Chronic Insulin Administration Elevates Blood Pressure in Rats

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Abstract To examine the relative contribution of dietary glucose and infused insulin on blood pressure, we administered a 4% glucose supplement (in drinking water) with and without insulin infusion (15.8 nmol [2.2 U]/d via osmotic minipump) to male Sprague-Dawley rats (n = 6). We also tested the effect of the sympatholytic agent clonidine on rats receiving glucose and insulin. Blood pressure and heart rate were recorded via a novel radio telemetry system. Experiments were performed using a crossover design with three animals receiving treatment and three receiving vehicle for 10 days. After a 10-day washout period, the groups were reversed, and the experiment was repeated. Blood samples for insulin and glucose were drawn throughout the study. Systolic and diastolic blood pressures increased (by 6.0 ± 1.2 and 2.2 ± 1.3 mm Hg, respectively) in the animals given glucose alone in association with an increase in plasma insulin. However, blood pressure increased more rapidly and to a greater extent, systolic by 8.6 ± 0.7 mm Hg and diastolic by 2.9 ± 1.1 mm Hg, during the insulin treatment that raised plasma insulin above the levels observed during glucose feeding alone. Heart rate increased equally during both treatments. The average change in blood pressure and average plasma insulin during the infusion were correlated (r = .72, P = .009). Blood pressure dropped during the week following discontinuation of the insulin infusion. On rechallenge with insulin and glucose, blood pressure again rose and then decreased after termination of the insulin and glucose administration. Clonidine prevented the rise in blood pressure and heart rate. We conclude that modest dietary carbohydrate supplementation induces chronic hyperinsulinemia and elevated blood pressure in the rat. The blood pressure response is exaggerated by exogenous insulin administration and prevented by sympathetic blockade. These data suggest that hyperinsulinemia can contribute to a sympathetically mediated rise in blood pressure in normal rats.

Key Words • glucose • hypertension, experimental • insulin • telemetry

Epidemiologic evidence suggests a provocative relationship between blood pressure and hyperinsulinemia.1–5 In white essential hypertensive subjects, the level of blood pressure has been shown to correlate with either fasting or postprandial plasma insulin levels. Moreover, the drop in plasma insulin that occurs with diet or exercise in obese subjects correlates with the drop in blood pressure resulting from these maneuvers. In addition, studies in prehypertensive human subjects suggest that hyperinsulinemia precedes the development of hypertension.7–10 Whether these associations between circulating insulin levels and blood pressure reflect a causal relationship is unknown, especially because blood pressure does not correlate with insulin in all ethnic groups.11

If insulin participates in blood pressure regulation, then administration of insulin should increase blood pressure. Acute administration of insulin increases blood norepinephrine levels and decreases urinary sodium levels.12,13 However, insulin administered acutely may cause vasodilation and not increase blood pressure.14 Data regarding the effect of chronic, exogenous hyperinsulinemia on blood pressure are controversial. Hall and colleagues15 were unable to increase blood pressure in dogs given high doses of insulin for 28 days under conditions of euglycemia. The same group could induce hypertension with chronic administration of insulin and dextrose in rats.16 To determine whether chronic hyperinsulinemia can elevate blood pressure, we administered insulin via osmotic minipumps to normal rats for 10 days and used an implanted transmitter device to determine blood pressure in freely moving animals. Previous studies may be less accurate because blood pressure measurements required tethering or restraint. Our results demonstrate that physiological elevations in circulating insulin, induced by either high glucose feeding or by exogenous insulin infusion, can induce elevated blood pressure in the rat.

Methods

Animals Six adult male Sprague-Dawley rats (Simonsen), weighing 250 to 300 g, were adapted to the environmentally controlled vivarium (23 ± 1°C, 30% to 40% humidity) with a 12-hour light/dark photocycle (lights on 7 AM). Rats were housed individually in polycarbonate cages (22 × 20 × 46 cm) on pine-shaving bedding with free access to food and water at all times. The animals in our laboratory are cared for in accordance with the guidelines of the Committee on Care and Use of Laboratory Animals.

Hardware The transmitter (TA11PA-C40, Data Sciences) consisted of a cylindrical electronics package (2.5 cm long, 1.5 cm in diameter) with a single-ended catheter (8 cm) attached. These devices transmit the frequency-modulated blood pressure and heart rate for a period of up to 6 months. Signals from the transmitters were received via an AM receiver (Data Sciences model CTR 86) and multiplexed with a consolidation matrix (BCM-100). A PC-based (IBM 80386–compatible) recording and analysis system (Dataquest III, Data Sciences) demodu-
lating the signal and converted the raw telemetered data into common units (eg, millimeters of mercury, beats per minute). This system recorded blood pressure (systolic, diastolic, and mean), heart rate, and activity of six animals every 10 minutes on a 24-hour basis. The transmitters were calibrated at the factory and checked for "0" pressure (variations of ±2 mm Hg were accepted) before implantation.

