Insulin Reverses Hypertension and Hypothalamic Depression in Streptozotocin Diabetic Rats

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SUMMARY Daily subcutaneous injections of lente insulin reduced the hypertension and bradycardia which developed consistently in streptozotocin diabetic rats. Insulin-treated rats also became less hyperglycemic, drank less water, and gained weight faster than untreated diabetic controls. Behavioral and tachycardiac effects elicited by electrical stimulation of the ventromedial hypothalamus while the rats were awake were similar, but attendant pressor responses were larger in those that had been treated with insulin. Under subsequent urethane anesthesia, pressor and sympathetic responses to hypothalamic stimulation, as well as pressor responses to tyramine and vasopressin, were augmented in insulin-treated rats. A generalized increase in cardiovascular reactivity caused by insulin seemed unlikely since pressor responses to norepinephrine were unaltered. Enhanced hypothalamic responsiveness was considered due to improvement of diabetic encephalopathy rather than to direct CNS stimulation by insulin because the injected insulin had mostly dissipated by the time pressor responses were recorded. By showing that insulin treatment produced changes opposite to those occurring during induction of diabetes our results suggest that insulin can alleviate cardiovascular and hypothalamic dysfunction in streptozotocin-induced diabetes. (Hypertension 5: 34-40, 1983)

KEY WORDS • cardiovascular reactivity • hypertension • insulin • streptozotocin-induced diabetes • vasopressin • ventromedial hypothalamus

INSULIN has long been used for treatment of diabetes mellitus yet its effects on cardiovascular or central nervous system (CNS) complications remain doubtful. Despite frequent descriptions of "diabetic encephalopathy," the existence of CNS lesions specific for diabetes mellitus has recently been questioned. Insulin treatment improves slowing of peripheral nerve conduction in diabetic patients, and prevents impairment of nerve conduction and axonal protein transport in streptozotocin diabetic rats; however, the neuropathy in alloxan diabetic rats is unaffected. The manner in which cardiovascular complications are altered also appears contradictory. Although insulin may be considered beneficial because it prevents cardiovascular changes, or restores vascular prostacyclin to normal in streptozotocin diabetic rats, it may also aggravate atherosclerotic complications by stimulating lipid synthesis and smooth muscle proliferation in arterial walls.

Because rats with streptozotocin-induced diabetes develop mild hypertension and bradycardia together with reduced pressor responsiveness to hypothalamic stimulation, we proposed that with increased susceptibility to hypertension, concurrent hypothalamic depression prevents further elevation of blood pressure. The present studies were done to determine how the cardiovascular, neural, and hypothalamic changes occurring in streptozotocin-induced diabetes would be affected by insulin treatment.

Methods

Thirty-three male Sprague-Dawley rats, weighing 157 ± 1 g and about 6 weeks old, were purchased from SASCO Inc. (Omaha, Nebraska).

Induction of Experimental Diabetes

Two weeks after they arrived in our laboratory all rats were anesthetized with methoxyflurane (Metofane by inhalation) for injection into the tail vein either of streptozotocin (50 mg/kg dissolved in pH 4.5 citric buffer) in 23 rats, or of equivalent amounts of the solvent in 10 others. Those injected with streptozotocin were given hypertonic glucose (3 ml of a 10%...
solution) by gavage after 4 and 10 hours to avoid fatalities from severe hypoglycemia. All the rats survived and were thereafter housed in individual cages kept in an air-conditioned room with unrestricted access to food and water. Body weight, systolic pressure, and heart rate were then measured once a week for 4 weeks.

**Insulin Treatment in Diabetic Rats**

After 4 weeks, nondiabetic controls were discarded while diabetic rats were selectively assigned into two groups to equalize group averages for body weight, systolic pressure, heart rate, and blood glucose. From then on, 13 rats were given daily subcutaneous injections of lente insulin (Squibb) while the other 10 received similar injections of isotonic (0.9%) sodium chloride solution. Daily injections were always given between 3:00 and 5:00 p.m. in a volume of 0.5-0.7 ml, and urine or blood samples, when required, were collected before the next injections were given. Initial doses of insulin (daily per rat) started with 2 U and, depending on extent of glycosuria or hyperglycemia, were adjusted up to 10 U; final injections were given the day before hypothalamic electrodes and aortic catheters were implanted. During this period, body weight and fluid intake were measured, and urine and blood samples were collected once a week. Urinary glucose concentrations were estimated using enzymatic test strips (Tes-Tape, Lilly). Blood samples (100 μl of mixed arterial and venous blood) were collected by cutting the tail while the rats were anesthetized with methoxyflurane, and blood glucose content was measured either enzymatically or by using an Eyetone reflectance colorimeter to determine color changes produced on Dextrostix reagent strips (Ames Company, Elkhart, Indiana).

