Streptozotocin Diabetic Rats Are Hypertensive Despite Reduced Hypothalamic Responsiveness

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SUMMARY To determine whether diabetes predisposes rats to hypertension, tail-cuff systolic pressures were measured in male rats made diabetic by pretreatment with streptozotocin. From Weeks 2 through 7, diabetic rats weighed less but had higher systolic pressures than nondiabetic ones. Further comparisons made while the rats were anesthetized with urethane showed that pressor and sympathetic nerve responses to ventromedial hypothalamic stimulation, as well as pressor responses to injected vasopressin, were significantly reduced in the diabetic group. A generalized reduction of cardiovascular reactivity was considered unlikely because systemic pressor responses to norepinephrine and tyramine were unimpaired. Yet reflex bradycardia elicited by norepinephrine was enhanced indicating that baroreceptor resetting had not occurred. Thus, diabetic rats were characterized by hypertension, narrowed pulse pressure, bradycardia with increased reflex responses to norepinephrine, and reduced pressor responses to hypothalamic stimulation and to vasopressin. The successful induction of diabetes was confirmed not only by the presence of hyperglycemia, hypoinsulinemia, glycosuria, and abnormal glucose tolerance, but also by reductions in pancreatic weight, insulin, and β-cell content. Although our results suggest that diabetic rats are predisposed to become hypertensive, other mechanisms such as hypothalamic depression may be activated to restrict further elevations in blood pressure. (Hypertension 4:556-565, 1982)

KEY WORDS • baroreceptor buffering • blood pressure regulation • experimental diabetes • hypertension • pressor responsiveness • sympathetic nervous system • vasopressin • ventromedial hypothalamus

HYPERTENSION is generally believed to be more prevalent among diabetics than nondiabetics,1,2 but why this might be so is uncertain. Previous attempts to record blood pressure in rats with experimental diabetes have yielded equivocal results. Although blood pressure in normotensive rats is usually elevated by either alloxan2-4 or streptozotocin,5,6 it has also been found unaffected7 or even slightly decreased by streptozotocin.8 In spontaneously hypertensive rats (SHR), blood pressure falls during the first few weeks after alloxan diabetes is induced,4,5,9 but several months later becomes even higher than before.3

While trying to characterize the cardiovascular changes occurring in normotensive rats that had been pretreated with streptozotocin, we found the ensuing diabetes accompanied by mild hypertension. Both blood pressure10 and blood sugar11 are elevated during hypothalamic stimulation, and although such effects have often been studied separately, concurrent pressor and hyperglycemic responses were recently elicited in the same rats by stimulation of the median forebrain bundle,12 which interconnects various hypothalamic nuclei. Because this suggested that streptozotocin diabetic rats may show signs of hypothalamic dysfunction, terminal measurements were made to determine whether pressor and sympathetic responses to hypothalamic stimulation had also been altered.

Methods

Most experiments were done on 22 male Sprague-Dawley rats that weighed 160 ± 1 g and were about 6 weeks old (purchased from SASCO Inc, Omaha, Nebraska). Later, additional experiments involving periodic determinations of plasma glucose and insulin were done on 12 other rats obtained from the same source.

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Induction of Experimental Diabetes

All rats were transiently anesthetized with methoxyflurane (Metofane) for injection of either streptozotocin or its vehicle into the tail vein. Of 22 rats, 14 received intravenous (i.v.) streptozotocin, 50 mg/kg, while eight others received equivalent amounts of the solvent (pH 4.5 citric buffer) alone. Those treated with streptozotocin were given hypertonic glucose (3 ml of a 10% solution) by gavage routinely 4 and 10 hours later to avoid fatalities from severe hypoglycemia. All the rats survived and were thereafter housed six to eight per cage in an air-conditioned room with unrestricted access to food and water.

