The Cardiac Renin-Angiotensin System in STZ-Induced Diabetes

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To determine the effect of diabetes on the cardiac renin-angiotensin system, we compared angiotensin II binding density and renin, angiotensinogen, and type 1 angiotensin II (AT1) receptor mRNA levels in hearts of Sprague-Dawley rats 14 days after the administration of streptozotocin (STZ), in vehicle-treated control rats, and in STZ-administered rats made euglycemic with insulin. Myocardial angiotensin II receptor density, determined using an in situ autoradiographic technique, was increased significantly in hyperglycemic diabetic rats in comparison with control rats and euglycemic diabetic rats (P < 0.01) as a result of an increase in both AT1 and AT2 (type 2 angiotensin II) subtypes. The myocardial AT1 receptor mRNA level, determined by slot blot hybridization, was also significantly greater in the hyperglycemic diabetic rats (P < 0.005). Neither plasma renin concentration nor cardiac renin or angiotensinogen mRNA levels differed among the three study groups. In an additional experiment, control and diabetic rats were infused with angiotensin II (200 ng·kg⁻¹·min⁻¹ i.p. for 7 days) or vehicle. Plasma renin concentration decreased significantly, whereas no significant changes occurred in cardiac renin or angiotensinogen steady-state mRNA levels. As in the first experiment, levels of AT1 receptor mRNA were significantly greater in the diabetic rats. Thus, myocardial angiotensin II receptor density is increased in diabetic rats in association with an increase in steady-state AT1 receptor mRNA levels, an abnormality that appears to be independent of changes in the circulating renin-angiotensin system. Diabetes 43:1180-1184, 1994

The identification of multiple components of the renin-angiotensin system in diverse organs (1) has led to the hypothesis that this system plays an autocrine or paracrine role in the regulation of local tissue functions. The existence of a cardiac renin-angiotensin system was initially suggested by biochemical identification of renin, angiotensin-converting enzyme, and angiotensin II receptors in cardiac tissue (2-4). More recently, mRNAs for renin (5), angiotensinogen (5,6), and the type 1 angiotensin II (AT1) receptor (7) have been identified in rat heart, an essential requisite for the postulated intracardiac biosynthesis of these proteins. A physiological role for the cardiac angiotensin receptor is suggested by both positive inotropic and chronotropic effects of angiotensin II (8,9). Angiotensin II also stimulates protein synthesis and cell growth in cultured chick heart cells (10) and causes vasoconstriction of coronary arteries (11). Moreover, a role for angiotensin II in a variety of pathophysiological states is suggested by the beneficial effects conferred by treatment with angiotensin-converting enzyme inhibitors (12).

Myocardial dysfunction occurs frequently in diabetes, even in the absence of coronary artery disease, systemic hypertension, or valvular heart disease (13), suggesting a primary diabetic cardiomyopathy (14). In the streptozotocin (STZ)-induced diabetic rat, a model of type I diabetes, abnormalities of cardiac function may be seen as early as 7 days after induction of diabetes (15). Because abnormalities of both circulating and local tissue renin-angiotensin systems have been described in diabetic rats (16-20), this study was undertaken to characterize the cardiac renin-angiotensin system in the STZ-induced diabetic rat model. Plasma renin levels, cardiac angiotensinogen, renin and angiotensin receptor mRNA levels, and cardiac angiotensin II receptor density were compared in control, hyperglycemic diabetic, and euglycemic diabetic rats.

RESEARCH DESIGN AND METHODS

All experiments were performed in male Sprague-Dawley rats (150-200 g, Bantin Kingman, Fremont, CA) that were maintained on standard rat diet and tap water ad libitum with 12 h light/dark cycles in a quiet environment.

**Experiment 1.** Diabetes (n = 20) was induced by intravenous administration of STZ (Sigma, St. Louis, MO), 60 mg/kg body wt, dissolved in sodium citrate buffer (0.1 M, pH 4.5). Control rats (n = 11), which were matched for age and weight, received an equal volume of the vehicle. Animals were considered to be diabetic if blood glucose levels were ≥29 mmol/L 72 h after the injection. Diabetic rats were further divided into two subgroups: one in which a fixed dose (2 U/day) of human recombinant insulin (Humulin N) was administered twice daily (at 8:00 a.m. and 8:00 p.m.) in doses (6-10 U/day) adjusted to achieve euglycemia (n = 10). Blood glucose levels were measured every other day by tail vein sampling (Accu-chek bG, Bio-Dynamics, Boehringer Mannheim, Indianapolis, IN). Rats were killed by decapitation 14 days after the administration of STZ, and trunk blood was collected in EDTA and immediately chilled. The plasma was separated by centrifugation and stored at −20°C for later determination of plasma glucose and renin concentrations. The heart was quickly removed, rinsed in phosphate-buffered saline, and frozen in liquid nitrogen for either RNA isolation or angiotensin II receptor assay.