**Chronic Cardiovascular Measurements in Awake Rats**

Systolic pressure and heart rate were recorded weekly with a tail-cuff method using a photoelectric sensor (IITC Inc, Landing, New Jersey) that allows indirect measurement without preheating in awake rats. 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 counting arterial pulsations recorded for 5 seconds with the cuff deflated and multiplying by 12.

**Direct Recording of Responses to Hypothalamic Stimulation in Awake Diabetic Rats**

Upon completing 4 weeks of daily insulin (or saline) injections, each rat was anesthetized with sodium pentobarbital (40 mg/kg IP) and prepared for hypothalamic stimulation. A 0.5 mm diameter concentric stainless steel electrode (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. For recording blood pressure, the outer end of an indwelling catheter inserted into the right femoral artery was passed subcutaneously to emerge at the nape of the neck. The next day, each rat was kept in a round open-topped cage, awake but partly restrained by a harness-and-swivel arrangement; a chest harness from the rat was attached through a steel spring to a slip-ring swivel (Airflyte Electronics, Bayonne, New Jersey) placed above the cage. Soon after pressor responses to hypothalamic stimulation had been recorded while the rat was awake, the rat was anesthetized with urethane (80 mg/100 g IP) and an additional catheter was inserted into 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 drugs. Intensity of hypothalamic stimulation was graded by using 10-second trains of 50–200 μA biphasic currents.

For recording sympathetic nerve activity, the inferior cervical ganglion was started over a bipolar stainless steel electrode ( uninsulated tips 1 mm apart). Nerves and electrode tips were immersed in mineral oil to prevent drying tissue. Spontaneous respiratory movements were abolished by paralyzing skeletal muscles with decamethonium bromide (Syncurine, 0.2 mg/100 g IV) and connecting the rat to an artificial respirator. Spike potentials were amplified (Grass P15AC amplifier) and recorded continuously on magnetic tapes which were later played back into an amplitude analyzer (F. Haer and Company, Brunswick, Maine) to convert individual spikes into uniform pulses. Number of individual pulses per second was counted with a rate analyzer and 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.

**Brain Histology, Drugs, and Statistics**

After each experiment, 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. 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. to locate lesion sites.

Drugs used were tyramine hydrochloride, norepinephrine bitartrate (Levophed; Sterling Drug Inc) and arginine vasopressin (Parke-Davis). Doses, in terms of weight of the respective salt per 100 g body weight, were: 20 and 40 μg for tyramine, 100 and 200 ng for norepinephrine, and 2.5 and 5.0 μU for vasopressin.

Data expressed as averages ± SEM from control versus insulin-treated groups were analyzed using two-tailed t tests for comparing means of independent samples, and differences at a 5% level were considered significant. An analysis of variance was used to examine all other comparisons and for F ratios significant at 5% or less, a multiple range test was applied to determine significance of differences between pairs of means.

Results

Induction of Diabetes and Hypertension

With Streptozotocin

During the first 4 weeks following streptozotocin injection, all rats showed a prominent glycosuria (with urinary glucose concentrations ranging from 0.5% to 2%) and toward the end, blood glucose levels were consistently high (average 311 ± 17 mg/100 ml). Body weight, instead of continuing to increase as it did in the nondiabetic group, declined with F ratios for weekly changes being significant at 5% (table 1) so that average body weight at the end of 4 weeks was lower (p < 0.05) than at the beginning. Systolic pressures were high even on the first week and they stayed at about 140 mm Hg thereafter (table 1). Of 23 rats, those that had systolic pressures exceeding 140 mm Hg numbered 6 on Week 1, 12 on Week 2, and 11 on Weeks 3 and 4. By contrast, nondiabetic rats had lower systolic pressures which consistently averaged about 120 mm Hg. Heart rates (beats per minute, bpm) during the same period fell progressively in both groups with averages during the last 2 weeks being significantly higher in control than in diabetic rats (table 1).

