Hyperglycemia and Angiotensin-Mediated Control of the Renal Circulation in Healthy Humans


Abstract—Type 1 and type 2 diabetics have an enhanced renal vasodilator response to angiotensin-converting enzyme (ACE) inhibition despite suppressed plasma renin activity (PRA), indicating possible activation of the intrarenal renin angiotensin system. To investigate the role of hyperglycemia, we evaluated the renal hemodynamic response to ACE inhibition in 9 healthy subjects in high-salt balance after steady-state hyperglycemia (8.4±1 mmol/L) was achieved via intravenous glucose administration. Renal plasma flow (RPF) and glomerular filtration rate (GFR) responses to captopril and to angiotensin II (Ang II) were measured as paraminohippuric acid and inulin clearances. Hyperglycemia produced a significant increase in RPF of 117 mL·min⁻¹·1.73 m⁻² after 90 minutes but not GFR. Administration of captopril at a dose of 25 mg during glucose infusion led to an increase in RPF of 173±24 mL·min⁻¹·1.73 m⁻² (P<0.01) but did not significantly change RPF in the absence of hyperglycemia (7±21 mL·min⁻¹·1.73 m⁻²). Captopril did not alter GFR in the presence or absence of hyperglycemia. Ang II infusion during hyperglycemia decreased RPF by 45±16 mL·min⁻¹·1.73 m⁻², and this was significantly enhanced by captopril (−98±26 mL·min⁻¹·1.73 m⁻², P<0.05). In contrast, there was no enhancement of the vasoconstrictor response to Ang II in the absence of hyperglycemia. PRA did not change with hyperglycemia. Enhancement of renal vasodilation during hyperglycemia by captopril without alteration of PRA suggests activation of the intrarenal renin angiotensin system. (Hypertension. 1999;33[part II]:559-564.)

Key Words: renal blood flow • glomerular filtration rate • hyperglycemia • sodium • renin

There has been a recent rekindling of interest in the state of the renin angiotensin system (RAS) in diabetes mellitus and its regulation of renal vascular tone. In addition to the fact that angiotensin-converting enzyme (ACE) inhibitors have a major impact on the natural history of diabetic nephropathy,1–3 there is some suggestion that polymorphisms of the genes of the renin cascade may also influence the development of renal disease.4 The state of activation or suppression of the RAS as assessed by plasma renin activity (PRA) can be misleading.5 Low-renin states have been described in both type 1 and type 2 diabetics at one extreme.6–8 While other studies have reported elevated PRA.9–11 In animal models, there is more evidence supportive of the presence of an intrarenal RAS that may be inappropriately normal or frankly activated despite a suppressed PRA.12–13

As an alternative approach to assessing the state of the renin system and its contribution to maintaining renal vascular tone, a series of studies have examined the renal vascular response to pharmacological interruption of the renin system in type 1 and type 2 diabetics. These studies have revealed an enhanced renal vasodilator response to the administration of ACE inhibitors and angiotensin II (Ang II) antagonists even when PRA was low, suggesting an intrarenal locus of RAS activation.14–17

In a recent study in patients with type 1 diabetes, moderate hyperglycemia over a 12-hour period led to a progressive increase in PRA and renal vasoconstriction compared with the prior euglycemic period.18 This suggests that hyperglycemia may activate the RAS. The present study was designed to assess whether elevated blood glucose levels influence PRA and Ang II–mediated renal vascular responses in healthy subjects. Our hypothesis was that stable hyperglycemia would activate the intrarenal RAS and thus enhance the renal vasodilator response to ACE inhibition by captopril. To determine whether the mechanism of action of captopril is mediated by blockade of the RAS as opposed to other pathways, we determined the presence or absence of enhancement of Ang II-induced vasoconstriction after captopril administration.

Methods

Subjects

Nine normal subjects (7 men and 2 women; age range, 24 to 59 years; body mass index (BMI) range, 21 to 32 kg/m²) were studied.
After an outpatient physical and laboratory evaluation, written informed consent was obtained from each patient. The studies were in accordance with guidelines and regulations of the Institutional Review Board of the Brigham and Women’s Hospital and Harvard Medical School. The subjects were free of cardiovascular, renal, and endocrine disease and had normal mean arterial pressures (range, 72 to 92 mm Hg), fasting blood glucose (range, 4.1 to 5 mmol/L), and fasting insulin levels (range, 14 to 158 pmol/L).