**Experiment 2.** The influence of circulating angiotensin II on the cardiac renin-angiotensin system was investigated in 10 diabetic rats treated with 2 U/day of insulin and 10 control rats. Ten days after administration of STZ or vehicle, rats were anesthetized with sodium pentobarbital, 50 mg/kg i.p., and an osmotic minipump (model 2001, Alza, Palo Alto, CA) was implanted, releasing the insulin or vehicle (10 mg/kg per day) for 7 days.
was implanted intraperitoneally. Five rats from each subgroup received angiotensin II (Human Angiotensin II, Peninsula, Belmont, CA) at an infusion rate of 200 ng \( \cdot \) kg\(^{-1} \cdot \) min\(^{-1}\), and the other 5 rats received the vehicle. This concentration and infusion rate of angiotensin II has been reported to increase the sensitivity of angiotensin II receptors in the rat adrenal gland without increasing blood pressure (21). Angiotensin II was dissolved in saline containing bovine serum albumin (48 mg/ml) to avoid loss of peptide by adherence to the osmotic membrane. Rats were infused for 7 days. After infusion, rats were killed by decapitation, and the hearts were handled as described above.

Plasma glucose and renin concentrations. Glucose was determined using a YSI model 23A glucose autoanalyzer (Yellow Springs, OH). Plasma renin concentration was determined as the rate of angiotensin I generation in plasma incubated for 2 h at pH 6.5 in the presence of excess rat angiotensinogen using the method of Menard and Catt (22).

RNA isolation. Total cellular RNA was isolated from frozen tissue by a modification of the guanidine thiocyanate method of Chirgwin (23) as described previously (24). The resultant RNA pellet was dissolved in sterile water and quantified by ultraviolet absorbance at 260/280 nm. RNA integrity was verified by agarose gel electrophoresis.

Probe synthesis. Rat angiotensinogen and renin antisense \([\text{32P]}\) cRNA probes were synthesized as described previously (17). The AT1 probe (a gift of Kenneth Bernstein, Emory University, Atlanta, GA) (7) was synthesized by nick translation (N.5500, Amersham, Arlington Heights, IL) using the HindIII-Not I cDNA fragment of CalBS. A \(28^\text{S}\) -labeled oligonucleotide complementary to bases 4011–4036 of human \(28^\text{S}\) ribosomal RNA was prepared as described by Barbu and Dautry (25).

mRNA analysis. Angiotensinogen, renin, and angiotensin receptor mRNAs were quantitated by slot blot hybridization as described previously (17,24). Autoradiographs were obtained by exposure to Cronex XT (Du Post, Wilmington, DE) with an intensifying screen at \(-80^\circ\)C for 7–8 days and were scanned with a laser densitometer. Duplicate blots were prepared and hybridized with the \(28^\text{S}\) ribosomal RNA probe as a means to detect RNA degradation and loading artifacts.

In situ angiotensin II receptor assay. The distribution and density of cardiac angiotensin II receptors were assessed by both film and emulsion autoradiography using a modification (4) of the in situ receptor binding assay of Allen et al. (26) using \(^{[32\text{P}]}\) cRNA probes. The ATI and AT2 receptor mRNAs were quantitated by slot blot hybridization as described previously (17,24). Autoradiographs were obtained by exposure to Cronex XT (Du Post, Wilmington, DE) with an intensifying screen at \(-80^\circ\)C for 7–8 days and were scanned with a laser densitometer.

Film autoradiography, the slides were exposed to LKB-Ultradyn film for 7–12 days at room temperature. Films were processed with Kodak emulsion. The background number of grains, present outside the tissue sections, was subtracted. Specific binding was calculated by subtracting nonspecific binding, as determined by incubating adjacent sections with 10 \(\mu\)mol/l unlabeled \([\text{Sar}^1,\text{Ile}^8]\) angiotensin II, from total binding.

Binding to ATI and AT2 receptors was calculated by subtracting nonspecific binding from the binding in the presence of PD123177 (10 \(\mu\)mol/l) and losartan (10 \(\mu\)mol/l), respectively. The combination of losartan and PD123177 inhibited specific binding almost completely. Values are means \(\pm\) SE of 6 rats in each group.