Changes Caused by Insulin Treatment in Diabetic Rats

Rats treated with insulin resumed gaining weight while those that were untreated continued losing it. At the third and fourth weeks, the insulin-treated diabetic rats actually became heavier than the untreated controls (table 2). Insulin-treated rats drank much less water daily. Blood glucose levels remained consistently higher than normal in both groups, but were significantly lower among insulin-treated than among untreated diabetics during the last 3 weeks. Four of the insulin-treated rats died: two during the night from unknown causes and two others following severe convulsions possibly caused by hypoglycemia. Both of the cardiovascular effects produced during induction of diabetes diminished with insulin treatment: systolic pressures became lower while heart rates became faster (table 2).

Responses to Hypothalamic Stimulation in Awake Diabetic Rats

Insulin-treated rats showed wider pulse pressures and higher heart rates than the untreated controls. Average heart rates were 310 ± 19 in untreated rats and 385 ± 11 (p < 0.005) in insulin-treated ones; corresponding values for pulse pressure (mm Hg) were 32 ± 4 and 61 ± 4 (p < 0.001) respectively. There were no significant differences in basal pressures. Average pressures in insulin-treated rats were: 138 ± 5 systolic, 97 ± 5 mean, and 77 ± 6 diastolic; corresponding averages in untreated controls were 125 ± 5, 103 ± 3, and 92 ± 4 respectively. As the strength of stimulating currents applied to the ventromedial hypothalamus was increased, behavioral effects became progressively more pronounced and the following phases could be identified: in Phase I, the rats sniffed, hyperventilated, or looked around without moving their bodies; in Phase II, they moved about the cage and occasionally picked up food; in Phase III, they stood up on both hindlegs with forelegs resting on the side of the cage; and in Phase IV, they attempted to climb out. Accompanying increases in blood pressure and heart rate also became proportionally larger as stimulus strength was increased. In both rat groups, current thresholds for eliciting any given behavioral phase were almost identical and there were no signifi-
TABLE 2. Effects of Daily Insulin Treatment in Streptozotocin Diabetic Rats

<table>
<thead>
<tr>
<th>Variable measured</th>
<th>Rat group</th>
<th>Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Body weight</td>
<td>control</td>
<td>249 ± 8</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>249 ± 11</td>
</tr>
<tr>
<td>Fluid intake</td>
<td>control</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>265 ± 15*</td>
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<tr>
<td>Blood glucose</td>
<td>control</td>
<td>308 ± 27</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>313 ± 22</td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>control</td>
<td>142 ± 5</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>142 ± 5</td>
</tr>
<tr>
<td>Heart rate</td>
<td>control</td>
<td>311 ± 10</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>311 ± 8</td>
</tr>
</tbody>
</table>

*Significantly different from corresponding value for the control group; data expressed as averages ± SEM from 10 control and 9-13 insulin-treated diabetic rats.

Cardiovascular and Sympathetic Nerve Effects of Hypothalamic Stimulation in Anesthetized Diabetic Rats

There was no significant difference in baselines for frequency of sympathetic nerve firing (spikes/sec) which averaged 18.5 ± 1 in untreated and 16.5 ± 1 (p > 0.1) in insulin-treated rats. Cardiovascular differences were similar to those obtained when these same rats were awake: pulse pressures were wider, and heart rates were higher in insulin-treated than in untreated rats. Pulse pressures averaged 35 ± 3 in the untreated and 64 ± 4 (p < 0.001) in the insulin-treated group while corresponding heart rates were 316 ± 16 and 358 ± 9 (p < 0.05) respectively. Baseline systolic (127 ± 6 in untreated and 136 ± 11 in insulin-treated rats; p > 0.4) and mean (102 ± 4 in untreated and 90 ± 8 in insulin-treated rats; p > 0.1) pressures did not differ, but diastolic pressures were significantly lower in insulin-treated rats (67 ± 6) than in untreated controls (89 ± 3; p < 0.01).

FIGURE 1. Cardiovascular responses to electrical stimulation of the ventromedial hypothalamus in awake streptozotocin diabetic rats. Upper two tracings of phasic and mean femoral pressure (mm Hg) respectively, and lowest tracing of heart rate (bpm). Panel A is from an untreated control and panel B from an insulin-treated rat. Arrows indicate start of 10-second periods of hypothalamic stimulation and accompanying Roman numbers represent the behavioral phase elicited.

