Deficiency in angiotensin AT1a receptors prevents diabetes-induced hypertension

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Wichi RB, Farah V, Chen Y, Iriigoyen MC, Morris M. Deficiency in angiotensin AT1a receptors prevents diabetes-induced hypertension. Am J Physiol Regul Integr Comp Physiol 292: R1184–R1189, 2007. First published November 22, 2006; doi:10.1152/ajpregu.00524.2006.—The renin-angiotensin system has been implicated in the etiology of the cardiovascular complications of diabetes. Our studies extend these findings to show a specific role for angiotensin AT1a receptors in mediating diabetes-induced hypertension. Male angiotensin AT1a knockout (AT1aKO) and wild-type (AT1aWT) mice with arterial telemetric catheters were injected with streptozotocin (STZ; 150 mg/kg ip). The STZ dose was selected on the basis of a dose-response experiment in C57/Bl mice. Blood glucose, water intake, body weight, blood pressure (BP), and heart rate (HR) were measured over a 2-wk period. Estimates of BP and HR variance (BPV and HRV) and their low- and high-frequency domains were also determined. STZ induced similar levels of hyperglycemia and glycemic control (1, 23). However, there are interactions with the vasodilator peptide, ANG (1–7) (21, 56). We reported that streptozotocin (STZ) diabetes caused activation of angiotensin-converting enzyme-2 (ACE2), a proteolytic enzyme involved in formation of the vasodilator peptide, ANG (1–7) (21, 56). We reported that STZ-induced insulin deficiency or insulin resistance produced by a high fructose diet activated the RAS, as seen by ACE activity, ANG II levels, and brain expression of ANG AT1a mRNA (14, 15).

Type 1 diabetes is genetically linked and normally occurs early in life. It is related to a deficit in pancreatic function, resulting in a reduction in circulating insulin. Animal models have been developed that rely on selective pancreatic toxins. For example, STZ is toxic to pancreatic β-cells, producing a syndrome similar to Type 1 diabetes in both rats and mice. The cardiovascular outcomes in rats are decreased heart rate and increased or decreased arterial pressure, as well as baroreflex dysfunction (9, 10, 19, 27). There is less information in mice although studies document variable changes in blood pressure, as well as vascular and renal damage after STZ treatment (20, 26, 36).

The objective of our study was to explore the role of ANG AT1a receptors in the cardiovascular consequences of a model of Type 1 diabetes. We present the hypothesis that a deficiency in ANG AT1a receptors will attenuate or block diabetes-induced cardiovascular changes. Insulin deficiency was induced in angiotensin AT1a wild-type (AT1aWT) control and AT1a knockout (AT1aKO) mice with vascular radiotelemetric probes. Blood pressure (BP) and heart rate (HR) as well as cardiovascular; heart rate; blood pressure; renin-angiotensin system; mice; autonomic function; streptozotocin; diabetes

DIABETES MELLITUS IS A RISK factor for the development of cardiovascular disease, such as hypertension, atherosclerosis, and congestive heart failure. Mortality rates are greater in diabetic compared with nondiabetic persons, and cardiovascular disease in diabetic patients further increases the risk. The hallmark symptom for diabetes is hyperglycemia, resulting from β-cell damage (Type 1) or insulin resistance (Type 2). Hyperglycemia is thought to be a major determinant in the development of long-term complications of diabetes, and treatment is focused on strict glycemic control (1, 23). However, there are interactions with the renin-angiotensin system (RAS), which contribute to diabetes-induced organ damage (8, 23, 43, 53).

The RAS plays an important role in the regulation of blood pressure and volume, mainly via the actions of ANG II. Most of the cardiovascular effects of ANG II are mediated by AT1 receptors, which are present in vasculature, brain, heart, and other tissues. AT1 receptors exist as two subtypes, AT1a and AT1b, in mice and rats (33). Studies of the functional role of AT1a receptors have relied on gene deletion (30) or gene silencing (5) methods, because it is not possible to pharmacologically distinguish AT1 receptors. ANG AT1a-deficient mice show low blood pressure, altered renal and autonomic function, and increased sodium sensitivity (6, 30, 45, 46, 49, 50).

