Impact of Hyperglycemia on the Renin Angiotensin System in Early Human Type 1 Diabetes Mellitus

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Abstract. It has been demonstrated previously that moderate hyperglycemia without glucosuria can increase plasma renin activity and mean arterial pressure in young healthy males with early uncomplicated type 1 diabetes mellitus. This study was conducted to extend these observations by testing the hypothesis that mild to moderate hyperglycemia can affect renal function by increasing renin angiotensin system (RAS) activity in diabetic humans. The study included 10 men and women with early, uncomplicated type 1 diabetes (duration <5 yr), all ingesting a controlled sodium and protein diet. They were studied on four separate occasions, during a subdepressor dose of the angiotensin II (AngII) receptor blocker losartan, and during graded AngII infusion, 1.5 and 2.5 ng/kg per min, while euglycemic (blood glucose 4 to 6 mmol/L) and again while hyperglycemic without glucosuria (blood glucose 9 to 11 mmol/L), according to a randomized crossover design. Outcome measures included mean arterial pressure (MAP), GFR, effective renal plasma flow (ERPF), renal vascular resistance (RVR), filtration fraction (FF), and urine sodium excretion (UNaV) at baseline and in response to the above maneuvers. During hyperglycemic conditions, MAP was significantly higher compared with euglycemia, as were RVR and FF. After the administration of losartan, a significant renal and peripheral depressor effect was noted, with decreases in MAP, RVR, and FF, whereas during euglycemia the responses to losartan were minimal. AngII infusion resulted in elevations in MAP, RVR, and FF and a decline in UNaV during both glycemic phases, but the responses during hyperglycemia, most significantly at the 1.5 ng/kg per min infusion rate, were blunted. These data support the hypothesis that hyperglycemia affects renal function by activating the RAS. The mechanism remains obscure, but these contrasting responses may provide a link between the observations that maintenance of euglycemia and blockade of the RAS prevent or delay diabetic kidney disease, and furthermore, may clarify the mechanism whereby high glucose promotes renal disease progression in diabetes.
blockade and AngII infusion in comparison to those occurring during euglycemic conditions. Subjects were studied on a controlled sodium and protein diet. Renal hemodynamic function was assessed using classic inulin and para-aminohippurate (PAH) clearance techniques. The target plasma glucose concentration during the hyperglycemic phase of the experiment was chosen to avoid glucosuria, thus preventing an osmotic diuresis and changes in extracellular fluid volume that could affect the outcome measures. In both phases of the experiment, blood glucose was maintained by a modified glucose clamp technique that has been described previously (3,6).

Materials and Methods

Subjects

The study consisted of 10 diabetic subjects, nine males and one female, mean age 23 ± 2. Four of the 10 subjects had participated in a previous study from this laboratory (7), and their characteristics were therefore similar. In brief, they were all insulin-dependent, and studied within 5 yr of diagnosis (mean 3.5 ± 0.6 yr). They were otherwise healthy nonsmokers who were normotensive and nonobese, on no medications except for insulin, and without evidence of retinopathy, microalbuminuria, a decrease in creatinine clearance, or an orthostatic decline in BP, as determined by a qualified internist. They were well-controlled metabolically, with the HbA1C less than 10% in all cases, mean 7.5 ± 1.0%. The study was performed with the approval of the University of Toronto Human Subjects Review Committee, and with the informed written consent of each subject.

Each subject was instructed to adhere to a 200 mmol sodium, 1.5 g/kg protein diet for 7 d before each study, and compliance was ascertained by measurement of 24-h urine sodium (UNaV) and urea excretion on the sixth day before each study day. Subjects were considered properly prepared for study if the excretion of sodium was 180 to 220 mmol in 24 h. No subjects were excluded on this basis. All subjects refrained from caffeine for 48 h before each study.

