Mechanisms of albuminuria in the chronic nitric oxide inhibition model

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Mechanisms of albuminuria in the chronic nitric oxide inhibition model. Am J Physiol Renal Physiol 279: F1060–F1066, 2000.—Chronic nitric oxide (NO) inhibition causes hypertension and renal injury. Concomitant salt overload promotes massive albuminuria. We investigated the mechanisms whereby these treatments impair glomerular permselectivity. Adult male Munich-Wistar rats received either a standard-salt (SS; 0.5% Na) or high-salt (HS; 3.1% Na) diet and either no treatment or the NO inhibitor Nω-nitro-arginine methyl ester (L-NAME). At 30 days, albuminuria was moderate, the density of fixed anionic sites at the glomerular basement membrane (GBM), estimated by cationic ferritin binding, declined by ~35%, and the fractional clearance of 70-kDa neutral dextran (ϕ) rose moderately in rats receiving L-NAME and SS. Rats given L-NAME and HS exhibited massive albuminuria, whereas ϕ was nearly tripled. Depletion of GBM anionic sites was also seen in these rats. The GBM was thicken in both L-NAME-treated groups. These abnormalities were largely reversed after cessation of treatments. These results indicate that chronic L-NAME treatment promotes reversible albuminuria by impairing both glomerular size and charge selectivity. These effects likely reflect functional rather than structural disruption of the glomerular wall.

kidney glomerulus; kidney physiopathology; capillary permeability; sodium; dietary

CHRONIC NITRIC OXIDE (NO) inhibition promotes severe and progressive arterial hypertension and renal structural injury, consisting of glomerular ischemia, glomerulosclerosis, and interstitial expansion (4, 34). In addition, urinary albumin losses are markedly increased in these animals (4, 39), reaching nephrotic levels when NO inhibition is associated with dietary salt overload (14, 39). The mechanisms underlying the severe impairment of the glomerular barrier observed in this model have not been investigated.

Under normal circumstances, restriction to the filtration of macromolecules occurs mostly at the glomerular basement membrane (GBM) and the slit membrane (10, 12, 32). Macromolecules can be hindered by size, due to the existence of restrictive “pores” in the GBM and, particularly, in the slit membrane (8, 31, 36). In addition, because most of these molecules are negatively charged, they are repelled by the fixed anionic sites distributed throughout the glomerular wall, especially in the GBM (12, 32).

The pathogenesis of proteinuria in clinical and experimental renal disease may involve a glomerular size defect (6, 38), the depletion of fixed negative charges at the glomerular wall (7), or an association between these two abnormalities (28). In the present study, we examined rats subjected to chronic inhibition of NO synthesis, alone or in combination with dietary salt excess, to determine if: whether the massive albuminuria associated with this model is mediated by a size defect, by a charge defect, or by a combination of both mechanisms; and: whether the increased glomerular permeability observed in this model can be reversed on discontinuation of NO inhibition or, instead, reflects permanent damage to the capillary wall.

METHODS

Forty adult male Munich-Wistar rats, obtained from a local colony at the University of Sào Paulo and weighing 240-260 g, were used in this study. The animals were maintained in groups of three or four at 23 ± 1°C on a 12:12-light-dark cycle, with free access to food and water. All rat food was purchased from Harlan Teklad (Madison, WI). All experimental procedures were conducted in accordance with our institutional guidelines.

Experimental groups. Rats were distributed among four experimental groups as described below. Standard-salt (SS; n = 8) untreated control rats received ad libitum tap water and rat chow containing standard amounts of sodium (0.5%) and protein (25%) during 4 wk. SS+NAME (n = 8) rats were treated like SS rats but received the NO inhibitor Nω-nitro-L-arginine methyl ester (L-NAME; 700 mg/µl) in the drinking
water, corresponding to an expected ingestion of ~70 mg·kg⁻¹·day⁻¹. This treatment was maintained for 4 wk. High-salt (HS; n = 8) untreated rats received ad libitum tap water and rat chow containing 25% protein and high (3.1%) sodium content during 4 wk. HS+NAME (n = 8) rats were treated like HS rats but received l-NAME in the drinking water for 4 wk. Because HS rats drink twice as much water as SS rats, l-NAME was dissolved in the drinking water at 350 mI/l to ensure that the l-NAME dose was similar between the HS+NAME and SS+NAME groups. To ascertain the reversibility of the glomerular functional abnormalities associated with treatments, an additional group was studied (HS+NAME-REC), consisting of eight rats receiving a HS diet and l-NAME as described above during 4 wk. At the end of this period, all treatments were discontinued. Rats were then allowed to recover during the ensuing 4 wk.

