Angiotensin Responsiveness in Hyperfiltering and Nonhyperfiltering Diabetic Rats

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ABSTRACT
Renal and systemic responses to angiotensin II were studied in hyperglycemic diabetic rats (streptozotocin, 60 mg/kg, iv) and vehicle-injected controls at 24 h, 1 wk, 2 mo, or at 6 to 12 mo. In normal rats, the GFR was less than 0.80 mL/min per 100 g body wt (0.57 ± 0.02 mL/min per 100 g body wt; range: 0.40 to 0.79 mL/min per 100 g body wt; N = 45). Hyperfiltration (GFR ≥ 0.80 mL/min per 100 g body wt) was observed in all diabetic rats studied at 1 wk (GFR, 1.03 ± 0.07 mL/min per 100 g body wt; N = 5; P < 0.001 versus control). However, at earlier and later times, GFR was elevated in only 8 of 18 of the diabetic rats (44%), with an overall prevalence of 56% (13 of 23). Mean arterial pressure, plasma glucose, urine volume, and filtration fraction were not different in hyperfiltering diabetic rats compared with nonhyperfiltering diabetic rats or normal controls. Angiotensin II (12.5 ng/kg per minute iv) had no effect on GFR in normal rats or nonhyperfiltering diabetic rats, but it normalized GFR in hyperfiltering diabetic rats (0.74 ± 0.05 mL/min per 100 g body wt). In contrast with the renal effects of angiotensin II, blood pressure responses were similar in hyperfiltering and nonhyperfiltering diabetic rats. The findings that angiotensin II infusion caused a greater fall in GFR in hyperfiltering diabetic rats than in nonhyperfiltering diabetic rats, but that blood pressure responses were similar, suggests a localized abnormality in angiotensin responsiveness in the kidneys. The mechanism appears to be distinct from the previously described reduction in glomerular angiotensin II receptor sites and may involve changes in the local production or degradation of angiotensin peptides.

Key Words: Renal vascular reactivity, diabetes, insulin, angiotensin receptors

Insulin-dependent diabetes mellitus causes predictable changes in renal function in both humans and laboratory animals. Early in the course of diabetes in humans and in experimental animals, there is a rise in GFR. It is believed that hyperfiltration may lead to later glomerular pathology and progressive renal impairment (1–4). The mechanism(s) leading to diabetic hyperfiltration are not known, but many factors have been implicated. The level of plasma glucose, duration of diabetes, genetic constitution, and hypertension are factors that have been suggested to contribute to the development of diabetic kidney disease in humans and animal models (1,5).

Multiple abnormalities in hormone systems have been reported in diabetic glomeruli, suggesting that the cause of hyperfiltration may involve a complex interaction between vasodilator and vasoconstrictor factors. Perhaps the best studied is the renin-angiotensin system. One week after the induction of diabetes with streptozotocin, the number of specific glomerular angiotensin II receptor sites was reduced by one third (6,7). This finding could not be explained by homologous down-regulation because plasma angiotensin II levels were not elevated and suggested an alternate mechanism for the receptor deficit (7). However, when the plasma renin-angiotensin system was stimulated either by sodium depletion or by intrarenal infusion, hyperfiltration was corrected, suggesting that the reduced receptor density relative to the availability of endogenous angiotensin II may play a role in the hyperfiltration (8).

This investigation was undertaken to study the relationship between glomerular hyperfiltration and angiotensin II responsiveness in diabetic rats over a 1-yr interval after the induction of diabetes. We hypothesized that angiotensin II responsiveness would be blunted in hyperfiltering diabetic rats because angiotensin II receptors were reduced in number early in the course of the disease. To our surprise, we found that angiotensin II caused greater reductions in GFR in hyperfiltering diabetic rats than in nonhyperfiltering diabetic rats or normal controls.
The relationship between the duration of diabetes, hyperfiltration, and systemic vascular and renal responses to angiotensin II was examined.

METHODS

Adult male Sprague-Dawley rats were given ad libitum access to standard Purina rat chow (Ralston Purina Co., Chicago, IL) (sodium ash content, 0.42%) and tap water. Diabetes was induced by the rapid iv injection of streptozotocin (60 mg/kg, iv) in citrate buffer into the jugular vein of anesthetized rats (pentobarbital sodium, 45 mg/kg, ip), and the diabetic state was confirmed by positive urinary glucose (Ames dipstick; Miles Inc., Elkhart, IN) at 24 h and by plasma glucose measurements at the time of study. Plasma glucose was measured by the hexose kinase method.

