renal surface. The left ureter was cannulated with PE-10 polyethylene tubing for urine collection. After about 2.5 h of anesthesia, urine was collected for 30 to 40 min for the determination of flow rate and inulin concentration. Hydraulic pressures in superficial glomerul (PsOd), tubes, and arterioles were determined with a servo-nulling device (Model V; Instrumentation for Physiology and Medicine, San Diego, CA). Whole-kidney filtration fraction (PF) was determined by the simultaneous collection of blood samples from the left femoral artery and renal vein and the assessment of the respective 14C activities to calculate inulin extraction. Blood samples were obtained from the renal vein with a sharpened glass micropipette, 40- to 45-μm OD. Plasma and urine 14C activities were measured in a scintillation counter (Beckman Instruments, Schiller Park, IL). RPF was calculated as RPF = GFR/FF. Renal vascular resistance (RVR) was estimated by the expression RVR = MAP(1 - Ht)/RPF, where Ht represents arterial hematocrit. At the end of the experiment, the renal tissue was perfusion fixed in situ with Dubosq-Brazil solution after a brief washout with saline. After fixation, two midcortical slices of the right kidney were embedded in paraffin, and 2- to 3-μm-thick sections were stained by the periodic acid–Schiff reaction for examination by light microscopy. Additional sections were stained with Masson trichrome or silver methenamine.

Long-Term Studies

Eight rats from Group SHAM, 8 from Group NX, 12 from Group NX + NAME, and 8 from Group NX + NAME + L were monitored for up to 21 days of treatment. Awake systemic arterial pressure, evaluated by a tail-cuff method (18), and urinary albumin excretion rate were examined at Days 7 and 21. At the end of the study, blood was collected from a tail vein in Groups SHAM, NX, and NX + NAME for the determination of PRA and plasma creatinine concentration. Rats of all groups were then anesthetized with sodium pentobarbital, 50 mg/kg ip; the remnant kidneys were perfusion fixed, weighed, and prepared for histologic examination as described above.

Analytical

The urinary albumin excretion rate was determined by a radial immunodiffusion technique (19). PRA was measured by an enzymatic technique (20), adapted for small samples. Total plasma protein concentration was determined by the biuret reaction (21).

Histologic Techniques

The extent of GS was evaluated quantitatively as described previously (22). Briefly, a score was attributed to each glomerulus according to the extent of sclerotic injury: 0, intact glomeruli; 1, lesions affecting 25% or less of the glomerular area; 2, lesions affecting 25 to 50% of the glomerular area; 3, lesions affecting 50 to 75% of the glomerular area; and 4, lesions involving more than 75% of the glomerular area. A GS index (GSI) was calculated for each rat as the weighted average of all individual glomerular scores obtained. At least 150 glomeruli were examined for each rat. The extent of glomerular collapse and glomerular necrosis was also quantitatively evaluated according to criteria described previously (11) and detailed in the Results section. The frequency of each type of glomerular lesion was expressed as a percentage of the total number of glomeruli examined. To assess the extent of interstitial expansion, the fraction of renal cortex occupied by interstitial tissue staining positively for collagen was quantitatively evaluated in Masson-stained sections by a point-counting technique (23) in 25 consecutive microscopic fields, with the same ocular grid and the same magnification as for determination of glomerular tuft volume (Vg).

The average Vg of each rat was estimated by the method of Weibel (24), after light microscopic examination at a final magnification of ×320 under a 100-point ocular grid. The corresponding microscopic field covered an area of 135,532 μm². The mean glomerular random cross-sectional area (Acr) was determined for each rat by averaging individual values for at least 50 randomly sampled glomerular tuft profiles (56, 1499)
Renal Ablation and Nitric Oxide

on average). Individual glomerular values were calculated by counting points falling within the glomerular area. $V_Q$ was then calculated as: $V_Q = 1.25 \cdot (A_Q)^{3/2}$. Collapsed glomeruli were not considered in the determination of $V_Q$.

Statistics

One-way analysis of variance with six preplanned pairwise comparisons according to the Bonferroni method was used in this study (26). A paired $t$ test was also used where appropriate. Statistical significance was considered at $P$ levels of 0.05 or less. Because percentages and albumin excretion rates were not normally distributed, log transformation (for albuminuria) and arc sine transformation (for percentages) were performed before statistical analysis.