Three days before admission, the subjects were placed on a high-salt diet (200 mmol of sodium per day), and a 24-hour urine sample was collected daily for the measurement of sodium. The subjects were admitted for the study after they were in high-sodium balance, ie, when 24-hour urine sodium was >150 mmol. Each subject was admitted to the General Clinical Research Center the evening before the study day and was maintained on the high-salt diet throughout the study period. The subjects were studied on a high-salt diet because previous studies have shown that the abnormalities of renin-angiotensin–mediated renovascular control were unmasked by a high-salt diet in diabetics. For comparison, subjects on a low-salt diet were obtained from a prior study.

Protocol Sequence
Three studies were carried out in each subject, on 3 separate days. Each protocol began 1 hour after the onset of the paraminohippuric acid (PAH) infusion. On day 1 (glucose-only day), the subjects received a loading dose of 20% dextrose (8.2 mg · kg⁻¹ · min⁻¹) for 15 minutes. Blood glucose was monitored every 15 minutes with a glucometer. After the loading period, the rate of glucose infusion was adjusted to achieve a target blood glucose (8.4 to 9.5 mmol/L) over a period of 4 hours. The rate of infusion of 20% dextrose was adjusted as described previously. Urine samples were checked by dipstick for glycosuria and remained negative throughout the study period. After day 2 (Ang II/captopril/Ang II day), angiotensin II amide (Hypertensin, Ciba-Geigy) was administered intravenously at 3 ng · kg⁻¹ · min⁻¹ for 45 minutes with an electronic infusion pump (Harvard Apparatus Co Inc). This was followed by treatment with an ACE inhibitor, captopril, at a dose of 25 mg orally. Ninety minutes later, Ang II was again infused intravenously. Blood pressure was measured every 5 minutes with a Critikon Dinamap automated blood pressure monitor throughout the baseline PAH infusion and every 2 minutes during Ang II infusion. On day 3 (glucose/Ang II/captopril/Ang II day), glucose was infused to maintain a target blood glucose of 8.4 to 9.5 mmol/L over a period of 4 hours, as described above. One hour after initiation of glucose infusion, Ang II and captopril were administered sequentially, as on day 2.

Renal Function Studies
After an overnight fast of 8 hours, 3 intravenous catheters were placed in the arms at least 2 hours before infusions. Two catheters were used for the infusions and the other for obtaining blood samples. PAH and inulin were infused and clearances reflected renal plasma flow (RPF) and glomerular filtration rate (GFR) respectively as described previously.

Laboratory Procedures
Blood samples were collected on ice, spun immediately, and the plasma stored at −80°C until the time of assay. Serum and urinary sodium and potassium levels were measured by flame photometry with lithium as an internal standard. Serum creatinine, PAH, and inulin were measured by an autoanalyzer technique. PRA was measured with a radioimmunoassay with use of a commercially available kit.

Statistical Analysis
The primary end point studied was the magnitude of change in RPF and GFR in response to captopril treatment. All data are expressed as mean±SEM. Statistical differences in 2 sample data were assessed by t test. Differences among ≥3 variables were determined by ANOVA and the Fisher protected least significant difference test. The null hypothesis was rejected when the p value was less than 0.05. BMI was calculated as weight (in kilograms) divided by height (in square meters). The relationships between variables were determined by regression analysis.

Results
Renal Hemodynamic Response to Sustained Hyperglycemia on Glucose-Only Infusion Day
Figure 1 shows the relationship between plasma glucose concentration, insulin level, and rate of glucose infusion. Fasting plasma glucose averaged 5±0.2 mmol/L in the study subjects. After the priming period of glucose infusion, plasma glucose concentration reached a steady-state level of 8.4±1 mmol/L (Figure 1A). Periodic checks by dipstick of urine voided during the glucose infusion did not detect glycosuria. Plasma insulin levels rose gradually, lagging behind the glucose curve, and reached stable levels after 60 to 90 minutes of glucose infusion (Figure 1B). There was a positive correlation between insulin levels during glucose infusion and fasting plasma glucose (r=0.8, P<0.05). In sodium-replete subjects used in this study, glucose infusion on the glucose-only day produced a significant increase in RPF from 562±37 to 679±27 mL · min⁻¹ · 1.73 m² after 105 minutes (a change of +117 mL · min⁻¹ · 1.73 m²) but did not significantly change over the rest of the glucose infusion period (Figure 2). There was no significant change in GFR during glucose infusion (Figure 2).
Renal Hemodynamic Responses to Captopril During Normoglycemia and Hyperglycemia in Subjects on a High-Salt Diet

The Table compares baseline RPF with that obtained during glucose infusion and captopril treatment. In the absence of glucose infusion, administration of captopril at a dose of 25 mg did not alter baseline RPF (766 ± 21 mL min⁻¹ m⁻²) (Figure 3). In contrast, RPF increased by 173±24 mL min⁻¹ m⁻² in response to captopril treatment during glucose infusion. Administration of captopril during glucose infusion led to a further increase in RPF (56 mL min⁻¹ m⁻², P<0.05) compared with the rise in RPF in response to glucose alone (Figure 3). There was no significant effect of captopril on GFR in the presence or absence of hyperglycemia (12.2±3 versus 10.2±2 mL min⁻¹ m⁻²).