Diabetes is associated with activation of the RAS in both humans and animals, and changes in the RAS have been linked to cardiovascular dysfunction (9, 15, 34, 40, 55). It is well accepted that antagonists of the RAS are the method of choice for management of hypertensive diabetics (8, 23). Epidemiological studies showed that treatment with ANG receptor blockers (ARBs), losartan, or candesartan, lowered blood pressure and also reduced the incidence of new cases of diabetes (32, 41, 57). This is important as it illustrates a possible long-term, protective role of drug therapies, which target the RAS. Likewise, in animals, ARBs reduced blood pressure in diabetic hypertensive rats (34, 47). Awad et al. (3) demonstrated that diabetes is associated with increased renal production of ANG II. A recent study showed that streptozotocin (STZ) diabetes caused activation of angiotensin-converting enzyme-2 (ACE2), a proteolytic enzyme involved in formation of the vasodilator peptide, ANG (1–7) (21, 56). We reported that STZ-induced insulin deficiency or insulin resistance produced by a high fructose diet activated the RAS, as seen by ACE activity, ANG II levels, and brain expression of ANG AT1a mRNA (14, 15).

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variance in time and frequency (autoregressive spectral analysis) domains were determined.

METHODS

Animals and protocols. The first objective was to establish the appropriate dose of STZ to produce hyperglycemia. This was accomplished using a dose-response test (100, 150, and 200 mg/kg ip STZ) in fasted C57BL/J male mice. Mice were injected with citrate buffer or STZ and measurements (body weight, water intake, blood glucose, plasma osmolality, and hematocrit) were made 6 days later. These mice did not have telemetry probes.

Cardiovascular experiments were performed in male AT1aKO mice and AT1aWT, weighing 25–30 g. The original breeding pairs for the AT1aKO colony were derived from the strain developed by Coffman and colleagues (30) (Duke University, Durham, NC). F2 progeny of C57BL/10 mated with SV129/) were used for experiments, such that all mice have the same genetic and environmental background. Mice were housed individually at 22°C with a 12:12-h dark-light cycle (0500–1700, lights on). Mice were given ad libitum access to tap water and standard chow. Radiotelemetry catheters were inserted into the aorta via the carotid artery and experiments were begun 7–10 days later. STZ was injected intraperitoneally into mice that had been fasted for 6 h (150 mg/kg in 10 mM citrate buffer, pH 4.5). BP, HR, body weight, water intake, and blood glucose were measured before and after STZ injection. All of the mice had telemetric probes. Wright State University’s Laboratory Animal Care and Use Committee approved all of the experiments.

Cardiovascular measurements. Under ketamine-xylazine anesthesia (120:20 mg/kg im), radiotelemetry catheters (model TA11PA-C20, Data Sciences International, St. Paul, MN) were inserted into the carotid artery using methods described previously (16). Mice were housed in individual cages with minimal manipulations and under low noise levels. The value of radiotelemetry is that it provides accurate, low-stress measurements of cardiovascular parameters. Blood pressure was measured at 500 Hz for 30-min periods during the light period at baseline and 1, 7, and 14 after STZ injection. In a second experiment, BP was recorded 30 min before and 10 min after injection of captopril (10 mg/kg ip). The decrease in BP after injection was considered for the analysis. Data were analyzed using software (CODAS, Dataq Instruments, Akron, OH) that detects beat-by-beat values of systolic arterial pressure.

Variance and autoregressive spectral analysis. Heart rate variability (HRV) and blood pressure variability (BPV) were evaluated using BP recordings (continuous 30 min, 5,000 Hz) made at baseline and 14 days after STZ injection. Overall variability of HR and BP was assessed in the time domain by means of variance. HRV and BPV fluctuations were assessed in the frequency domain using autoregressive spectral analysis, as described elsewhere (7, 11, 16, 42). Briefly, HRV and BPV series were divided in segments of 300 beats and overlapped by 50%; a spectrum was obtained for each of the segments via the Levinson-Durbin recursion, with the model order chosen overlapped by 50%; a spectrum was obtained for each of the segments

Glucose and insulin measurements. Glucose was measured using an Accu-Check Advantage Blood Glucose Monitor (Roche Diagnostic, Indianapolis, IN). Mice were fasted for 6 h, and blood samples were taken using a razor cut on the tip of the tail. Plasma insulin levels were measured by radioimmunoassay in 20-μl aliquots of plasma using a commercial kit (Linco Research, St. Charles, MO).

