Short- and Long-Term Effect of Angiotensin II Receptor Blockade in Rats with Experimental Diabetes

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ABSTRACT

The short- and long-term effects of specific angiotensin II (All) receptor blockade on the evolution of glomerular injury in moderately hyperglycemic diabetic rats were studied. Three groups of animals were used, a control group, a group of diabetic rats treated with insulin, and a group of insulin-treated diabetic rats receiving the All receptor antagonist losartan in drinking water. After 4 to 6 wk of observation, diabetic rats showed higher systolic blood pressure and GFR than normal controls. Losartan treatment prevented both systolic blood pressure and GFR rise. Three other groups of rats, similarly treated for a 1-yr period, were used for renal functional and morphologic evaluation. Diabetic animals had higher urinary protein excretion and glomerulosclerosis incidence than did normal controls. Losartan significantly prevented proteinuria and glomerulosclerosis. Evaluation of the sieving properties of the glomerular membrane by Ficoll fractional clearance showed an important increase in the filtration of this marker in diabetic animals, as compared with that in controls, and almost complete prevention of this change in losartan-treated animals. Theoretical analysis of fractional clearance data with a hetero-porous model of glomerular size-selectivity showed that in diabetic animals the size of membrane pores was increased uniformly, as compared with that in controls. These changes were completely prevented by the All receptor antagonist. The results presented here strongly indicate that reduction of All activity plays a crucial role in the preservation of glomerular structure and function and suggest that the favorable effects previously observed with angiotensin-converting enzyme inhibition in this model depend directly on the reduction of All activity.

Key Words: Glomerulosclerosis, glomerular size-selectivity, hyperglycemia, membrane pores, proteinuria

More than 30% of patients with insulin-dependent (Type I) diabetes develop progressive nephropathy (1). Patients who are more likely to progress to overt diabetic glomerulopathy have higher than normal GFR in the early phase of the disease (2). Hyperfiltration, subsequent proteinuria, and glomerular structural injury occur with time in rats with streptozotocin-induced diabetes, a model that has been extensively used to clarify the pathophysiology of diabetic nephropathy (3–6). Previous studies have documented that normalization of glomerular hypertension in streptozotocin diabetic rats by the angiotensin I–converting enzyme (ACE) inhibitor enalapril prevented proteinuria and subsequent glomerular injury (7,8). Whether the beneficial effect of ACE inhibition on glomerular function is solely the consequence of the inhibition of angiotensin II (All) biologic activity or whether other mechanisms may possibly be implicated is still matter of speculation. The possibility that ACE inhibitors reduce proteinuria because they increase kinin activity (9,10) rests on animal experiments showing that in rats with passive Heymann nephritis the serine protease inhibitor aprotinin, which blocks the synthesis of bradykinin, prevented ACE inhibitor–mediated reduction of proteinuria (11). The recent availability of All antago-
nists highly specific for AT_{1} receptors and orally active allows us to address this question. Another important effect of ACE inhibitors in human insulin-dependent diabetes is that they lower urinary albumin excretion and effectively ameliorate size-selective properties of glomerular barrier (12,13). Once again, whether this favorable effect of ACE inhibition is due to reduced AT_{1} activity or to modulations of other hormonal systems is not yet understood. Evidence against a direct role for AT_{1} in modulating glomerular permeability derives from the observation that II infusion did not reverse the reduction of proteinuria induced by long-term ACE inhibition (14).

In this study, we investigated the short- and long-term effects of a nonpeptide AT_{1} receptor blocker that does not interfere with bradykinin synthesis and metabolism (15,16) in streptozotocin-induced diabetes. We evaluated the action of this compound on systolic blood pressure (SBP), proteinuria, renal function, and morphology. We also studied the effect of all blockade on glomerular membrane size-selective properties in control and experimental groups at the end of the long-term study. Glomerular permeability function was evaluated with Ficoll as a tracer, instead of the commonly used dextran (17,18), and by an analysis of experimental data with previously established theoretical models (17,18). As recently reported (19), at variance with dextran, Ficoll macromolecules have a globular configuration; thus, they can be considered as ideal, neutral spheres that pass across the glomerular membrane.