Renal Function Measurements

GFR and effective RPF were measured as described previously (9). In brief, rats were anesthetized with thiobutabarbital (100 mg/kg, ip) and placed on a heated dissection table to maintain a core body temperature at 37°C. Surgical preparation included the placement of a tracheostomy (PE 240 or PE 260; Clay Adams, Parsippany, NJ), femoral artery and femoral vein catheters (PE 50), and bilateral ureteral catheters (PE 10). During the surgery, a constant infusion of normal saline was delivered at 3.3 mL/h, followed by a constant infusion at 1.20 mL/h. At the completion of surgery, 125Iiothalamate (25 μCi) and 131I-hippuran (60 μCi) were given as a bolus (0.1 mL), followed by a constant infusion (iothalamate, 0.063 μCi/min; hippuran, 0.150 μCi/min). Mean arterial pressure (MAP) was monitored with a Statham P23 ID transducer (Gould Instruments, Oxnard, CA) connected to a Grass polygraph (Grass Instruments, Quincy, MA). Thirty minutes later, timed urine samples were collected for three 10-min periods with midpoint plasma sampling (0.35 mL). At the end of each experiment, urine and plasma samples (10 to 25 μL) were counted for 125I and 131I. Two-kidney GFR, RPF (uncorrected for extraction fraction), and filtration fraction were calculated as the average of three collection periods.

In other experiments, the effects of angiotensin II on renal hemodynamics were studied. After baseline measurements were completed (clearance period 1), angiotensin II (6.25 ng/kg per minute iv) was infused. Fifteen minutes after the initiation of angiotensin infusion, the clearance measurements were repeated (period 2). A third clearance measurement was performed 15 min after the initiation of an infusion of a higher dose of angiotensin II (12.5 ng/kg per minute iv). In preliminary studies in normal and diabetic rats, repeated clearance measurements (up to six per rat) gave stable values.

Establishing Normal Values for GFR

Hyperfiltration was defined after the upper limit of GFR was established in a group of 45 normal rats of ages ranging from 6 wk to 14 mo. In every instance, GFR was less than 0.80 mL/min per 100 g body wt (mean ± SE: 0.57 ± 0.02 mL/min per 100 g body wt; range, 0.40 to 0.79 mL/min per 100 g body wt), which is taken as the upper limit of normal for GFR. Rats older than 4 mo had reductions in the mean GFR/100 g body wt (age < 4 mo, GFR: 0.63 ± 0.02; N = 31; age 8 to 14 mo, GFR: 0.46 ± 0.03 mL/min per 100 g body wt, N = 14; P < 0.01) and in the upper limit of GFR/100 g body wt (age < 4 mo, GFR: 0.44 to 0.79; age 8 to 14 mo, GFR: 0.38 to 0.63 mL/min per 100 g body wt). We chose a conservative approach of defining hyperfiltration as the GFR/100 g body wt that exceeded values measured for all rats, regardless of age.

Protocols

Three separate protocols were performed on individual groups of rats. The first protocol examined the relationship between body weight, age, and GFR. Five normal rats (age control) and five littermates injected with streptozotocin (untreated diabetics) were studied at 1 wk. Because untreated diabetic rats routinely lost 20 to 30 g of body weight over the first week, a third group of normal rats were studied as a control for body weight. These rats were selected from the 45 animals used to establish the normal range of GFR to exactly match the weights of the 1-wk diabetic rats ± 2 g of body weight.

The second protocol examined the effects of insulin on GFR in diabetic rats at 1 wk. Ten normal age-matched controls were studied on the same day as 1-wk diabetic rats (N = 10). Twenty-four hours after the injection of streptozotocin, diabetes was documented by the measurement of plasma glucose concentration. Half of the rats were injected with isophane insulin (5 to 10 U/24 h, sc) to normalize plasma glucose levels.

The third protocol studied the effects of the duration of diabetes on renal and systemic hemodynamics in the basal state and in response to angiotensin II infusions. In brief, after the baseline determination of MAP, GFR, RPF, and urine flow rates, rats received a graded infusion of angiotensin II and the measurements were repeated. Normal controls (N = 10) or untreated diabetic rats (N = 23) were studied at either 1 day, 1 wk, 2 mo, or 6 to 12 mo after the induction of diabetes. Because of a power interruption during the angiotensin II infusion portion of the protocol, one diabetic rat and its control did not complete the protocol and have been eliminated from the data analysis of the angiotensin II protocols. Because body weight and kidney weight increase at a different rate...
In normal and untreated diabetic rats, angiotensin II sensitivity was assessed by examination of the data both as GFR and RPF adjusted for body weight and as a percent change in renal hemodynamics from baseline.