RESULTS

Short-Term Studies

Renal and systemic functional and hemodynamic parameters at 7 days of NO synthase inhibition are displayed in Table 1. Body growth was moderately retarded in rats with reduced renal mass (Groups NX, NX+NAME, and NX+NAME+L). Kidney weight did not differ significantly among groups at this stage, indicating marked hypertrophy of renal mass in rats with NX. As described previously (1), blood pressure was elevated in NX rats, reaching 128 ± 4 mm Hg (110 ± 2 in SHAM; $P < 0.05$). Mean arterial pressure was also further elevated in rats receiving $l$-NAME (142 ± 6 mm Hg; $P < 0.05$ versus SHAM), although the difference relative to Group NX failed to reach statistical significance. Blood pressure remained at levels slightly above control in Group NX+NAME+L (124 ± 3 mm Hg; $P < 0.05$ versus NX+NAME; $P > 0.05$ versus SHAM). Whole-kidney GFR fell by 40% after NX (0.68 ± 0.07 mL/min in Group NX versus 1.12 ± 0.04 in SHAM; $P < 0.05$), indicating the occurrence of renal functional hypertrophy, because renal mass was reduced by 67% in these rats. The administration of $l$-NAME or Losartan promoted no further alteration in GFR (0.71 ± 0.08 in Group NX+NAME and 0.71 ± 0.04 in Group NX+NAME+L; $P > 0.4$ versus Group NX). Only Group NX+NAME exhibited a significant elevation in filtration fraction compared with control. As described previously for the ablation model (1), $P_{OC}$ was considerably elevated in Group NX (66 ± 2 mm Hg versus 53 ± 1 in sham-operated rats; $P < 0.05$). Chronic $l$-NAME treatment increased $P_{OC}$ to extremely high levels in Group NX+NAME (86 ± 4 mm Hg). Losartan treatment restored $P_{OC}$ to values similar to those seen in Group NX (67 ± 2 mm Hg; $P < 0.05$ versus SHAM and NX+NAME). Plasma protein concentration was slightly but significantly increased in Group NX+NAME (6.1 ± 0.1 g/dL versus 5.6 ± 0.1 in SHAM). RVR increased predictably in Group NX (38.1 ± 3.7 mm Hg/mL per minute versus 16.2 ± 0.6 in controls; $P < 0.05$). $l$-NAME treatment overload promoted a further numerical increase in RVR (45.0 ± 4.5 mm Hg/mL per minute; $P > 0.05$ versus NX). PRA tended to be higher than control in Groups NX and NX+NAME (7.6 ± 1.6 and 7.3 ± 3.8 ng of angiotensin I (AI)/mL per hour versus 3.8 ± 0.5 in controls; $P > 0.05$), although these differences failed to reach statistical significance.

Kidneys were uniformly perfused in all groups studied. In rats observed at 1 wk, and more often in those studied at 3 wk after ablation, microscopic examination of renal tissue disclosed four types of parenchymal injury: (1) GS, which appeared as hyaline deposits, associated with collapse of capillary loops in a portion of the glomerular area (Figure 1a) or, less often, in its entirety (Figure 1c). These areas were usually associated with adhesions to Bowman's capsule and stained in blue by the Masson technique, suggesting collagen deposition. Many of these areas were distinctly delimited from adjacent healthy tissue (Figure 1b), in contrast with the segmental sclerotic lesions previously described in renal ablation and other experimental models (1–3). (2) Glomerular collapse (Figure 2), consisting of reduction in tuft size, closure of capillary loops, and thickening of the basement membrane without adhesions to Bowman's capsule. (3) Glomerular necrosis, appearing as very sharply delineated hyalinized areas that stained negatively for silver methenamine and red with Masson trichrome. Lysis of necrotic material, with formation of microaneurysms, occurred frequently in these areas (Figure 3a). Other lesions stained blue by the Masson technique, suggesting collagen deposition and incipient organization (Figure 3b). (4) Interstitial expansion