METHODS

Forty-eight male Sprague-Dawley rats (Charles River, Calco, Italy) of 250 to 275 g body wt were used in these studies. Animal care and procedure were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (EEC Council Directive 86/609, OJ L 358, December 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985). All animals were allowed free access to standard rat chow (containing 20% of protein by weight) and to tap water. Animals were divided into six groups of eight rats. Groups 1 and 4 received no specific therapy and are later referred to as control groups. Groups 2, 3, 5, and 6 were made diabetic by an iv tail injection of streptozotocin (Sigma Chemical Co., St Louis, MO), 60 mg/kg, under thiopental sodium anesthesia (50 mg/kg ip). Induction of diabetes was confirmed, 2 days later, by the measurement of tail blood glucose (BG) level with a reflectance meter (Miles Ames Division, Miles Laboratories Inc., Elkhart, IN). Diabetic rats received daily evening injections of insulin (Ultratard HM, Novo Nordisk Farmaceutici S.r.l., Rome, Italy) in doses individually adjusted to maintain BG level between 200 and 400 mg/dL. BG levels were monitored at least once a week in all diabetic rats and occasionally in control animals for comparison. Groups 2 and 5 received no treatment other than insulin. Groups 3 and 6 were given insulin and treated with the AT_{1} receptor blocker losartan (losartan potassium salt; Du Pont Merck, Wilmington, DE) (20) at a daily dose of 30 to 35 mg/kg in the drinking water. The concentration of losartan in the drinking water was adjusted according to the water intake in order to maintain a constant daily dose of the drug.

Animals of Groups 1, 2, and 3 underwent whole-kidney function evaluation after 4 to 6 wk of observation (short-term study). The remaining Groups, 4, 5, and 6, were monitored for 12 months (long-term study), at which time whole-kidney function and glomerular permeability function were evaluated by the solute clearance technique. Rats were then euthanized, kidneys were removed, and renal tissue was processed for morphologic studies. During the observation period, awake SBP was measured every 2 wk and at monthly intervals for the short- and long-term study, respectively, by the tail cuff method (21). The urinary protein excretion rate was monitored at the same time intervals.

Whole-Kidney Functional Studies

In all animals of Groups 1 to 3, renal clearance studies were performed as previously described (22). Briefly, after BG measurement, rats were anesthetized with thiopental sodium (60 mg/kg body wt ip) placed on a temperature-regulated table, and tracheotomized. A catheter was inserted into the left femoral artery for periodic blood sampling and continuous blood pressure monitoring with an electronic transducer (Battaglia Rangoni, Bologna, Italy). A catheter was also placed in the left femoral vein for the infusion of clearance markers. Urine was collected by bladder incannulation. After the completion of surgery, a bolus of 2 mL/kg of 5% inulin and 0.2% p-aminohippurate (PAH) in normal saline was infused as a priming load, followed by a sustaining infusion of the same solution at the rate of 3 mL/h/kg. Fifty to 60 min after the bolus infusion, three timed clearance periods of about 30 min each were started. Arterial blood samples were obtained before and at the end of each clearance period for the evaluation of plasma inulin and PAH. Urine collection was started 2.5 min after the first blood sample to allow for the transit time from Bowman’s space to the tip of the bladder catheter.

In six animals of Group 4 and in five animals of both Groups 5 and 6, the clearances of inulin, PAH, and Ficoll were measured. At variance with previous studies, we used native Ficoll molecules instead of the radiolabeled compound (17–19). As for the short-
term study, anesthetized rats received a priming load (1 mL/kg) of 10% inulin and 0.4% PAH in saline solution through the left femoral vein. A continuous infusion of inulin (5%) and PAH (0.2%) was then started at a constant rate of 2 mL/h·kg. At the end of the inulin and PAH bolus infusion, animals were also given a priming load of Ficoll 70 (130 mg/kg; Pharmacia Fine Chemicals, Uppsala, Sweden) over a 10-min period (1 mL/kg), followed by a continuous infusion of a Ficoll solution (25 mg/kg) at 1 mL/h·kg. Forty to 50 min after the Ficoll bolus infusion, three timed clearance periods of about 30 min were performed. Arterial blood and urine samples were collected as described previously for the evaluation of inulin, PAH, and plasma protein concentration.

