Functional and Structural Correlates of Glomerulosclerosis after Renal Mass Reduction in the Rat

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Abstract. Previously, it was shown that 5/6 renal mass reduction by surgical excision (RK-NX) results in a marked reduction of glomerulosclerosis (GS) at 6 wk compared with the conventional 5/6 renal ablation by infarction (RK-I) model. To determine the pathogenetic correlates of the striking differences in GS, radiotelemetrically measured BP; single nephron function; glomerular volume; the temporal expression of mRNA for renin, transforming growth factor-β, and platelet-derived growth factor-B; and plasma renin concentration were compared between RK-NX, RK-I, and sham-operated control rats. Hypertension only developed in the RK-I model, was present at 3 d after infarction, and was correlated with both an increased expression of renin mRNA by Northern analysis and elevated plasma renin concentration. Structural (glomerular volume) and functional (single nephron blood flow and GFR) indices of the compensatory adaptive response were significantly but similarly increased in the RK-NX and RK-I rats compared with sham-operated controls, indicating that these adaptations per se are not responsible for the initiation of GS after 5/6 renal mass reduction. Glomerular capillary pressure ($P_{GC}$) was also significantly increased in both RK-I (56 ± 2 mmHg) and RK-NX rats (50 ± 0.9 mmHg) compared with controls (46 ± 0.8 mmHg, $P < 0.01$), but the increase was significantly greater in RK-I versus RK-NX rats ($P < 0.05$) consistent with the higher BP in RK-I rats. These data indicate that differences in renin probably account for the early divergence of BP (and $P_{GC}$) responses between RK-I and RK-NX models. Transforming growth factor-β and platelet-derived growth factor-B mRNA expression in pooled RNA from kidneys from each group showed increases at 21 d along with early evidence of glomerular injury in the RK-I group but not in the RK-NX group, consistent with their postulated roles as molecular mediators of GS, but only in rats with pathologic glomerular hypertension.

The progressive nature of chronic renal disease has been extensively investigated using the 5/6 renal ablation model (right uninephrectomy and infarction of two-thirds of the left kidney) (1–5). These animals, over time, develop a syndrome of systemic and glomerular hypertension (HTN), proteinuria, and progressive glomerulosclerosis (GS) of the initially normal remnant nephrons. Data in both experimental and human glomerular disease have indicated that increased accumulation of extracellular matrix may be the final common pathway in the pathogenesis of GS (6,7). It has been postulated that increased expression of the growth factors transforming growth factor-β (TGF-β) and platelet-derived growth factor-B (PDGF-B), mediated by the growth effects of angiotensin II (AngII) on glomerular mesangial cells, results in glomerular extracellular matrix synthesis and eventual GS (8–12). However, significant controversy persists as to the mechanisms responsible for the initiation of this pathogenetic sequence of events (1–5,13–16). It has been suggested that the adaptations associated with the compensatory hypertrophy response per se may be maladaptive and result in eventual GS (2–5,13–16). Some investigators have ascribed a primary pathogenetic importance to the increased glomerular capillary pressures and flows associated with the compensatory increases in function, the so-called hemodynamic theory (2,3,15,16), while other investigators have blamed the cellular events and increased growth factor expression associated with the structural glomerular hypertrophy response (4,11–14). Since both glomerular HTN and glomerular hypertrophy coexist in the infarction model (RK-I) and precede the development of GS, it has been difficult to separate the individual contribution of these two pathogenetic mechanisms. Similarly, the beneficial effects of a low protein diet in this model have not allowed the relative importance of the two pathogenetic pathways to be ascertained, as both hemodynamic and structural components of the compensatory hypertrophy response are abrogated by a low protein diet (2–5,16–19). The beneficial effects of angiotensin-converting enzyme inhibition have likewise been ascribed to its hemodynamic effects (reduction in $P_{GC}$) or alternatively to the blockade of the growth effects of AngII (2–5,11,12,14,16,19).

We have recently demonstrated that when equivalent approximately 5/6 renal mass reduction (RMR) is achieved by surgical excision (RK-NX) instead of infarction, there is a marked reduction in GS at 6 wk compared to the traditional infarction (RK-I) model (21). The present studies were per-
formed to define the pathogenetic correlates of these striking differences in GS between the two models. Radiotelemetric BP monitoring, single nephron function, glomerular hypertrophy, the temporal expression of renin, TGF-β and PDGF-B mRNA, and plasma renin concentration were examined in these two models of RMR and compared to sham-operated control rats.

Materials and Methods

Male Sprague Dawley rats (200 to 300 g body wt) were fed a standard (24%) protein diet (Purina, St. Louis, MO) and synchronized to a 12/12 h light (6 a.m. to 6 p.m.) and dark (6 p.m. to 6 a.m.) cycle. Animal experimentation was conducted in accord with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. All rats received food and water ad libitum throughout the study. After measurement of basal serum creatinine ($S_{Cr}$), all rats underwent either sham surgery or approximately 5/6 renal ablation (right nephrectomy and either infarction [RK-I] or surgical excision [RK-NX] of 2/3 of the left kidney). $S_{Cr}$ was again measured at 3 d to stratify for RMR.

