Effect of Angiotensin II Antagonist Eprosartan on Hyperglycemia-Induced Activation of Intrarenal Renin-Angiotensin System in Healthy Humans

Suzette Y. Osei, Deborah A. Price, Lori M.B. Laffel, Maria C. Lansang, Norman K. Hollenberg

Abstract—We have previously reported that hyperglycemia in healthy human subjects increased the renal vasodilator response to the angiotensin-converting enzyme inhibitor captopril. This observation raised intriguing possibilities relevant to the pathogenesis of nephropathy in patients with diabetes mellitus. To ascertain whether the effect of captopril was indeed mediated by a reduction in angiotensin II (Ang II) formation, we performed another study in which an Ang II antagonist, eprosartan, was used in place of captopril. Nine healthy subjects were studied in high sodium balance (ie, sodium intake 200 mmol/d). On the first day, the subjects received 600 mg eprosartan orally, and renal plasma flow (RPF) and glomerular filtration rate (GFR) were measured. Glucose was infused intravenously on the second and third study days to increase plasma glucose to a level below the threshold for glycosuria (8.8 mmol/L).

Eprosartan at a dose of 600 mg or placebo was administered randomly on the second or third study day 1 hour after initiation of glucose infusion. RPF increased (by 76 ± 7 mL/min · 1.73 m², P < 0.01) in response to sustained moderate hyperglycemia and then increased further (by 147 ± 15 mL/min · 1.73 m², P < 0.01) when eprosartan was administered during hyperglycemia. Eprosartan, conversely, did not affect RPF and GFR in normoglycemic subjects. GFR was not affected by either hyperglycemia or eprosartan. Neither plasma renin activity nor plasma Ang II concentration changed during hyperglycemia, suggesting that the hormonal responses responsible for the enhanced renal vasodilator response to eprosartan occurred within the kidney. The enhancement of the renal vasodilator effect of eprosartan during hyperglycemia is consistent with activation of the intrarenal renin-angiotensin system. (Hypertension. 2000;36:122-126.)

Key Words: hemodynamics ■ kidney ■ glomerular filtration rate ■ hyperglycemia ■ sodium ■ angiotensin II

The efficacy of blockade of the renin-angiotensin system (RAS) in retarding the progression of renal disease in patients with type 1 and type 2 diabetes has focused attention on the potential interaction between glycermia and the RAS in the pathogenesis of diabetic nephropathy.1,2 Consistent with this view, we have recently shown enhanced renal vasodilator responses to captopril administration during sustained moderate hyperglycemia.3 Because this occurred without an alteration in the plasma renin activity (PRA) in sodium-replete healthy humans, this finding was suggestive of activation of the intrarenal RAS by hyperglycemia. On the other hand, it is possible that the enhancement of the renal vasodilator action during hyperglycemia of angiotensin-converting enzyme (ACE) inhibition was mediated at least in part by other vasodilator mediators, such as bradykinin, and prostaglandin release.4,5 To determine the specific role on angiotensin II (Ang II) in the alteration of renal hemodynamics induced by hyperglycemia, we compared the effects of the Ang II antagonist eprosartan on renal plasma flow (RPF) and glomerular filtration rate (GFR) in healthy humans maintained on a high-salt diet, when the subjects were normoglycemic or moderately hyperglycemic.

Methods

Subjects
After an outpatient physical and laboratory evaluation, 9 healthy men aged 26 to 46 years with a body mass index of 21.4 to 34.5 kg/m² were selected for the study. The subjects were free of cardiovascular, renal, and endocrine disease and had normal blood pressure (mean arterial pressure 68 to 102 mm Hg), fasting blood glucose (4.2 to 5.0 mmol/L), and insulin (29 to 156 pmol/L) levels. The baseline characteristics of the study subjects are shown in Table 1. The studies were in accordance with the guidelines and regulations of the Institution Review Board of Brigham and Women's Hospital and Harvard Medical School. After obtaining written informed consent, the subjects were placed on a high-salt diet (200 mmol sodium/d) 3 days before admission, and 24-hour urine samples were collected daily for measurement of...
sodium. The subjects were admitted for studies in high sodium balance (ie, 24-hour urine sodium >150 mmol/L). Each subject was admitted to the General Clinical Research Center (GCRC) the evening before the study day and maintained on a high-salt diet throughout the study period. A high-salt diet suppresses the endogenous RAS and blunts the renal vasodilator response to captopril and eprosartan in normal individuals. Therefore, it is easier to demonstrate activation of the RAS under conditions of high salt balance.