Graded-size Ficoll molecules in plasma and urine samples collected during the first clearance period were separated by gel-permeation chromatography on an Ultrogel AcA34/44 column (1.6 × 40 cm; Pharmacia) as previously described (17,18). Column calibration was performed with six Ficoll fractions of known molecular weight (range, 17,500 to 132,000) kindly provided by Dr. K. Granath (Pharmacia). Effective molecular radii for Ficoll in eluted fractions were calculated according to Ficoll diffusion coefficients measured by Oliver et al. (19) using quasielastic light scattering. According to these observations, the relationship between weight-average molecular weight (MW) and effective molecular radius (r) is

\[ r = 0.421 \times (M_W)^{0.427} \]

where r is given in Å.

During sample separation, fractions of approximately 2 mL were automatically collected and Ficoll concentration was subsequently assayed by the anthrone method of Scott and Melvin with slight modifications (23,24). Fractional clearance of Ficoll was calculated as \( c_F = (U/P_F)/(U/P)_{inulin} \), where \( U/P_F \) and \( U/P_{inulin} \) are the urine-to-plasma concentration ratios of Ficoll and inulin, respectively. Ficoll molecules with an effective molecular radius of 28 to 60 Å were considered. For molecules smaller than 28 Å, plasma concentration was lower than the detection limit because of filtration during the clearance period, whereas molecules larger than 60 Å in radius had too low concentrations in urine samples.

Theoretical Analysis of Membrane Pore Structure

Fractional clearance data were analyzed by use of a theoretical model previously described in detail (17,25). The adopted theoretical model considers the glomerular capillary wall to be perforated by a continuous distribution of cylindrical pores and allows the estimation of intrinsic membrane permeability properties in terms of pore-size distribution parameters. As shown previously (17,18,24,26), we have assumed that the sizes of membrane pores are log-normally distributed. This distribution is characterized by the mean \( \mu \) and the SD of the corresponding normal probability distribution. It has been showed recently (19) that Ficoll fractional clearance values are better simulated assuming that the lognormal pore-size distribution is in parallel with a nonselective "shunt" pathway that does not restrict the passage of test macromolecules across the membrane. This so called "lognormal plus shunt" model is then characterized by three independent parameters: \( u \), \( s \) and \( \omega \). The first two parameters define the lognormal distribution, whereas \( \omega \) represents the fraction of the filtrate volume that would pass through the shunt if plasma protein were absent (17,19). Assuming both models to be accurate, we computed optimal membrane parameter values by nonlinear fitting of the group-mean Ficoll sieving coefficients. Optimal membrane parameter values were calculated minimizing the following sum of squared errors:

\[ \sum_{i=1}^{m} (\theta_{exp,i} - \theta_{calc,i})^2 \]

where \( \theta_{exp,i} \) and \( \theta_{calc,i} \) are, respectively, the measured and calculated fractional clearance values, \( \theta_{calc} \) is the standard error of \( \theta_{exp} \), and m is the number of datum points. In addition to the membrane pore-size parameters, the theoretical model adopted is based on another freely adjustable parameter, the ultrafiltration coefficient (Kf). This parameter was calculated for the two-kidney glomerular population by use of a theoretical model of glomerular ultrafiltration (17,27) and the measured GFR determinants.

Other Analyses

Inulin and PAH concentrations in plasma and urine samples were measured by previously described methods (28,29). GFR, RPF, and filtration fraction (FF), measured as inulin and PAH clearances and their ratio, respectively, were calculated by the use of standard formulas. Protein concentration in arterial plasma was determined in duplicate by the technique of Lowry et al. (30). Protein concentration in 24-h urine samples was measured by the Coomassie blue G dye–binding method (31).