Four sets of studies were performed in separate sets of animals to: (1) characterize the structural and functional adaptations at the whole kidney level at 3 wk as well as for the assessment of morphologic injury; (2) define the determinants of the hyperfiltration response at the single nephron level at 3 to 4 wk; (3) define the temporal expression of mRNA for renin and the growth factors TGF-β and PDGF-B over the initial 3 wk after RMR; and (4) examine the temporal changes in plasma renin concentration (PRC) after RMR.

Whole Kidney Studies

At the time of renal ablation or sham surgery, radio transmitters (Data Sciences International, St. Paul, MN) were installed (21–24). The rats were anesthetized with sodium pentobarbital (45 mg/kg, intraperitoneally), and the sensor’s catheter was inserted into the aorta below the level of the renal arteries. The radio frequency transmitter was fixed to the peritoneum. The signals from the pressure sensor were converted and temperature compensated and sent via the radio frequency transmitter to the telemetry receiver. The receiver was connected to a BCM-100 consolidation matrix that transmitted the signals to the Dataquest IV acquisition system (Data Sciences International). Systolic BP in each animal was continuously recorded at 10-min intervals throughout the course of 3 wk, each reading representing the average BP during a 5-s sampling period. Tail vein blood samples were obtained at 3 d for measurement of $S_{Cr}$ as an index of the degree of renal mass reduction. After 3 wk, tail vein $S_{Cr}$ and 24-h urine collections for protein excretion were obtained. The rats were then anesthetized with intravenous sodium pentobarbital (40 mg/kg), tracheostomy was performed using polyethylene (PE-200) tubing, and the rats were surgically prepared for measurement of inulin clearance and renal blood flow (RBF) as described previously (21–26). In brief, a carotid artery was cannulated with PE-50 tubing and connected to a Windograf (model 40-8474; Gould, Glen Burnie, MD) for continuous recording of mean arterial pressure. A femoral vein was cannulated with PE-50 tubing and a priming dose of inulin in 150 mM NaCl was administered, followed by a continuous maintenance infusion of 150 mM NaCl containing inulin at 0.055 ml/min to maintain the plasma concentration of inulin at approximately 50 mg/dl and for replacement of surgical and ongoing fluid losses. The left ureter was then cannulated with polyethylene tubing for collection of urine samples. A 1.0-mm R series flow probe (Transonic Systems, Ithaca, NY) was placed around the left renal artery for measurement of RBF by a flowmeter (Transonic Systems), as described previously (21–26). At the conclusion of the surgery, a 150 mM NaCl bolus equal to 1% of body weight was administered. Two 20-min clearances of inulin were obtained. Blood samples were obtained at the midpoint of each urine collection. The kidneys were then perfusion-fixed at their ambient BP and subsequently processed for morphologic and morphometric studies.

Morphologic Methods. Transverse sections of the kidney through the papilla were fixed in situ by perfusion for 5 min at the measured BP with 1.25% glutaraldehyde in 0.1 M cacodylate buffer. Sections were cut at a thickness of 2 μm and stained with hematoxylin and eosin and periodic acid-Schiff. Sections were evaluated systematically in each kidney for evidence of glomerular injury in a blinded manner by standard morphologic methods (21–24). At least 100 glomeruli in each animal, and usually more, were evaluated for the presence of lesions of segmental sclerosis (collapsed capillaries with obliteration of the capillary lumina, frequently accompanied by a fibrinous adhesion between the glomerular tuft and Bowman’s capsule) and/or necrosis (fibrinoid necrosis of part or all of the glomerular tuft with loss of architecture often accompanied by capillary thrombosis, karyorrhexis, fibrin leakage into the Bowman space, and proliferation of the parietal epithelium of Bowman’s capsule). The severity of glomerulosclerosis was expressed as the percentage of glomeruli exhibiting such injury.

Morphometric Methods. Glomerular volume was measured by area perimeter analysis (Bioquant System IV software; R&M Biometrics, Nashville, TN). The cross-sectional area ($A_{CS}$) of 75 consecutive glomerular profiles contained in one kidney section for each animal was measured using a digitizing pad as described previously (21,23,24). The mean glomerular volume ($V_{G}$) was then calculated from the respective mean $A_{CS}$ as $V_{G} = \beta \kappa (A_{CS})^{3/2}$, where $\beta = 1.38$ is the size distribution coefficient and $\kappa = 1.1$ is the shape coefficient for glomeruli idealized as spheres (27,28).