Materials

[125]Iothalamate was purchased from Iso-Tex Diagnostics (Friendswood, TX), and [131]IIoipuran was purchased from Syncor International (Garden City, NY). All chemicals were purchased at the highest grade available.

Data Analysis

Data are presented as the mean ± standard error. Differences between groups were tested by use of analysis of variance for parametric and nonparametric data with the SAS program (SAS Institute, Cary, NC) on an IBM (IBM, Purchase, NY) PC computer. Where repeated observations were made on the same rats, data were analyzed by repeated measures analysis of variance performed at each level of the repeated factor with the Student-Newman-Keuls multiple comparison test. Where appropriate, the Kruskal-Wallis test was used. Separate analyses were performed to determine the relationships between the duration of diabetes and the presence of hyperfiltration on angiotensin responses. The null hypothesis was rejected when P < 0.05.

RESULTS

The effects of diabetes on body weight, plasma glucose, and renal hemodynamics at 1 wk are shown in Table 1. Diabetic rats lost 5% of their body weight during the first week, compared with a 9% gain in body weight in age-matched controls (P < 0.05). Although MAP was reduced in diabetic rats, the value did not reach statistical significance. RPF and GFR were significantly elevated in diabetic rats compared with either age-matched or weight-matched controls. The increases in RPF and GFR were significant when data were analyzed either as GFR or RPF per rat or per 100 g body wt.

The effects of insulin replacement on GFR were determined. The administration of insulin to five diabetic rats normalized plasma glucose concentrations and reverse hyperfiltration at 1 wk when compared with normal littermates or untreated diabetic rats (Figure 1).

The relationship between the duration of diabetes and GFR is shown in Figure 2. Each rat was studied once at the time indicated on the abscissa. The stippled area represents the normal range of GFR in 45 rats undergoing measurements in our laboratory (see Methods). GFR measurements of 10 of 45 control rats that were studied on the same days as the diabetic rats are indicated on the left side of the figure. At 1 wk after the induction of diabetes, all rats studied had elevated GFR (1.03 ± 0.07 ml/min per 100 g body wt; P < 0.001). At earlier (1 day) and later times (2 mo or 9 to 12 mo), only 8 of 18 rats exhibited hyperfiltration. When all of the diabetic rats (1 day, 1 wk, 2 mo, and 6 to 12 mo) were analyzed by GFR, 10 diabetic rats had normal GFR and 13 diabetic rats had increased GFR. Body weights were similar in nonhyperfiltering and hyperfiltering diabetic rats (nonhyperfiltering: 390 ± 36 [N = 10] versus hyperfiltering: 324 ± 9 g body wt [N = 13], P = not significant [NS]), and the differences in GFR were significant when the data were not factored for body weight (nonhyperfiltering: 2.13 ± 0.17 versus hyperfiltering: 2.72 ± 0.14 ml/min; P < 0.01). Compared with normal rats, MAP was equally reduced in diabetic rats with normal or elevated GFR (Figure 3A). Plasma glucose was also equally elevated in diabetic rats with and without hyperfiltration. This finding was further substantiated by the observation that the amount of polyuria was similar in the hyperfiltering and non-

### Table 1. Renal function in 1 wk diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>BWI (g)</th>
<th>BWF (g)</th>
<th>Glucose (mg/dL)</th>
<th>MAP (mm Hg)</th>
<th>RPF (ml/min per 100 g body wt)</th>
<th>RPF (ml/min per 100 g body wt)</th>
<th>GFR (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic (N = 5)</td>
<td>361 ± 9</td>
<td>342 ± 7</td>
<td>388 ± 34</td>
<td>96 ± 8</td>
<td>2.52 ± 0.15 °</td>
<td>8.64 ± 0.61 °</td>
<td>1.03 ± 0.07 °</td>
</tr>
<tr>
<td>Weight control</td>
<td></td>
<td>343 ± 8</td>
<td>106 ± 11</td>
<td>106 ± 3</td>
<td>1.96 ± 0.13</td>
<td>6.75 ± 0.52</td>
<td>0.71 ± 0.03</td>
</tr>
<tr>
<td>(N = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.42 ± 0.13</td>
</tr>
<tr>
<td>Age control</td>
<td></td>
<td>352 ± 15</td>
<td>384 ± 14</td>
<td>104 ± 10</td>
<td>1.64 ± 0.11</td>
<td>6.25 ± 0.33</td>
<td>0.67 ± 0.04</td>
</tr>
<tr>
<td>(N = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.57 ± 0.13</td>
</tr>
</tbody>
</table>