Body weights were measured weekly. At various times, extent of glycosuria was estimated by using enzymatic test strips (Tes-Tape, Lilly) to determine glucose concentrations in urine samples collected from rats that had been treated with streptozotocin. During terminal measurements of pressor responsiveness (while the rats were anesthetized with urethane; see below) a 100 μl sample of arterial blood was collected for enzymatic measurement of plasma glucose content. After each experiment, the pancreas was removed, weighed, and extracted with acid ethanol. Krebs bicarbonate buffer containing bovine serum albumin (2 mg/ml) was used to prepare various dilutions of the pancreatic extract, which were then assayed using antiinsulin serum and [1/25]-labeled insulin. Insulin content was expressed as μg/g wet weight of pancreas by comparing diluted samples with standard solutions containing pork crystalline insulin (Lilly). Additionally, the pancreas from two rats in each group (i.e., control and streptozotocin-treated) was fixed with Bouin’s fluid and embedded in paraffin. The number of β-cells was then determined using an indirect immunoperoxidase method.

Chronic Cardiovascular Measurements in Awake Rats

On Weeks 2, 3, 4, 5, and 7 following pretreatment with streptozotocin (or its vehicle), systolic pressure and heart rate were recorded. Instead of our usual tail-cuff method, a photoelectric sensor (IITC Inc, Landing, New Jersey) was used, which allows indirect measurement in awake rats without preheating. After confinement in the rat holder for 30 minutes at a room temperature of 27°C, tail pulsations registered with this sensor were large enough for accurate estimation of systolic pressure in most rats. During validation of this method, tail-cuff systolic pressures in eight rats averaged 128 ± 5 mm Hg compared to an average of 130 ± 5 mm Hg for femoral systolic pressure recorded simultaneously from indwelling catheters. By recording arterial pulsations and cuff pressure on separate channels of the recorder, systolic pressure was determined as the level at which pulsations reappeared during gradual deflation of the cuff. Each measurement was obtained by averaging five individual readings. Heart rate was calculated by multiplying arterial pulsations recorded for 5 seconds by 12.

Direct Recording of Responses to Hypothalamic Stimulation in Awake Rats

At 8 weeks after pretreatment, each rat was anesthetized with intraperitoneal (i.p.) sodium pentobarbital (40 mg/kg) and prepared for hypothalamic stimulation. A concentric stainless steel electrode 0.5 mm in diameter (NE-100, custom-made by Rhodes Medical Instruments, Woodland Hills, California) was placed in the ventromedial hypothalamus at stereotaxic coordinates anteroposterior 6.0, lateral 1.0, and dorsoventral −3.7, and fixed to the skull with stainless-steel screws and dental cement. For recording blood pressure, an indwelling catheter was inserted into the right femoral artery with its outer end passed subcutaneously to emerge at the nape of the neck. One day later, each rat was kept in a round open-topped cage, awake but partly restrained by a harness-and-swivel arrangement; a harness wrapped around the rat’s chest was attached by a steel spring to a slip-ring swivel (Airflyte Electronics, Bayonne, New Jersey) placed above the cage. Wires inserted in the steel spring were then connected to the hypothalamic electrode on one end, and to a square-wave stimulator (Grass S-88 with PSIU6 constant current unit) on the other end. Tygon tubing inside the spring was used to connect the indwelling femoral catheter to a pressure transducer (Statham P23Gb) located beside the cannula swivel. A correction factor (obtained by dividing height of the fluid column by the ratio between the densities of mercury and water) was added to the transducer calibration to compensate for hydrostatic pressure resulting from the difference in height between the transducer and the rat. Aside from pulsatile and mean femoral pressures, heart rates were recorded simultaneously by triggering a biotachometer with the phasic pressure signal from the transducer. Hypothalamic stimulation was graded by pulsing 10-second trains of biphasic currents (100 cps frequency, 1 msec pulse duration) starting at 20 μA and increasing by 10 μA increments until 140 μA.

Sympathetic and Pressor Responsiveness in Anesthetized Rats

Soon after pressor responses to hypothalamic stimulation had been recorded while the rat was awake, it was anesthetized with urethane (80 mg/100 g i.p.), and additional catheters were inserted into the left femoral artery for blood sampling and the left femoral vein for drug injections. Pulsatile femoral pressure and sympathetic nerve activity were then recorded continuously to determine responsiveness not only to graded hypothalamic stimulation but also to intravenously injected adrenergic drugs. Intensity of hypothalamic stimulation was graded by using 10-second trains of biphasic currents (100 μA) of different intensities. Drug injections consisted of tyramine (20 and 40 μg/100 g), norepinephrine (50, 100, and 200 ng/100 g), and vasopressin (2.5 and 5 mU/100 g).