Renal Function Studies
After an overnight fast, the study subjects had 3 intravenous catheters placed in their arms at least 2 hours before the infusions for the renal function studies were initiated. Two catheters were used for the infusions, and a third in the opposite arm was used for obtaining blood samples. RPF and GFR were determined from the measurement of para-aminohippuric acid (PAH) and inulin clearances, respectively, as previously described.

Protocol Sequence
Three studies were carried out in each subject on 3 separate days. Each protocol began 1 hour after the onset of the PAH/inulin infusion. On day 1, eprosartan (SmithKline Beecham Pharmaceuticals) was administered at a dose of 600 mg PO, and PAH and inulin clearances and hormones were measured at regular intervals. On the next 2 study days, glucose was infused intravenously in all the study subjects. Eprosartan or placebo was administered in random order to the subjects on either day 2 or day 3 one hour after the glucose infusion had been initiated. Thus, all subjects received either glucose and eprosartan or glucose and placebo on day 2 or day 3. Eprosartan and placebo pills were sent to the GCRC by the pharmacist, and the study subjects, research nurse, physician, and technicians conducting the clearance and hormonal assays were all blinded to the content of the pill administered on either day 2 or day 3. Blood pressure was measured every 5 minutes with a Critikon Dinamap automated blood pressure monitor throughout the infusion protocols.

Glucose Infusion Protocol
On days 2 and 3, a loading dose of 20% dextrose was administered at a rate of 8.2 mg · kg⁻¹ · min⁻¹ to each study subject for 15 minutes. After the loading period, the rate of infusion of 20% dextrose was adjusted to achieve a target blood glucose concentration of ≈8.8 mmol/L (ie, below the renal threshold) over a period of 4 hours, as previously described. Blood glucose was monitored every 15 minutes with a glucometer. Urine samples were checked by dipstick for glycosuria and remained negative throughout the study period.

Laboratory Procedures
Blood samples were collected on ice and spun immediately, and the plasma was stored at −80°C until the time of assay. Urine sodium was measured by flame photometry with lithium as an internal standard. PAH and inulin were measured by an autoanalyzer technique as previously described. PRA, aldosterone, and Ang II were measured by radioimmunoassay with commercially available kits at baseline and 225 minutes after administration of glucose or eprosartan.

Statistical Analysis
The primary end point studied was the magnitude of change in RPF and GFR in response to eprosartan treatment. All data are expressed as mean±SEM. The values of RPF and GFR were compared with the subjects’ baseline values by paired t test. Differences among ≥3 variables were determined by ANOVA and the Fisher protected least significant difference test. The null hypothesis was rejected for a value of P<0.05. Body mass index (BMI) was calculated as weight (kg)/height (m²). The following equations were used for conversion of units of measurement: glucose concentration in mmol/L = mg/dL × 0.055, and insulin concentration in pmol/L = mU/L × 7.18.

Results

Hemodynamic and Hormonal Responses to Sustained Hyperglycemia
Figure 1 shows the relationship between plasma glucose concentration, insulin, and the rate of glucose infusion (Figure 1A) and the effect of glucose on renal hemodynamics (Figure 1B). Fasting plasma glucose averaged 4.5±0.09 mmol/L in the study subjects. After the loading period, plasma glucose concentration increased to a steady-state level of 8.8±0.4 mmol/L within 30 minutes (Figure 1A). Plasma insulin increased from a fasting level of 77.8±13 pmol/L to a steady-state level of 461±98 pmol/L (Figure 1A). There was no significant change in mean arterial pressure in response to glucose infusion (Table 2). Moderate sustained hyperglycemia from glucose infusion produced a significant increase in RPF from 593±17 to 668±20 mL · min⁻¹ · 1.73 m⁻² after 180 minutes, a change of 76 mL · min⁻¹ · 1.73 m⁻² (Figure 2), but did not affect GFR (Figure 1B, Table 2). PRA and plasma Ang II levels were not affected by the infusion of glucose alone (Table 3).