Renal Hemodynamic Responses to Ang II Infusion

The renovascular response to Ang II at a dose of 3 ng kg⁻¹ min⁻¹ before captopril was significantly reduced during hyperglycemia (−45±16 mL min⁻¹ m⁻²) compared with normoglycemia (−98±26 mL min⁻¹ m⁻²) (P<0.05) (Figure 4A). The renovascular responses to infused Ang II were compared before and after captopril treatment. In subjects on a high-salt diet, there was no enhancement of Ang II–mediated vasoconstriction by captopril in the absence of hyperglycemia (−160±36 versus −129±55) (Figure 4A). During hyperglycemia, Ang II–mediated vasoconstriction was enhanced by captopril (−45±16 versus −98±26 mL min⁻¹ m⁻²).

Renal Plasma Flow Measurements on Different Study Days

<table>
<thead>
<tr>
<th>Study Day</th>
<th>RPF Measurement</th>
<th>Glucose</th>
<th>Captopril (Normoglycemia)</th>
<th>Captopril + Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Baseline</td>
<td>562±37</td>
<td>586±34</td>
<td>578±46</td>
</tr>
<tr>
<td>2</td>
<td>Baseline</td>
<td>679±26*</td>
<td>595±40</td>
<td>735±44*</td>
</tr>
</tbody>
</table>

Data are mean±SEM; n=9. RPF was measured by PAH clearance (mL min⁻¹ m⁻²). See “Methods” for details.

*P<0.05 compared with baseline renal plasma flow by t test.

Figure 3. Change in RPF at 195 minutes during the glucose infusion and captopril treatment.

Figure 4. The presence or absence of enhancement of Ang II–mediated vasoconstriction after captopril. In subjects on a low-salt diet, captopril-induced renal vasodilation is associated with striking enhancement of the renal vascular response to Ang II, probably indicating that the dominant mechanism responsible for renal vasodilation is a reduction of Ang II formation by captopril. The enhancement is not evident in subjects on a high-salt diet, in which captopril induces little or no renal vasodilation (A). Note that during hyperglycemia, despite the high-salt diet, captopril induced a substantial enhancement of the renal vasoconstrictor response to Ang II (A), probably reflecting activation of the intrarenal RAS by hyperglycemia.
Plasma Renin Activity
PRA was not altered when glucose was infused alone (Figure 5A). Treatment with captopril increased PRA from 0.3 ± 0.1 to 3.4 ± 1.1 ng · mL⁻¹ · hr⁻¹ (P < 0.05) in the absence of hyperglycemia (Figure 5B). There was a small but significant increase in PRA from 0.4 ± 0.1 to 1.4 ± 0.8 ng · mL⁻¹ · hr⁻¹ in response to captopril treatment during glucose infusion.

Volume Expansion and Change in Serum Potassium
There was a small but significant decrease in the hematocrit from 38.3 ± 1 to 37.1 ± 1% on the glucose-only day and from 37.2 ± 1 to 36.2 ± 1% on the glucose/Ang II/captopril/Ang II day. Serum potassium decreased from a baseline value of 4.0 ± 0.1 to 3.7 ± 0.1 mmol/L at the end of the glucose-only day (P < 0.05). Similarly, serum potassium decreased from 4.2 ± 0.1 mmol/L at the beginning to 3.9 ± 0.1 mmol/L at the end of the glucose/Ang II/captopril/Ang II day (P < 0.05).

Discussion
In the present study, moderate hyperglycemia (≈8.4 mmol/L) increased RPF by 20% but did not significantly alter GFR. This level of hyperglycemia below the renal threshold for glycosuria was chosen to reduce the possibility of changes in RPF that could result from osmotic diuresis and tubuloglomerular feedback mechanisms. Renal hemodynamic response to glucose infusion in normal subjects has been variable. For example, Brochner-Mortensen reported a 14% increase in GFR at a plasma glucose level of 10 mmol/L, whereas Christiansen et al. observed a 6% rise in GFR and no change in RPF at a plasma glucose level of 11 mmol/L. It is not unusual for a change in RPF not to be accompanied by a corresponding change in GFR during glucose infusion. The difference between the renal hemodynamic response in the present study and those reported by other investigators may have resulted from the subrenal threshold level of glycemia and the fact that we controlled the dietary salt intake in our subjects. Another possible explanation for the failure of GFR to increase with RPF in our study is that hyperglycemia may have altered PAH extraction.