Statistical analysis. Results are expressed as means ± SE. Statistical analyses were performed using two-way repeated-measures ANOVA, followed by Newman-Keuls post hoc test. The relationship between BP and glycemia was evaluated by calculating the Pearson correlation coefficient using data from AT1aWT and AT1aKO mice. The change in mean arterial pressure (MAP) produced by captopril was tested with Student’s t-test. Differences were considered to be significant at P < 0.05.

RESULTS

An STZ dose-response experiment was performed in C57BL/J male mice (Table 1). Results showed that body weight was decreased with the 200 mg/kg STZ dose. Water intake and blood glucose were increased with the 150 and 200 mg/kg STZ doses. There were no changes in osmolality or hematocrit, even at the highest dose, suggesting that STZ-induced diabetes did not change volume status.

STZ produced time-dependent increases in blood glucose and water intake, which were similar between AT1aWT and AT1a KO (Fig. 1A and Table 2). Plasma insulin levels were significantly decreased after STZ treatment in AT1aWT (162 ± 35 vs. 25 ± 12 μU/ml, baseline vs. STZ, P < 0.01) and AT1a KO (140 ± 19 vs. 40 ± 18 μU/ml, baseline vs. STZ, P < 0.05). ANOVA for plasma insulin showed significant main effects of treatment (basal vs. STZ) ([F(1.8) = 27.98, P < 0.001].

Blood pressure was lower in AT1aKO, as documented in previous studies (7, 30) (group effect, P < 0.001). STZ diabetes increased blood pressure in AT1aWT but not in AT1aKO (Fig. 1B). Peak MAP levels in AT1aWT reached 124 ± 6 mmHg (Fig. 1B) (P < 0.001). There were no changes in HR in either group (Fig. 1C). A correlative relationship was noted between blood glucose and BP (r = 0.75, P < 0.02).

To examine the role of the RAS in the BP changes, we tested the effect of acute treatment with a converting enzyme inhibitor, captopril. Figure 2 illustrates the percentage change in MAP after treatment with captopril (10 mg/kg, ip). Captopril significantly reduced MAP in AT1aWT (125 ± 10 to 106 ± 12 mmHg) but caused no change in AT1aKO (89 ± 7 to 86 ± 9 mmHg). ANOVA for repeated measures showed significant main effects of treatment ([F(1.6) = 45.7, P < 0.001] and drug × group interactions [F(1.6) = 26.8, P < 0.02].

Table 1. Body weight, water intake, blood glucose, plasma osmolality, and hematocrit measured 6 days after STZ injection in C57BL/J male mice

<table>
<thead>
<tr>
<th>STZ Dose, mg/kg</th>
<th>Buffer (n = 8)</th>
<th>100 (n = 5)</th>
<th>150 (n = 6)</th>
<th>200 (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>24 ± 0.6</td>
<td>25 ± 0.7</td>
<td>23 ± 0.7</td>
<td>19 ± 1.1*</td>
</tr>
<tr>
<td>Water intake, ml/24 h</td>
<td>4 ± 0.5</td>
<td>3 ± 0.5</td>
<td>11 ± 0.7*</td>
<td>9 ± 0.7*</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>194 ± 18</td>
<td>243 ± 54*</td>
<td>327 ± 27*</td>
<td>343 ± 12</td>
</tr>
<tr>
<td>Osmolality, mOsm/kg</td>
<td>348 ± 28</td>
<td>336 ± 6</td>
<td>349 ± 12</td>
<td>343 ± 12</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>39 ± 2</td>
<td>46 ± 5.5</td>
<td>46 ± 2.3</td>
<td>41 ± 1.6</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. For body weight, there was significant main effect of treatment ([F(4.1) = 16.65, P < 0.001]. For water intake, there was significant main effect of treatment ([F(5.5) = 17.66, P < 0.001]). For blood glucose, there was significant main effect of treatment ([F(5.4) = 23 P < 0.001]. For osmolality and hematocrit, there were no difference changes.

*P < 0.001 vs. saline; n = 5–9 animals/group. STZ, streptozotocin.

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To study autonomic modulation of the heart and vasculature, we determined the effect of STZ-induced diabetes on BPV and HRV in time and frequency domains (Fig. 3, Table 3). For BPV, there were significant reductions in BPV and its LF oscillatory component under baseline conditions in AT1aKO compared with AT1aWT (Fig. 3). In AT1aWT, STZ decreased BPV by 50% (19 ± 0.5 vs. 8 ± 1 mmHg², before and after STZ) and the LF domain by 75% (12 ± 1.5 vs. 4 ± 0.9 mmHg², before and after STZ) (Fig. 3). There were no differences in HF at baseline or after STZ (data not shown).

