Evaluating Hyperfiltration with Glycine in Hypertensive Rats with Renal Ablation

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SUMMARY Hypertension-induced renal damage is mediated by increased glomerular pressure and flow. These alterations have been evaluated by the renal response to protein or amino acids. To test this assumption, we studied glomerular hemodynamic responses to glycine infusion in rats with reduced renal mass, with and without Goldblatt hypertension. The left kidney was ablated by two thirds in 12 rats, and in 5, hypertension was induced by clipping the right renal artery. Seven normal, unmanipulated rats served as controls. Micropuncture was performed in the left kidney during control and 15% glycine infusion periods, 45 days after surgery. Arterial pressure was higher in hypertensive rats (160.3 mm Hg) than in controls (103.8 mm Hg) and rats with renal ablation (125 mm Hg; p<0.05). Higher values of single-nephron glomerular filtration rate and single-nephron plasma flow in rats with renal ablation (63.0, 223.7 nl/min) and hypertension (46.1, 239.7 nl/min) than in controls (28.8, 94.9; p<0.05) demonstrated the presence of hyperfiltration. However, glomerular pressure was elevated only in hypertensive rats (40.1 mm Hg), when compared to controls (32.7 mm Hg; p<0.05) and rats with renal ablation (33.4 mm Hg; p<0.05). Glycine increased single-nephron glomerular filtration rate and single-nephron plasma flow in control rats by 76 and 65%; rats with renal ablation had only partial responses, 35% and 23%, respectively, whereas in hypertensive rats the response was completely abolished. Glycine detected hyperfiltration and unmasked a dysfunction of preglomerular vessels that was greater in hypertensive rats and could contribute to the rise in glomerular pressure and flow and thereby to glomerular damage.

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KEY WORDS • glomerular hemodynamics • amino acid vasodilation • glomerular hypertension • preglomerular vessel dysfunction

IT is now well accepted that increased glomerular capillary pressure and flow can lead to severe glomerular damage. Numerous investigators have demonstrated this association in different experimental conditions in which reduction of renal mass is associated with high protein diet or systemic hypertension.1-4 Dworkin et al.2 described in detail the glomerular lesions induced by hyperfiltration in uninephrectomized rats with deoxycorticosterone-salt hypertension. These included marked dilation of capillary loops, mesangial proliferation, rupture of the capillary wall with extravasation of plasma and formed elements of blood to Bowman's space giving rise to microaneurysms, and the presence of segmental sclerosis in as much as 30% of the glomeruli. We found very similar hemodynamic and structural changes in Goldblatt hypertensive rats with partial ablation of the unclipped kidney.

In our study, structural changes were absent in normotensive controls with equal reductions in renal mass, in spite of similar degrees of hyperfiltration, but without increase in glomerular pressure. This finding suggested that the rise in glomerular pressure rather than in flow determined the structural damage.5

Short-term protein intake is known to produce a vasodilator response in the kidneys6, 7 that appears to be mediated by glucagon release,7 although other humoral factors have been proposed.8 Studies in experimental animals and in humans, using oral protein intake or amino acid infusion, showed a rapid increase in glomerular filtration rate (GFR) and renal plasma flow that reached a maximum after 60 to 120 minutes.5-8 This response was reproduced successfully by Baylis9 in rats surgically prepared for micropuncture using 15% glycine infusion. In humans, it has been used to evaluate the presence of hyperfiltration, assuming that
preexisting elevation of glomerular pressure and flow will prevent any further rise in GFR\(^5\); this assumption, however, has not been tested directly by measuring the glomerular hemodynamic response to amino acid infusion under conditions associated with hyperfiltration. Therefore, the purpose of this study was to test if the response to glycine infusion can predict the presence of hyperfiltration by directly measuring glomerular hemodynamics in normal control rats and in rats with hyperfiltration induced by partial ablation of one kidney with and without Goldblatt hypertension.

**Materials and Methods**

Reduction of renal mass was induced in 12 Wistar rats weighing 150 g. Under light ether anesthesia, partial renal ablation (RA) was induced by ligating three or four branches of the left renal artery to reduce renal mass by two thirds; in five rats a 0.2-mm internal diameter silver clip was placed in the right renal artery to induce systemic hypertension (HT). Systolic blood pressure was measured in the tail of conscious rats before and every 2 weeks after the clip was placed, using a programmed electrosphygmomanometer and a MK-IV physiograph (Narco Biosystems, Houston, TX, USA). Forty-five days after surgery rats underwent micropuncture studies on the partially ablated left kidney.