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt (g)</th>
<th>Left Kidney Wt (g)</th>
<th>MAP (mm Hg)</th>
<th>GFR (mL/min)</th>
<th>FF</th>
<th>$P_{OC}$ (mm Hg)</th>
<th>$P_{prot}$ (g/dL)</th>
<th>RVR (mm Hg/mL per minute)</th>
<th>PRA (ng of AI/mL per hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>266 ± 3</td>
<td>1.05 ± 0.02</td>
<td>110 ± 2</td>
<td>1.12 ± 0.04</td>
<td>0.31 ± 0.01</td>
<td>53 ± 1</td>
<td>5.6 ± 0.1</td>
<td>16.2 ± 0.6</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>NX</td>
<td>253 ± 6</td>
<td>1.00 ± 0.08</td>
<td>128 ± 4</td>
<td>0.68 ± 0.07</td>
<td>0.34 ± 0.01</td>
<td>66 ± 2</td>
<td>5.5 ± 0.2</td>
<td>38.1 ± 3.7</td>
<td>7.6 ± 1.6</td>
</tr>
<tr>
<td>NX+NAME</td>
<td>249 ± 6</td>
<td>0.95 ± 0.03</td>
<td>142 ± 6</td>
<td>0.71 ± 0.08</td>
<td>0.38 ± 0.02</td>
<td>86 ± 4</td>
<td>6.1 ± 0.1</td>
<td>45.0 ± 4.5</td>
<td>7.3 ± 3.8</td>
</tr>
<tr>
<td>NX+NAME+L</td>
<td>256 ± 3</td>
<td>0.90 ± 0.03</td>
<td>124 ± 3</td>
<td>0.71 ± 0.04</td>
<td>0.37 ± 0.02</td>
<td>67 ± 2</td>
<td>6.0 ± 0.2</td>
<td>36.7 ± 4.1</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Results are expressed as mean ± 1 SE. Abbreviations: MAP, mean arterial pressure; FF, filtration fraction; $P_{OC}$, glomerular hydraulic pressure; $P_{prot}$, plasma total protein concentration; ND, not determined.

$P < 0.05$ versus SHAM.

$P < 0.05$ versus NX.

$P < 0.05$ versus SHAM and NX+NAME.

$P < 0.05$ versus NX+NAME.
Figure 1. (a) Segmental GS typically found in Groups NX and NX+NAME. Deposition of a hyaline material, staining blue by the Masson technique, is readily seen in association with collapse of the capillary loops and an extensive adhesion to Bowman's capsule. Masson trichrome in 3-μm-thick section. (b) Segmental GS in a sharply delineated area of the glomerular tuft. Hyalinization, collapse of the capillary loops, and adhesion to Bowman's capsule are seen in the sclerosed area. This type of lesion was observed exclusively in Group NX+NAME. Masson trichrome in 3-μm-thick section. (c) Global sclerosis of the glomerular tuft. Masson trichrome in 3-μm-thick section. (Figure 4), consisting of focal enlargement of the interstitial area, with cell infiltration and deposition of a collagen-like material, frequently in association with tubular atrophy and vacuolization. Extensive, sometimes confluent areas of interstitial injury and tubular atrophy were observed in more severely affected animals. Microvascular lesions ranged from simple thickening of the arteriolar wall to nearly complete luminal obstruction and fibrinoid necrosis of the vessel.

Parameters quantifying glomerular injury at 1 wk of NX are shown in Table 2. The urinary albumin excretion rate increased markedly in Group NX (74.7 ± 11.2 mg/24 h versus 0.9 ± 0.1 in SHAM; \(* P < 0.05\)). Simultaneous L-NAME treatment did not worsen albuminuria in Group NX+NAME (60.3 ± 3.8 mg/24 h; \(* P > 0.2\) versus NX). Losartan treatment attenuated albuminuria in Group NX+NAME+L (20.3 ± 8.8 mg/24 h; \(* P < 0.05\) versus NX and NX+NAME). The prevalence of glomeruli with GS or necrotic lesions was very small in all groups at this stage, whereas the percentage of collapsed glomeruli was slightly increased in Group NX+NAME (1.7 ± 0.5% versus 0.1 ± 0.1 in SHAM; \(* P < 0.05\)). The fraction of renal parenchyma occupied by interstitial tissue was higher in Groups NX (7.1 ± 1.4% versus 1.2 ± 0.1 in SHAM; \(* P < 0.05\)) and NX+NAME (6.6 ± 1.2%; \(* P < 0.05\) versus SHAM). Losartan treatment limited interstitial expansion (3.3 ± 0.6% in Group NX+NAME+L; \(* P > 0.2\) versus SHAM).