Subjects were studied on four separate occasions, twice during a euglycemic state and twice during a moderately hyperglycemic state, randomly determined. The time interval between the four investigations was a minimum of 10 d and a maximum of 3 wk. They were admitted to the Toronto Hospital the evening before each study day. An 18-gauge peripheral venous cannula was inserted into a left antecubital vein for infusions of insulin and glucose. Blood glucose was measured every hour (Accucheck), and the insulin infusion was varied to maintain euglycemia (4.0 to 6.0 mmol/L) or moderate hyperglycemia without glucosuria (9 to 11 mmol/L). Subjects were thus rendered euglycemic or hyperglycemic for 18 h: from 9 p.m. until the conclusion of the study on the following day, as described previously (3). All studies were conducted at 8:30 a.m., after an overnight fast, with the subjects lying supine in a warm quiet room.

Procedures

Part 1. This portion of the study was conducted twice, once during euglycemic conditions and once while hyperglycemic. A second 18-gauge peripheral venous cannula was inserted into an antecubital vein for infusions of insulin and PAH, and a third cannula was inserted into the contralateral arm for blood sampling. Each subject voided and then drank 800 ml of water in the first 45 min to induce a water diuresis. Two hundred milliliters was ingested in each hour of the protocol to maintain an adequate urine output for collection of spontaneously voided samples. Hemodynamic parameters (MAP, heart rate) were measured throughout the study by an automated sphygmomanometer (Dinamap), and recorded once in each half hour of the protocol. Renal hemodynamics were measured using inulin and PAH clearance techniques, as described previously (3,7). In brief, three timed urine collections of 20 min duration each were obtained by spontaneous voiding for determination of baseline GFR and effective renal plasma flow (ERPF). At the end of this period, losartan (Cozaar®, Merck, Sharpe, and Dohme) was administered at a subpressor dose of 25 mg. During each hour, for 3 h, blood was collected for inulin, PAH, and hematocrit (Hct), and urine was collected for inulin, PAH, and urine sodium excretion (UNaV).

Part 2. This portion of the study was also conducted twice, once during euglycemic conditions and once while hyperglycemic. A second 18-gauge peripheral venous cannula was inserted into the left antecubital vein and connected to a Harvard infusion pump (Harvard Apparatus) for infusions of insulin, PAH, and AngII. A third cannula was inserted into the contralateral arm for sampling. Arterial pressure and pulse rate were measured noninvasively every 30 min by an automatic BP recorder (Dinamap). A solution of AngII (Hypertensin®, Ciba Geigy) (2.5 mg/vial) was prepared by dissolving the diluent in normal saline to produce a concentration of 0.5 mg/ml. Two hundred and fifty milliliters of normal saline was then added to 0.2 ml of AngII to produce a concentration of 400 ng/ml. AngII was infused at two doses, 1.5 and 2.5 ng/kg per min, each dose for 30 min. Subjects remained supine except to void. Blood was collected once during each AngII infusion period for inulin, PAH, Hct, and urine was collected for inulin, PAH, and UNaV. MAP was also measured at the midpoint of each infusion. A further collection of both blood and urine was obtained at the end of the AngII infusion, after a 30-min recovery period.

Sample Collection and Analytical Methods

Blood samples collected for inulin and PAH determinations were immediately centrifuged at 3000 rpm for 10 min at 4°C. Plasma was separated, placed on ice, and then stored at −70°C before the assay. Urine sodium concentration was measured by a flame photometry method. Urine samples collected for inulin and PAH were promptly alkalinized by addition to 4 ml of urine 23 μl of 4 M NaOH to prevent formation of an adduct between PAH and glucose (8). Inulin concentrations in plasma and urine were measured by a modified method of Walser et al. (9), and PAH concentration was measured by a spectrophotometric method according to Brun (10). The mean of the final two clearance periods before each maneuver represent baseline GFR and ERPF, expressed per 1.73 m². Filtration fraction (FF) represented the ratio of GFR to ERPF. Renal blood flow (RBF) was calculated by dividing the ERPF by (1-Hct). RVR was derived by dividing MAP by the RBF.