Micropuncture Studies to Define the Determinants of Single Nephron Hyperfiltration

These rats underwent sham or RMR surgery as described above, but without the placement of radio transmitters. At 3 to 4 wk, the rats were anesthetized with inactin (100 mg/kg, intraperitoneally) and placed on a temperature-regulated (37°C) micropuncture table and prepared for micropuncture studies as described previously (29). Left kidney GFR, proximal tubular pressure ($P_{s}$), stop flow pressures ($P_{SF}$), peritubular capillary (first order) pressures ($P_{PC}$), and SNGFR were determined using 3H inulin. Nephrons were mapped by injection of 0.9% NaCl stained with fast green dye. Only nephrons clearly within the normal remnant parenchyma and well away from scar areas were selected for micropuncture. Three to five proximal tubular collections were obtained in each rat. Similarly, 3 to 4 $P_{UF}$ measurements with a continuously recording Servomull system were made in each rat. Simultaneous femoral arterial and renal vein collections were obtained to determine inulin extraction and whole kidney filtration fraction (17,30). Afferent arterial colloid oncotic pressure ($\pi_{A}$) was calculated using the femoral arterial plasma protein concentration and the Landis–Pappenheimer equation. $P_{GC}$ was calculated as PSF + $\pi_{A}$. Other equations used were: glomerular plasma flow ($Q_{G}$) = SNGFR/FF; glomerular blood flow (GFB) = $Q_{G}/1 - Hct$; efferent arteriolar blood flow (EABF) = GFB - SNGFR; afferent, efferent, and total renal arteriolar resistance ($R_{A} = AP - P_{GCB}/GFB \times (7.982 \times 10^{10})$; $R_{E} = P_{GC} - P_{PC}/EABF \times (7.982 \times 10^{10})$; $R_{TA} = R_{A} + R_{E}$, respectively; net ultrafiltration pressure ($P_{UF}$) = $P_{GC} - P_{T} - \pi_{A}$; and ultrafiltration coefficient ($K_{f}$) = SNGFR/$P_{UF}$ (29–31).
**Temporal Expression of Growth Factors**

These rats underwent sham or RMR surgery and installation of radio transmitters for BP radiotelemetry as described for rats undergoing renal hemodynamic studies. Separate sets of sham (n = 8), RK-NX (n = 9), and RK-I (n = 9) rats each were sacrificed at 3, 7, and 21 d, and the kidneys were harvested for Northern blot analysis for renin, TGF-β, and PDGF-B mRNA expression. The infarcted areas (scars) and the immediately adjacent areas were excised quickly, and the remaining kidney tissue was placed into liquid nitrogen.

**Northern Blot Analysis.** RNA isolation was performed by a modification of the method of Chirgwin et al. (32). One hundred micrograms of this “non-scar” renal tissue was homogenized with guanidinium isothiocyanate. The RNA was isolated by isopycnic centrifugation over CsCl, and its concentration was measured spectrophotometrically at 260/280 nm using the molar extinction coefficient of nucleic acids. A standardized aliquot of RNA (20 μg) was separated by electrophoresis on a formaldehyde agarose denaturing gel and transferred to a Nytran® membrane by capillary transfer. Lanes were loaded with pooled RNA from each of the three groups of rats sacrificed at each of the three separate time points. The RNA was immobilized to the membrane by ultraviolet cross-linking.

The membrane was successively hybridized with four parts (15 ml of Formamide, 0.6 ml Denhardt’s solution, 1.5 ml of 1 M phosphate buffer, 7.5 ml of 20× SSC, 1.5 ml of 205 SDS, 2.4 ml of diethylypyrocatecholate water, and 1.5 ml of salmon sperm DNA)/blot and one part 50 dextran sulfate solution with 32 P-dCTP-labeled cDNA probes (renin [gift of Kevin R. Lynch, University of Virginia, Charlottesville, VA], TGF-β, PDGF-B, and GAPDH, which was used as the housekeeping gene [American Type Culture Collection, Manassas, VA]). The cDNA was radiolabeled using a random prime labeling kit (Pharmacia, Piscataway, NJ). After each hybridization, the membrane was washed and placed in an x-ray cassette for the requisite exposure time. Autoradiograms were quantified using computerized densitometry (Bioquant Systems IV software; R&M Biometrics) and corrected for protein loading. This was accomplished by factoring the relative density of the various mRNA autoradiograms by the GAPDH mRNA autoradiogram (the values in sham controls were arbitrarily assigned a value of 1).

**Temporal Changes in PRC**

PRC measurements were performed at 3 and 21 d after RMR. These rats underwent sham or RMR surgery and installation of radio transmitters for BP radiotelemetry as described for rats undergoing renal hemodynamic studies. For rats that were to undergo PRC measurements at 3 d, the right or left femoral artery was catheterized with flexible Tygon® tubing at the time of RMR surgery. The distal end of the catheter was tunneled through the subcutaneous tissue and exteriorized at the back of the neck. The catheter was flushed and filled with a heparin dextrose solution and plugged with a straight blunted pin. For the rats undergoing PRC measurements at 21 d, the placement of the femoral artery catheter was performed 2 d earlier under sodium pentobarbital (45 mg/kg, intravenously) anesthesia. Blood samples were obtained from rats resting quietly in restrainers. Blood was drawn into chilled ice-cold heparinized plastic syringes (25,26). Blood samples were centrifuged at 4°C, and the plasma was stored at −20°C until analyzed. The rats were sacrificed after blood samples were obtained.