TABLE 3. Stimulus Thresholds and Cardiovascular Responses during Different Behavioral Phases Elicited by Electrical Stimulation of the Ventromedial Hypothalamus in Awake Diabetic Rats

<table>
<thead>
<tr>
<th>Behavioral phase</th>
<th>Current strength (μA)</th>
<th>Pressor response (mm Hg)</th>
<th>Tachycardia (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>I</td>
<td>30 ± 0</td>
<td>33 ± 3</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>II</td>
<td>46 ± 4</td>
<td>44 ± 3</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>III</td>
<td>59 ± 7</td>
<td>60 ± 4</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>IV</td>
<td>69 ± 7</td>
<td>69 ± 4</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>F ratio</td>
<td>8.68</td>
<td>21.41</td>
<td>16.19</td>
</tr>
</tbody>
</table>

Data presented as averages ± SEM from 10 control and nine insulin-treated rats. Pressor responses (increases in mean pressure) and tachycardia expressed as changes from baselines given in the text. For averages within each group, with f1 = 3 and f2 = 22, F ratios of 3.05 or more are significant at 5% and of 4.82 or more at 1%.

*Indicates significant differences from corresponding averages of the control group.
Subsequent electrical stimulation of the ventromedial hypothalamus increased not only blood pressure but also the rate of sympathetic nerve firing. For each 10-second period of stimulation, neural firing accelerated immediately to attain peak levels within the first seconds after which it subsided slightly, but still stayed well above baseline level (fig. 2). Blood pressure began to rise soon after neural firing was increased, and magnitude of both effects was directly proportional to strengths of currents used for hypothalamic stimulation. Instead of the tachycardia that was elicited while the same rats were awake, hypothalamic stimulation during urethane anesthesia caused bradycardia almost equally in both groups. With 50 μA currents, magnitude of neural and pressor responses was the same, but for all other current strengths both responses were larger in insulin-treated than in control rats, and with 100 and 150 μA currents the differences between rat groups were significant (table 4).

Graded doses of norepinephrine, tyramine, or vasopressin produced dose-related increases in blood pressure accompanied by irregular changes in heart rate. There was no significant difference between rat groups in magnitude of pressor or bradycardiac responses to norepinephrine, or of heart rate changes produced by tyramine or vasopressin (table 5). Pressor responses elicited with either tyramine or vasopressin were, however, appreciably larger in insulin-treated than in control rats.

Verification of Hypothalamic Electrode Sites

Although insulin-treated diabetic rats were invariably heavier than the untreated controls, by the time terminal experiments were done the wet weight of brains removed did not vary greatly (average of 1.80 ± 0.01 g for the controls as compared with 1.82 ± 0.07 g for insulin-treated rats p > 0.5). Similarly, 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 6.25 ± 0.07, lateral 1.04 ± 0.06, and dorsoventral −3.37 ± 0.14 for the control group, with corresponding values of 6.31 ± 0.07, 0.95 ± 0.07, and −3.31 ± 0.14 respectively for the insulin-treated group. None of the differences between groups was significant.

Discussion

Daily treatment with insulin evidently reverses cardiovascular and hypothalamic dysfunction in streptozotocin diabetic rats. All rats developed hypertension and bradycardia during the first 4 weeks after streptozotocin was injected (table 1), and upon subsequent treatment with insulin both effects were reduced (table 2). Furthermore, electrical stimulation of the ventromedial hypothalamus elicited larger pressor and sympathetic nerve responses from insulin-treated rats than from untreated controls (table 4). Inasmuch as these changes are opposite to those produced during induction of diabetes, our results imply that insulin treatment restores normal cardiovascular and hypothalamic function.

Previous studies have varied widely in types of insulin used as well as dosage or routes of administration. Within 18 hours following single insulin injections in streptozotocin diabetic rats, plasma glucose begins to rise and when good diabetic control is attained many animals die from extreme hypoglycemia. During insulin treatment lasting for several months, Rasch obtained good control with daily doses (U/kg) of 7.8 ± 2.3 and poor control with doses of 1.6 ± 0.2.