Statistical Analyses

Results are presented as mean ± SEM. Comparison of all parameters at baseline was made using paired t tests. Differences in responses to losartan during euglycemic and hyperglycemic conditions were determined by two-way repeated-measures ANOVA and Bonferroni correction. Differences in response to AngII infusion were similarly determined. All statistical analyses were performed using the statistical package SAS (SAS Institute, Inc., Cary, NC)

Results

The baseline characteristics of the subjects during the losartan experiment are shown in Table 1. A comparison was also made of these parameters during the AngII infusion study, and
similar results were obtained. No significant differences were noted between studies, except for plasma glucose, as planned.

The responses of MAP, GFR, ERPF, RBF, RVR, FF, and UNaV to losartan during both euglycemia and hyperglycemia are shown in Table 2. Baseline MAP was significantly elevated during the hyperglycemic phase of the experiment, consistent with a pressor effect of glucose. GFR was unchanged, and RVR and FF were significantly elevated. UNaV was not significantly different between phases of the experiment. During the hyperglycemic phase of the experiment, the response to losartan was striking and included significant elevations in ERPF and RBF, and reductions in MAP, RVR, and FF, effectively abolishing the hyperglycemia-mediated pressor effect. No significant changes in these parameters were noted during euglycemia (Figures 1 to 3). The UNaV response to losartan did not differ between phases of the experiment.

The responses of MAP, GFR, ERPF, RBF, RVR, FF, and UNaV to graded AngII infusion are shown in Table 3. Once again, baseline MAP was significantly elevated during the hyperglycemic phase, as were RVR and FF. UNaV was not significantly different. In both phases of the experiment, graded AngII infusion resulted in significant increases in MAP, RVR, and FF, and decreases in UNaV. However, the MAP, RVR, FF, and UNaV responses to AngII infusion were significantly blunted during hyperglycemic conditions at the 1.5 ng/kg per min infusion rate (Figure 4). Recovery values did not differ significantly from baseline.

**Discussion**

Epidemiologic studies have shown a close relationship between glucose control and renal disease in diabetic patients (1). The mechanism by which hyperglycemia promotes progression of chronic renal disease is unknown, but a previous study from this laboratory has implicated glucose-mediated activation of the RAS. The current study examined the renal hemodynamic

### Table 1. Baseline measures (losartan study)\(^a\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Euglycemia</th>
<th>Hyperglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>80 ± 4</td>
<td>81 ± 3</td>
</tr>
<tr>
<td>UNa (mmol/d)</td>
<td>198 ± 14</td>
<td>211 ± 21</td>
</tr>
<tr>
<td>Urea (mmol/d)</td>
<td>324 ± 16</td>
<td>331 ± 20</td>
</tr>
<tr>
<td>UNaV (μmol/min)</td>
<td>362 ± 38</td>
<td>354 ± 29</td>
</tr>
<tr>
<td>Hct</td>
<td>0.407 ± 0.009</td>
<td>0.401 ± 0.01</td>
</tr>
<tr>
<td>Plasma insulin (pmol/L)</td>
<td>99 ± 13</td>
<td>94 ± 12</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>5 ± 0.2</td>
<td>10 ± 1(^b)</td>
</tr>
</tbody>
</table>

\(^a\) UNa, 24-h urine sodium excretion; Urea, 24-h urine urea excretion; UNaV, urine sodium excretion; Hct, hematocrit.

\(^b\) P < 0.05 versus value obtained during euglycemia.