**Surgeries**

**Blood Pressure Transmitters**

Catheters were inserted into the abdominal aorta, and the transmitter was attached to the abdominal wall. After a 12-hour fast, the animal was placed under anesthetic (ketamine, 0.1 mL/100 g body wt 1P; xylazine, 0.01 mL/100 g body wt 1P) and antibiotic (penicillin, 0.15 mL per rat), the abdomen was shaved, an incision was made from the xiphisternum to the pelvis, and the abdomen was opened along the linea alba. The abdominal aorta was exposed, dissected free from the vena cava just posterior to the renal veins, and occluded with clamps above and below the site of implant. The transmitter catheter was inserted into the aorta just anterior to the bifurcation of the iliac arteries, directed toward the heart. The catheter was held in place using cyanoacrylate cement (Vetbond, 3M) and a fiber patch. The transmitter was placed into the abdominal cavity and secured to the body wall with sutures as the incision was closed.

**Osmotic Minipumps**

Osmotic minipumps (Alzet model 2001) were implanted into subcutaneous tissue on the backs of animals lightly anesthetized with methoxyflurane (Pitman-Moore). The skin was shaved and washed, and an incision was made adjacent to the site chosen for pump placement. A subcutaneous pocket was made, the filled pump (717.5 nmol [100 U]/mL Humulin BR or normal saline) inserted, and the wound closed. Mini-pumps were replaced after 5 days so each animal had two implants per experiment because preliminary studies indicated that insulin delivery by this method in control rats declined after 5 days.

**Experimental Design**

Experiments were performed using a crossover design with three animals receiving treatment and three receiving vehicle for 10 days. After a 10-day washout period, the groups were reversed, and the experiment was repeated. Blood samples for insulin and glucose were drawn on days -1, 1, 2, 5, 6, 7, 9, 10, and 11 relative to pump insertions. Insulin (15.8 nmol [2.2 U] Humulin BR per day) was administered with a 4% glucose supplement in the drinking water once blood pressure was stabilized (usually 1 to 2 weeks). In the first set of studies, the effect of dietary glucose was compared with dietary glucose and insulin infusion. After treatment with insulin and a washout period, animals were rechallenged with insulin. In the second set of studies the effect of clonidine (1 µg/mL; ~25 µg/kg per day) administration was examined in animals receiving insulin and dietary glucose. Body weight and food and water intake were measured daily between 1 PM and 3 PM.

**Blood Collection and Insulin and Glucose Analysis**

Blood samples (0.5 mL) were collected from nonfasted restrained rats by tail amputation between 3 PM and 4 PM. Glucose was measured by a glucose oxidase method (Beckman Glucose Analyzer II, Beckman Instruments). Insulin was measured by a charcoal precipitation radioimmunoassay. Statistical Analysis

A proprietary program (DATAQUEST DOSORT, Data Sciences) was used to extract the telemetry data for each individual animal from raw data files. Data were analyzed using either paired t tests, Pearson's correlation, or general linear models ANOVA with a Fisher's exact test for individual comparisons. Data are presented as mean±SEM with P<.05 considered significant.

**Results**

**Metabolic Results**

Afternoon (3 to 4 PM) insulin levels increased for both the insulin- and saline-infused animals (Fig 1A). Mean plasma insulin levels for the 10-day period were 457±25 pmol/L (63.7±3.5 µU/mL) for the insulin-infused and 225±22 pmol/L (31.4±3.0 µU/mL) for the saline-infused rats (insulin>saline, F1,10=47.9, P<.0001) compared with 57±9 pmol/L (8.0±1.3 µU/mL) for the baseline periods. Mean plasma glucose levels for the 10-day period were 3.8±0.2 mmol/L (67.7±2.8 mg/dL) for the insulin-infused and 5.0±0.1 mmol/L (90.9±1.8 mg/dL) for the saline-infused rats (saline>insulin, F1,10=38.2, P<.0001) compared with 4.6±0.1 mmol/L (82.4±1.8 mg/dL) for the baseline periods (Fig 1B).