Hemodynamic Responses to Eprosartan During Normoglycemia and Hyperglycemia
Administration of eprosartan at a dose of 600 mg did not cause a significant change in RPF (544±13 versus 561±20 mg/L).

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**TABLE 1. Baseline Characteristics on First Study Day**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>High-Salt Diet (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>37.7±2.2</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>117±5</td>
</tr>
<tr>
<td>Diastolic</td>
<td>71±4.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.8±1.4</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L</td>
<td>4.5±0.08</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L</td>
<td>76.3±11</td>
</tr>
<tr>
<td>24-h urine sodium, mmol/L</td>
<td>293±34</td>
</tr>
<tr>
<td>PRA, ng · L⁻¹ · s⁻¹</td>
<td>0.18±0.05</td>
</tr>
<tr>
<td>Plasma aldosterone, pmol/L</td>
<td>3±0.33</td>
</tr>
</tbody>
</table>

Values are mean±SEM for baseline measures in 9 healthy adult subjects in high salt balance.
The administration of eprosartan 1 hour after the initiation of glucose infusion resulted in a significant increase in RPF (562 ± 12 mL/min/1.73 m², P < 0.05), with a peak change of 147 mL/min/1.73 m² (Table 2, Figure 2). The peak change in RPF was significantly greater in response to eprosartan than in response to placebo during hyperglycemia (P < 0.01). Eprosartan did not affect GFR or mean arterial pressure during sustained hyperglycemia (Table 2).

**Hormonal Responses to Eprosartan During Normoglycemia and Sustained Hyperglycemia**

In the absence of glucose infusion, treatment with eprosartan resulted in an increase in the PRA by 180% compared with baseline (0.05 ± 0.01 versus 0.14 ± 0.003 ng·L⁻¹·s⁻¹, P < 0.05) and an increase in plasma Ang II levels by 15% versus baseline (16.0 ± 1.3 versus 18.4 ± 2 pmol/L, P < 0.05) (Table 3). When eprosartan was administered to hyperglycemic subjects, PRA was 6-fold higher compared with baseline (0.5 ± 0.09 versus 3.1 ± 1.1 ng·L⁻¹·s⁻¹, P < 0.05). Ang II levels were 1.5-fold higher than baseline levels (18.5 ± 2 versus 28.7 ± 5.5 pmol/L, P < 0.05) in response to eprosartan treatment under conditions of sustained hyperglycemia (Table 3).

**TABLE 2. Effects of Glucose and Eprosartan on Renal Hemodynamics and Mean Arterial Pressure**

<table>
<thead>
<tr>
<th>Study day</th>
<th>RPF, mL/min/1.73 m²</th>
<th>GFR, mL/min/1.73 m²</th>
<th>MAP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>544 ± 13</td>
<td>119 ± 6</td>
<td>91 ± 4</td>
</tr>
<tr>
<td>Eprosartan/normoglycemia</td>
<td>561 ± 20</td>
<td>118 ± 6</td>
<td>87 ± 4</td>
</tr>
<tr>
<td>2 and 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>593 ± 17</td>
<td>118 ± 8</td>
<td>86 ± 4.7</td>
</tr>
<tr>
<td>Placebo+hyperglycemia</td>
<td>668 ± 20*</td>
<td>121 ± 7</td>
<td>84 ± 4</td>
</tr>
<tr>
<td>Baseline</td>
<td>562 ± 12</td>
<td>117 ± 7</td>
<td>89 ± 4</td>
</tr>
<tr>
<td>Eprosartan+hyperglycemia</td>
<td>709 ± 26*</td>
<td>122 ± 6</td>
<td>84 ± 6</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of values obtained after 180 minutes of glucose infusion. RPF, GFR, and mean arterial pressure (MAP) were measured in 9 healthy adults in high salt balance (ie, 24-h urine sodium >150 mEq). See Methods for protocol of glucose infusion and eprosartan treatment.