Long-Term Studies

Parameters evaluating renal function and systemic hemodynamics after 3 wk of ablation are shown in Table 3. Body weight was slightly and not significantly lower in Group NX (275 ± 7 g body wt versus 296 ± 5 in the SHAM Group; \(* P > 0.05\)). By contrast, body growth was clearly stunted in Group NX+NAME (238 ± 8 g; \(* P < 0.05\) versus SHAM and NX). Losartan treatment restored body growth in Group NX+NAME+L (272 ± 5 g; \(* P > 0.05\) versus SHAM). Nearly 33% of rats (4 of 12) in Group NX+NAME died spontaneously before renal functional or morphologic examination could be performed. Mortality was zero in the other groups. As expected, the plasma creatinine concentration was high in Group NX compared with that in sham-operated controls (1.14 ± 0.07 mg/dL versus 0.55 ± 0.02 in SHAM; \(* P < 0.05\)). Concomitant L-NAME treatment exacerbated creatinine retention (1.50 ± 0.10 mg/dL; \(* P < 0.05\) versus NX). Losartan treatment restored plasma creatinine concentration to levels similar to those seen in Group NX (1.06 ± 0.16 mg/dL in Group NX+NAME+L; \(* P < 0.05\) versus NX+NAME). As described previously, systemic arterial pressure, evaluated by a tail-cuff method, was markedly elevated at this stage in Group NX.
Figure 3. (a) Segmental necrosis of the capillary tuft, with lysis of necrotic tissue and formation of a microaneurysm. Masson trichrome in 3-μm-thick section. (b) Segmental necrosis of the capillary tuft. The necrotic tissue (arrow) was almost completely replaced by material staining blue by the Masson technique, suggesting collagen deposition. Masson trichrome in 3-μm-thick section.

Figure 4. Focal expansion of the interstitial area, with cell infiltration, deposition of a collagen-like material, and tubular atrophy. Masson trichrome in 3-μm-thick section.

(161 ± 6 mm Hg versus 123 ± 2 in controls; P < 0.05). L-NAME treatment aggravated arterial hypertension in Group NX+NAME (191 ± 7 mm Hg; P < 0.05 versus SHAM and NX). In losartan-treated rats, tail-cuff pressure decreased to levels only slightly above control (132 ± 8 mm Hg; P < 0.05 versus NX and NX+NAME). PRA was close to control in Group NX (4.7 ± 0.8 ng of AI/mL per hour versus 4.4 ± 0.6 in SHAM; P > 0.05). PRA increased numerically in Group NX+NAME (9.8 ± 3.8 ng of AI/mL per hour; P > 0.05 versus control), although this difference failed to attain statistical significance. Of note, PRA varied widely in this group, ranging from 1.3 to 26.3 ng of AI/mL per hour.

A quantitative evaluation of renal structural injury 3 wk after renal ablation is given in Table 4. As in rats studied at 1 wk, the urinary albumin excretion rate was markedly increased relative to control (61.6 ± 13.4 mg/24 h versus 1.7 ± 0.1 in SHAM; P < 0.05). Albuminuria of similar magnitude appeared in Group NX+NAME (61.5 ± 3.9 mg/24 h; P < 0.05 versus SHAM). Simultaneous treatment with losartan limited but did not prevent albuminuria in Group NX+NAME+L (31.0 ± 10.2; P > 0.05 versus SHAM). Perfused kidney weight was slightly lower in Group NX than in SHAM (1.28 ± 0.04 g versus 1.54 ± 0.02; P < 0.05), again indicating considerable renal hypertro-
TABLE 2. Quantitative assessment of renal parenchymal injury 1 wk after NX*