### Table 2. Renal and peripheral responses to losartan\(^a\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>2 Hours</th>
<th>3 Hours</th>
<th>Baseline</th>
<th>2 Hours</th>
<th>3 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>88 ± 2</td>
<td>85 ± 2</td>
<td>85 ± 2</td>
<td>95 ± 2(^b)</td>
<td>83 ± 2(^c,d)</td>
<td>79 ± 1(^c,d)</td>
</tr>
<tr>
<td>GFR (ml/min)(^c)</td>
<td>119 ± 4</td>
<td>114 ± 5</td>
<td>118 ± 5</td>
<td>118 ± 5</td>
<td>110 ± 5</td>
<td>113 ± 5</td>
</tr>
<tr>
<td>ERPF (ml/min)(^c)</td>
<td>710 ± 25</td>
<td>673 ± 33</td>
<td>672 ± 37</td>
<td>586 ± 26(^b)</td>
<td>706 ± 48(^c,d)</td>
<td>794 ± 32(^c,d)</td>
</tr>
<tr>
<td>RBF (ml/min)(^c)</td>
<td>1203 ± 91</td>
<td>1108 ± 68</td>
<td>1111 ± 71</td>
<td>983 ± 59(^b)</td>
<td>1346 ± 88(^c,d)</td>
<td>1304 ± 50(^c,d)</td>
</tr>
<tr>
<td>FF</td>
<td>0.16 ± 0.01</td>
<td>0.17 ± 0.005</td>
<td>0.18 ± 0.009</td>
<td>0.20 ± 0.01(^b)</td>
<td>0.16 ± 0.01(^c,d)</td>
<td>0.14 ± 0.006(^c,d)</td>
</tr>
<tr>
<td>RVR (mmHg/L per min)</td>
<td>75 ± 4</td>
<td>77 ± 4</td>
<td>78 ± 4</td>
<td>90 ± 5(^b)</td>
<td>72 ± 4(^c,d)</td>
<td>69 ± 2(^c,d)</td>
</tr>
<tr>
<td>UNaV (μmol/min)</td>
<td>362 ± 38</td>
<td>335 ± 61</td>
<td>343 ± 54</td>
<td>324 ± 29</td>
<td>290 ± 25</td>
<td>265 ± 62</td>
</tr>
</tbody>
</table>

\(^a\) MAP, mean arterial pressure; ERPF, effective renal plasma flow; RBF, renal blood flow; FF, filtration fraction; RVR, renal vascular resistance. Other abbreviations as in Table 1.

\(^b\) P < 0.05 versus baseline value during euglycemia.

\(^c\) P < 0.05 versus baseline value.

\(^d\) P < 0.05 versus response to losartan during euglycemia.

\(^c\) Corrected per 1.73 m\(^2\).
responses to AngII infusion and RAS blockade during hyperglycemia of short duration in young healthy subjects with early uncomplicated type 1 diabetes. The rationale reflected the possibility that the pattern of renal responsiveness to AngII and to losartan at different blood glucose levels might provide a mechanism that could help to clarify the impact of poor glucose control on the initiation and progression of diabetic renal disease.

The first major observation was that in both phases of the experiment, moderate hyperglycemia without glucosuria of several hours duration was associated with a renal and peripheral pressor effect, manifested by increases in MAP, RVR, and FF, similar to a previous study from this laboratory (3). Other studies using animal and human models have examined the BP effect of hyperglycemia. In a study by Brands and Hopkins that used the streptozotocin rat model to determine the contribution of poor glucose control to diabetes-related hypertension, it was demonstrated that in short-term experimental diabetes, decreasing the insulin infusion to produce hyperglycemia resulted in corresponding elevations in arterial pressure, in spite of a
natriuresis. The hypertensive and natriuretic effects of poor glycemic control were completely reversible with restoration of insulin therapy and normalization of blood glucose (11). The authors hypothesized that the MAP increase may be secondary to the loss of an insulin-mediated vasodilator effect when the insulin dose was lowered to produce hyperglycemia. The results of the current set of experiments tend to support the existence of hyperglycemia-mediated increases in MAP, but not the mechanism proposed by Brands. The method used to produce hyperglycemia did not result in a decline in plasma insulin concentrations, but rather in equivalence of values during the phases of the study. In addition, in human subjects, insulin-mediated vasodilation occurs with higher levels of insulin than were apparent in the present study (12,13); therefore, it is unlikely that insulin concentrations played a part in the present findings.