**Laboratory Analyses**

Urinary protein was measured by the quantitative sulfosalicylic acid method with human serum albumin serving as standard. Serum creatinine was measured using a creatinine analyzer (Beckman Instruments, Fullerton, CA) (21,24). Insulin in urine and plasma filtrates was determined spectrophotometrically by the diphendylamine method as described previously (21–26). GFR was calculated using standard formulas. PRC was determined by incubating plasma with excess homologous renin substrate, in the presence of inhibitors of converting enzyme and angiotensinase, and determining the amount of Ang II generated during the incubation period, as described previously (25,26). PRC was expressed in units of nanograms of Ang II per milliliter plasma per hour incubation with substrate (i.e., ng AngII/ml per h).

**Statistical Analyses**

All results are expressed as mean ± SEM. Statistical analysis was performed using ANOVA followed by Student-Newman-Keuls test or by Kruskal-Wallis nonparametric ANOVA followed by Dunn multiple comparison test as appropriate (33). A P value of <0.05 was considered statistically significant.

**Results**

**Whole Kidney Studies**

Table 1 shows that the baseline body weight, S_Cr, and 24-h urine protein excretion were not different between the three groups. S_Cr, at 3 d was significantly increased in the two groups that had undergone RMR compared with the sham group, but was not different between the RK-NX and the RK-I groups, indicating comparable RMR. The final body weight was not significantly different between the three groups. The RK-I rats had significantly higher BP compared to both the sham and the RK-NX rats, but the BP of the RK-NX rats was not significantly different from the sham control rats. By 3 wk, evidence of glomerular injury, although still relatively mild, was only present in the RK-I rats in the form of a modest but significant increase in 24-h protein excretion rates as well as the percentage of glomeruli that exhibited histologic evidence of glomerular injury. These indices were not different between RK-NX and sham control groups.

Figure 1, A and B, provides the data for the functional and structural indices of the renal hypertrophy response at 3 wk after RMR. Kidney weight and glomerular volume (Figure 1A) were significantly increased in the two RMR groups compared to the sham rats but were not significantly different from each other. By 3 wk after RMR, the RBF and GFR of the remnant left kidneys of both RMR groups had increased such that no significant differences were seen between them and the intact left kidney of the sham-operated controls (Figure 1B).

**Single Nephron Studies**

The body weights, S_Cr, and urine protein excretion data of the rats undergoing micropuncture studies are presented in Table 2 and were essentially similar to Table 1. Also provided are the whole kidney RBF and GFR data for these groups. Table 3 presents the renal functional data at the single nephron level for the sham control and the two RMR groups. Both single nephron plasma flow and GFR were significantly greater in the two RMR groups compared with the sham controls but were not significantly different from each other. Mean arterial...
pressure (AP) under anesthesia was significantly greater in the RK-I compared with the RK-NX and sham rats, consistent with the average systolic BP recorded in the unanesthetized rats of the three groups during the whole kidney studies. All components of renal vascular resistance (total, afferent, and efferent) were reduced in both RMR groups compared to sham controls; however, the reduction in $R_A$ in the RK-I group did not reach statistical significance. However, it should be noted that the $R_A$ in the RK-I group is expected to be somewhat elevated due to the superimposition of autoregulatory afferent vasoconstriction, albeit impaired, in response to the significantly higher AP in this group. The $P_{GC}$ and $P_{UF}$ were significantly greater in both RMR groups compared to the sham controls, but the increase was significantly greater in the RK-I compared to the RK-NX group. By contrast, the increase in $K_f$ in the two RMR groups compared to the sham rats was statistically significant only for the RK-NX rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Body Weight (g)</th>
<th>$S_{cr}$ (µmol/L)</th>
<th>Proteinuria (g/d)</th>
<th>3rd Body Weight (g)</th>
<th>$S_{cr}$ (µmol/L)</th>
<th>Proteinuria (g/d)</th>
<th>$K_f$ (mL/min/kg)</th>
<th>$P_{GC}$ (µm² x 10⁻⁶)</th>
<th>$P_{UF}$ (µm² x 10⁻⁶)</th>
<th>Glomerular Injury (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (n=8)</td>
<td>250 ± 7.3</td>
<td>34.2 ± 1.8</td>
<td>0.004 ± 0.0007</td>
<td>245 ± 4.9</td>
<td>33.6 ± 0.6</td>
<td>0.003 ± 0.0005</td>
<td>27.8 ± 2.5</td>
<td>67.6 ± 2.3</td>
<td>67.6 ± 2.3</td>
<td>0.002 ± 0.0005</td>
</tr>
<tr>
<td>RK-NX (n=10)</td>
<td>245 ± 4.9</td>
<td>32.0 ± 3.5</td>
<td>0.002 ± 0.0009</td>
<td>240 ± 7.3</td>
<td>33.6 ± 0.6</td>
<td>0.003 ± 0.0005</td>
<td>27.8 ± 2.5</td>
<td>67.6 ± 2.3</td>
<td>67.6 ± 2.3</td>
<td>0.002 ± 0.0005</td>
</tr>
<tr>
<td>RK-I (n=7)</td>
<td>240 ± 7.3</td>
<td>32.0 ± 3.5</td>
<td>0.002 ± 0.0009</td>
<td>240 ± 7.3</td>
<td>33.6 ± 0.6</td>
<td>0.003 ± 0.0005</td>
<td>27.8 ± 2.5</td>
<td>67.6 ± 2.3</td>
<td>67.6 ± 2.3</td>
<td>0.002 ± 0.0005</td>
</tr>
</tbody>
</table>