RESULTS

Experiment 1. Blood glucose increased promptly after administration of STZ and remained significantly greater than control (22.8 \(\pm\) 1.7 vs. 82.2 \(\pm\) 0.7 mmol/l, \(P < 0.0001\)) in the rats that received low-dose insulin therapy. Hyperglycemia was completely ameliorated (6.9 \(\pm\) 1.0 mmol/l) in the rats that received the higher doses of insulin. Rats treated with the higher doses of insulin gained weight rapidly, reaching values not significantly different from control at the end of the experimental period (286 \(\pm\) 10 vs. 297 \(\pm\) 4 g, respectively), whereas the rate of growth was significantly slower in rats treated with low-dose insulin (245 \(\pm\) 6 g, \(P < 0.0001\)). Plasma renin concentration did not differ significantly among the three experimental groups (control 3.7 \(\pm\) 0.4, low-dose 4.2 \(\pm\) 0.8, high-dose 3.7 \(\pm\) 0.3 ng \(\cdot\) I\(^{-1}\) \cdot\) s\(^{-1}\)).

Myocardial angiotensin II receptor density, as reflected by the number of silver grains, was increased significantly in hyperglycemic diabetic rats in comparison to control rats and euglycemic diabetic rats; this increase reflected differences in both AT1 and AT2 receptor subtypes (Fig. 1). Regional analysis of angiotensin II binding to interventricular septum and right and left ventricles indicated that angiotensin II receptor density was increased to the same extent in all regions of diabetic hearts. Similar differences were observed by measurements of optical density performed on film autoradiograms using computerized microdensitometry (control rats: 30.5 \(\pm\) 0.3 optical density units; diabetic rats: 35.6 \(\pm\) 0.2 optical density units).

Myocardial AT1 receptor mRNA levels were significantly greater in hyperglycemic rats than in the control or euglycemic diabetic rats (Table 1). In contrast, cardiac renin and angiotensinogen mRNA levels in the diabetic rats treated either with low- or high-dose insulin therapy did not differ.
Cardiac renin, AT1 receptor, angiotensinogen mRNA, and 28 S ribosomal RNA levels in control, low-dose, and high-dose insulin-treated diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Renin mRNA</th>
<th>AT1 mRNA</th>
<th>AG mRNA</th>
<th>28S mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>2.4 ± 0.2</td>
<td>1.03 ± 0.2</td>
<td>5.4 ± 0.2</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>Diabetes + insulin (2 U/day)</td>
<td>5</td>
<td>2.4 ± 0.3</td>
<td>1.96 ± 0.1*</td>
<td>5.4 ± 0.4</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>Diabetes + insulin (6–10 U/day)</td>
<td>5</td>
<td>1.9 ± 0.2</td>
<td>1.38 ± 0.2</td>
<td>4.8 ± 0.2</td>
<td>3.1 ± 0.2</td>
</tr>
</tbody>
</table>

Data are means ± SE of the values obtained after intravenous administration of STZ (60 mg/kg) or vehicle. Data are expressed in arbitrary scanner units (S.U.). Intergroup comparisons were done by ANOVA. AG, angiotensinogen. *P < 0.005 vs. control; †P < 0.05 vs. diabetes + insulin (2 U/day).

significantly from control levels (Table 1). Levels of 28S ribosomal RNA also did not differ significantly among the study groups (Table 1), further indicating the specificity of the changes in AT1 receptor mRNA.

**Experiment 2.** As in experiment 1, AT1 receptor mRNA levels were increased in the diabetic animals (Fig. 2, Table 2). No significant differences were observed for either renin or angiotensinogen mRNA levels in response to diabetes or to angiotensin II infusion. Chronic angiotensin II infusion induced a significant decrease in plasma renin concentration (Table 2), indicating effective suppression of the circulating renin-angiotensin system.

**DISCUSSION**

The results of this study demonstrate that myocardial angiotensin II receptors are increased in rats 2 weeks after induction of diabetes. This increase was associated with an increase in steady-state AT1 receptor mRNA levels, suggesting increased gene expression as the likely mechanism. Moreover, these alterations appear to reflect the metabolic disturbances accompanying the diabetic state, because neither increased angiotensin II binding nor AT1 receptor mRNA levels were observed in rats treated with insulin in doses sufficient to achieve euglycemia. Further evidence for the specificity of this effect was the absence of concomitant changes in the expression of other genes of the renin-angiotensinogen system in the diabetic animals.

We have recently shown that at least two high-affinity receptor sites are present in both myocardium and conduction tissue obtained from rat heart (4). The presence of high-affinity receptors in the myocardium can account for the positive inotropic effect of angiotensin in isolated muscle preparations of mammalian heart (8). Angiotensin II also has a positive chronotropic effect, but this may occur indirectly as a consequence of stimulation of cardiac efferent sympathetic activity (28) and/or vagal inhibition (29).