<table>
<thead>
<tr>
<th>Group</th>
<th>Ualb·V (mg/24 h)</th>
<th>V₉ (10⁶ μm³)</th>
<th>GSI</th>
<th>%INT</th>
<th>%COLL</th>
<th>%NECR</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>0.9 ± 0.1</td>
<td>0.83 ± 0.03</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>NX</td>
<td>74.7 ± 11.2b</td>
<td>1.31 ± 0.09a</td>
<td>1.2</td>
<td>0.8</td>
<td>3.1</td>
<td>1.54</td>
</tr>
<tr>
<td>NX+NAME</td>
<td>60.3 ± 3.8b</td>
<td>1.32 ± 0.05b</td>
<td>2.0</td>
<td>1.0</td>
<td>6.6</td>
<td>1.7</td>
</tr>
<tr>
<td>NX+NAME+L</td>
<td>20.3 ± 8.6c,d</td>
<td>1.03 ± 0.06c,d</td>
<td>0.9</td>
<td>0.5</td>
<td>3.3</td>
<td>0.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results expressed as mean ± 1 SE. Abbreviations: Ualb·V, urinary albumin excretion rate; V₉, glomerular volume; %INT, fraction of renal cortical tissue occupied by interstitium; %COLL, frequency of collapsed glomeruli; %NECR, frequency of glomeruli exhibiting segmental necrotic lesions.
<sup>b</sup> P < 0.05 versus SHAM.
<sup>c</sup> P < 0.05 versus NX.
<sup>d</sup> P < 0.05 versus NX+NAME.

TABLE 3. Renal and systemic functional and hemodynamic parameters 3 wk after NX*.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt (g)</th>
<th>CREAT (mg/dL)</th>
<th>TCP (mm Hg)</th>
<th>PRA (ng of AI/mL per hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>296 ± 5</td>
<td>0.55 ± 0.02</td>
<td>123 ± 2</td>
<td>4.4 ± 0.6</td>
</tr>
<tr>
<td>NX</td>
<td>275 ± 7</td>
<td>1.14 ± 0.07b</td>
<td>161 ± 6b</td>
<td>4.7 ± 0.8</td>
</tr>
<tr>
<td>NX+NAME</td>
<td>238 ± 8b,c</td>
<td>1.50 ± 0.10b,c</td>
<td>191 ± 7b,c</td>
<td>9.8 ± 3.8</td>
</tr>
<tr>
<td>NX+NAME+L</td>
<td>272 ± 6a</td>
<td>1.06 ± 0.16b,d</td>
<td>132 ± 8d</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results expressed as mean ± 1 SE. Abbreviations: CREAT, plasma creatinine concentration; TCP, tail-cuff pressure; ND, not determined.
<sup>b</sup> P < 0.05 versus SHAM.
<sup>c</sup> P < 0.05 versus NX.
<sup>d</sup> P < 0.05 versus NX+NAME.

TABLE 4. Quantitative assessment of renal parenchymal injury 3 wk after NX*.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ualb·V (mg/24 h)</th>
<th>Left Kidney Wt (g)</th>
<th>V₉ (10⁶ μm³)</th>
<th>GSI</th>
<th>%INT</th>
<th>%COLL</th>
<th>%NECR</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>1.7 ± 0.1</td>
<td>1.54 ± 0.02</td>
<td>0.89 ± 0.03</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>NX</td>
<td>61.6 ± 13.4b</td>
<td>1.28 ± 0.04b</td>
<td>1.91 ± 0.11b</td>
<td>5.9</td>
<td>1.5</td>
<td>5.9</td>
<td>1.0</td>
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<tr>
<td>NX+NAME</td>
<td>61.5 ± 3.9b</td>
<td>1.02 ± 0.04b,c</td>
<td>1.55 ± 0.06b,c</td>
<td>25.8</td>
<td>4.7b,c</td>
<td>12.9</td>
<td>2.8b,c</td>
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<tr>
<td>NX+NAME+L</td>
<td>31.0 ± 10.2</td>
<td>1.19 ± 0.06b,d</td>
<td>1.33 ± 0.11b,c</td>
<td>3.6</td>
<td>1.1</td>
<td>4.2</td>
<td>0.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results expressed as mean ± 1 SE. Abbreviations: Ualb·V, urinary albumin excretion rate; V₉, glomerular volume; %INT, fraction of renal cortical tissue occupied by interstitium; %COLL, frequency of collapsed glomeruli; %NECR, frequency of glomeruli exhibiting segmental necrotic lesions.
<sup>b</sup> P < 0.05 versus SHAM.
<sup>c</sup> P < 0.05 versus NX.
<sup>d</sup> P < 0.05 versus NX+NAME.