*RMR, renal mass reduction; $S_{cr}$, serum creatinine; RK-NX, 5/6 renal mass reduction by surgical excision; RK-I, 5/6 renal ablation by infarction.*

Figure 1. (A) Left kidney weight and glomerular volume for the sham (○), 5/6 renal ablation by infarction (RK-I) (■), and 5/6 renal mass reduction by surgical excision (RK-NX) (△) rats at 3 wk after sham ablation or 5/6 renal ablation. *P < 0.01 versus sham. (B) Renal blood flow (RBF) and GFR for the left kidney of the same three groups of rats sacrificed at 21 d. There were no significant differences between the groups. The mean arterial pressure under anesthesia for the three groups was as follows: sham, 120.8 ± 2.8 mmHg; RK-NX, 115.3 ± 5.1 mmHg; RK-I, 132.9 ± 4.9 mmHg. $P < 0.05$ RK-NX versus RK-I.
Table 2. Basal and final data of rats that underwent single nephron function studies at 3 to 4 wk after RMR\(^a\)

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial</th>
<th></th>
<th></th>
<th></th>
<th>Final (3 to 4 wk)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body Weight (g)</td>
<td>$S_\text{Cr}$ ((\mu)mol/L)</td>
<td>Proteinuria (g/d)</td>
<td></td>
<td>Body Weight (g)</td>
<td>Proteinuria (g/d)</td>
<td>RBF(^b) (ml/min per kg)</td>
<td>GFR(^b) (ml/min per kg)</td>
<td></td>
</tr>
<tr>
<td>Sham ((n = 10))</td>
<td>228 ± 5.3</td>
<td>28.3 ± 3.5</td>
<td>0.003 ± 0.0006</td>
<td></td>
<td>30.9 ± 3.5</td>
<td>0.005 ± 0.001</td>
<td>32.6 ± 2.5</td>
<td>3.9 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>RK-NX ((n = 10))</td>
<td>242 ± 8.2</td>
<td>29.2 ± 1.8</td>
<td>0.003 ± 0.0005</td>
<td></td>
<td>74.3 ± 5.3(^c)</td>
<td>0.007 ± 0.001</td>
<td>28.9 ± 4.2</td>
<td>2.7 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>RK-I ((n = 10))</td>
<td>236 ± 6.6</td>
<td>26.5 ± 2.7</td>
<td>0.003 ± 0.0005</td>
<td></td>
<td>76.9 ± 3.5(^c)</td>
<td>0.029 ± 0.006(^c)</td>
<td>23.5 ± 2.1</td>
<td>2.2 ± 0.3(^d)</td>
<td></td>
</tr>
</tbody>
</table>

\(a\) RBF, renal blood flow. Other abbreviations as in Table 1.
\(b\) The measurements are for only the intact left kidneys of sham control rats and the remnant left kidney of RK-NX and RK-I rats.
\(c\) \(P < 0.01\) compared to sham.
\(d\) \(P < 0.05\) compared to sham.
\(e\) \(P < 0.01\) compared to sham and RK-NX.

Table 3. Single nephron function at 3 wk after RMR\(^a\)

| Group          | \(\bar{\text{AP}}\) (mmHg) | \(Q_\text{A}\) (nl/min) | SNGFR (nl/min) | \(R_\text{TA} \times 10^{10}\) (dyn-s-cm\(^{-5}\)) | \(R_\text{A} \times 10^{10}\) (dyn-s-cm\(^{-5}\)) | \(R_\text{E} \times 10^{10}\) (dyn-s-cm\(^{-5}\)) | \(P_{\text{GC}}\) (mmHg) | \(\Delta P\) (mmHg) | \(P_{\text{UF}}\) (mmHg) | \(K_{\text{F}}\) (nl/s per mmHg) |
|----------------|-----------------|-----------------|----------------|-----------------|-----------------|-----------------|----------------|----------------|----------------|----------------|----------------|
| Sham (\(n = 10\)) | 104.6 ± 2 | 183 ± 18 | 38 ± 1.8 | 2.6 ± 0.3 | 1.6 ± 0.2 | 1.0 ± 0.1 | 46 ± 0.8 | 34.5 ± 0.8 | 16.7 ± 0.4 | 0.04 ± 0.002 |
| RK-NX (\(n = 10\)) | 104.3 ± 3 | 427 ± 39\(^b\) | 78 ± 9.7\(^c\) | 1.2 ± 0.2\(^b\) | 0.7 ± 0.15\(^b\) | 0.5 ± 0.08\(^b\) | 50 ± 0.9\(^b\) | 39.1 ± 1.1\(^c\) | 21.7 ± 0.4\(^b\) | 0.06 ± 0.008\(^c\) |
| RK-I (\(n = 10\)) | 135.8 ± 3\(^e\) | 440 ± 64\(^b\) | 73 ± 9.0\(^b\) | 1.6 ± 0.2\(^c\) | 1.0 ± 0.16 | 0.6 ± 0.09\(^c\) | 56 ± 1.1\(^b,d\) | 43.5 ± 0.6\(^c,d\) | 26.2 ± 1\(^b,d\) | 0.05 ± 0.006 |