Micropuncture studies under euvolemic conditions were undertaken in seven normal rats, in six RA rats, and in five with RA and HT. Anesthesia was induced with Inactin, 100 mg/kg i.p., and rats were surgically prepared as previously described. In brief, jugular vein, femoral artery, left ureter, and bladder were catheterized. The left kidney was exposed through a flank incision, placed in a Lucite holder, and packed with thin-flowing silicone (Xantopren, Bayer). The surface was then covered with Ringer's solution. A femoral artery catheter was used to take periodic blood samples and monitor mean arterial pressure. During surgery, rats received an infusion of plasma (1% of body weight) through a jugular catheter. Immediately after a bolus injection of 100 mg of inulin in 0.5 ml of Ringer's solution, an infusion of 2% inulin in Ringer's solution was started at a rate of 2.2 ml/hr. A period of 60 minutes was allowed for equilibration.

The following experimental protocol was performed in all rats. After measuring glomerular hemodynamics during control, a glycine infusion was started by adding glycine to the inulin infusion solution to obtain a final concentration of 15% without changing the infusion rate. Thirty minutes were allowed for equilibration before repeating micropuncture measurements.

Samples of proximal tubular fluid were obtained during 3 minutes from six different nephrons after inserting an oil block for determining flow rate and inulin concentration to calculate single-nephron glomerular filtration rate (SNGFR). Inulin was measured in plasma and urine collected from the left ureter to calculate whole-kidney GFR. Using a continuous-recording Servo Null micropipet transducer (Model 4A, Instrumentation for Physiology and Medicine, San Diego, CA, USA), intratubular pressure was measured in additional proximal tubules under free-flow (FFP) conditions and after stopping tubular flow with an oil block (stop-flow pressure, SFP); hydrostatic pressure was also measured in peritubular capillaries.

Colloid osmotic pressure in glomerular capillaries was estimated from the protein concentration in femoral artery (preglomerular) and in blood obtained by puncturing surface efferent arterioles (postglomerular). The estimates of afferent and efferent plasma protein concentration permitted calculation of single-nephron filtration fraction and initial glomerular capillary plasma flow rate (SNPF), using equations given elsewhere. Effective filtration pressure and ultrafiltration coefficient \(K_f\) were calculated with the equation given by Blantz. The concentration of inulin in tubular fluid, plasma, and urine, and protein concentrations in plasma from the efferent arteriole and femoral artery were determined as previously described. Results are expressed as means ± SEM. Statistical analysis was performed by Student's t test for comparing the changes induced by glycine in the same group and by one-way analysis of variance followed by computation of modified t values and multiple pairwise comparisons according to the method of Bonferroni for group comparisons. Statistical significance was defined as \(p<0.05\).

**Results**

Table 1 shows the results obtained in the three groups of rats. Partial ablation of the left kidney was followed by a slight but significant elevation of arterial pressure compared to normal control rats. The procedure produced a considerable reduction of renal mass as indicated by a 75% lower value of GFR; in the glomerular microcirculation, however, RA-induced hyperfiltration was disclosed by a 119% higher value of SNGFR. Elevation of SNGFR was associated with a similar increase in SNPF of 138% and in the \(K_f\) of 132%. Glomerular hydrostatic pressure estimated as SFP, glomerular capillary pressure (Poc), transcapillary hydrostatic pressure gradient (ΔP), or effective filtration pressure (EFP) was not different than in normal controls. The higher values of SNPF were associated with renal vasodilation as indicated by the lower values of renal resistance, although only the differences in total and efferent resistance were significant. The association of RA and constriction of the right renal artery produced severe hypertension in every rat. Mean arterial pressure in this group was significantly higher than in the other two. In rats with RA-HT, GFR was similarly reduced by 58% and hyperfiltration was evident in the glomerular microcirculation; indeed, SNGFR and SNPF were 61% and 151% higher, respectively, than in normal controls. In the presence of systemic hypertension, the elevation of SNGFR was associated with a significant increment in glomerular pressure estimated as SFP, PGC, EFP, and (ΔP) and with lower but not significantly different values of \(K_f\) when compared with RA rats. In this group, the higher
values of Poc and SNPF indicated an abnormal response of renal vascular resistance to systemic hypertension; in fact total resistance was lower than in controls, although only the difference in afferent resistance was significant, and values were not different from those of RA rats in which systemic pressure was normal.

Glycine infusion produced quite different effects on glomerular hemodynamics in the three groups of animals. In normal controls it induced a remarkable rise in GFR; whole-kidney GFR rose 36% and SNGFR 76%. The rise in SNGFR was due to a concomitant elevation of SNPF, since the other determinants of SNGFR did not change. In fact SNPF changed in the same proportion as SNGFR, 65%; therefore filtration fraction did not vary. Although there was a significant rise in glomerular pressure estimated either as SFP or Poc, ΔP did not change because of a simultaneous increase in FFP produced by the increase in tubular flow rate secondary to the rise in SNGFR. The $K_f$ tended to rise during glycine infusion, but the change did not reach statistical significance. Renal vasodilator response to glycine was due mainly to a fall in afferent resistance, since the fall in effenter resistance was not significant.