Both SS and HS regimens were instituted 1 wk before initiation of l-NAME treatment.

Renal functional studies. For assessment of renal hemodynamics and glomerular permeability to dextran, eight rats from each group were anesthetized with inactin (100 mg/kg body wt ip) and placed on a temperature-controlled surgical table. Rectal temperature was maintained at 37 ± 0.5°C. The left femoral artery was catheterized with PE-50 polyethylene tubing for determination of baseline hematocrit, for continuous monitoring of mean arterial pressure (MAP) by a Statham P23Db pressure transducer connected to a chart recorder (model A8200, Anamed Instruments, São Paulo, Brazil) and for collection of blood samples during the experiment. After tracheotomy, both jugular veins were catheterized with PE-50 tubing for determination of baseline hematocrit, for continuous monitoring of mean arterial pressure (MAP) by a Statham P23Db pressure transducer connected to a chart recorder (model A8200, Anamed Instruments, São Paulo, Brazil) and for collection of blood samples during the experiment. After tracheotomy, both jugular veins were catheterized with PE-50 tubing. Homologous plasma (to replace surgical losses) was infused through the left jugular, while a saline solution, containing inulin at 25 mg/ml and 14C-labeled neutral dextran, 70 kDa molecular mass, at 0.1 μCi/ml, was infused through the right jugular at 1.5 ml/h throughout the experiment. The left ureter was catheterized with PE-10 tubing. About 2.5 h after anesthesia, two timed (20-in) urine collections were performed for determination of flow rate, inulin concentration, and radioactive dextran activity. A 50-μl blood sample was collected at the middle of each period to assess the glomerular filtration rate (GFR) by inulin clearance and to determine the 70-kDa neutral dextran clearance (Cp). Plasma and urinary inulin concentrations were measured by the anthrone technique, whereas the activities of 14C were determined in a beta counter (Beckman Instruments, Shiller Park, IL). The fractional dextran clearance, assumed to be identical to the glomerular sieving coefficient for this compound (7), was calculated as ϕ = Cp/GFR-100.

Ultrastructural studies. At the end of each experiment, the abdominal aorta was ligated above, while an 18-gauge needle connected to a PE-50 polyethylene tubing was inserted below, the emergence of the renal arteries. A vent was made on the renal vein. The kidneys were then perfused with saline solution containing 2 mg of cationic ferritin (CF; Sigma, St. Louis, MO) after a brief washout with pure saline. After excision of the kidneys, 1-mm³ fragments of renal cortex were fixed in 2% glutaraldehyde in phosphate buffer (0.1 M, pH 7.4) for 2 h at room temperature. The fragments were rinsed in 0.9% sodium chloride solution containing 17.8 mg of sucrose/ml and buffered to pH 7.3 with sodium bicarbonate. Postfixation was carried out in 1% OsO4 in 0.08 M cacodylate buffer at pH 7.3 for 1 h. After being rinsed in 0.9% sodium chloride solution, the fragments were immersed in 0.5% aqueous uranyl acetate for 12–18 h. After dehydration in an ethanol series starting at 70%, the specimens were embedded in Araldite. Sections (0.5 μm) were stained with 1% toluidine blue and 1% azur II. Silver urathin sections were contrasted with uranyl acetate and lead citrate and examined under a Jeol 1010 transmission electron microscope at 80 kV.

The glomerular ultrastructure was examined at a final magnification of ×80,000, and at least three micrographs that included properly oriented GBM sections were taken for each rat. The density of fixed anionic sites at the GBM, indirectly assessed by the binding of CF, was quantified by superimposing a 300-point grid on enlarged glomerular micrographs. The fraction of the GBM area covered by CF (%CF) was then estimated as %CF = F/T, where F is the number of points hitting areas of CF deposition and T represents the total of points hitting the GBM. The average thickness of the GBM was estimated by randomly superimposing a test line on enlarged photographs of the glomerular wall and measuring the segment of this line comprised within the GBM area (1).

Urinary protein analysis. Four weeks after treatments were started, rats were placed in metabolic cages and urine was collected during 24 h. Those rats whose treatments were discontinued after 4 wk (HS+NAME-REC) were subjected to the same procedure at the end of an additional 4 wk. Total urinary protein concentration was measured by the sulfosalicylic method, whereas urinary albumin concentration was determined by radial immunodiffusion (24).