There were no significant differences in HRV between AT1aWT and AT1aKO under baseline conditions (Table 3). STZ treatment did not alter HRV in AT1aWT. However, in AT1aKO, STZ produced marked increases in HRV and its HF oscillatory component, changes greater than 200% (Table 3).

**DISCUSSION**

A study was conducted to determine the role of ANG AT1a receptors in the hypertension associated with a model of Type 1 diabetes. Major findings are 1) hypertension produced by STZ diabetes was absent in mice lacking ANG AT1a receptors even in the face of a marked hyperglycemia and 2) autonomic modulation of the vasculature and heart was altered differentially in AT1aWT and AT1aKO.

There is much experimental evidence to show that diabetes produced by insulin deficiency is associated with a myriad of cardiovascular and metabolic effects. In humans, diabetes is associated with hypertension which is correlated with other pathologies such as stroke, infarction, and heart failure (23, 53). Most studies on the cardiovascular effects of STZ diabetes

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**Table 2. Body weight and water consumption under basal conditions and 1, 7, and 14 days after induction of STZ diabetes**

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT1aWT</td>
<td>33±1.5</td>
<td>33±1.4</td>
<td>30±1.2</td>
<td>30±1.2</td>
</tr>
<tr>
<td>AT1aKO</td>
<td>34±0.5</td>
<td>31±1.0</td>
<td>31±0.5</td>
<td>30±0.7</td>
</tr>
<tr>
<td>Water intake, ml/24 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT1aWT</td>
<td>4±0.5</td>
<td>5±1</td>
<td>28±4*</td>
<td>48±2*</td>
</tr>
<tr>
<td>AT1aKO</td>
<td>7±2</td>
<td>10±3</td>
<td>32±8*</td>
<td>50±8*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. For body weight, there was a significant main effect of time \(F(3,27) = 8.5, P < 0.001\) with no group or interaction effect. For water intake, there was a significant effect of time \(F(3,24) = 80.4, P < 0.001\) with no group or interaction effect. *\(P < 0.01\) vs. basal; \(n = 6\) animals/group.

AT1aKO, STZ produced marked increases in HRV and its HF oscillatory component, changes greater than 200% (Table 3).
have been conducted in rats, with results showing decreased, increased, or unchanged blood pressure (9, 19, 27, 37, 48). A comprehensive study in inbred mice strains also showed that STZ produced variable changes in blood pressure (26). Our results in the C57BL/SV129 hybrid showed that STZ diabetes produced a gradual increase in blood pressure along with hyperglycemia.

Our studies are based on the hypothesis that the RAS is important in the development of diabetes-induced cardiovascular pathologies. There is clinical and experimental data that show that diabetes is associated with activation of the RAS (14, 40). Moreover, the therapeutic use of ACE inhibitors or angiotensin receptor blockers documented the clinical benefits in diabetic hypertensive patients (13, 32, 41, 57). Thus, it was a logical step to examine the time course of diabetes-induced cardiovascular changes in a genetic strain that lack ANG AT1a receptors. Results showed that induction of diabetes in AT1aKO mice produced no change in blood pressure, supporting a role for the ANG AT1a receptor subtype in mediating the cardiovascular changes. On the opposite side, accentuation of the RAS enhanced the cardiovascular complications of diabetes (29).

To further examine the role of the RAS in the STZ diabetic syndrome, we measured the blood pressure-lowering effect of captopril. There was a marked decrease (almost 20%) in AT1aWT but no change in AT1aKO. Plasma ACE activity was also increased in STZ diabetic animals (14). This is consistent with the study of Crespo et al. (9), which showed that hypertension in STZ diabetic rats was associated with increased ACE activity in the vasculature. Long-term treatment of STZ diabetic mice with an ACE inhibitor reduced blood pressure, whereas losartan therapy in diabetic rats reduced blood pressure and improved renal function (36, 47). The data taken together suggest that activation of the RAS plays a key role in diabetes-associated hypertension.

Cardiovascular data were further analyzed using spectral analytical approaches. These methods have been used successfully to provide information on autonomic modulation of the circulation under different genetic and pathological conditions (7, 15, 16, 18, 44). In the present study, we measured HRV and BPV and their frequency components (LF and HF) at baseline and 2 wk after induction of diabetes.