° Abbreviations: BWI, initial body weight; BWF, body weight at time of measurements.

* P < 0.01 versus weight control and age control.

* P < 0.05 versus weight control and age control.
The effects of insulin replacement on renal hemodynamics. When compared with 10 normal rats (five weight control and five age control), diabetic rats had significantly elevated GFR. The administration of insulin (5 to 10 U sc daily) normalized both plasma glucose concentrations and GFR in 1-wk diabetic rats. The number in the bars represents the number of individual rats studied in each group. MAP was 109 ± 4 mm Hg in normal rats, 94 ± 7 mm Hg in untreated diabetic rats, and 87.0 ± 4 mm Hg in the insulin-treated rats (normal versus insulin-treated diabetic rats, P < 0.05; normal versus untreated diabetic rats, P = NS; untreated versus insulin-treated diabetic rats, P = NS). RPF was similarly elevated in the untreated diabetic rats (2.47 ± 0.19 ml/min per 100 g body wt (BW)) compared with normal controls (1.84 ± 0.11 ml/min per 100 g body wt) or insulin-treated diabetic rats (1.81 ± 0.17 ml/min per 100 g body wt) (untreated diabetic rats versus normal controls or insulin-treated diabetic rats, P < 0.01).

Additional experiments were performed to test the hypothesis that alterations in renal vascular responses could contribute to the pathogenesis of hyperfiltration. Angiotensin II was infused at a nonpressor dose that raised MAP by approximately 20 mm Hg (12.5 ng/kg per minute). These studies were done on the same rats depicted in Figure 2. Nine control rats and 22 diabetic rats completed the protocol. Normal rats and nonhyperfiltering diabetic rats had virtually identical responses to angiotensin II, whether expressed as GFR and RPF normalized for body weight (Figure 4) or as percent change from baseline (independent of body weight) (Table 2). At a low dose, angiotensin II had no effect on MAP or GFR and caused a very modest reduction in RPF (Figure 4). In contrast, the same dose of angiotensin II caused a striking reduction in RPF in hyperfiltering diabetic rats, which was associated with a significant fall in GFR toward the levels seen in control or nonhyperfiltering diabetics. At the higher dose, angiotensin II had no effect on GFR in normal or in nonhyperfiltering diabetic rats, but it normalized GFR and RPF in the hyperfiltering diabetic rats. Table 2 shows the changes in GFR and RPF in response to the high dose of angiotensin II, expressed as a percentage of the baseline. GFR decreased only in the diabetic, high-GFR group (P < 0.01). RPF decreased in all groups (P < 0.01), but the percent decrease was significantly greater in the hyperfiltering diabetic rats compared with the nonhyperfiltering group. The filtration fraction was significantly increased in all groups with the higher dose of angiotensin II (P < 0.01).

The effects of angiotensin II on renal resistance (RR) were estimated by the formula RR (arbitrary units [A.U.]) = MAP/RPF (Table 3). RR was reduced by ~60% in hyperfiltering compared with nonhyperfiltering diabetics. The only parameter that correlated with hyperfiltration was RPF (Figure 3B). Nonhyperfiltering diabetic rats had normal RPF, but RPF was elevated by 45% in hyperfiltering diabetic rats (P < 0.01). The filtration fraction was not different from control in hyperfiltering and nonhyperfiltering diabetic rats, suggesting that increased RPF was an important factor associated with hyperfiltration.