The composition of urinary proteins was estimated by SDS-PAGE, utilizing an adaptation of the discontinuous system described by Laemmli (23). The volume of each urine samples or standard solutions placed in each well was individually adjusted so that its calculated protein content was always 15 μg. Protein standards (Pharmacia, Uppsala, Sweden) ranged from 10 to 100 kDa. Purified rat IgG was used as an additional high-molecular-weight standard. The plates were stained with 0.1% Coomassie blue R-250 (Pharmacia), dissolved in 25% methanol, 10% acetic acid in water.

Statistics. Differences among groups were assessed by one-way ANOVA, with posttest pairwise comparisons between groups according to the Bonferroni formulation (37). Because urinary albumin excretion rate (UalbV) and ϕ were not normally distributed, log transformation of these parameters was performed before statistical analysis. Interaction between dietary salt and l-NAME treatment was assessed by performing two-way ANOVA of the SS, SS+NAME, HS, and HS+NAME groups. The Pearson correlation coefficient was calculated to assess linear correlation between log (UalbV) and either %CF, GBM thickness (λ), or log ϕ. For all statistical tests, P ≤ 0.05 was considered significant.

RESULTS

As expected, individual water consumption was about twice as high in HS (76 ± 6 ml/day) and HS+NAME (74 ± 2 ml/day) as in SS (35 ± 3 ml/day) and SS+NAME (38 ± 3 ml/day) rats. Thus l-NAME intake was 78 ± 6 and 76 ± 2 mg·kg⁻¹·day⁻¹ in SS+NAME and HS+NAME rats, respectively. These values were not significantly different when compared by using the unpaired Student’s t-test. Individual food consumption was 20 ± 2 in the SS group, 18 ± 1 in the SS+NAME group, 18 ± 2 in the HS group, and 11 ± 2 g/day in the HS+NAME group. The respective daily sodium intakes, estimated from the known composition of the diets (0.5% Na for SS and 3.1% for HS) were therefore 4.3 ± 0.4 mmol/day in SS, 3.9 ± 0.2 mmol/day in SS+NAME, 24 ± 3 mmol/day in HS, and 15 ± 4 mmol/day in HS+NAME rats.
Table 2. Renal functional and systemic hemodynamic parameters

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>GFR, ml/min</th>
<th>C_F, ml/min</th>
<th>ϕ, \times10^{-2}</th>
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<tr>
<td>SS</td>
<td>109 ± 1</td>
<td>1.35 ± 0.03</td>
<td>9.0 ± 0.4</td>
<td>6.9 ± 0.2</td>
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<tr>
<td>SS + NAME</td>
<td>160 ± 4*</td>
<td>0.80 ± 0.07*</td>
<td>9.8 ± 1.1</td>
<td>12.5 ± 3.3</td>
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<tr>
<td>HS</td>
<td>115 ± 3</td>
<td>1.24 ± 0.05</td>
<td>9.8 ± 0.6</td>
<td>7.9 ± 0.5</td>
</tr>
<tr>
<td>HS + NAME</td>
<td>166 ± 7*</td>
<td>0.72 ± 0.03*</td>
<td>17.5 ± 1.9*</td>
<td>24.2 ± 2.5†</td>
</tr>
<tr>
<td>HS + NAME-REC</td>
<td>118 ± 3‡</td>
<td>1.21 ± 0.05‡</td>
<td>12.0 ± 0.7†</td>
<td>10.1 ± 2.6‡</td>
</tr>
</tbody>
</table>

Values are as means ± SE; n = 8 rats/group. MAP, mean arterial pressure; GFR, glomerular filtration rate; C_F, renal clearance of 70-kDa neutral dextran; ϕ, renal fractional clearance of 70-kDa neutral dextran (C_F/GFR); SS, standard salt; HS, high salt; NAME, N^\*/-nitro-L-arginine methyl ester; REC, recovery. *P < 0.05 vs. respective untreated control. †P < 0.05 vs. respective SS; ‡P < 0.05 HS + NAME-REC vs. HS + NAME.

Renal functional studies. Renal and systemic hemodynamic parameters measured at 4 wk of treatment are displayed in Table 1. As described previously (14, 34, 39), MAP was markedly elevated in rats receiving L-NAME at either dietary salt regimen. Blood pressure returned to control levels 30 days after treatments were withdrawn (HS+NAME-REC). GFR was markedly decreased in the SS+NAME and HS+NAME group. Interruption of treatments was associated with normalization of GFR after 4 wk. The fractional dextran clearance, i.e., ϕ, was increased by 81% in the SS+NAME compared with the SS group. However, ϕ more than tripled in the HS+NAME compared with the HS group. This abnormality was almost entirely reversed after treatments were interrupted.