Figure 1 illustrates the response to glycine in the other two groups. In rats with RA, in which renal vasodilation was already present, the response to glycine was partially suppressed; in fact, SNGFR increased only 35% and SNPF 23%. Other determinants of SNGFR remained unchanged, and total GFR did not change; renal resistances tended to fall, but only efferent resistance decreased significantly. In the group with RA-HT, the presence of hyperfiltration and glomerular hypertension was associated with complete suppression of the response to glycine, whole-kidney and single-nephron GFR remained unchanged, and no significant change was observed in any of the determinants of SNGFR. SNPF remained elevated, and glomerular pressure was not affected by glycine. Renal resistances did not decrease but tended to rise, although the changes were not significant.

Discussion

It is presently accepted that increased glomerular pressure and flow constitute an important mechanism of progression of renal damage in different experimental conditions, including several forms of experimental hypertension with reduction of renal mass.1-4 In the present study, partial ablation of one kidney clearly induced hyperfiltration, as demonstrated by the elevated values of SNGFR and SNPF. However, only in rats with systemic hypertension was there an increase in hydrostatic glomerular capillary pressure. This association of hyperfiltration and glomerular hypertension appears to be critical for the development of renal damage.

### Table 1. Hemodynamic Determinants of Glomerular Filtration in Normal Control Rats and in Rats with Partial Renal Ablation or Renal Ablation and Goldblatt Hypertension

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Glycine</th>
<th>Control</th>
<th>Glycine</th>
<th>Control</th>
<th>Glycine</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>103.8±5.8</td>
<td>108.4±4.7</td>
<td>125±2.6†</td>
<td>120±2.7</td>
<td>160.1±10.5§∥</td>
<td>159.9±11.1†∥</td>
</tr>
<tr>
<td>GRf (ml/min)</td>
<td>1.04±.08</td>
<td>1.41±0.13</td>
<td>0.30±0.1</td>
<td>0.32±0.08</td>
<td>0.44±0.07∥</td>
<td>0.37±0.07§∥</td>
</tr>
<tr>
<td>SNGFR (nl/min)</td>
<td>28.8±2.0</td>
<td>50.8±3.9*</td>
<td>63.0±4.5†</td>
<td>85.1±7.93‖</td>
<td>46.1±5.2†</td>
<td>45.6±4.5#</td>
</tr>
<tr>
<td>SNPF (nl/min)</td>
<td>94.9±7.1</td>
<td>156.8±13.0*</td>
<td>223.7±14.3†</td>
<td>275.9±22.7*</td>
<td>241.9±26.3‡</td>
<td>249±29.7</td>
</tr>
<tr>
<td>SNFF</td>
<td>0.31±0.02</td>
<td>0.33±0.03</td>
<td>0.27±0.02</td>
<td>0.30±0.01</td>
<td>0.20±0.02$</td>
<td>0.25±0.05</td>
</tr>
<tr>
<td>$\rho_a$ (mm Hg)</td>
<td>15.6±0.11</td>
<td>14.4±0.72</td>
<td>17.0±0.8</td>
<td>14.5±1.1</td>
<td>17.7±1.3</td>
<td>16.8±1.5</td>
</tr>
<tr>
<td>$\rho_e$ (mm Hg)</td>
<td>27.5±1.2</td>
<td>26.6±0.32</td>
<td>26.8±0.9</td>
<td>25.2±1.5</td>
<td>24.9±1.0</td>
<td>26.1±1.5</td>
</tr>
<tr>
<td>FFP (mm Hg)</td>
<td>9.8±0.9</td>
<td>14.0±1.9*</td>
<td>10.0±0.7</td>
<td>9.5±0.6</td>
<td>10.8±1.2</td>
<td>10.2±1.6</td>
</tr>
<tr>
<td>Pct (mm Hg)</td>
<td>11.6±1.1</td>
<td>15.8±1.7*</td>
<td>11.5±0.5</td>
<td>10.2±0.7</td>
<td>13.4±1.4</td>
<td>11.5±1.3</td>
</tr>
<tr>
<td>SFP (mm Hg)</td>
<td>27.0±1.2</td>
<td>34.1±2.6*</td>
<td>26.5±1.4</td>
<td>28.2±1.4</td>
<td>35.5±2.1‡</td>
<td>33.1±2.0</td>
</tr>
<tr>
<td>Poc (mm Hg)</td>
<td>42.7±1.1</td>
<td>48.3±2.3*</td>
<td>43.4±1.3</td>
<td>43.0±2.1</td>
<td>50.9±1.8§</td>
<td>50.0±5.4</td>
</tr>
<tr>
<td>$\Delta P$ (mm Hg)</td>
<td>32.7±1.3</td>
<td>34.7±1.6</td>
<td>33.4±1.7</td>
<td>33.5±1.7</td>
<td>40.1±2.5§</td>
<td>39.8±2.7</td>
</tr>
<tr>
<td>AR (dync-sec-cm⁻²)</td>
<td>3.11±0.58</td>
<td>1.81±0.42*</td>
<td>1.77±0.3</td>
<td>1.57±0.4</td>
<td>2.22±37†</td>
<td>2.79±0.69</td>
</tr>
<tr>
<td>ER (dync-sec-cm⁻²)</td>
<td>1.85±0.24</td>
<td>1.16±0.25</td>
<td>0.77±0.12†</td>
<td>0.8±0.21‡</td>
<td>0.91±0.21</td>
<td>1.24±0.39</td>
</tr>
<tr>
<td>TR (dync-sec-cm⁻²)</td>
<td>4.96±0.79</td>
<td>3.08±0.6</td>
<td>2.58±0.39†</td>
<td>2.37±0.56</td>
<td>3.14±0.56</td>
<td>4.04±1.1</td>
</tr>
<tr>
<td>EFP (mm Hg)</td>
<td>10.9±1.6</td>
<td>13.2±2.1</td>
<td>12.5±2.4</td>
<td>13.6±1.1</td>
<td>18.9±2.7†</td>
<td>18.3±2.2</td>
</tr>
<tr>
<td>$K_f$ (nl/sec/mm Hg)</td>
<td>0.044±0.01</td>
<td>0.074±0.02</td>
<td>0.102±0.03†</td>
<td>0.128±0.04</td>
<td>0.046±0.01</td>
<td>0.042±0.01</td>
</tr>
</tbody>
</table>