Figure 2. The temporal course of GFR after the induction of diabetes. GFR was studied over a 12-mo interval after the induction of diabetes. The stippled band represents the range of GFR measurements of 45 normal rats, ages 2 to 14 mo (0.40 to 0.79 ml/min per 100 g body wt (BW)). The data shown for control measurements were performed on the same days as the measurements in diabetic rats and are representative of the entire group. At 1 wk after the induction of diabetes, all rats studied had elevated GFR (P < 0.001). At earlier (1 day) and later (2 mo or 6 to 12 mo) times only 8 of 18 rats hyperfiltered.
A low-dose angiotensin II infusion significantly increased RR, but RR remained significantly lower in the hyperfiltering group. A high-dose angiotensin II infusion further increased RR in both groups to virtually identical values (diabetic, normal GFR: 87.7 ± 9.3 versus diabetic, high GFR: 89.4 ± 8.7 A.U./body wt; P = NS). The percent increase in RR compared with the baseline (no drug) period was greater in hyperfiltering than in nonhyperfiltering diabetic rats after high-dose angiotensin II infusion (P < 0.01).

DISCUSSION

Many theories have been presented to explain the cause of glomerular hyperfiltration in diabetes mellitus, but there is a paucity of data regarding some important basic aspects of the hyperfiltration phenomenon. Central among these remain the role of the duration of diabetes and the potential role of alterations in the renin-angiotensin system in the pathogenesis of diabetic nephropathy. The experiments presented here studied the prevalence of hyperfiltering diabetic rats. A low-dose angiotensin II infusion significantly increased RR, but RR remained significantly lower in the hyperfiltering group. A high-dose angiotensin II infusion further increased RR in both groups to virtually identical values (diabetic, normal GFR: 87.7 ± 9.3 versus diabetic, high GFR: 89.4 ± 8.7 A.U./body wt; P = NS). The percent increase in RR compared with the baseline (no drug) period was greater in hyperfiltering than in nonhyperfiltering diabetic rats after high-dose angiotensin II infusion (P < 0.01).

Figure 3. Comparison of renal and systemic hemodynamics in diabetic rats. Diabetic rats were divided into two groups: diabetics with normal GFR (DM, normal GFR) and diabetics with increased GFR (DM, increased GFR). Results of MAP, plasma glucose concentration, and urine output are depicted in Panel A. Panel B shows GFR, RPF, and filtration fraction. The numbers in the bars indicate the number of rats studied in each group. BW, body weight.

Figure 4. The effects of angiotensin II infusion on anesthetized diabetic (DM) and control rats. After the placement of femoral arterial and venous catheters, baseline MAP, GFR, and RPF were measured. Fifteen minutes later, angiotensin II was infused at 6.25 ng/kg per minute, and the measurements were repeated. A third set of measurements was made after an infusion of angiotensin at 12.5 ng/kg per minute. Nine control rats, 10 nonhyperfiltering diabetic rats, and 12 hyperfiltering diabetic rats were studied. The asterisks represent significant differences between the various groups at a single-dose level of angiotensin II. Compared with baseline measurements, angiotensin II caused significant increases in MAP and filtration fraction (FF) and reductions in RPF in all groups. GFR was unchanged at either dose level in normal or nonhyperfiltering diabetic rats but was significantly reduced in hyperfiltering diabetic rats (P < 0.01).
TABLE 2. Percent change in GFR and RPF in response to angiotensin II (12.5 ng/kg per minute)

<table>
<thead>
<tr>
<th>Group</th>
<th>% Change in GFR</th>
<th>% Change in RPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (N = 9)</td>
<td>1 ± 10</td>
<td>-31 ± 9º</td>
</tr>
<tr>
<td>Diabetic, Normal GFR (N = 10)</td>
<td>13 ± 9</td>
<td>-27 ± 7º</td>
</tr>
<tr>
<td>Diabetic, High GFR (N = 14)</td>
<td>-28 ± 5º</td>
<td>-48 ± 4º</td>
</tr>
</tbody>
</table>

º P < 0.01 versus baseline.
º P < 0.05 versus normal and P < 0.01 versus diabetic, normal GFR group.
º P < 0.05 versus normal and diabetic, normal GFR groups.

Hyperfiltration in untreated streptozotocin diabetic Sprague-Dawley rats and the relationship between hyperfiltration and changes in angiotensin II responsiveness.

The role of the duration of diabetes on GFR was studied in untreated diabetic rats. At 1 wk, all diabetic rats had increased GFR, but at the other times, (1 day, 2 mo, 6 to 12 mo) approximately half of the diabetic rats “ hyperfiltered.” Simple clinical parameters could not account for hyperfiltration because MAP, plasma glucose, urine output, filtration fraction, and body weight were not different in hyperfiltrating and nonhyperfiltrating diabetic rats. Hyperfiltrating diabetic rats had a significant increase in RPF without changes in filtration fraction, suggesting that the increases in GFR could be largely explained by the increases in RPF.