UalbV (Table 2) was increased 20-fold in the SS+NAME compared with the SS group. Albuminuria was massively increased in the HS+NAME group, reaching values more than 100 times as high as in the HS group. Although albuminuria was similarly elevated in the HS+NAME-REC group at 4 wk of treatment (284 ± 64 mg/24 h immediately before interruption), it decreased to levels not statistically different from baseline 4 wk after treatments were ceased.

Ultrastructural studies. Virtually no abnormalities were noted in electron microscopic sections from the SS+NAME group. In the HS+NAME group, simplification of pedicles was observed in most electron microscopic sections. However, severe podocyte injury and denudation of the external aspect of the GBM, such as described in other models (25, 28), were not observed.

L-NAME treatment significantly decreased the density of anionic sites at the GBM (%CF), as estimated by the extent of CF deposition (Table 2 and Fig. 1). %CF was numerically lower in the HS+NAME compared with the SS+NAME group, although this difference did not reach statistical significance. CF deposition at the endothelial layer also appeared consistently reduced in this group compared with control (Fig. 1). After withdrawal of treatments, the CF deposition density at the glomerular wall was nearly completely restored.

Table 2. Albuminuria and glomerular ultrastructural parameters

<table>
<thead>
<tr>
<th></th>
<th>UalbV, mg/24 h</th>
<th>%CF</th>
<th>λ, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>1.3 ± 0.1</td>
<td>20.7 ± 1.4</td>
<td>146 ± 6</td>
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<tr>
<td>SS + NAME</td>
<td>32.0 ± 8.0*</td>
<td>13.2 ± 1.8</td>
<td>188 ± 12*</td>
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<td>HS</td>
<td>2.2 ± 0.3</td>
<td>19.0 ± 1.0</td>
<td>159 ± 4</td>
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<tr>
<td>HS + NAME</td>
<td>233.2 ± 41.2†</td>
<td>9.50 ± 1.7*</td>
<td>206 ± 9*</td>
</tr>
<tr>
<td>HS + NAME-REC</td>
<td>10.2 ± 2.9‡</td>
<td>17.1 ± 1.3§</td>
<td>172 ± 9§</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 rats/group. UalbV, urinary albumin excretion rate; %CF, percent glomerular basement membrane (GBM) area occupied by CF; λ, GBM thickness. *P < 0.05 vs. respective untreated control. †P < 0.05 vs. respective SS. ‡P < 0.05 HS + NAME-REC vs. HS + NAME.

Fig. 1. Representative electron micrographs of the glomerular wall in 3 of the experimental groups examined in this study. A: high-salt (HS; control, 30 days of treatment). Dense cationic ferritin (CF) deposition is seen at the endothelium (End) and at the glomerular basement membrane (GBM), mostly at the lamina rara interna. Ep, epithelium. B: HS N^*/-nitro-L-arginine methyl ester (L-NAME; HS+NAME; 30 days of treatment). Rarefaction of CF deposition is clearly seen at both the endothelium and the GBM. In addition, the GBM appears thickened compared with control. C: HS+NAME-REC (30 days after interruption of treatments). CF binding increased, whereas GBM thickness decreased, toward control values.
There was a significant increase in $\lambda$ in both the SS+NAME and HS+NAME groups, reaching values $\sim 30\%$ higher than in the respective controls (Table 2 and Fig. 1). After cessation of treatments, $\lambda$ decreased toward normal values.

Correlation analysis and two-way ANOVA. There was a strong positive linear correlation between log ($U_{alb/V}$) and log $\varphi$ ($r = 0.73, P < 0.0001$). A similar correlation was found between log ($U_{alb/V}$) and $\lambda$ ($r = 0.62, P < 0.0001$). A negative, equally tight linear correlation was observed between log ($U_{alb/V}$) and the density of GBM anionic sites (%CF; $r = 0.78, P < 0.0001$) (Fig. 2).