Mechanisms thought to underlie renal vasodilation during hyperglycemia have been reviewed. They include extracellular and plasma volume expansion, vascular hyposensitivity to Ang II and norepinephrine, and increased production of vasodilator mediators such as prostaglandin E₂ and prostacyclin. Other biochemical changes during hyperglycemia that have an effect on renal hemodynamics are hyperinsulinemia, hyperosmolality, and potassium depletion. Although moderate elevations of plasma insulin levels in healthy humans have been shown to increase RPF without affecting GFR, the mechanism is unclear. This effect of insulin was shown to be independent of the release of prostaglandins and nitric oxide. In contrast, others have demonstrated that inhibition of prostaglandin synthesis prevents insulin-mediated vasodilation. Moderate hyperglycemia may have influenced renal hemodynamics in the present study through hyperinsulinemia and extracellular volume expansion. It is unlikely that hypertonicity played a significant role, because the calculated osmolarity was minimally increased (4 to 5 mOsm/kg) at the steady-state levels of plasma glucose achieved.

An important observation in the present study was the enhancement of the renal vasodilator response to captopril during moderate hyperglycemia in these subjects in high-salt balance. The contribution of the RAS to the regulation of renal hemodynamics depends on salt balance. On a high-salt diet, there is suppression of the endogenous RAS and minimal renal vasodilation after the administration of ACE inhibitors. The renal vasodilator response to captopril observed in subjects maintained on a high-salt diet was unexpected. Possible mechanisms underlying the renal vasodilator response to captopril include blockade of production of Ang II due to hyperglycemia-mediated activation of the kidney RAS and the release of vasodilators such as bradykinin and prostaglandins. We have previously distinguished between these 2 possible mechanisms by determining the presence or absence of enhancement of the vasoconstrictor action of infused Ang II by captopril. The enhancement of Ang II–mediated vasoconstriction by captopril is suggestive of a decrease in local Ang II and supports the view that captopril causes vasodilation at least in part by blockade of the RAS. In contrast, the absence of enhancement would point to captopril-mediated release of vasodilator mediators such as bradykinin, prostaglandins, and nitric oxide. Our observation of an increased vasoconstrictor response to Ang II infusion after captopril treatment is therefore consistent with decreased local Ang II levels and, by inference, hyperglycemia-mediated activation of the RAS. Studies are currently underway to better characterize the interaction between Ang II and glucose with use of specific Ang II receptor antagonists.

Another interesting finding of this study was the blunted renovascular response to the initial Ang II infusion during...
hyperglycemia compared with the response in the absence of glucose. It is possible that hyperglycemia-mediated renal vasodilation may have masked the full vasoconstrictor effect of Ang II. On the other hand, hyperglycemia has been shown to decrease vasoconstrictor responses through downregulation of Ang II receptors.35

In the present study, hyperglycemia for 4 hours was not associated with a significant change in PRA. In contrast, elevated plasma renin was observed in type 1 diabetics after 12 hours of moderate hyperglycemia compared with a prior euglycemic period.18 The difference in the PRA response between our healthy subjects and diabetics could be attributed to the duration of hyperglycemia and intrinsic differences between the diabetic and normal state. Administration of captopril increases PRA by blocking the feedback inhibition of Ang II on renin (short feedback loop). Although there was an increase in PRA when captopril was administered to subjects during hyperglycemia, the magnitude of the increase was less compared with what was observed after captopril administration in the euglycemic state. A possible mechanism for the blunted response of PRA to captopril treatment during hyperglycemia is that hyperglycemia may have altered the metabolism of intrarenal renin or decreased its release into the circulation, as has been demonstrated previously.36

The renovascular responses to moderate hyperglycemia in healthy subjects in the present study may provide some insights into the pathogenesis of diabetic nephropathy. A similar enhancement of renal vasodilation during ACE inhibition has been observed in type 1 and 2 diabetics in high-salt balance and appropriately suppressed PRA and plasma Ang II.14,16 Moreover, the renal vasoconstrictor response to Ang II in diabetics is enhanced by captopril, thus reflecting a fall in local Ang II.14,16 Local activation of the RAS has been linked to renal hemodynamic and other Ang II effects that promote diabetic renal disease.37 A potential significance of activation of intrarenal RAS in diabetes is suggested by studies showing that ACE inhibitors, and more recently Ang II antagonists, modify the natural history of diabetic renal disease. Thus, blockade of intrarenal Ang II formation may be an avenue for therapeutic intervention.38 Further studies would be required to unravel the mechanisms underlying the interaction between glycermia and the RAS and how these may influence renovascular responses in diabetes.

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References


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