For recording sympathetic nerve activity, the abdominal plexus was exposed, and the inferior nerve
bundle (emerging from the celiac ganglion and accompanying the superior mesenteric artery) was placed over a bipolar stainless steel electrode (uninsulated tips 1 mm apart). Nerves and electrode tips were immersed in mineral oil to prevent tissue drying. To reduce noise during recording, spontaneous respiratory movements were abolished by paralyzing skeletal muscles with decamethonium bromide (Syncurine, 0.2 mg/100 g i.v.) and connecting the rat to an artificial respirator. Spike potentials were amplified (Grass P15AC amplifier), monitored on a storage oscilloscope, and recorded continuously on magnetic tape. Tapes were later played back into an amplitude analyzer (F. Haer and Co., Brunswick, Maine) to delete background noise and convert individual spikes into uniform pulses. The low-level control of the window discriminator was routinely set to filter background noise persisting after crushing the nerve. The number of individual pulses per second was counted with a rate analyzer whose output was recorded as a histogram on an ink-writing recorder converted to digital form using a computer interface, and printed by a programmed calculator. 21

**Determinations of Plasma Glucose, Insulin, and Intravenous Glucose Tolerance Tests**

To allow repeated blood sampling, 12 other rats were studied. As described above, six were injected with streptozotocin while the rest received equivalent amounts of the vehicle. Once a week for the next 3 weeks, the rats were fasted overnight, anesthetized with methoxyflurane, and their tails cut for bleeding. On the fifth week, after overnight fasting, an indwelling cannula was inserted into a femoral vein while the rats were anesthetized with sodium pentobarbital. A 4 mg/100 g i.p. The cannula was used during intravenous glucose tolerance tests to slowly inject a 50% solution of D-glucose (0.2 ml/100 g) and then to collect four 150 µl samples (at 0, 5, 20, and 60 minutes) of blood. Plasma concentrations of glucose were measured enzymatically, 13 and those of insulin by radioimmunoassay using a double antibody system. 22

**Histology, Drugs, and Statistics**

After each experiment involving hypothalamic stimulation, a 0.5 mA direct current was passed through the hypothalamic electrode for 10 seconds to produce a small lesion at its tip. Through a thoracotomy, a 15-gauge needle was inserted via the left ventricle into the ascending aorta, and 10% formalin was perfused into the brain as described by Wolf. 23 The whole brain was then removed, weighed, and stored in formalin (containing 1% potassium ferricyanide) until sectioning. Transverse sections (40 µ) stained with cresyl violet were compared with the atlas by Pellegrino et al. 17 to locate lesion sites. Kidneys removed from two control and five diabetic rats were sectioned, stained (with hematoxylin-eosin and para-aminosalicylic acid, or Jones stain 24), and examined under light microscopy.

Drugs used were tyramine hydrochloride, norepinephrine bitartrate (Levophed), and arginine vasopresine. All doses are expressed in terms of the respective salts.

Data expressed as averages ± SEM from control vs streptozotocin-treated groups were analyzed using two-tailed t tests for comparing means of independent samples; 25 differences at a 5% level (p < 0.05) were considered significant. Analysis of variance was used to examine for possible changes occurring at different weeks within each group (tables 1, 2, and 3); for F-ratios significant at 5% or less, Duncan's multiple range test 26 was applied to determine significance of differences between pairs of means.

**Results**

**Chronic Effects of Streptozotocin Pretreatment**

All but one of the rats treated with streptozotocin became diabetic. Following streptozotocin injection in 14 rats, urine samples tested with enzymatic test strips showed approximate glucose concentrations of 0.25% in nine rats during the first day, and of 0.5% in 13 rats during the second day. Rats with glycosuria subsequently failed to gain weight as rapidly as the controls, so that from Weeks 2 through 7 their body weights were considerably lower. Thus, F-ratios for changes in body weight during this period were significant in control, but not in streptozotocin-treated rats (table 1). The only rat that did not show glycosuria also gained weight normally; consequently, it was considered non-diabetic and excluded from the streptozotocin-treated group.