As a compromise, we began with 2 U of lente insulin daily and then increased doses in each rat depending on changes in body weight, fluid intake, and blood sugar. From the second through the fourth week, the doses ranged from 4 to 10 U per rat per day. Whether normoglycemia was ever attained was undetermined because glucose levels were always elevated in blood samples collected just before the next insulin injection.
TABLE 4. Sympathetic and Cardiovascular Responses to Electrical Stimulation of the Ventromedial Hypothalamus in Urethane-anesthetized Diabetic Rats

<table>
<thead>
<tr>
<th>Current strength (µA)</th>
<th>Neural firing (spikes/sec)</th>
<th>Pressor response (mm Hg)</th>
<th>HR response (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>50</td>
<td>6.4 ± 2</td>
<td>12.4 ± 5</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>100</td>
<td>48.1 ± 11</td>
<td>81.9 ± 9*</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>150</td>
<td>75.7 ± 9</td>
<td>107.3 ± 11*</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>200</td>
<td>95.0 ± 9</td>
<td>113.5 ± 8</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>F ratio</td>
<td></td>
<td></td>
<td>36.72</td>
</tr>
</tbody>
</table>

Data obtained from the same rats and presented as in table 3. All values represent average ± SEM changes from baselines given in the text. *Indicates significant differences from corresponding averages of the control group.

(i.e., 24 hours after the last injection) even though averages were significantly lower in insulin-treated than in control rats.

Possibly related to the reversal of hypertension and bradycardia described here, insulin has been blamed for causing hypotension22 and tachycardia in diabetic patients,23 as well as tachycardia in alloxan diabetic rabbits.24 How hypotension is produced is unknown, but tachycardia has been attributed to decreased plasma volume.25

Other than stopping hypertension and bradycardia, the most prominent effect of insulin treatment was on pressor responsiveness to hypothalamic stimulation. In addition, although pressor responses to injected norepinephrine were unaffected, those to tyramine and vasopressin were larger in insulin-treated than in untreated diabetic rats (see tables 4 and 5). A generalized increase in peripheral cardiovascular reactivity caused by insulin does not seem responsible for the following reasons: augmented pressor responses to hypothalamic stimulation were always accompanied by greater increases in sympathetic nerve firing (table 4), pressor responses to norepinephrine were unaltered (table 5), and in isolated perfused rat tails, insulin attenuates vasoconstrictor responses to norepinephrine.26 Enhancement of hypothalamic pressor responses must have been due mainly to increased sympathetic nerve firing. And since plasma catecholamine concentrations are elevated by insulin,27 if norepinephrine in nerve endings increases similarly, then enhanced responsiveness to hypothalamic stimulation, as well as to tyramine, could also result from an increased release of endogenous norepinephrine.

A partial, but similar, restoration by insulin of vasoconstrictor responses was recently observed by Cavaliere and Taylor28 in Kyoto-Wistar rats with alloxan-induced diabetes. After pitting the spinal cord, they showed that responses to sympathetic outflow stimulation and to tyramine, which were depressed in untreated diabetic rats, were restored to normal in insulin-treated ones. Despite differences in methodology, their findings generally resemble ours except on responses to norepinephrine which they found greatly depressed in alloxan-diabetic rats whether treated with insulin or not.
Entirely different mechanisms may be changing pressor responsiveness to vasopressin. Plasma vasopressin becomes elevated in diabetic patients as well as hypertensive rats, and upon finding pressor responses to vasopressin reduced in streptozotocin diabetic rats we suggested that reduced responsiveness may be due to receptor saturation. An alternative mechanism based on impaired baroreceptor buffering has been proposed to explain enhancement of pressor responses to vasopressin after intracerebroventricular injection of angiotensin, or in salt-sensitive genetically hypertensive rats. Whatever the cause may be, our findings of larger pressor responses to vasopressin in insulin-treated than in untreated diabetic rats (table 5) supports the conclusion that cardiovascular dysfunction in diabetes can be effectively reversed by insulin.

Although insulin modifies electrical activity of certain hypothalamic neurones, and insulin receptors are widely distributed in the CNS, direct actions on the CNS seem improbable because insulin was last injected 36 hours before pressor responses were recorded. Perhaps pressor and sympathetic responsiveness to hypothalamic stimulation increased following chronic insulin treatment because of improvement of the diabetic encephalopathy. Even though streptozotocin-induced diabetes in rats does not completely resemble clinical diabetes in man, our findings imply that appropriate control of hyperglycemia with insulin could reduce the cardiovascular complications of diabetes mellitus.

Acknowledgment

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