Reduced HRV is associated with cardiovascular pathologies (2, 42). Clinical and experimental studies have shown that spectral power in the HF range of HRV reflects parasympathetic activity (2, 42); however, the interpretation of the LF range is controversial (2, 42, 44). Diabetes in humans is associated with a reduction in HRV (24, 31, 35), which is thought to be detrimental to cardiovascular health. In rats, there is evidence for a reduction in HRV and its HF component in association with insulin-deficient diabetes (19, 28). However, in our study of a short time course, STZ treatment produced no change in either HR or HRV in control WT mice.

In contrast to the lack of effect in the controls, there were marked increases in HRV and its HF domain in STZ diabetic AT1aKO mice. These findings are in accordance with the idea that the chronic blockade of RAS exerts protective effects on the heart. In both heart failure (12) and diabetes (38), autonomic control was improved after blockade of the RAS. Kontopoulos et al. (38) showed that when diabetic patients were treated with an ACE inhibitor, there was an increase in HRV and parasympathetic activity with the suggestion that this reduced cardiac risk. Indeed, there is much evidence to suggest that an increase in vagal tonus or HRV are biomarkers of improved cardiovascular prognosis (39, 51). The increase in HRV in the AT1a KO with induction of diabetes supports the idea that antagonism of the RAS system, whether by drugs or genetics, is beneficial.

Consistent with our previous report (7), BPV was lower in mice lacking ANG AT1a receptors. Pharmacological intervention with angiotensin receptor blockers also decreased BPV stabilization.

Table 3. Heart rate variability in time (variance) and frequency domains

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Pulse Interval, ms²</th>
<th>Variance</th>
<th>LF</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT1aWT</td>
<td>Basal</td>
<td>78±12</td>
<td>35±5</td>
<td>30±5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>STZ</td>
<td>76±11</td>
<td>17±3</td>
<td>45±9</td>
<td></td>
</tr>
<tr>
<td>AT1aKO</td>
<td>Basal</td>
<td>38±3</td>
<td>27±7</td>
<td>17±2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>STZ</td>
<td>87±13*</td>
<td>31±5</td>
<td>45±10*</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Low-frequency (LF) power oscillations (0.1 to 1.0 Hz) and high-frequency (HF) power of oscillations (1.0 to 5.0 Hz) under basal conditions and 14 days after induction of STZ diabetes. For HRV, there were significant main effects of time [F(1,9) = 11.1, P < 0.009] and time × group interactions [F(1,9) = 12.5, P < 0.007]. For LF, there were no differences. For HF, there was a significant main effect of time [F(1,9) = 17.7, P < 0.003]. *P < 0.02 vs. basal AT1aKO; n = 5–6 animals/group.
(22, 25). Treatment with STZ produced a prominent reduction in BPV in controls with no effect in AT1aKO. These data are consistent with studies in STZ diabetic rats, which showed reduced BPV (19, 52). The lowered BPV observed in both AT1aKO and diabetic AT1aWT mice is likely associated with increased sympathetic input to the vasculature. A test of pharmacological adrenergic antagonists in AT1aKO mice showed an enhanced depressor response (7). The absence of a further decrease in BPV in AT1aKO treated with STZ may be related to the possibility that the autonomic nervous system is operating at maximal sympathetic modulation. Increase in sympathetic activity is seen as a bad prognostic marker in cardiovascular diseases like hypertension and heart failure (39).

It is well accepted that BPV and its LF oscillations are reflective of sympathetic modulation of the circulation (4, 17, 54). However, one must consider whether the changes in status are acute or chronic. For example, acute stress increased BPV, while chronic stress produced a decrease (16). Likewise, acute blockade of adrenergic systems reduced BPV in the basal state and reduced the response to stress (17). It is interesting to note that the BPV response in type-1 diabetes was opposite to that seen in a dietary model of glucose intolerance (15). When mice consumed fructose, there was an increase in BPV in both time and frequency domains. However, the changes were phasic, seen only with the onset of the dark cycle when mice are active.

In conclusion, studies in ANG AT1aKO mice support a role for this pressor receptor subtype in mediating the hypertension of STZ diabetes. The data further support the beneficial actions of antagonism of the RAS in ameliorating the cardiovascular effects of STZ diabetes. The data further support the beneficial actions of antagonism of the RAS in ameliorating the cardiovascular effects of STZ diabetes.

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