The results of recent studies using converting enzyme inhibitors have led to speculations about the possible role of the cardiac renin-angiotensin system in conditions such as chronic congestive heart failure (30), acute myocardial in-

**FIG. 2.** A: northern hybridization of a radiolabeled AT1 receptor cDNA probe to rat cardiac RNA. Total cardiac RNA was fractionated by electrophoresis using a 1% agarose gel, followed by capillary transfer to a nylon membrane, hybridization, and autoradiography. B: slot blot hybridization of radiolabeled AT1 receptor cDNA with 4 μg samples of total RNA extracted from control and diabetic rat hearts from experiment 2 (Table 2).
mRNA levels in control and insulin-treated diabetic rats infused with either vehicle or angiotensin II

TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Weight (g)</th>
<th>Heartbody ratio (×1,000)</th>
<th>Glucose mmol/l</th>
<th>PRC (ng·kg⁻¹·s⁻¹)</th>
<th>Renin mRNA (S.U.)</th>
<th>AT1 mRNA (S.U.)</th>
<th>AG mRNA (S.U.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + vehicle</td>
<td>5</td>
<td>301 ± 6</td>
<td>3.38 ± 0.10</td>
<td>6.0 ± 0.2</td>
<td>4.98 ± 1.8</td>
<td>5.3 ± 0.9</td>
<td>5.6 ± 0.7</td>
<td>4.4 ± 0.6</td>
</tr>
<tr>
<td>Control + ANG II</td>
<td>5</td>
<td>301 ± 11</td>
<td>3.57 ± 0.08</td>
<td>7.0 ± 0.3</td>
<td>1.08 ± 0.4*</td>
<td>5.3 ± 1.1</td>
<td>9.6 ± 1.3*</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>Diabetes + vehicle</td>
<td>4</td>
<td>238 ± 51</td>
<td>3.20 ± 0.07</td>
<td>23.2 ± 0.51</td>
<td>5.92 ± 2.0</td>
<td>4.9 ± 1.3</td>
<td>15.0 ± 3.3*</td>
<td>3.6 ± 0.7</td>
</tr>
<tr>
<td>Diabetes + ANG II</td>
<td>5</td>
<td>224 ± 67</td>
<td>3.76 ± 0.13</td>
<td>24.1 ± 1.55</td>
<td>0.56 ± 0.33</td>
<td>5.1 ± 0.7</td>
<td>17.0 ± 3.3*</td>
<td>4.2 ± 0.5</td>
</tr>
</tbody>
</table>

Data are means ± SE of the values obtained 14 days after intravenous administration of STZ (60 mg/kg) or vehicle and 7 days after intraperitoneal infusion of angiotensin II (ANG II) (200 ng·kg⁻¹·min⁻¹) or vehicle. Plasma glucose and plasma renin concentration (PRC) were measured on trunk blood obtained at the time of killing. Rats received a low dose of insulin (2 U/day) to maintain a moderate hyperglycemic state. RNA data are expressed in arbitrary scanner units (S.U.). Intergroup comparisons were done by ANOVA. AG, angiotensinogen. *P < 0.05 vs. control infused with vehicle; †P < 0.0001 vs. respective control; ‡P < 0.025 vs. respective control; §P < 0.05 vs. diabetic infused with vehicle; ¶P < 0.025 vs. diabetic infused with vehicle; ¶¶P = 0.07 vs. respective control.

In contrast with the increased expression of AT1 receptor in cardiac tissue noted in this study, we previously found that both angiotensin II receptor number and AT1 receptor mRNA levels were decreased in the kidneys of diabetic rats (38). Normal levels of renin mRNA in the kidney and decreased levels of angiotensinogen mRNA in the liver (19–19) have been reported to occur in STZ-induced diabetic rats, whereas other studies have found increased levels of renin mRNA in the kidneys of rats with genetically induced diabetes (20), as well as increased angiotensinogen mRNA in the kidneys (19) and decreased angiotensinogen mRNA in adipose tissue of STZ-induced diabetic rats (18). Although these apparently divergent observations are difficult to resolve, they seem to indicate a propensity for tissue-specific changes in local renin-angiotensin systems in diabetes.

In this study, chronic infusion of angiotensin II in doses sufficient to suppress plasma renin concentration did not alter renin or angiotensinogen mRNA levels in either control or diabetic rats, indicating that the expression of these genes in the heart is not under the influence of circulating angiotensin II. Similarly, the increased levels of AT1 receptor mRNA that characterized the diabetic rats were not affected by angiotensin II infusion. Thus, the alteration in angiotensin II receptors is more likely to reflect abnormal angiotensin II generation in cardiac tissue or primary abnormalities of angiotensin II receptor gene expression induced by diabetes.

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