The calories consumed from the drinking water during the infusions were 10.5±0.8 kJ (2.5±0.2 kcal)/100 g body wt for the saline-infused and 9.2±0.4 kJ (2.2±0.1 kcal) for the insulin-infused animals (not significantly different). This amounted to 11% to 12% of the total caloric intake in both groups: 76.6±1.3 kJ (18.3±0.3 kcal)/100 g body wt for saline-infused and 72.4±1.7 kJ (17.3±0.4 kcal) for insulin-infused. The weight change in both groups was not significantly different.
Hemodynamic Results

Systolic blood pressure increased in both the insulin-infused (by 8.6±0.7 mm Hg) and saline-infused (by 6.0±1.2 mm Hg) animals (Fig 2A). The rise was greater and appeared earlier in the insulin-infused animals than in the saline-infused animals (F1,100=38.9, P<.0001). Diastolic blood pressure (Fig 2B) only modestly increased in the saline-infused animals at the end of the infusion. Insulin-infused animals had an earlier and greater increase in diastolic blood pressure (by 2.9±1.1 mm Hg) than the saline-infused animals (by 2.2±1.3 mm Hg) (F1,100=38.9, P<.0001). Heart rate (Fig 2C) increased gradually, but similarly, for both groups of rats during the infusion period (F1,100=0.18, P=NS). The average change in blood pressure and average plasma insulin during the infusion were correlated (Fig 3, r=0.72, P=.009).

After this initial study, animals were again given insulin and glucose. Blood pressure dropped during the week following discontinuation of the insulin infusion. On rechallenge with insulin and glucose, blood pressure again rose (by 8.0±3.6 mm Hg for systolic blood pressure) and then decreased after termination of the insulin and glucose administration.

Effect of Clonidine

Animals consuming clonidine from their drinking water had lower systolic (Fig 4A, F1,100=319.2, P<.0001) and diastolic blood pressures (Fig 4B, F1,100=104.8, P<.0001) and heart rate (Fig 4C, F1,100=254.2, P<.0001). Blood pressure and heart rate rebounded to above baseline levels after removal of clonidine from the drinking water (Fig 4).

Discussion

Our data demonstrate that physiological hyperinsulinemia induced by insulin infusion and/or glucose feeding is accompanied by elevated blood pressure and heart rate in normal rats. Use of telemetry allowed constant monitoring of these hemodynamic variables without manipulating the animals, which allowed us more sensitivity and accuracy in detecting blood pressure changes in response to perturbation. Other techniques to measure blood pressure require that the animal be restrained or tethered to obtain readings, so measurements reflect the animal's response to the blood pressure measurement as well as to the experimental manipulation. Telemetry is not subject to that confounding variable and therefore is a highly useful technique to measure blood pressure in animal models.

In the present study, hyperinsulinemia appears to directly affect changes in blood pressure. Glucose administration increased circulating insulin levels, which increased even further with insulin infusion. The increase in blood pressure was more rapid and greater in the rats with combined insulin infusion and glucose administration than in those receiving glucose alone. Heart rate increased equally in both groups. Plasma insulin levels directly correlated with the mean increase in blood pressure, suggesting a causal relation between insulin and blood pressure. Furthermore, both blood
pressure and heart rate decreased after cessation of treatment and increased after rechallenge, indicating that the hemodynamic changes followed the time course of changes in circulating insulin.

Although there was no significant increase in blood glucose in animals receiving glucose alone, there was a significant drop in blood glucose in the insulin-infused animals. It is possible that this may have contributed to the rise in blood pressure in the insulin-infused animals; however, the insulin and saline groups had identical increases in heart rate, which suggests the differences in glucose did not cause a counterregulatory response. Hypoglycemia induced by acute insulin infusion increases plasma glucagon, growth hormone, cortisol, epinephrine, and, especially when glucose levels fall below 2.2 mmol/L (40 mg/dL), norepinephrine. Severe hypoglycemia (1.2 mmol/L, 22 mg/dL) has been associated with elevated blood pressure. In general, however, acute hypoglycemia is not accompanied by an increase in blood pressure.

The rise in blood pressure during the saline infusion and glucose administration suggests that either the hyperinsulinemia or the ingestion of glucose elevated blood pressure. Diets rich in simple carbohydrates have been found to lead to an increase in blood pressure in rats. When sucrose (10% wt/vol in the drinking water) was fed to spontaneously hypertensive and Wistar-Kyoto rats, blood pressures measured using tail-cuff plethysmography were elevated by 3 to 4 weeks in both strains. When weaning Sprague-Dawley rats were given 8% sucrose, blood pressures (assessed by tail-cuff plethysmography and femoral catheter) and heart rates were elevated after 5 weeks. In these studies approximately 50% of the caloric intake was uncomplexed carbohydrate. In our study simple sugars accounted for 11% to 12% of the caloric intake, significantly less than in previous studies but enough to induce mild, chronic hyperinsulinemia. The use of telemetry allowed us to demonstrate that these increases in insulin could be associated with chronic elevations of blood pressure.