\(a\) \(\bar{\text{AP}}\), mean arterial pressure; SNGFR, single-nephron GFR; \(R_\text{TA}\), total renal arterial resistance; \(P_{\text{GC}}\), glomerular capillary pressure; \(R_\text{A}\), afferent arteriolar resistance; \(R_\text{E}\), efferent arteriolar resistance; \(Q_\text{A}\), glomerular plasma flow; \(P_{\text{UF}}\), ultrafiltration pressure; \(K_{\text{F}}\), ultrafiltration coefficient.
\(b\) \(P < 0.01\) compared to sham.
\(c\) \(P < 0.05\) compared to sham.
\(d\) \(P < 0.05\) compared to RK-NX.
\(e\) \(P < 0.01\) compared to sham and RK-NX.
Studies of Temporal Expression of mRNA for Renin, TGF-β, and PDGF-B

Figure 2 illustrates the average systolic BP in the rats that underwent Northern blot analysis at each time point (3, 7, and 21 d). The RK-I rats had significantly increased systolic BP at all time points compared to both the sham and RK-NX rats. By contrast, no significant differences were seen in systolic BP between the RK-NX and sham rats at any of the time points. Northern blot analysis was used to measure the temporal expression of renin, TGF-β, and PDGF-B at 3, 7, and 21 d. Figure 3 shows the relative density units/sham controls for the mRNA expression of renin, TGF-β, and PDGF-B. Renin mRNA expression was increased in the RK-I rats at 3 and 7 d compared to the sham and RK-NX rats but was subsequently suppressed at 21 d. By contrast, the RK-NX rats exhibited no increase in renin mRNA expression at any time point, but rather a temporary suppression on day 7. The temporal pattern of TGF-β and PDGF-B mRNA expression was characterized by a marked increase in both at 21 d in only the RK-I group in whom glomerular injury had started to develop, whereas only a modest increase in TGF-β but not PDGF-B mRNA expression was noted at all time points in the RK-NX rats.

Temporal Changes in PRC after RMR

Table 4 presents the radiotelemetrically measured average systolic BP and the PRC data for the separate sets of sham, RK-NX, and RK-I rats, each of whom underwent PRC measurements at 3 and 21 d. As can be noted, changes in PRC after RMR were directionally similar to the changes in tissue renin mRNA expression. PRC was significantly increased in the RK-I rats at 3 d compared to both the sham and the RK-NX rats. At 21 d, PRC of RK-I rats was not significantly different than sham rats but was still significantly higher than that of the RK-NX rats. Average systolic BP showed an excellent correlation with PRC at 3 d (Figure 4) ($r=0.84$, $P<0.0001$), but not at 21 d ($r=0.36$, $P>0.09$).

Discussion

The functional and structural adaptations after severe RMR have been postulated to be maladaptive and responsible for the proteinuria and progressive GS that subsequently develop in these animals (2–4,11–16). Support for these conclusions has primarily been obtained by studies in the RK-I model, which develops hypertension soon after RMR (1–5,20–24). However, reduction of an equivalent amount of renal parenchyma by surgical excision (RK-NX model) instead of infarction does not result in the development of hypertension at least during

Figure 2. Average systolic BP (mmHg) for the sham (□), RK-I (■), and RK-NX (□) sacrificed at each of the time points (3, 7, and 21 d). *$P<0.01$ versus sham and RK-NX rats.