Morphologic Studies

At the end of the long-term study, midcoronal slices of the left kidney were processed for light microscopic examination. Fragments of the kidneys were immersion fixed in Dubosq-Brazil fluid, as described previously (32), and embedded in paraaffin. Sections 3 μm thick were stained with Masson's trichrome, with hematoxylin and eosin, and by the periodic-acid Schiff technique. Sections including superficial and juxtaglomerular glomeruli were evaluated. At least 80
glomeruli were examined for each animal, and the extent of glomerular damage was expressed as the percentage of glomeruli with focal or global sclerotic lesions. Tubular changes (atrophy, cast formation, and tubular dilation) and interstitial fibrosis and inflammation were graded from 0 to 4+ (0, no changes; 1, changes affecting <25% of sample; 2+, changes affecting 25 to 50% of sample; 3+, changes affecting 50 to 75% of sample; 4+, changes affecting >75% of sample). Kidney tissue specimens were analyzed by the same pathologist blind to the nature of the experimental groups.

Statistical Analysis

All results are expressed as mean ± s. Data were analyzed by one-way or two-way analysis of variance as appropriate. Significance level of difference between individual group means, subjected to the analysis of variance, was established by use of the Tukey-Cicchetti test for multiple comparisons (33). Estimates of renal injury by morphologic studies were compared with the Mann-Whitney test for non-parametric data. Statistical significance was defined as P < 0.05. Values for urinary protein excretion, which were not normally distributed, were subjected to logarithmic transformation before statistical analysis.

RESULTS

Short-Term Studies

Values for body weight, kidney weight, SBP, and whole-kidney functional parameters measured 4 to 6 wk after streptozotocin injection are shown in Table 1. Both untreated and losartan-treated diabetic rats had numerically lower body weights than did normal control rats, but differences did not reach statistical significance. Left kidney weight was significantly higher in both diabetic groups as compared with that in controls (P < 0.05). The two groups of diabetic rats also had comparable levels of BG during all observation periods. Values of BG at time of whole-kidney function evaluation averaged 90 ± 9 mg/dL in control Group 1, 320 ± 82 mg/dL in diabetic Group 2, and 317 ± 65 mg/dL in losartan-treated Group 3. SBP was significantly elevated (P < 0.01) in diabetic animals as compared with that in controls (see Table 1). Losartan treatment was effective in controlling SBP. In diabetic rats, GFR was significantly (P < 0.01) higher than that measured in normal controls (see Table 1). Losartan administration to diabetic rats completely prevented hyperfiltration; actually, GFR in these animals was comparable to that measured in controls. RPF and FF levels were significantly elevated in untreated diabetic animals as compared with those in normal controls (P < 0.01 and P < 0.05, respectively). In losartan-treated diabetic animals, RPF and FF levels were comparable to those in normal rats.

Long-Term Studies

Mean body weights at the end of the 12-month observation period were comparable in both diabetic groups, although they did not reach that of control animals (Table 2). Serial values of BG concentrations and SBP during 1 yr of observation are given in Figure 1. Stable and comparable moderate hyperglycemia was maintained throughout the duration of the experiment in both diabetic groups. Diabetic rats exhibited significantly higher values of SBP than did controls (Figure 1). Losartan administration to diabetic rats completely prevented this increase in SBP. Urinary protein excretion rates measured during the long-term study are reported in Figure 2. In all three groups, a significant elevation in urinary protein excretion with time was observed (P < 0.01). By the end of the study, proteinuria was significantly higher in diabetic animals than in controls (263 ± 133 and 153 ± 118 mg/24 h, respectively). By contrast, despite comparable hyperglycemia, diabetic rats given losartan had significantly lower urinary protein excretion than did untreated diabetics through the ob-