Two-way ANOVA indicated that both L-NAME and salt overload exerted a strong influence on albuminuria and $\varphi$. In addition, a significant degree of interaction was observed between L-NAME and dietary salt leading to an approximate 20-fold increase in $U_{alb/V}$. The relatively moderate albuminuria observed in this group may have resulted from two distinct, independent abnormalities: 1) a charge defect, as indicated by the 35% reduction in the density of CF binding sites (α-CF) at the GBM observed in these animals; and 2) a size defect, as suggested by the 81% increase in $\varphi$ compared with control values.

Concomitant L-NAME treatment and dietary salt excess aggravated the glomerular barrier dysfunction already observed in SS+NAME rats, raising albuminuria to nephrotic levels at 30 days of treatment. These

DISCUSSION

Chronic NO inhibition resulted in a 20-fold increase in $U_{alb/V}$. The relatively moderate albuminuria observed in this group may have resulted from two distinct, independent abnormalities: 1) a charge defect, as indicated by the 35% reduction in the density of CF binding sites at the GBM observed in these animals; and 2) a size defect, as suggested by the 81% increase in $\varphi$ compared with control values.

Concomitant L-NAME treatment and dietary salt excess aggravated the glomerular barrier dysfunction already observed in SS+NAME rats, raising albuminuria to nephrotic levels at 30 days of treatment. These
two maneuvers interacted strongly to augment albuminuria, as revealed by two-way ANOVA. One possible mechanism for this cooperation might be a disruption of the glomerular size-selective properties, because $\varphi$ more than tripled in HS+NAME rats compared with HS. Accordingly, two-way ANOVA disclosed a specific interaction between L-NAME and salt overload to increase $\varphi$. Theoretically, this effect could have resulted from the concomitant decrease in GFR, which would tend to increase $\varphi$ by enhancing the relative importance of transcapillary diffusion (7, 30). However, even a 50% decrease in GFR would be predicted to augment $\varphi$ by no more than 50% (7), as opposed to the 206% increase observed in the HS+NAME relative to the HS group. In addition, GFR was reduced by a similar extent in rats receiving SS and L-NAME, which exhibited a much smaller increase in $\varphi$, suggesting that a true size defect occurred in the HS+NAME group. Impairment of glomerular size selectivity might result from the appearance of large nonselective openings at the glomerular wall, creating a “shunt pathway” for the passage of albumin and other macromolecules. Because the urinary excretion of IgG was negligible compared with that of albumin, the radius of these hypothetical “large pores” is unlikely to have substantially exceeded 60 Å (the approximate radius of the IgG molecule). Accordingly, we were unable to detect podocyte detachment from the GBM, or any epithelial abnormality other than foot process “fusion” that might constitute an anatomic basis for a large, unselective shunt pathway, as shown in other proteinuric conditions (6, 28). The finding of a size defect in L-NAME-treated rats in the absence of an obvious ultrastructural abnormality of the glomerular wall suggests an alteration of the molecular composition and/or conformation at the GBM and/or the slit membrane. The increase in $\lambda$ found in L-NAME-treated rats and the tight correlation observed between $\lambda$ and $U_{aib}V$ suggest that disruption of the GBM architecture may indeed have occurred in these animals.

In HS+NAME rats, the density of anionic charges at the GBM (%CF) was numerically decreased compared with in SS+NAME rats, falling to 50% of baseline. This difference between results in HS+NAME and SS+NAME rats did not attain statistical significance. It must be noted, however, that even a small (hence difficult to detect) further loss of GBM negative charge may have exerted a disproportionate effect on albuminuria, because the passage of polyanions is expected to grow exponentially, with linear decreases in the fixed anion density at the glomerular wall (9). Accordingly, in this study log ($U_{aib}V$) exhibited a strong negative linear correlation with %CF. Moreover, it should be stressed that, due to its large molecular dimensions, the penetration of CF into the GBM was always mostly confined to the lamina rara interna. Because the density of anionic sites may be higher at the outermost layers of the GBM (12), it is conceivable that more severe charge depletion occurred in this group than revealed by examination of CF deposition. In addition, albuminuria may have been aggravated in L-NAME-treated rats by the loss of anionic sites at the endothelial layer. Finally, it must be noted that anionic depletion of the GBM was most likely associated with loss of charged molecules such as glycosaminoglycans, which may have contributed to loosen the GBM meshwork, thus increasing $\lambda$ and impairing both the charge- and the size-selective properties of the glomerular wall (18).