The elevated blood pressure observed during glucose administration is also likely to be sympathetically mediated. When Young and Lansberg provided 8% sucrose in the drinking water of female Sprague-Dawley rats for 3 days, they found increased [H3]norepinephrine turnover in the hearts of the rats. Walgren et al compared the effects of diets supplemented 50% (by calorie) with fructose, sucrose, dextrose, or corn starch on sympathetic nervous system activity in female Sprague-Dawley rats. After 10 days on the diet, sympathetic activity was increased in both cardiac tissue and interscapular brown adipose tissue in all groups. In these studies, caloric intake was restricted so all animals consumed the same number of calories. Inhibiting carbohydrate but not fat absorption from the intestine blocked the increase in sympathetic activity. The adrenal may contribute to the increase in sympathetic activity. With food freely available, glucose-fed male Sprague-Dawley rats were found to have greater urinary epinephrine than cornstarch-fed rats. Adrenal demedulation, which reduced urinary epinephrine to near zero, prevented the increase in blood pressure in the glucose-fed rats. Central stimulation of sympathetic activity may also contribute to the increase in blood pressure with carbohydrate feeding. Electrical stimulation of the ventromedial hypothalamus resulted in greater pressor and sympathetic nerve responses in sucrose-treated rats. Acute glucose infusion directed to the brain was found to increase plasma norepinephrine without raising plasma insulin, glucose, or epinephrine. Thus, simple sugars may have direct effects to enhance central sympathetic activity and blood pressure independent of insulin.

However, physiological hyperinsulinemia itself appears to increase blood pressure in the rat. Brands et al infused insulin at a rate of 7.2 μmol (1.0 mU)/kg per minute (=0.47 U per rat per day) into Sprague-Dawley rats that were coinfused with dextrose at 12.2 mmol (22 mg)/kg per minute (=10.3 g per rat per day). The average increase in mean arterial pressure for 5 days was 8 mm Hg. Chow intake decreased approximately 50% during the infusions, perhaps due to the calories (=172 kJ, 41 kcal/d) derived from the large amount of dextrose infused. Thus, in this study it was difficult to...
separate the effect of the insulin versus the effect of the dextrose infusion on blood pressure.16 Tomiyama et al16 infused insulin into young (5-week-old) Dahl salt-sensitive and salt-resistant rats for 3 weeks. Blood glucose remained normal without glucose supplementation. The salt-sensitive rats had elevated blood pressure (tail-cuff and direct measurements) and plasma norepinephrine and a transient increase in sodium retention when they were given salt in their diet. This study suggested that insulin itself could promote a rise in blood pressure in animals with a tendency for salt-sensitive hypertension.33 In the present study, both insulin- and saline-infused animals consumed the same amounts of carbohydrate. However, the insulin-infused animals had a faster and greater rise in pressure, suggesting that insulin itself had an effect on blood pressure.

Hyperinsulinemia promotes sympathetic activation in the absence of hypoglycemia. Activation of sympathetic nerve activity has been demonstrated during a hyperinsulinemic, euglycemic clamp.14,36 Treatment of the insulin-infused animals with the a2-agonist clonidine in our study resulted in complete suppression of the increases seen in blood pressure and heart rate, suggesting that there was a sympathetic component contributing to the hypertension associated with hyperinsulinemia. In rats fed 66% fructose in their diet, clonidine prevented the hypertension but not the insulin resistance associated with fructose feeding. Reaven and colleagues (Hwang et al)37 have suggested that the hyperinsulinemia in this model stimulates sympathetic nervous system activity that contributes to the hypertension but not the insulin resistance. Insulin also increases renal tubal sodium reabsorption and alters Ca2+ and Mg2+ fluxes to increase intracellular Ca2+ and decrease intracellular Mg2+, which promote hypertension.36,38 Acutely, insulin induces vasodilation in some vascular beds and vasoconstriction in others39 and chronically may alter vascular remodeling processes through its effects as a growth factor.36

In summary, dietary glucose supplementation led to an increase in plasma insulin, blood pressure, and heart rate in normal rats. Chronic infusion of insulin in combination with oral glucose supplementation led to further increases in plasma insulin and blood pressure, which were prevented by sympathetic inhibition. These studies suggest that physiological hyperinsulinemia, induced endogenously or exogenously, enhances blood pressure and sympathetic activity in the rat. Whether similar mechanisms are operative in hyperinsulinemic humans with hypertension remains to be determined.

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