*Values are means ± SEM. NL = normal; RA = renal ablation; RA-HT = renal ablation and hypertension; MAP = mean arterial pressure; GFR = glomerular filtration rate; SN = single nephron; SNPF = SN plasma flow; SNFF = SN filtration fraction; $\rho_a$, $\rho_e$ = afferent and efferent oncotic pressure; FFP = free-flow tubular pressure; Pct = peritubular capillary pressure; EFP = effective filtration pressure; $K_f$ = ultrafiltration coefficient.

*p<0.05 glycine vs control; †p<0.05 NL vs RA; §p<0.05 NL vs RA-HT; $p<0.05$ RA vs RA-HT; ‡p<0.05 Gly NL vs Gly RA; ¶p<0.05 Gly NL vs Gly RA-HT; #p<0.05 Gly RA vs Gly RA-HT.
 Previous studies, including one from our laboratory in rats with the same type of hypertension as in the present study, demonstrated that hyperfiltration by itself, that is, the increment in flow to glomerular capillaries alone, does not produce structural damage, whereas the association of increased glomerular pressure and flow induces severe damage to glomerular capillaries. Studies in humans have suggested that the rise in GFR and renal plasma flow induced by acute protein intake or an amino acid infusion represents the "renal function reserve" and may therefore be used for indirect detection of alterations in glomerular hemodynamics, assuming that preexisting elevation of glomerular pressure and flow will prevent any rise in GFR. The recent demonstration by Baylis that this response can be induced with 15% glycine infusion in rats surgically prepared for micropuncture allows the possibility to test this assumption directly by measuring the glomerular hemodynamic response in conditions associated with hyperfiltration and without associated glomerular hypertension.

Our results in control rats confirmed the findings of Baylis, glycine produced an increment in SNGFR that was entirely due to the rise in SNPF, since the other determinants of SNGFR did not change. This would indicate that the response to glycine is determined mainly by the ability of preglomerular vessels to dilate and to increase flow to glomerular capillaries in response to a stimulus such as glycine. In the presence of hyperfiltration associated with RA, where renal resistances were already decreased, there was only a partial response, suggesting a functional impairment of preglomerular vessels that limits the ability to dilate in response to glycine infusion.

Arterial hypertension is commonly associated with structural and functional alterations of preglomerular vessels; vascular hypertrophy and sclerosis of preglomerular vessels are usually present after long-standing hypertension, and autoregulation is frequently impaired. In our study, the presence of arterial hypertension in RA-HT was associated with a greater degree of functional impairment of preglomerular vessels, as indicated on one hand by the total inability to dilate in response to glycine infusion, and on the other by failure to constrict efficiently and prevent the rise in glomerular capillary pressure, which resulted in the combination of hyperfiltration and glomerular hypertension. This favored the development of glomerular structural damage.

In summary, renal function reserve, as estimated by the response to glycine, is determined by the functional integrity of preglomerular vessels. Hyperfiltration alone is associated with functional impairment of these vessels that limits their response to glycine infusion. Complete blunting of the response to glycine suggests a greater functional derangement associated with hyperfiltration and increased glomerular pressure.

References

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