The functional and structural abnormalities of the glomerular wall in rats undergoing chronic NO inhibition, particularly when associated with salt overload, may have resulted from the marked glomerular hypertension observed in these animals (4), which is aggravated by dietary salt overload (14, 14). Intracapillary hypertension may directly enhance the filtration of proteins by increasing the force that governs the passage of macromolecules across the glomerular wall (8, 29). Besides this direct physical effect, glomerular hypertension may damage the endothelial (13, 27) and epithelial (25, 33) layers of the glomerular wall, thus facilitating the traffic of macromolecules. Mechanical stress may also affect the GBM and the slit membrane, increasing the size of the pores, or equivalent structures, that function as pathways for macromolecule filtration (35). Moreover, the density of anionic charges in the GBM was shown to decrease in response to elevations in perfusion pressure (16, 40). In rats receiving chronic NO inhibitors, angiotensin II receptor blockers lower glomerular pressure and strongly attenuate albuminuria, regardless of whether the rats are concomitantly given salt overload (14, 34). These observations suggest that intracapillary hypertension may indeed mediate the increase in glomerular permeability observed in these rats. However, it must be noted that, in other experimental models, severe glomerular hypertension is not necessarily associated with massive albuminuria. In rats with diabetes mellitus, despite persistent glomerular hypertension, albuminuria remains low for nearly 1 yr before exceeding 100 mg/day (15). In rats with puromycin aminonucleoside nephrosis, albuminuria reaches nephrotic levels at a time when glomerular pressure is normal, then nearly disappears when glomerular pressure rises (2). Therefore, factors in addition to glomerular hypertension may have also participated in the pathogenesis of the massive albuminuria associated with this model.

A host of experimental findings obtained in several microcirculatory territories suggests that local, continuous NO production may play an important physiological role by directly restricting the capillary permeability to macromolecules. In murine (22) and feline (20) mesenteric circulations, acute inhibition of NO synthesis led to marked extravasation of albumin, indicating a corresponding increase in capillary leakiness. Conversely, the administration of a NO donor or a cGMP analog prevented albumin leakage induced by histamine or other inflammatory mediators (17). In rat coronary circulation (11), intravenous infusion of L-NAME markedly increased vascular permeability, an effect unrelated to the concomitant elevation of blood pressure. Evidence that NO limits microvascular per-
meability to macromolecules has also been obtained in the human lung (5) and in the rat hind paw (19). The results obtained in the present study suggest that this effect of NO may occur at the glomerular microcirculation as well. The mechanisms by which NO inhibition might enhance capillary permeability are poorly understood. In intestinal capillaries, NO inhibition may disorganize the endothelial cytoskeleton and, consequently, disrupt cell-cell junctions, leading to the formation of intercellular leaks and allowing the passage of large molecules (3). NO inhibition may also trigger inflammatory events such as leukocyte adherence (21) and mast cell degranulation (22). We have recently shown preliminary evidence (26) of lymphocyte and macrophage infiltration in the renal parenchyma of rats receiving chronic l-NAME treatment and that this abnormality is markedly aggravated by dietary salt overload. These events may contribute to increased glomerular permeability in this model, perhaps owing to the release of inflammatory mediators by these infiltrating leukocytes. Whether this effect involves a glomerular cytotoxic action, resulting in deficient synthesis of GBM/slit-membrane constituents, is presently unknown.

The massive albuminuria observed at 30 days of combined HS and l-NAME treatment was almost completely reversed 30 days after this regimen was stopped. Recovery of the glomerular wall properties was accompanied by near normalization of the GBM negative charge density and of the sieving coefficient for 70-kDa dextran, indicating that both the size and charge defects observed at 30 days of HS+NAME treatment were reversed. This finding is consistent with the view that a functional defect (for instance, impaired biosynthesis of glomerular wall constituents), rather than permanent structural damage, was responsible for the severe glomerular barrier dysfunction observed in this study. Restoring NO synthesis and/or normalization of glomerular hemodynamics may have governed this recovery process. It remains to be established whether rats surviving l-NAME and HS treatment for substantially longer than 30 days would also recover or whether the process leading to albuminuria in these animals would perpetuate itself if treatments were maintained for a sufficiently long period. Equally undetermined is whether recovery would still occur if the l-NAME dose employed in this study were substantially higher.

In summary, the present observations suggest that the albuminuria observed in the chronic NO inhibition model is associated with a size defect as well as with depletion of electronegative charges at the GBM. The massive albuminuria that develops when salt overload is superimposed on NO inhibition involves exacerbation of the size and, in all likelihood, the charge defect. Albuminuria almost completely disappears on interruption of treatments, suggesting that it results from a glomerular functional, rather than structural, defect.

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