Systemic and renal responsiveness to angiotensin II was compared in hyperfiltrating and nonhyperfiltrating diabetic rats. Although MAP was equally reduced in hyperfiltrating and nonhyperfiltrating diabetic rats compared with normal controls, blood pressure responses to angiotensin II infusion were virtually identical in all groups studied. In marked contrast with the blood pressure responses, angiotensin II caused a striking fall in RPF and GFR in hyperfiltrating diabetic rats, with only a modest reduction in nonhyperfiltrating diabetic and normal control rats. RR was ~60% lower in hyperfiltrating than in nonhyperfiltrating diabetic rats. Angiotensin II infusion reduced GFR and RPF and raised RR to a greater extent in the hyperfiltrating than in the nonhyperfiltrating diabetic rats when expressed in absolute values or when compared as percent change from baseline. These data indicate that renal vasoreactivity differed in hyperfiltrating and nonhyperfiltrating diabetic rats but that systemic vascular responses were virtually identical. These findings are in contrast with previous experiments that examined renal responsiveness to a synthetic thromboxane agonist in diabetic rats. As compared with normal controls, the renal responses to the thromboxane agonist were blunted in diabetic rats (see reference 15). In this study, renal responses to angiotensin II in hyperfiltrating diabetic rats were increased.

Several important and unexpected observations were made in these studies. First, despite similar clinical characteristics, only half of diabetic rats hyperfiltered. Although hyperfiltration was universal at 1 wk, some rats hyperfiltered as early as 1 day or as late as 12 months after the onset of the diabetes. Because the number of rats studied was relatively small, it is not possible to conclude from these studies that hyperfiltration is most common at 1 wk. Most interestingly, however, when all times studied were considered together, the prevalence of hyperfiltration approximated 50%. The incidence of hyperfiltration parallels the incidence of diabetic nephropathy in patients with juvenile-onset diabetes mellitus and may be compatible with other (possibly genetic) factors that may govern the susceptibility to hyperfiltration in hyperglycemic subjects. There was no apparent relationship between increased GFR and simple

TABLE 3. Effects of angiotensin II on RR in hyperfiltrating and nonhyperfiltrating diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>BW° (g)</th>
<th>RRC/100 g body wt (A.U./body wt)</th>
<th>RRL/100 g body wt (A.U./body wt)</th>
<th>RRH/100 g body wt (A.U./body wt)</th>
<th>RRC (A.U.)</th>
<th>RRL (A.U.)</th>
<th>RRH (A.U.)</th>
<th>% Change RRC</th>
<th>% Change RRL</th>
<th>% Change RRH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic, normal GFR (N = 10)</td>
<td>390</td>
<td>55.0</td>
<td>68.1</td>
<td>87.7</td>
<td>229.4</td>
<td>279.5</td>
<td>347.8</td>
<td>27.2</td>
<td>17.5</td>
<td>70.0</td>
</tr>
<tr>
<td>Diabetic, high GFR (N = 12)</td>
<td>326</td>
<td>±36</td>
<td>±5.6</td>
<td>±6.0</td>
<td>±7.3</td>
<td>±48.7</td>
<td>±50.6</td>
<td>±55.4</td>
<td>±7.8</td>
<td>±17.5</td>
</tr>
<tr>
<td>P Value</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

° Abbreviations: body wt, body weight at time of measurements; RRC, renal resistance during no drug period; RRL, renal resistance during angiotensin infusion (6.25 ng/kg per minute); RRH, renal resistance during angiotensin infusion (12.5 ng/kg per minute); % Change RRL = 100 (RRL – RRC)/RRC; % Change RRL = 100 (RRH – RRC)/RRC.
clinical parameters (i.e., blood glucose level, urine output), suggesting a multifactorial etiology.