Systolic pressures were appreciably elevated in streptozotocin-treated rats by Week 2, and this slight elevation persisted through Week 7 (table 1). By contrast, accompanying changes in heart rate were transient; averages in streptozotocin-treated rats were initially lower but increased gradually to almost equal those in the controls by Week 7. Because these results suggested that cardiovascular status had already been altered by streptozotocin, additional experiments were done to determine whether responses to hypothalamic stimulation were also affected.

**Behavioral and Cardiovascular Effects of Hypothalamic Stimulation in Awake Rats**

When pulsatile arterial pressures were recorded directly from indwelling femoral catheters on Week 8, average systolic (134 ± 8 mm Hg in control and 132 ± 4 mm Hg in streptozotocin-treated rats; p > 0.5) and mean (110 ± 3 mm Hg in control and 103 ± 4 mm Hg in streptozotocin-treated rats; p > 0.2) pressures were almost equal, but diastolic pressures in streptozotocin-treated rats (99 ± 3 mm Hg) were significantly higher (p < 0.03) than those in the control group (88 ± 3 mm Hg). On the other hand, pulse...
any significant differences in magnitude either of tachycardia during each phase, or of pressor responses during Phases 2 and 3 (table 2). During Phases 3 and 4, however, pressor responses were smaller in the streptozotocin-treated than in the control group (fig. 1), thereby implying that while neither behavioral nor tachycardic responses had been affected, pressor responsiveness was selectively diminished.

Cardiovascular and Sympathetic Nerve Effects of Hypothalamic Stimulation in Anesthetized Rats

To allow recording of sympathetic nerve activity during hypothalamic stimulation, all rats were anesthetized with urethane and the splanchnic nerves prepared as described above. Frequency of sympathetic nerve firing (spikes/sec) was initially almost the same (p > 0.5), averaging 17.6 ± 2.4 in the streptozotocin-treated group and 16.7 ± 3.1 in the control group. Baselines for femoral blood pressure were slightly lower than those recorded while the same rats were awake, but still showed a similar relationship between groups. Systolic pressures did not differ significantly (i.e., 121 ± 6 mm Hg in control and 128 ± 4 mm Hg in streptozotocin-treated rats; p > 0.2), but mean and diastolic pressures in streptozotocin-treated rats (102 ± 4 and 88 ± 5 mm Hg respectively) were higher (p < 0.05) than those for the control group (87 ± 5 and

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**Table 1. Chronic Effects of Streptozotocin Pretreatment**

<table>
<thead>
<tr>
<th>Variable measured</th>
<th>Rat group</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>7</th>
<th>F ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>Control</td>
<td>249 ± 4</td>
<td>262 ± 5</td>
<td>275 ± 8</td>
<td>294 ± 7</td>
<td>316 ± 11</td>
<td>12.11</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>186 ± 11*</td>
<td>201 ± 12*</td>
<td>199 ± 14*</td>
<td>192 ± 15*</td>
<td>195 ± 20*</td>
<td>ns</td>
</tr>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td>Control</td>
<td>113 ± 3</td>
<td>114 ± 5</td>
<td>127 ± 4</td>
<td>125 ± 2</td>
<td>124 ± 2</td>
<td>3.30</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>136 ± 4*</td>
<td>141 ± 7*</td>
<td>142 ± 3*</td>
<td>145 ± 3*</td>
<td>149 ± 3*</td>
<td>ns</td>
</tr>
<tr>
<td>Heart rate (/min)</td>
<td>Control</td>
<td>—</td>
<td>368 ± 14</td>
<td>346 ± 14</td>
<td>358 ± 11</td>
<td>340 ± 9</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>—</td>
<td>345 ± 9</td>
<td>313 ± 5*</td>
<td>332 ± 12</td>
<td>341 ± 7</td>
<td>2.77</td>
</tr>
</tbody>
</table>

Data are expressed as averages ± SEM obtained from eight control and 13 streptozotocin-treated rats. In analyzing data within each group, with f1 = 3 and f2 = 32-63, F-ratios equal to or greater than 4.46 are significant at 1%. *p < 0.05 as compared with corresponding averages of the control group.