<p>| TABLE 1. Systemic and whole-kidney functional parameters at 4 to 6 wk after induction of diabetesa |
|---------------------------------|----------------|----------------|----------|----------|----------|---------|---------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Body Wt (g)</th>
<th>Left Kidney Wt</th>
<th>BG (mg/dL)</th>
<th>SBP (mm Hg)</th>
<th>GFR (mL/min/100 g)</th>
<th>RPF (mg/min)</th>
<th>FF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control</td>
<td>8</td>
<td>493 ± 37</td>
<td>1.91 ± 0.12</td>
<td>90 ± 9</td>
<td>128 ± 8</td>
<td>0.74 ± 0.07</td>
<td>2.39 ± 0.13</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>2 Diabetic</td>
<td>8</td>
<td>462 ± 38</td>
<td>2.22 ± 0.28b</td>
<td>320 ± 82c</td>
<td>160 ± 10d</td>
<td>1.13 ± 0.11d</td>
<td>2.82 ± 0.10d</td>
<td>38 ± 3*</td>
</tr>
<tr>
<td>3 Losartan Treated</td>
<td>8</td>
<td>455 ± 56</td>
<td>2.19 ± 0.29b</td>
<td>317 ± 65e</td>
<td>120 ± 14</td>
<td>0.76 ± 0.10</td>
<td>2.22 ± 0.10</td>
<td>33 ± 4</td>
</tr>
</tbody>
</table>

a Values are mean ± SD. N, number of animals.

b P < 0.05 versus control.

c P < 0.01 versus control.

d P < 0.01 versus control and losartan treated.

* P < 0.05 versus control and losartan treated.
TABLE 2. Systemic and whole-kidney functional parameters at 12 months after induction of diabetes

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Body Wt (g)</th>
<th>Left Kidney Wt (g)</th>
<th>UprotV (mg/day)</th>
<th>Ca (g/dL)</th>
<th>GFR (mL/min·100 g)</th>
<th>RPF (mL/min·100 g)</th>
<th>FF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Control</td>
<td>8</td>
<td>770 ± 93</td>
<td>2.20 ± 0.31</td>
<td>153 ± 118</td>
<td>5.6 ± 0.3</td>
<td>0.74 ± 0.07</td>
<td>2.15 ± 0.10</td>
<td>33 ± 3</td>
</tr>
<tr>
<td>5 Diabetic</td>
<td>8</td>
<td>644 ± 51</td>
<td>2.35 ± 0.16</td>
<td>263 ± 133</td>
<td>5.1 ± 0.3</td>
<td>0.70 ± 0.09</td>
<td>2.40 ± 0.22</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>6 Losartan Treated</td>
<td>8</td>
<td>638 ± 76</td>
<td>2.54 ± 0.27</td>
<td>108 ± 70</td>
<td>5.5 ± 0.7</td>
<td>0.74 ± 0.08</td>
<td>2.16 ± 0.09</td>
<td>33 ± 4</td>
</tr>
</tbody>
</table>

* Values are mean ± SD. N, number of animals; UprotV, urinary protein excretion; Ca, plasma protein concentration. Ca, GFR, RPF, and FF are the mean of six animals of Group 4 and five animals of Groups 5 and 6.

** P < 0.05 versus control.

*** P < 0.05 versus control and losartan treated.

Figure 1. Alternative monthly average values for awake SBP and BG concentration during 12-month observation in controls and untreated and losartan-treated diabetic rats. SBP was significantly increased in diabetic rats as compared with losartan-treated and control animals at all times. Both diabetic groups had comparable and above-normal elevation of BG throughout the study. Values are mean ± SD. **P < 0.01 versus losartan treated and control at the same time; ***P < 0.01 versus diabetic and losartan-treated group at the same time.

Figure 2. Serial values of urinary protein excretion rate during 12-month observation in controls and untreated and losartan-treated diabetic rats. Diabetic rats developed progressive proteinuria, with values being significantly higher than those in nondiabetic control rats. In losartan-treated rats, urinary protein excretion was significantly prevented. Values are mean ± SD. **P < 0.05 and ***P < 0.01 versus control and losartan-treated group.

Final urinary protein excretion in losartan-treated diabetics averaged 108 ± 70 mg/24 h, a value numerically lower than that in controls, but the difference did not reach statistical significance. Plasma protein concentration and glomerular hemodynamic parameters were comparable among the three groups (Table 2). FF was numerically lower in diabetic animals than in controls and losartan-treated animals, but the difference did not reach statistical significance. Thus, despite 1 yr of moderate hyperglycemia, diabetic rats do not develop a significant fall in renal function nor did All blockade affect glomerular filtration.