A second interesting finding was the observation that angiotensin II had a greater vasoconstrictor effect in the renal microcirculation of hyperfiltering diabetic rats compared with nonhyperfiltering diabetic rats and normal controls. This occurred despite similar systemic pressor responses in all groups. Although increased vascular reactivity to angiotensin II has been described in some nonrenal tissues in diabetes (10,11), the finding of increased intrarenal vasoconstriction was somewhat unexpected, in view of the previous descriptions of reduced angiotensin II receptor sites in diabetic glomeruli (6,7). Nevertheless, the observation that hyperfiltration can be reversed by increases in angiotensin II concentrations is compatible with the previous work of Bank et al. (6,12). However, in those studies, angiotensin II effects were not compared in hyperfiltering and nonhyperfiltering diabetic rats. The differential responsiveness to angiotensin II in diabetic rats with normal or elevated GFR suggests that the abnormalities in either the local production or signaling pathways for angiotensin II are present in hyperfiltering diabetic rats, but not in diabetic rats with normal GFR.

These experiments indicate that the changes in the renin-angiotensin system in diabetes are more complex than previously appreciated. The complexity is likely to be twofold: there is a heterogeneity of responses to the diabetic state by individual subjects, and there are multiple biochemical abnormalities that can occur that contribute to diabetic renal disease. For example, multiple abnormalities in prostaglandin production (13,14) and renal responses to thromboxane analogs have been recently identified in glomeruli from diabetic rats (15). In this study, renal responses to angiotensin II in hyperfiltering diabetic rats were increased.

There is an apparent paradox in the observations that angiotensin-converting enzyme inhibitors reverse proteinuria and histopathologic lesions in diabetic rats (16) and the observation that angiotensin II infusion reverses diabetic hyperfiltration. In normal kidneys, angiotensin II constricts both the afferent and efferent arterioles, with efferent arteriolar constriction being the dominant action. In hyperfiltering diabetic rats, there is increased single-nephron glomerular plasma flow, reduced afferent arteriolar resistance, normal to low efferent arteriolar resistance, and elevated glomerular capillary pressures. In hyperfiltering diabetic rats, angiotensin-converting enzyme inhibitors have been shown to significantly lower transglomerular capillary pressures by lowering MAP and by causing small (but not significant) decreases in efferent arteriolar resistance and single-nephron blood flow (16). Angiotensin II infusions are likely to reverse hyperfiltration by constricting the afferent and efferent arterioles, thus limiting single-nephron blood flow. Glomerular capillary pressure could be increased or decreased, depending on the relative constriction of the afferent and efferent arterioles. Therefore, the mechanisms of reversing hyperfiltration are different for angiotensin-converting enzyme inhibition and angiotensin infusions. In addition, the biochemical basis of the protection afforded by angiotensin-converting enzyme inhibition is not yet understood because these drugs have a myriad of actions, including the potentiation of bradykinin, a vasodilator.

Although little is known about the nature of the variability of individual responses to the diabetic state, several mechanisms have been identified at the tissue level. Changes in the plasma renin angiotensin system cannot account for hyperfiltration, because it has been previously shown that PRA and angiotensin II levels are virtually identical in 1-wk diabetic rats and non diabetic controls (7). It is possible, however, that changes in the production of active renin and angiotensinogen, or the activity of degradative pathways for mature angiotensin II in the kidney, may have a greater bearing on intrarenal vascular tone than either plasma hormone levels or the maximal number of angiotensin receptor sites, as measured by Scatchard plots. A low tissue level of angiotensin II relative to the number of available receptors would lead to a reduced number of occupied receptors compared with the number of available binding sites and could favor hyperfiltration. A shift in the distribution of angiotensin II receptors by either a change in subtype or a shift in the activity of receptors in the afferent and efferent arterioles is also possible. Molecular approaches have been used to assess the local concentrations of the components of the renin-angiotensin system in diabetes. Correa-Rotter and coworkers recently demonstrated a decrease in systemic renin activity without a change in renal renin content or renin and angiotensinogen mRNA, supporting the hypothesis that the intrarenal renin-angiotensin system may be dissociated from the systemic expression of these hormones in diabetic rats (17). Similar data were obtained in studies investigating mRNA for renin and angiotensinogen in kidneys (18) or the white adipose tissue (19) of streptozotocin diabetic rats. However, other studies have found that renal angiotensinogen mRNA levels are increased in diabetes (20). Additional studies using probes for other key components of the renin-angiotensin system (i.e., prorenin, angiotensin-converting enzyme) will be helpful in further exploring this observation. Alternatively, intrarenal angiotensin II levels may be reduced in diabetes if pathways for the degradation of mature peptide were stimulated. Other experiments aimed at studying the balance between intrarenal angiotensin II production...
and degradation would help to clarify the relationship between diabetic hyperfiltration and the intrarenal renin-angiotensin system.

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