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**Table 2. Stimulus Thresholds and Cardiovascular Responses during Behavioral Phases Elicited by Ventromedial Hypothalamic Stimulation in Awake Rats**

<table>
<thead>
<tr>
<th>Behavioral phase</th>
<th>Current strength (µA)</th>
<th>Pressor response (mm Hg)</th>
<th>Tachycardia (/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>I</td>
<td>30 ± 3</td>
<td>30 ± 3</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>63 ± 8</td>
<td>64 ± 5</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>III</td>
<td>82 ± 11</td>
<td>80 ± 6</td>
<td>19 ± 5</td>
</tr>
<tr>
<td>IV</td>
<td>93 ± 12</td>
<td>100 ± 6</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>F-ratio</td>
<td>9.03</td>
<td>40.43</td>
<td>15.31</td>
</tr>
</tbody>
</table>

Data presented as averages ± SEM. Pressor responses (e.g., increases in mean arterial pressure) and tachycardia expressed as changes from baselines given in the text. For data within each group, with f1 = 3 and f2 = 20-32 F-ratios equal to or greater than 4.46 are significant at 5% while those equal to or greater than 4.94 are significant at 1%. *p < 0.05 as compared with corresponding averages for the control group.
71 ± 5 mm Hg respectively). Similarly, heart rates remained slower ($p < 0.01$) in streptozotocin-treated (348 ± 17 /min) than in control rats (409 ± 10 /min).

Electrical stimulation of the ventromedial hypothalamus increased not only mean blood pressure but also the rate of sympathetic nerve firing. For each 10-second period of stimulation, neural firing accelerated immediately to attain maximal elevation during the first 5 seconds, after which it subsided slightly but still stayed well above baseline level (fig. 2). Mean blood pressure began to rise soon after neural firing was increased, and the magnitude of both effects appeared directly related to current strength used for hypothalamic stimulation. These effects were smaller in streptozotocin-treated than in control rats, and for 100 and 150 μA currents, differences between rat groups were

![Figure 1. Cardiovascular responses to electrical stimulation of the ventromedial hypothalamus in awake rats. Upper two tracings are of phasic and mean femoral pressure (mm Hg) respectively, and the lowest tracing is of heart rate (/min). The first two panels (A) are from a control rat, and last two panels (B) are from one treated with streptozotocin. Arrows indicate start of 10-second periods of hypothalamic stimulation, and accompanying roman numerals represent the behavioral phase elicited.](image)

**Table 3. Sympathetic and Cardiovascular Responses to Ventromedial Hypothalamic Stimulation in Urethane-Anesthetized Rats**

<table>
<thead>
<tr>
<th>Current strength ($μA$)</th>
<th>Neural firing (spikes/sec)</th>
<th>Pressor response (mm Hg)</th>
<th>HR response (/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>50</td>
<td>9.5 ± 6</td>
<td>1.3 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>100</td>
<td>78.8 ± 7</td>
<td>29.6 ± 7*</td>
<td>26 ± 5</td>
</tr>
<tr>
<td>150</td>
<td>97.7 ± 11</td>
<td>53.1 ± 8*</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>200</td>
<td>107.6 ± 8</td>
<td>84.8 ± 12</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>F-ratio</td>
<td>27.99</td>
<td>20.76</td>
<td>20.13</td>
</tr>
</tbody>
</table>

*Data obtained from the same rats and presented as in table 2. All values represent average changes from baselines given in the text.*

*$p < 0.05$ as compared with corresponding averages for the control group.
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Figure 2. Pressor and neural effects of hypothalamic stimulation after induction of urethane anesthesia. Tracings from top to bottom are of: phasic femoral pressure (mm Hg), histogram showing frequency of sympathetic nerve firing (spikes/sec), and original analog signal of sympathetic nerve activity. Panel A is from a control rat and B is from one treated with streptozotocin. Small arrows indicate where the histogram went off scale because increases in firing frequency were too high. Large arrows indicate onset of 10-second periods of hypothalamic stimulation with numbers signifying current strengths (μA) used for stimulation.

significant (table 3). Instead of the tachycardia that was elicited from the same rats when they were awake, hypothalamic stimulation during urethane anesthesia caused bradycardia almost equally in both groups.