phy in this group. Renal enlargement was more modest in Group NX+NAME (1.02 ± 0.04 g; P < 0.05 versus SHAM and NX), whereas Group NX+NAME+L exhibited intermediate values (1.19 ± 0.06 g; P < 0.05 versus SHAM and NX+NAME). Glomerular volume was nearly doubled in Group NX compared with that in sham-operated controls (1.91 ± 0.11 · 10⁶ μm³ versus 0.89 ± 0.03 in SHAM; P < 0.05). Glomerular hypertrophy was less pronounced in Group NX+NAME (1.55 ± 0.06 · 10⁶ μm³; P < 0.05 versus SHAM and NX) and only modest in Group NX+NAME+L (1.33 ± 0.11 · 10⁶ μm³; P < 0.05 versus SHAM). The development of GS, evaluated by the GSI, was evident in Group NX (5.9 ± 1.5 versus 0.1 ± 0.1 in SHAM; P > 0.05), although the difference relative to control was not statistically significant. GSI was over fourfold higher in Group NX+NAME (25.8 ± 4.7; P < 0.05 versus SHAM and NX). Losartan treatment reduced GSI to much lower levels, although still numerically higher than in SHAM (3.6 ± 1.1; P < 0.05 versus NX+NAME). Collapsed glomeruli were virtually absent in Groups SHAM and NX, but represented 4.4 ± 1.1% of the glomeruli examined in Group NX+NAME (P < 0.05 versus SHAM and NX). Tuft collapse appeared in only 0.9 ± 0.6% of the glomeruli in Group NX+NAME+L, a value not statistically different from that observed in the SHAM group. Segmental necrotic lesions were absent in the SHAM and NX groups. The frequency of necrotic glomeruli was 0.5 ± 0.4% in Group NX+NAME, a value not significantly different from that in SHAM or NX. A larger proportion of necrotic lesions occurred in Group NX+NAME+L (2.1 ± 0.7%; P < 0.05 versus SHAM and NX). The magnitude of interstitial injury correlated roughly with the
extent of GS: in Group NX, 5.9 ± 1.0% of the cortical parenchyma was occupied by interstitial tissue (3.1 ± 0.3% in SHAM; \(P > 0.05\)). This proportion rose to 12.9 ± 2.8% in Group NX+NAME (\(P < 0.05\) versus SHAM and NX), whereas losartan treatment largely prevented interstitial expansion in Group NX+NAME+L (4.2 ± 0.8%; \(P < 0.05\) versus NX+NAME). Mild microvascular lesions, consisting mostly of arteriolar wall thickening, appeared in Groups NX and NX+NAME+L. More severe lesions, such as complete luminal obstruction, fibrinoid necrosis of the vessel wall, and peritubular interstitial fibrosis, occurred predominantly in Group NX+NAME.

**DISCUSSION**

As described previously (1,3), renal ablation was associated in this study with marked renal hypertrophy and hyperfiltration, because left kidney weight and whole-kidney GFR decreased less than would be expected, given the initial reduction of renal mass. The superimposition of chronic NO synthase blockade had no effect on GFR and increased RVR only slightly, suggesting that NO has little participation in the renal hemodynamic adaptations associated with high-grade NX. Our findings are consistent with those recently reported by Griffin and associates (27). Those investigators examined the acute effect of NO synthase inhibition in rats with NX, uninephrectomy, or sham operation. They noted that the renal hemodynamic effects of NO synthase inhibition (systemic hypertension, renal vasoconstriction, and reduced GFR) were quite comparable in the three groups that they studied. We now show that, contrasting with the small effect of NO synthase inhibition on whole-kidney hemodynamics, chronic l-NAME treatment dramatically aggravates the glomerular hypertension characteristic of this model, indicating a profound derangement of the intrarenal microcirculation (most likely represented by afferent vasodilatation and/or efferent vasoconstriction).