Fractional clearance values for Ficoll molecules of graded sizes (from 28 to 60 Å) measured in control, diabetic, and losartan-treated animals are presented in Figures 3 and 4. These values are in good agreement with those recently reported in normal Munich Wistar rats (19) and significantly lower than those previously reported for neutral dextran macromolecules. In diabetic animals, Ficoll sieving coefficients were significantly higher than in the control group for all molecular radii studied (Figure 3). The effect of losartan treatment on Ficoll fractional clearance is depicted in Figure 4. In losartan-treated animals, Ficoll fractional clearances are significantly lower than in the diabetic group (Figure 4) and comparable to that measured in control animals.

The results of the theoretical analysis performed
Figures 3 and 4. Fractional clearance of Ficoll as a function of molecular radius measured in control Group 4 and in diabetic Group 5 and in losartan-treated Group 6 after 12 months of observation. Experimental values are shown as mean ± s. Curves represent average-group best fit to the data by use of the lognormal plus shunt pore-size distribution for an assumed value of $\Delta P = 45$ mm Hg. $^* P < 0.05$ vs. control group; $^{**} P < 0.01$ vs. control group.

As shown in Table 3, values of the sum of squared errors are lower for the lognormal plus shunt model.

In diabetic animals, as compared with controls, the pore-size distribution was shifted towards larger sizes, as reflected by an important increase in the value of $u$ and $\omega_0$. The same indication is offered by lognormal distribution alone, as shown by the increased value of the parameter $r^*$ (1%) reported in Table 3. As defined previously (17, 18), $r^*$ (1%) is the pore radius for which 1% of filtrate volume passes through pores larger than $r^*$ and it is a measure of the importance of the largest membrane pores. Thus, both of the assumed distributions show that the size of membrane pores is uniformly increased by diabetes. The treatment of diabetic animals with the AI receptor antagonist losartan induced a uniform reduction in membrane pore sizes. The selective portion of the assumed pore-size distribution was reduced in size to values lower than that in the control group (see values of $u$ in Table 3), and concomitantly, $\omega_0$ [or $r^*$ (1%) for the lognormal distribution] was significantly lower than in untreated diabetic rats and similar to nondiabetic controls. To give a more detailed description of these changes in membrane pore-size distributions among the three groups, the calculated lognormal probability distribution functions are reported in Figure 5. As shown in this graph, the size of the membrane pores is shifted towards larger pores in diabetic animals, as compared with controls, whereas in losartan-treated an-
animals, membrane pores are even smaller than in control rats.

After 1 yr of observation, four control rats out of eight developed focal and segmental glomerulosclerosis (Figure 6). The formation of tubular casts (mean score of tubular structural changes, 0.9; range, 0 to 2) and signs of interstitial fibrosis or inflammation (mean score, 0.6; range, 0 to 1) were observed in these animals. A significantly higher incidence of glomerulosclerosis \((P < 0.05)\) was observed in diabetic rats than in controls. Glomerular sclerosis and hyalinosis with segmental collapse of the glomerular tuft were detected in all untreated diabetic rats, affecting on average 9.7% of the glomerular population (range, 1.1 to 23.3%). Tubular structural changes (mean score, 1.5; range, 0 to 2) and interstitial fibrosis and inflammation (mean, 0.9; range, 0 to 2) were also significantly higher than in controls \((P < 0.05)\). In diabetic rats treated with losartan, glomerular sclerotic lesions and tubular and interstitial inflammation were limited. The mean value of glomeruli affected by sclerosis \((0.8\%); \text{range, 0 to 5}\%) was significantly lower than in untreated diabetics and even than in aging nondiabetic controls. Losartan treatment also significantly prevented tubular changes (mean score, 0.4; range, 0–2; \(P < 0.01\)) and interstitial damage (mean, 0.3; range, 0 to 1; \(P < 0.01\)) as compared with untreated diabetic animals.