Because it seemed possible that diminished pressor responsiveness to hypothalamic stimulation may in part be due to peripheral inhibition of cardiovascular reactivity by streptozotocin, responses to intravenously injected pressor agents were also measured. Graded doses of norepinephrine, tyramine, or vasopressin produced dose-related increases in blood pressure accompanied by irregular changes in heart rate. There were no significant differences in the magnitude of pressor responses to norepinephrine or tyramine, but reflex bradycardia elicited by norepinephrine was consistently more pronounced in streptozotocin-treated rats (table 4). By contrast, pressor responses to vasopressin were significantly smaller in streptozotocin-treated than untreated rats, while the attendant bradycardia occurred almost equally in both groups. Hence, these results indicate that, whereas pressor responses to hypothalamic stimulation and to vasopressin were inhibited selectively in streptozotocin diabetic rats, those to norepinephrine and tyramine were unaffected.

Hyperglycemia, Hypoinsulinemia, and Abnormal Glucose Tolerance Following Streptozotocin Pretreatment

Plasma glucose (mg/100 ml) determined in blood samples collected during the experiments above was consistently higher than normal not only in the diabetic (557 ± 31) but also in the control rats (259 ± 16; \( p < 0.001 \)). Since this implied that hyperglycemia was at

<table>
<thead>
<tr>
<th>Pressor agent</th>
<th>Dose (100 g)</th>
<th>Pressor response (mm Hg)</th>
<th>HR response (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>50</td>
<td>9 ± 1</td>
<td>-2 ± 3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>15 ± 1</td>
<td>-4 ± 4</td>
</tr>
<tr>
<td>Tyramine</td>
<td>20</td>
<td>13 ± 1</td>
<td>19 ± 7</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>21 ± 1</td>
<td>19 ± 7</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>2.5</td>
<td>24 ± 4</td>
<td>-13 ± 3</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>36 ± 3</td>
<td>-20 ± 2</td>
</tr>
</tbody>
</table>

\( *p < 0.05 \) as compared with corresponding averages for the control group.
least in part being caused by mechanisms unrelated to streptozotocin pretreatment, plasma glucose and insulin were measured weekly in other rats that had not been subjected to hypothalamic stimulation. During the first 3 weeks, diabetic rats always had higher plasma glucose and lower plasma insulin concentrations than the controls (table 5). Glucose tolerance tests done on the fifth week by measuring plasma glucose levels following intravenous injection of hypertonic D-glucose solution showed that the ensuing hyperglycemia was considerably lower in control than in diabetic rats. Conversely, plasma insulin levels became markedly elevated in control but not in diabetic rats (table 6). Thus, analysis of variance on the data for insulin showed that, whereas the F-ratio for control rats of 43.18 was highly significant at 1%, that for diabetic rats of 0.072 was not.

Postmortem Verification of Hypothalamic Electrode Sites and Diabetic State

Wet weight of brains removed from streptozotocin-treated rats (1.74 ± 0.02 g) was significantly lower (p < 0.001) than that of the controls (1.99 ± 0.03 g) but when expressed in terms of per kilogram of body weight, then diabetic rats had relatively larger brains (8.51 ± 0.48 g/kg) than the controls (5.74 ± 0.16 g/kg). This means that although brain weights had been reduced, the magnitude of reduction was disproporionate lower than that for the rest of the body. Yet, despite the resulting difference in brain size, sites of electrode placement were identical. Electrode tips were invariably located in the ventromedial hypothalamus adjacent to the fornix, median forebrain bundle, and anterior and lateral hypothalamic areas in all rats (fig. 3). Average stereotaxic coordinates (mm) determined by comparing transverse brain sections with the atlas were: anteroposterior 6.2 ± 0.08, lateral 1.12 ± 0.08, and dorsolateral -3.42 ± 0.19 for the control group, with corresponding values of 6.28 ± 0.08, 1.27 ± 0.09, and -3.41 ± 0.17 respectively for the

TABLE 5. Plasma Glucose and Insulin Levels in Anesthetized Rats

<table>
<thead>
<tr>
<th>Variable measured</th>
<th>Rat group</th>
<th>Weeks</th>
<th></th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/100 ml)</td>
<td>Control</td>
<td>67±4</td>
<td>89±4</td>
<td>64±7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>291±29*</td>
<td>361±30*</td>
<td>425±32*</td>
<td></td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>Control</td>
<td>1.49±0.48</td>
<td>1.85±0.88</td>
<td>0.81±0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.21±0.14*</td>
<td>0.18±0.11</td>
<td>0.06±0.03*</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as averages ± SEM from six control and six diabetic rats.