Chronic NO synthase inhibition markedly aggravated the renal structural lesions associated with NX. Rats subjected to NX alone had only mild renal injury 3 wk after nephrectomy, as would be expected given the relatively short period of observation. By contrast, nephrectomized rats also receiving l-NAME treatment exhibited advanced glomerular lesions, complete hyalinization of a large number of glomeruli, and creatinine retention, indicating the occurrence of functional and structural renal compromise. Renal injury may have been even worse in this group, given the relatively high proportion of animals that died prematurely and whose kidneys, in all likelihood more severely affected, could not be examined in detail. These findings suggest the existence of a strong pathogenetic interaction between NX and chronic NO synthase inhibition to promote GS, although the intimate mechanisms mediating this interaction are unclear. Aggravation of glomerular injury in the remnant kidney by concomitant l-NAME treatment may have resulted from the more severe systemic hypertension observed in these rats. This hypothesis is consistent with the finding that limiting hypertension with Losartan largely prevented GS in this group. Previous studies of NX have suggested that systemic hypertension may be central to the development of progressive GS (28). However, the relatively low dose of l-NAME used in this study led to hypertension comparable to that encountered in models such as chronic NO synthase inhibition (11,15,16), the spontaneously hypertensive rat (29), or the Milan hypertensive rat (30), none of which is characterized by the rapid development of severe GS. In fact, glomerular injury develops more readily in Milan normotensive rats than in the related hypertensive strain (30).

Glomerular hypertension has been implicated as a potential factor of glomerular injury in such disparate models as NX (1), diabetes mellitus (2), and DOC-salt hypertension (31), among others. Mechanical injury originating from high \(P_{oc}\) may help explain the progression of GS in this study. High-grade glomerular injury was associated with extremely elevated \(P_{oc}\) in Group NX+NAME, whereas concomitant losartan treatment lowered both \(P_{oc}\) and GS to levels similar to those seen with NX alone. However, we reported previously that glomerular hypertension of similar magnitude coexisted with much less GS in rats treated with l-NAME and salt overload (11), suggesting that nonhemodynamic factors may also have contributed to the progressive GS observed in Group NX+NAME. Recent evidence has suggested that glomerular hypertrophy, in association with glomerular hypertension (5) or as an independent risk factor (4), may also contribute to the development of GS. Marked glomerular hypertrophy was indeed demonstrated in Group NX, in agreement with previous observations in the remnant model (1,3). However, glomerular hypertrophy cannot explain the more severe GS found in Group NX+NAME, because glomerular tuft enlargement was less prominent in these animals.

GS was not the only conspicuous finding in the complex renal histologic picture observed in Group NX+NAME. Global collapse of the capillary tuft, consistent with severe glomerular ischemia and entirely distinct from GS, appeared in nearly 5% of glomeruli in this group, as previously described in intact rats receiving chronic l-NAME treatment (11,16). In addition to the large morphologic difference between collapsed and sclerotic glomeruli, the mechanisms underlying these two entities also appear to differ widely: although GS relates at least in part to increased transmission of arterial pressure to the glomerular microcirculation, hence to high \(P_{oc}\), tuft collapse is linked to afferent arteriolar occlusion and capillary hypoperfusion. The reasons for this nonuniform effect of chronic NO synthase blockade among glomeruli of the same kidney are unclear.

Collapsed glomeruli similar to those encountered in this study were described by Meyer and Rennke (32) in

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**References**

rats with partial nephrectomy. Correa-Rotter and coworkers (33) showed increased renin expression in association with these glomeruli, suggesting that they may constitute a source of uncontrolled renin production and inadequately high levels of angiotensin II activity. Indeed, we showed evidence that these glomeruli may influence the circulating levels of renin (11,16). These results lend further support to this concept, because the presence of collapsed glomeruli, almost exclusively confined to Group NX+NAME, was associated with severe arterial and glomerular hypertension and a trend toward elevated PRA at 3 wk of ablation. Nevertheless, it should be noted that enhanced renin production might result directly from NO synthase blockade, because NO has been reported to inhibit renin secretion from renal tissue (34), although conflicting results have been found by other investigators (35).