*P < 0.05 compared with baseline values on particular study day.
**TABLE 3. Effects of Glucose and Eprosartan on PRA and Ang II Levels**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>After 225 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eprosartan/normoglycemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA, ng·L⁻¹·s⁻¹</td>
<td>0.05±0.01</td>
<td>0.14±0.03</td>
</tr>
<tr>
<td>Ang II, pmol/L</td>
<td>16.0±1.3</td>
<td>18.4±1.9</td>
</tr>
<tr>
<td>Placebo/ hyperglycemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA, ng·L⁻¹·s⁻¹</td>
<td>0.2±0.05</td>
<td>0.2±0.02</td>
</tr>
<tr>
<td>Ang II, pmol/L</td>
<td>17.9±1.8</td>
<td>17.0±1.3</td>
</tr>
<tr>
<td>Eprosartan/ hyperglycemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA, ng·L⁻¹·s⁻¹</td>
<td>0.5±0.09</td>
<td>3.1±1.1*</td>
</tr>
<tr>
<td>Ang II, pmol/L</td>
<td>18.5±2.0</td>
<td>28.7±5.5*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. PRA and Ang II levels were measured in 9 healthy subjects in high salt infusion (24-h urine sodium >150 mEq). See Methods for protocol of glucose infusion and eprosartan treatment.

*P<0.05 compared with baseline values.

## Discussion

We have previously shown that moderate hyperglycemia not only increases RPF but also enhances substantially the renal vasodilator response to ACE inhibition with captopril. The enhanced renal vasodilator response to ACE inhibition could have reflected a range of mechanisms, including a reduction in Ang II formation, or an accumulation of vasodilators, such as prostaglandins, kinins, or NO. The latter possibility is suggested by the lack of alteration in PRA in response to hyperglycemia. On the other hand, a role for the RAS in the renovascular response to hyperglycemia was suggested by the observation that ACE inhibition not only increased RPF but also enhanced the renal vasoconstrictor response to Ang II infusion. These findings were consistent with a reduction in the intrarenal Ang II formation during sustained moderate hyperglycemia. To determine the specific role of Ang II as a mediator of changes in renal hemodynamic mechanisms during hyperglycemia, the present study was carried out in healthy adults who received the angiotensin receptor blocker eprosartan. Hyperglycemia resulted in a 12% increase in RPF. Administration of eprosartan at a dose of 600 mg PO caused a further 14% increase under hyperglycemic conditions but did not affect GFR. In contrast, eprosartan did not alter RPF and GFR in these salt-replete subjects under normoglycemic conditions. Eprosartan increased PRA under both normoglycemic and hyperglycemic conditions in subjects on a high-salt diet, a finding consistent with blockade of the intrarenal Ang II receptors that regulate PRA (ie, the short feedback loop). The observation that eprosartan produced a large increase in renal blood flow during hyperglycemia suggests that hyperglycemia leads to an increase in Ang II-mediated renal vascular tone. This is consistent with our previous observation with captopril.

There have been variable reports on the renal vascular response to blockade with the Ang II receptor antagonists under salt-replete conditions. For example, a single dose of losartan (50 mg) did not alter RPF under these conditions. In contrast, Ilson et al reported a significant increase in effective RPF after the administration of 350 mg eprosartan to healthy salt-replete subjects. In an earlier study, we observed renal vasodilation in response to eprosartan in subjects on a low salt diet and a smaller, but significant, increase in RPF in subjects on a high-salt diet. There was no significant change in RPF in response to eprosartan under normoglycemic conditions in the present study. The higher level of positive salt balance achieved in the present study may explain the lack of effect of eprosartan on RPF in our subjects. The 24-hour sodium excretion on the day before the renal hemodynamic assessment was almost 300 mmol sodium, which was associated with a striking reduction in PRA, to <0.1 ng·mL⁻¹·h⁻¹. Thus, the present study may have been performed under more stringent conditions of suppression of the renin system. Second, we have previously described a significant variation in the degree to which the renin system contributes to renal vascular tone in healthy individuals. That study was performed under conditions of low salt diet to activate the renin system. The possibility exists that the same variation occurs under conditions of a high-salt diet, which would lead to variable renal hemodynamic responses to blockade of the RAS.