\*p < 0.05 as compared with corresponding averages for the control group.

TABLE 6. Intravenous Glucose Tolerance Tests in Anesthetized Rats

<table>
<thead>
<tr>
<th>Variable measured</th>
<th>Rat groups</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/100 ml)</td>
<td>Control</td>
<td>100±9</td>
<td>526±59</td>
<td>246±12</td>
<td>131±16</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>407±12*</td>
<td>974±74*</td>
<td>552±48*</td>
<td>442±39*</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>Control</td>
<td>3.40±1.9</td>
<td>37.21±2.1</td>
<td>22.07±2.7</td>
<td>8.13±2.5</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.14±0.1</td>
<td>0.17±0.1*</td>
<td>0.21±0.2*</td>
<td>0.17±0.1</td>
</tr>
</tbody>
</table>

Averages ± SEM from five control and three diabetic rats.

\*p < 0.05 as compared with corresponding averages from control.
streptozotocin-treated group. None of the differences between groups was significant.

All the pancreatic indices that were measured postmortem confirmed induction of diabetes by our streptozotocin regimen (table 7). Pancreatic weight, insulin content, and number of β-cells were consistently lower, in streptozotocin-treated than in control rats. Kidneys from streptozotocin-treated rats generally tended to be larger than those from the controls, but no apparent differences were detected upon microscopic examination.

### Discussion

Together with mild hypertension, streptozotocin diabetic rats had the following cardiovascular characteristics: narrowed pulse pressure, slowed heart rate with increased reflex bradycardia in response to injected norepinephrine, and reduced responsiveness to hypothalamic stimulation. The narrowed pulse pressure would make endpoint detection during tail-cuff measurements difficult and may, therefore, account for previous failures to demonstrate the blood pressure elevation. In the diabetic rats that we studied, tail-cuff systolic pressures were consistently high from Wees 2 through 7, but when phasic arterial pressures were recorded later from indwelling catheters, only mean and diastolic pressures were elevated, while corresponding systolic pressures became almost the same as those of the control group. This apparent conversion of systolic to diastolic hypertension must be due, at least in part, to differences in experimental conditions during blood pressure recording.

By Week 7, all our rats had grown accustomed to repeated tail-cuff measurements so that any stresses thereby induced would have been minimal. On the other hand, subsequent recording of phasic pressures while the rats were attached to a chest harness was a novel and highly stressful procedure. Thus, although baseline heart rates did not differ during Week 7 (see table 1), heart rates were elevated, while corresponding systolic pressures were consistently high from Weeks 2 through 7, though large, were still essentially normal. Notwithstanding these arguments, renal dysfunction cannot be completely ignored because type I (juvenile onset) diabetes, which resembles the streptozotocin diabetic model, is usually associated with sodium-mediated, low-renin hypertension. An alternative explanation might be that hypovolemia caused by polyuria sufficed to stimulate release of endogenous vasopressin. Increased amounts of circulating vasopressin could cause receptor saturation and thereby explain why pressor responses to injected vasopressin were reduced (table 4). Even though plasma vasopressin was not measured in the present studies, others have shown that it becomes elevated not only in diabetic patients but also in various forms of experimental hypertension in rats.

Bradycardia occurs when diabetes is induced in normotensive rats with streptozotocin or in spontaneously hypertensive ones with alloxan. Although underlying mechanisms are obscure, the bradycardia seems unique only for drug-induced diabetes in rats since heart rates usually remain normal in diabetic patients.

As baroreceptor buffer mechanisms reset to operate at higher pressure levels during development of hypertension, reflex bradycardia elicited by pressor drugs should diminish in diabetic rats that become hypertensive. But since reflex bradycardia elicited by injected norepinephrine actually became more pronounced in diabetic than in control rats (table 4), the logical implication is that baroreceptor resetting must somehow be suppressed during streptozotocin-induced diabetes. In line with this, some diabetic patients have defective circulatory reflexes, and conversely, orally administered glucose inhibits baroreceptor function in patients with various cardiovascular disorders. Because the hypothalamus normally inhibits reflex bradycardia, it could be that baroreceptor resetting was prevented by concomitant hypothalamic depression.