Segmental fibrinoid necrosis of the glomerular tuft was evident in Group NX+NAME at 3 wk of ablation. These lesions were described previously in association with DOC-salt hypertension (31) and, more recently, chronic NO synthase inhibition (11,16). Some of these lesions evolved toward the formation of microaneurysms, whereas signs of collagen deposition and organization were visible in others. The finding of very sharply delineated scarred areas in a large proportion of glomeruli suggests that, in Group NX+NAME, GS may have partly resulted from the progressive organization of these necrotic areas. Consistent with this hypothesis is the finding that urinary albumin levels were similar between Groups NX and NX+NAME, despite major differences in the levels of GS: low or normal albumin output would be indeed expected if glomerular scarring were largely a consequence of ischemic injury. In addition, the frequency of necrotic lesions was higher in Group NX+NAME+L an unexpected finding given the very low degree of GS seen in this group. This observation might simply indicate that scattering of necrotic tissue is slower in these animals.

The adverse effects of simultaneous ablation and L-NAME treatment on renal structure were not confined to the glomerulus. A mild expansion of the cortical interstitial area, with cell infiltration and deposition of a collagen-like material, was evident in Groups NX and NX+NAME 1 wk after ablation. Although this process was not progressive in Group NX, its severity increased at 3 wk in Group NX+NAME, paralleling the evolution of GS. We had shown previously that renal interstitial fibrosis is one of the most striking features observed in rats undergoing chronic NO synthase inhibition and that this process is aggravated by concomitant salt overload (11). In the last two decades, several investigators emphasized the importance of chronic interstitial injury in the pathogenesis of progressive nephropathies (23,36–38). Risdon and coworkers (37) showed that, in chronic nephropathies, the degree of interstitial inflammation was a better predictor of renal functional deterioration than was the extent of glomerular injury, a finding subsequently confirmed by others (23,36,38). Interstitial fibrosis often represents the main manifestation of chronic renal disease, such as in cyclosporine toxicity (39) and adriamycin nephrosis (40). Thus, the development of more prominent interstitial expansion in Group NX+NAME may have contributed, in association with more severe glomerular injury, to the renal functional impairment observed in these animals.

The rapid organization of glomerular necrotic tissue and the development of chronic interstitial injury observed in Group NX+NAME suggest that renal cell proliferation may have been enhanced in these animals. Increased cell proliferation and the effect of growth factors may indeed underlie the progression of both GS (6) and interstitial fibrosis (36) in NX as well as in other experimental models. Because NO inhibits the proliferation of leukocytes (12,13), smooth muscle (14), and mesangial cells (9), chronic NO synthase inhibition may have favored mitosis and inflammation at the glomerular and interstitial areas. On the other hand, high-grade NX may facilitate mitogenesis at the glomerulus, as shown by Floege and coworkers (6), and at the interstitial area, as indicated by the modest interstitial expansion observed in this study with NX alone. Thus, the association of NX and NO synthase inhibition may have promoted glomerular and interstitial scarring to a higher extent than would have occurred with either of these maneuvers alone. Additional stimulus for renal cell proliferation may have resulted from activation of the renin-angiotensin system, suggested by the trend toward the higher levels of circulating renin observed in Group NX+NAME. Also consistent with this view is the finding that losartan treatment virtually prevented the development of GS and interstitial fibrosis in Group NX+NAME+L. Angiotensin II may trigger intracellular mechanisms that eventuate in the process of cell division (41) and may thus exert a promitogenic effect on mesangial cells (42), fibroblasts (43), and vascular smooth muscle cells (44). Accordingly, Johnson and associates (44) have recently shown prominent vascular smooth muscle proliferation and renal interstitial fibrosis, with comparatively less severe glomerular injury, in rats treated chronically with exogenous infusions of angiotensin II. Moreover, preliminary observations have indicated that inhibition of the renin-angiotensin system limits the development of chronic interstitial fibrosis in the remnant kidney model (45). Interstitial nephritis associated with long-term cyclosporine treatment can be ameliorated by angiotensin-conveting enzyme inhibitors (46) or losartan (47), in all likelihood via nonhemodynamic mechanisms.

In summary, chronic NO synthase inhibition worsens considerably the renal and structural consequences of NX, leading rapidly to pronounced GS, glomerular ischemia, and interstitial fibrosis. Severe glomerular hypertension, enhanced cell proliferation, and increased renin production are likely to participate in the pathogenesis of this process.
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REFERENCES


40. Bertani T, Cutillo P, Zeita C, Broggini M, Remuzzi G.