Renal hemodynamic responses to glucose infusion are variable in healthy and diabetic individuals. For example, Walczyk et al reported a 43% decrease in effective RPF and no change in GFR in response to elevation of plasma glucose to 30 mmol/L in healthy individuals. On the other hand, GFR was 6% higher but RPF was not affected in response to a glucose level of 11 mmol/L in healthy subjects studied by Christensen et al. In contrast, GFR increased by 5% and effective RPF increased by 8% when glucose was infused to achieve a blood level of ~15 mmol/L in diabetics in the same study. Contrary to the above response in diabetics, Mogensen did not observe a change in RPF in diabetics at a higher level of blood glucose (~38 mmol/L), although GFR decreased by 9%. On the basis of these studies, it is difficult to determine to what extent hyperglycemia, per se, and hormonal and vascular changes contribute to alterations in renal hemodynamics during glucose infusion in diabetic and healthy subjects.

Although previous studies have examined the effect of RAS blockade on renal hemodynamics in diabetes, our previous study and the present study are the only studies, to our knowledge, that examine the interaction between hyperglycemia and angiotensin blockade in healthy human subjects. Miller has shown that losartan caused a significant increase in effective RPF by ~200 mL·min⁻¹·1.73 m⁻² when it was administered to patients with early uncomplicated type 1 diabetes under hyperglycemic conditions (9 to 11 mmol/L). In contrast, there was no change in RPF when type 1 diabetics were treated with losartan under euglycemic conditions. A similar magnitude of increase in RPF was observed when type 2 diabetics with an average blood glucose level of 8.8 to 9.5 mmol/L were treated with irbesartan. The magnitude of increase in RPF in response to eprosartan treatment in the present study, ie, 70 mL·min⁻¹·1.73 m⁻², was similar to our previous observation in healthy subjects treated with captopril under moderate hyperglycemic conditions. The increase in RPF in healthy subjects was lower compared with previous results in diabetics and may be partly attributable to the level and/or duration of hyper-
glycemia. For example, in the study by Miller,18 type 1 diabetes patients received losartan after blood glucose had been maintained at 9 to 11 mmol/l for ≈12 hours. In contrast, eprosartan was administered 1 hour after the initiation of glucose infusion in the present study.

Further enhancement of RPF by eprosartan during moderate hyperglycemia is suggestive of activation of the intrarenal RAS. Potential mechanisms that may mediate this process include alterations in renal sympathetic nerve activity and hyperinsulinemia. Insulin has been shown to cause renal vasodilation in healthy humans in euglycemic clamp studies.20 However, it is unlikely that the enhancement of RPF by eprosartan was mediated by a further rise in insulin in the present study, because insulin levels did not change significantly on the hyperglycemia versus hyperglycemia/eprosartan treatment days. Although changes in renal sympathetic activity as well as other hormones as a result of hyperglycemia may lead to the activation of the intrarenal RAS, these specific mechanisms were not addressed by the present study.

The early stages of renal disease in diabetes are marked by glomerular hyperfiltration and, to a lesser extent, by increased RPF.21 These hemodynamic alterations are thought to precede glomerular and tubular hypertrophy and overt renal impairment. Factors implicated in these changes include plasma volume expansion, hyperinsulinemia, prostaglandins, and the RAS.22 The interaction between glycemia and the RAS and how that relates to diabetic nephropathy are not well understood. PRA is variable in the early stages of diabetes but tends to be suppressed in long-standing diabetes or established renal disease.21 Studies in animals have demonstrated activation of the intrarenal RAS with low PRA in response to hyperglycemia.23,24 Our observation that the renal vasodilator response to eprosartan is enhanced by hyperglycemia despite a lack of change in PRA is consistent with a similar activation of intrarenal RAS in humans.

The findings in the present study provide some insight into the potential role of alteration in renal hemodynamics by hyperglycemia in the pathogenesis of diabetic renal disease. Hyperglycemia-mediated alterations in renal hemodynamics have been shown to interact with polymorphisms of the ACE gene to determine the predisposition to nephropathy in type 1 diabetes.25 It is possible that activation of the intrarenal RAS, as indirectly demonstrated by the enhancement of renal vasodilation by eprosartan, is involved in this process. The present study does not address the contribution of prostaglandins, kinins, and increased insulin levels on hyperglycemia-mediated renal changes. It remains to be determined how these mediators interact with hyperglycemia and the RAS in the pathogenesis of diabetic nephropathy.

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