It was again noted that the hyperglycemia influenced renal hemodynamic function (a decline in ERPF and RBF with maintenance of GFR, resulting in an augmentation of RVR and FF). Glucose-induced hemodynamic changes have been detected in other organ systems in experimental and human diabetes. In a study by Kawai and colleagues (14) using Fischer 344 rats during bilateral carotid occlusion, hyperglycemia induced a progressive decrease in cerebral blood flow. In a study by Jin and Bohlen (15) using Zucker fatty diabetic rats, resting intestinal blood flow showed a significant decline after 30 min of hyperglycemia. Human studies support these findings. In a study by Giugliano and colleagues (16), acute hyperglycemia was induced in healthy nondiabetic subjects, and resulted in a significant decrease in leg blood flow. Reduced coronary flow reserve in patients with type 2 diabetes and angiographically normal coronary arteries has also been demonstrated (17,18). The renal circulation appears to be no less sensitive to the effects of hyperglycemia. The maintained GFR in conjunction with increases in RVR and FF observed in the current study indicate that most of the renal pressor effect of hyperglycemia appears to be at the efferent arteriole, which would normally result in an increased intraglomerular pressure with its attendant deleterious effects. A similar pattern of renal hemodynamic response has been noted during low dose AngII infusion (19).

The second major observation was that these hyperglycemia-mediated renal hemodynamic responses were abolished by administration of losartan. This supports the hypothesis that hyperglycemia exerts its renal pressor effect by activating the RAS. The fact that a subdepressor dose of losartan had little renal hemodynamic effect while subjects were euglycemic, but significantly reduced RVR and FF during hyperglycemia, suggests, but does not prove, a hyperglycemia-induced augmentation of intrarenal AngII levels. It is possible that a component of the renal effects may have been secondary to intrarenal changes in AngII, but since BP increased, an elevation in circulating AngII activity may have been operative. In fact, the renal changes could possibly be explained solely as a response to elevations in BP, which would be expected to cause an increase in RVR, mainly at the afferent arteriole, and an increase in FF. It can also be hypothesized that losartan reversed the hyperglycemia-mediated renal hemodynamic changes, not through its action on renal AngII receptors, but by its systemic BP-lowering effect. Whether the intrarenal RAS is activated by this maneuver cannot be discerned from this protocol, as the tissue RAS could not have been examined without a biopsy. This fact does not diminish the importance of the observation that RAS activation, whether systemic or intrarenal, can be induced by levels of hyperglycemia that are modest at best, and that are thought by some to constitute acceptable blood glucose control.
Other authors have suggested a link between hyperglycemia and activation of the RAS. In a study by O’Hare and colleagues (5), normotensive diabetic subjects were studied before and after an interval of improved metabolic control. Significant declines in BP and plasma renin concentration were noted to occur in parallel with improved blood glucose. Extracellular fluid volume was directly measured in that experiment, and no differences were noted between the periods. In a study by Nützi and colleagues (20) in healthy subjects, acute increases in plasma insulin and glucose after glucose loading were accompanied by decreases in plasma cortisol and aldosterone and elevations in PRA.

There is compelling evidence from studies using pharmacologic blockers of the RAS that the AngII-mediated increase in intraglomerular pressure is one of the major mechanisms in the progression of renal disease (2,21,22), and that reductions in glomerular pressure can ameliorate glomerular injury. The mechanism whereby increased glucose can activate the RAS is unknown. However, there are some clues. In a study by Woods et al. (4) in anesthetized dogs, the intrarenal infusion of glucose resulted in an increase in the renin secretion rate to greater than twice the control level, but only in filtering kidneys, thus implicating a tubuloglomerular feedback mechanism. It is possible that in the current study, hyperglycemia without glucosuria aggravated the normally enhanced proximal tubular sodium reabsorption by activating glucose-sodium cotransport mechanisms, ultimately reducing distal delivery and tubuloglomerular feedback activity and increasing renin secretion. Alternatively, increased insulin concentrations are known to increase sodium reabsorption, and could have acted in a similar manner. However, this appears to be a less attractive hypothesis, both because the antinatriuretic effect of insulin appears to be a distal phenomenon (31), and because no differences were detected in the plasma insulin concentrations between the phases of the experiment. Both of these mechanisms are difficult to defend, as no significant differences were noted in UNaV between phases of the experiment. It was demonstrated by Wang and colleagues, in opossum kidney cells (32), that high glucose stimulated expression of a fusion gene containing various lengths of the 5′ flanking regulatory sequence of the rat fused with the human growth hormone gene as a reporter, suggesting that hyperglycemia can modulate expression of the renal angiotensinogen gene in vivo. This phenomenon was time-dependent, with the greatest effect being observed during 2 d of incubation, and diminishing thereafter. It therefore may be possible that short-term hyperglycemia can activate the renal RAS through molecular rather than hemodynamic or physiologic mechanisms. The time course whereby such a phenomenon would occur in humans is unknown, and therefore such a mechanism must remain speculative. The results from the present study of whole kidney function in humans cannot offer any information that would clarify which of these putative mechanisms is operative. Although all of these mechanisms could account for the present findings by increasing renin secretion, they remain entirely hypothetical, and await further investigation.