Figure 3. Densitometric analysis of Northern blot. Relative densitometric values for each mRNA were corrected by dividing each value by that for the GAPDH mRNA in each blot. The values for renin, platelet-derived growth factor-B (PDGF-B), and transforming growth factor-β (TGF-β) mRNA expression are shown as the relative density units/controls (sham) at 3, 7, and 21 d after renal mass reduction in sham (□), RK-NX (□), and RK-I rats (■). Pooled RNA obtained from separate sets of sham ($n=8$), RK-NX ($n=9$), and RK-I ($n=9$) each, at the three time points after RMR, was used for the Northern blot studies. Repeated Northern analysis using the same pooled samples yielded similar results.
the first 6 wk after RMR (21,26,34–36). These data suggest that mechanisms in addition to a severe reduction in nephron number are responsible for the development of hypertension in the infarction model. The increased expression of renin mRNA at 3 and 7 d and the increased PRC at 3 d in the RK-I, but not in the RK-NX rats, are consistent with the results of previous studies and suggest an important role for the renin-angiotensin system in the initial pathogenesis of hypertension in the RK-I model (34–39). The excellent correlation between PRC and radiotelemetrically measured BP at 3 d provides strong support for such an interpretation. It should be noted that given the micropuncture data in the present study indicate that even marked increases in single nephron glomerular blood flow and filtration rate after severe approximately 5/6 RMR may not per se be sufficient to result in GS. These data additionally indicate that only modest increases in in the RK-I rats, the increase in renin mRNA expression in the noninfarct remnant renal tissue is probably insufficient to explain the increases in the PRC observed in these rats. It is likely that both renin mRNA expression and renin content were more markedly increased. This is probably insufficient to explain the increases in the PRC observed in these rats. It is likely that both renin mRNA expression and renin content were more markedly increased in the noninfarct remnant renal tissue than in more distant ones and contributing to the increased PRC (and BP) in these rats as well as affecting the function of other nephrons (38). However, the relative suppression of renin mRNA expression and the decline in the PRC by 21 d in the RK-I rats, which have also been noted in previous studies (34,35,37–39), suggest that additional renin-independent mechanisms, possibly related to hyperaldosteronism and/or volume expansion, play a role in maintaining the hypertension over time in RK-I rats (38,40). The lack of correlation between BP and PRC at 21 d is consistent with such an interpretation. However, it is noteworthy that despite the increased BP and the possible volume expansion present by 3 wk in the RK-I rats, the PRC was still not suppressed below that of sham controls and was significantly greater than in RK-NX rats. Such considerations suggest that despite the relative reduction in renin mRNA expression and the PRC, the renin-angiotensin system may nevertheless be pathogenetically important for the maintenance of sustained hypertension in RK-I rats.

Based on the initial micropuncture studies in the RK-I model, it had been suggested that the compensatory increase in single-nephron GFR (SNGFR) after RMR per se may be maladaptive, eventually resulting in glomerular injury and sclerosis (2,3). Subsequent studies have indicated that of the individual hemodynamic determinants of this augmentation in SNGFR, an increased $p_{GC}$ is the parameter of primary importance in the pathogenesis of the “hyperfiltration” glomerular injury (3,15,16). The micropuncture data in the present study indicate that even marked increases in single nephron glomerular blood flow and filtration rate after severe approximately 5/6 RMR may not per se be sufficient to result in GS. These data additionally indicate that only modest increases in $p_{GC}$, as seen in the RK-NX rats, are sufficient to achieve substantial single nephron hyperfiltration. No significant differences in pre- and postglomerular resistances were observed between the two groups. These data are therefore consistent with the interpretation that the greater $p_{GC}$ increase observed in the RK-I rats reflects glomerular transmission of the higher systemic pressures present in the RK-I rats, rather than being intrinsic to the hemodynamic adaptations that follow RMR. Moreover, our data indicate that such modest increases in $p_{GC}$ as seen in the RK-NX rats may not be pathologic, at least over the short term. These interpretations are additionally supported by our previous observations in the WKY strain of rats, which fail to develop HTN even after 5/6 renal ablation by infarction (29). These rats also exhibited modest elevations in $p_{GC}$ (approximately 5 mmHg) comparable to those observed in RK-NX rats.
in the present study and did not develop significant GS despite substantial single nephron hyperfiltration for up to 16 wk. Therefore, increases in $P_{GC}$ sufficient to be pathologic seem to be a superimposed consequence of systemic hypertension on the intrinsic adaptive changes of RMR. A similar conclusion was reached by Meyer and Rennke (41) after more limited RMR (41). Significantly greater systemic and glomerular HTN was noted in rats that had undergone 40% RMR by segmental renal infarction despite greater compensatory hyperfiltration in the normotensive uninephrectomized rats (50% RMR).

Some investigators have suggested that structural glomerular hypertrophy (and the associated cellular responses), rather than glomerular hyperfiltration (and the associated hemodynamic changes), may be the maladaptive process that eventually causes GS (11–14). Our data do not support such a postulate. Both RK-I and RK-NX rats exhibit comparable glomerular hypertrophy, despite the differences in GS that are observed by 6 wk between the two groups (21). However, these data do not exclude the potential of long-term adverse effects of glomerular hypertrophy because of the associated increases in glomerular capillary wall tension as predicted by the Laplace Law (Tension = Pressure × Radius) (28–30,42,43). Nevertheless, these data do suggest that glomerular hypertension per se may also be of limited pathologic import in the absence of pathologic glomerular hypertension. Additionally, the similarity of glomerular hypertrophy responses between the RK-NX and RK-I rats despite the early differences in renin mRNA expression and PRC do not support a primary role for AngII as a determinant of the glomerular hypertrophy response after RMR.