**DISCUSSION**

We have found that \(\text{AT}_{1}\) receptor blockade effectively reduced systemic blood pressure in rats with experimental diabetes studied 4 to 6 wk after streptozotocin injection. Actually, although untreated diabetic rats had significantly higher values of systemic blood pressure than did nondiabetic controls, blood pressure in losartan-treated diabetic rats and controls were comparable. Because the control of \(\text{BG}\) has been advocated as one of the mediators of changes in glomerular hemodynamics in diabetes (38), the experiments presented here have been per-

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**TABLE 3.** Group average optimal values of membrane pore-size parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>(\Delta P) (mm Hg)</th>
<th>Lognormal Plus Shunt Model</th>
<th>Lognormal Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(u) (Å)</td>
<td>(s)</td>
<td>(\omega \times 10^{-5})</td>
</tr>
<tr>
<td>4 Control</td>
<td>35</td>
<td>18.0</td>
<td>1.38</td>
</tr>
<tr>
<td>5 Diabetic</td>
<td>45</td>
<td>31.6</td>
<td>1.25</td>
</tr>
<tr>
<td>6 Losartan Treated</td>
<td>35</td>
<td>32.0</td>
<td>1.24</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>11.1</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>12.1</td>
<td>1.49</td>
</tr>
</tbody>
</table>

* Abbreviations: \(\Delta P\), assumed transmembrane hydraulic pressure difference; \(u\), \(s\), \(\omega\), and \(r'(1\%)\), membrane pore-size parameters described in the text; SSE, sum of squared errors.
formed maintaining a constant level of moderate hyperglycemia by individual daily sc administration of insulin. This maneuver resulted in comparable levels of BG in untreated and in losartan-treated diabetic rats throughout the duration of the experiment. Despite hyperglycemia, we found that GFR in losartan-treated animals was comparable to that in normal controls, whereas untreated diabetic rats had hyperfiltration.

Previous studies (7,8) have provided convincing evidence that ACE inhibitors effectively protected animals against proteinuria and glomerular structural abnormalities that invariably develop in streptozotocin diabetic rats with time. The mechanism(s) of the protective effect of ACE inhibitors in experimental diabetes is still debated, but one of the most plausible explanations is that ACE inhibitors selectively reduce efferent arteriolar resistance, thus reducing intraglomerular hypertension (7,39–41). In this study, we have documented that blocking All receptors by losartan effectively prevented abnormal glomerular permeability to macromolecules in diabetic rats as reflected by a significant lower proteinuria at various time intervals in losartan-treated versus untreated diabetic rats. Actually, although proteinuria progressively increased with time in untreated diabetic animals, losartan-treated diabetic rats were significantly protected and urinary protein levels in these animals were even numerically lower than in age-matched normal controls. In addition to the beneficial effect on proteinuria, All receptor blockade prevented glomerular and tubular structural changes. At the end of the long-term observation period, untreated diabetic rats showed major abnormalities of kidney tissue consisting in focal and segmental glomerulosclerosis with tubular atrophy and interstitial inflammation. Losartan-treated diabetic rats had less glomerular and tubulo-interstitial lesions than did both diabetic and normal controls.

To investigate whether All receptor blockade prevented proteinuria by effective amelioration of glomerular permeselective function, we measured the fractional clearance of Ficoll, polydisperse neutral macromolecules of graded molecular size. The choice of this tracer was based on the recent observation (19) that Ficoll, for its globular configuration, reflects glomerular filtration of circulating proteins more closely than the other conventionally used test molecules, the neutral dextrans, which are more elongated in shape. Fractional clearances of Ficoll were uniformly and significantly increased in diabetic animals, as compared with controls. In diabetic animals treated with losartan, fractional clearances of Ficoll were significantly lower than in untreated diabetics and comparable to values measured in normal controls.

We have also analyzed fractional clearance data with a heteroporous model of glomerular size-selec-
renal ablation model (35,42).

In conclusion, this study indicates that in experimental diabetic nephropathy: (1) development of proteinuria is associated with loss of glomerular membrane size-selective properties; (2) glomerular permeselective defect is due to an increase in the size of hypothetical membrane pores; and (3) treatment with the AT1 receptor antagonist losartan significantly prevents glomerular size-selective function and protects from glomerular and tubular structure.

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