Other explanations for the present findings were sought. It is known that protein intake can affect renal hemodynamic function and PRA in humans (33,34). However, the protein content of the diets was similar for all subjects, and the 24-h urea excretion was unchanged after all preparatory periods. Therefore, differences in protein intake can be excluded as a confounding variable. An unrecognized osmotic diuresis or osmotic fluid shift may have reduced extracellular fluid volume during hyperglycemic conditions, thus activating the RAS. It is known that the hemodynamic response to RAS blockade is blunted in sodium-replete conditions and enhanced by extracellular fluid volume contraction (35). A similar mechanism could account for the decreased renal response to AngII during hyperglycemia, in that a reduction in extracellular fluid volume would be expected to result in an augmentation of intrarenal AngII and downregulation of receptors. However, in the study by O’Hare et al. (5), and in the previous study from our laboratory, which used similar methodology (3), there was no evidence of intravascular volume depletion during the hyper-
activates the RAS cannot be obtained from this study of whole.

sponses to AngII infusion were blunted during hyperglycemia, abolished by losartan, indicating that they were mediated by

The renal and peripheral pressor effects of hyperglycemia were

creases BP and RVR, in conjunction with an increase in PRA. It is also possible that the baseline renal and peripheral pressor effects observed in this study while subjects were hyperglycemic were indicative of a glucose-mediated attenuation of endothelium-dependent vasodilation. There is substantial evidence that endothelium-dependent vasodilation is impaired in both experimental and human models of diabetes (36). Furthermore, hyperglycemia is known to attenuate endothelium-dependent vasodilation in studies in animals (37) and humans (38). This was considered unlikely first because the significant depressor response to RAS blockade during hyperglycemia appeared to implicate RAS activation, and second because RAS blockade was not shown to influence endothelial function in a large study of 91 subjects with stable type 1 diabetes (39). Taken together, the results from both of the maneuvers in the current study, losartan administration and AngII infusion, support the hypothesis that moderate short-term hyperglycemia without glucosuria results in renal and peripheral RAS activation. It is unlikely that any of these factors—protein intake, extracellular fluid volume changes, or endothelial dysfunction—could have contributed to the observed results.

It is interesting that the magnitude of the response to hyperglycemia was quite variable among subjects. It is well known only 30 to 40% of patients with type 1 diabetes will develop nephropathy, and that high glucose is a necessary but not sufficient factor in the development of this complication (1). It is tempting to speculate that the differences in susceptibility to nephropathy in patients with type 1 diabetes may relate to variations in the response of the RAS to hyperglycemia. This question cannot be answered by the present protocol, and would require longitudinal examination of a larger group of patients with type 1 diabetes.

In summary, the present study extends previous findings from this laboratory demonstrating that hyperglycemia increases BP and RVR, in conjunction with an increase in PRA. This set of experiments clearly establishes that hyperglycemia of short duration and of moderate magnitude results in an activation of the circulating, and possibly intrarenal, RAS. This was supported by the peripheral and renal hemodynamic responses to both losartan administration and AngII infusion. The renal and peripheral pressor effects of hyperglycemia were abolished by losartan, indicating that they were mediated by the RAS. The expected vasoconstrictor and excretory responses to AngII infusion were blunted during hyperglycemia, indicating previous activation of the system. Although a complete understanding of the mechanism by which hyperglycemia activates the RAS cannot be obtained from this study of whole kidney function, it provides a possible mechanism to explain the impact of hyperglycemia on the progression of diabetic renal disease.

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References


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