Our data similarly do not support a primary role for TGF-β and PDGF-B in the renal hypertrophy response after RMR. Although a modest increase in TGF-β mRNA was observed in RK-NX rats at all time periods, its relationship to compensatory hypertension is not clear. Comparable renal and glomerular hypertrophy was observed in the RK-I rats without an increase in TGF-β at 3 and 7 d after RMR. The marked increase in PDGF-B and TGF-β observed at 21 d in the present study in the RK-I group in whom glomerular injury has started to develop, but not in the RK-NX rats, suggests that such increased expression may be a consequence of glomerular hypertension and/or may be initiated by the early increases in renin (and AngII) at 3 and 7 d in the RK-I rats. Significant in vitro and in vivo evidence supports the potential of AngII to directly cause an increase in the expression of PDGF-B and TGF-β in the RK-I model through its “growth effects” on mesangial cells analogous to those on vascular smooth muscle cells (8–12,14,44). AngII is thought to initiate a molecular cascade by activating the immediate early genes c-fos and c-myc, followed by increases in the growth factors PDGF-B and TGF-β with resulting increases in extracellular matrix accumulation and GS. However, the present study does not allow a direct and independent evaluation of the role of increased AngII and/or aldosterone in the absence of glomerular hypertension, because the mechanical stress generated by glomerular capillary hypertension per se has the potential to alter glomerular mesangial and/or endothelial function and to initiate increased generation of cytokines, collagen synthesis, and GS (40,44–47). It is also possible that infarction per se may result in the generation of local factors other than AngII that may lead to increased PDGF-B and TGF-β expression and eventual GS in the absence of systemic and/or glomerular HTN. However, the absence of such GS in normotensive WKY rats despite infarction argues against such a possibility (29). Therefore, although the present data are consistent with the postulated roles of PDGF-B and TGF-β as mediators of glomerulosclerosis (6,7,10–12,14), they do not provide evidence that these growth factors are the primary initiators of the renal hypertrophy and/or GS in the absence of pathologic glomerular hypertension and/or increased AngII after 5/6 RMR. However, such interpretations regarding the role of TGF-β and PDGF-B in glomerular hypertrophy and injury are not definitive due to the limitations of the methods used in our study. Only pooled kidney tissue from rats from each group was used for the message expression studies. Moreover, the message expression was not localized within the kidney. Because the message from the glomeruli is only a small part of the total message in the whole kidney isolates, it is quite possible that specific time-dependent patterns of glomerular expression of TGF-β and PDGF-B after RMR may be different and therefore may not be detected by the methods used in the present study. Nevertheless, the temporal pattern of TGF-β and PDGF-B mRNA expression in the RK-I group with marked increases in both at 21 d is consistent with previous reports in the RK-I group (9,11,45). Increased glomerular endothelial TGF expression using in situ reverse transcription was observed by Lee et al. at 23 to 25 d, but not at 6 to 14 d after 5/6 renal ablation in rats fed a standard protein diet (45). However, modest increases in PDGF-B glomerular expression have been noted as early as 1 wk after 5/6 ablation by some investigators (9,11).

Collectively, these data support the hypothesis that pathologic glomerular hypertension is likely to be the primary initiator of intraglomerular cellular events such as increased PDGF-B and/or TGF-β expression, which eventually culminate in glomerulosclerosis. Furthermore, these data support the postulate that such pathologic glomerular hypertension is a consequence of enhanced transmission of systemic hypertension and is not intrinsic to the glomerular hemodynamic adaptations that follow severe RMR. Renal autoregulatory mechanisms that normally serve to protect glomerular capillaries from transmission of systemic hypertension have been demonstrated to be impaired in rats with remnant kidneys (24,25,48,49), and although renal autoregulation is comparably impaired in both RK-I and RK-NX rats (21), it should be noted that recent studies using continuous radiotelemetry in awake unanesthetized rats have demonstrated a marked exaggeration in the amplitude of BP fluctuations in hypertensive RK-I rats (22–24). Therefore, the impact of impaired autoregulation on glomerular pressures is likely to be much greater in the hypertensive RK-I rats than the normotensive RK-NX rats, and the relatively modest but significant differences in $P_{GC}$ observed between RK-I and RK-NX rats under anesthesia are likely to significantly underestimate the ambient glomerular capillary exposure to hypertension in unanesthetized RK-I rats. The
excellent correlation between radiotelemetrically measured BP and GS in individual RK-I rats provides additional support for the dominant role of hypertensive mechanisms in the pathogenesis of GS after RMR (21–24,48). Therefore, the striking difference in the severity of glomerulosclerosis after comparable RMR by infarction or surgical excision appears largely to be a consequence of the presence or absence of systemic hypertension in these two models and is independent of the compensatory functional and structural adaptations after RMR.

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