Where responsiveness to hypothalamic stimulation was being depressed is more likely to be central rather than peripheral for two reasons. First, because unlike
alloxan-diabetic rats whose cardiovascular sensitivity is reduced.\(^4\)\(^5\) pressor responses to injected norepinephrine and tyramine were unchanged (table 4). And second, because acceleration in sympathetic nerve firing during hypothalamic stimulation was also reduced, which means that the site of inhibition was located above the site where nerve activity was being recorded. Clearly then, aside from the hypothalamus itself, possible sites of depression would include any part of the descending neural pathway between the hypothalamus and the peripheral nerves.

As Hashimoto\(^3\) observed in alloxan diabetic rats, we also found brain weight significantly reduced in streptozotocin diabetic ones. Nonetheless, since electrode placements in the hypothalamus were almost identical, differences between control and diabetic rats in hypothalamic responsiveness could not have been due to stimulation of different hypothalamic areas. Exactly how changes in brain weight or size might affect responsiveness to hypothalamic stimulation is debatable. Teardrop-shaped areas activated by currents passed through concentric electrodes\(^46\) would, in our experiments, have a maximum but constant diameter of 1.5 mm (calculated from size of the two poles and the distance between them). If all other variables are kept constant, and if magnitude of pressor responses is always proportional to number of neural elements activated, then identical electrical stimuli should activate more elements and elicit larger pressor effects after brain size has been reduced. Alternatively, if the decrease in brain weight was due to loss of myelin (which insulates neural elements from each other),\(^47\) then the ensuing reduction in insulation would increase current spread during stimulation and pressor effects would similarly be enhanced. Demyelination may have greater effects here than elsewhere since the hypothalamus normally contains fewer myelinated fibers than other brain areas.\(^48\) Yet neither explanation fits because instead of being enhanced, pressor responses to hypothalamic stimulation were actually inhibited.

Inhibition of pressor and sympathetic responses to hypothalamic stimulation has been noted in our laboratory twice previously, but under very different experimental conditions. During attempts to induce hypertension by chronic stimulation of the anterior or posterior hypothalamus, responsiveness diminished progressively because of neural damage resulting from prolonged passage of unidirectional electrical currents.\(^27\) Similarly, when hypertension developed following chronic exposure to shaker stress, responsiveness was also reduced probably because of adaptation within the brain.\(^30\) By contrast, brains from streptozotocin diabetic rats show no gross abnormalities,\(^49\) and hypothalamic depression during experimental diabetes can hardly be considered analogous to inhibition caused by physiologic adaptation. Selective reduction of pressor but not of behavioral or tachycardiac responses, as was seen here, implies that only those hypothalamic neurons concerned with blood pressure regulation were depressed during streptozotocin-induced diabetes.

Hypothalamic sensitivity could be reduced either by streptozotocin itself, or by the ensuing diabetic state. Of the many changes taking place during diabetes, those that could conceivably depress hypothalamic function include hypoinsulinemia, increases in brain glucose, and brain dehydration. Although brain insulin concentrations stay remarkably constant in streptozotocin diabetic rats,\(^50\) hypoinsulinemia would lower plasma catecholamines and sympathetic nerve activity.\(^51\) Increases in brain glucose could likewise be inhibitory since glucose inhibits electrical activity preferentially in certain hypothalamic neurons.\(^52\) Or perhaps sensitivity to electrical stimulation is reduced by brain dehydration inasmuch as reductions in cerebral metabolism resembling those produced by alloxan-diabetes also occur following severe dehydration.\(^53\)

In summary, our results indicate that, while streptozotocin diabetic rats are indeed more prone to become hypertensive, other mechanisms are concurrently activated to keep blood pressure from rising further. Hypothalamic responsiveness is reduced, and probably because of this, baroreceptor buffering increases. Whether hypothalamic depression occurs in diabetes as a protective compensatory mechanism or a harmful side-effect is a moot point.

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