Insulin in the Brain Increases Gain of Baroreflex Control of Heart Rate and Lumbar Sympathetic Nerve Activity

Mollie P. Pricher, Korrina L. Freeman, Virginia L. Brooks

Abstract—Chronic central administration of insulin increases the gain of baroreflex control of heart rate, but whether baroreflex control of the sympathetic nervous system is similarly affected is unknown. The sites and mechanisms by which brain insulin influences the baroreflex are also unclear. Therefore, the present study tested the hypothesis that acute infusion of insulin into the brain ventricles of urethane-anesthetized rats increases gain of baroreflex control of heart rate and lumbar sympathetic nerve activity and that this action is gender specific. Furthermore, to identify the location within the brain that mediates these effects, insulin was infused into either the lateral ventricle or the fourth ventricle. Lateral ventricular insulin infusion increased the gain of baroreflex control of heart rate (2.1 ± 0.3 to 4.0 ± 0.6 bpm/mm Hg; P < 0.05) and sympathetic activity (2.3 ± 0.3% to 4.8 ± 1.1% control/mm Hg; P < 0.05) within 60 to 90 minutes; however, the increase in heart rate gain was similar in males and females. Increases in the maximum of baroreflex control of heart rate (395 ± 10 to 452 ± 13 bpm; P < 0.05) and of sympathetic activity (156 ± 13% to 253 ± 22% control; P < 0.05) were also observed. In contrast, fourth ventricular insulin infusion failed to alter baroreflex function. In conclusion, increases in brain insulin act acutely in the forebrain to enhance gain of baroreflex control of heart rate and lumbar sympathetic nerve activity. (Hypertension. 2008;51[part 2]:514-520.)

Key Words: male and female rats • urethane anesthesia • mean arterial pressure • nitroprusside • phenylephrine

Numerous insulin-resistant conditions are associated with blunted baroreflex gain, suggesting that the 2 may be functionally related. In support of this hypothesis, obesity-induced decreases in insulin sensitivity and in baroreflex sensitivity have been found to normalize when obese individuals lose weight. Moreover, the impaired baroreflex function caused by pregnancy improves when pregnant rabbits are treated with the insulin-sensitizing drug rosiglitazone. Nevertheless, the mechanism by which insulin resistance impairs baroreflex gain is currently unclear.

Considerable research indicates that brain insulin influences neural control of the circulation. Intracerebroventricular (ICV) insulin infusion acutely increases the activity of multiple sympathetic nerves. Moreover, several days of central insulin administration enhance the sensitivity or gain of baroreflex control of heart rate (HR) by increasing reflex tachycardia. Insulin is not synthesized in significant amounts in the brain but is present in cerebrospinal fluid and brain, albeit at levels considerably less than in plasma. It gains access from plasma via a saturable transport mechanism across the blood-brain barrier. Insulin resistance appears to hinder transport of insulin into the brain, which leads to a fall in brain insulin levels, at least in obese and pregnant animals. Therefore, in insulin-resistant states, decreases in brain insulin may attenuate baroreflex function by reversing the normal effect of insulin to enhance or support baroreflex gain. However, despite the potential widespread pathophysiological impact of this possible mechanism, the sites and mechanisms by which insulin in brain improves baroreflex gain are virtually unexplored.

To begin to investigate the mechanisms by which insulin enhances baroreflex function, we tested the following hypotheses: (1) increases in brain insulin acutely increase baroreflex gain, similar to its sympathoexcitatory effect; (2) the increment in gain induced by insulin is greater in males than females, similar to the central appetite-suppressing action of insulin; (3) in addition to its effect on HR, insulin also improves gain of baroreflex control of lumbar sympathetic nerve activity (LSNA); and (4) insulin acts in the forebrain to increase baroreflex gain. To test these hypotheses, insulin was infused either into the lateral ventricle (LV) or the fourth ventricle (4V) of urethane-anesthetized rats instrumented for recordings of mean arterial pressure (MAP), HR, and LSNA.

Methods

Animals

Male (350 to 400 g) and female (240 to 280 g) Sprague-Dawley rats (Charles River Laboratories, Inc) were used for these experiments. All of the rats were housed for ≥5 days before experimentation in a room with a 12-hour:12-hour light/dark cycle, with food (LabDiet 5001) and water provided ad libitum. All of the procedures were conducted in accordance with the National Institutes of Health Guide for the Health and Use of Laboratory Animals and were approved by

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Table 1. Effect of Lateral ICV Insulin Infusion on MAP and HR in Male and Female Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Females (n=7)</th>
<th>Males (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Insulin</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>109±8</td>
<td>101±7</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>349±14</td>
<td>365±16</td>
</tr>
</tbody>
</table>

Table 1 represents the effect of lateral intracerebroventricular (ICV) insulin infusion on mean arterial pressure (MAP) and heart rate (HR) in male and female rats. The data is presented as mean ± standard error of the mean. The table shows that there is no significant difference in MAP and HR between control and insulin groups in either sex. The data was analyzed using ANOVA and post-hoc Tukey's test.

The institutional animal care and use committee of the Oregon Health & Science University.

Surgery
Rats were deprived of food, but not water, the night before the experiment. Anesthesia was induced with 5% isoflurane in 100% oxygen and was maintained with 2% isoflurane in oxygen. Throughout the surgery and experiment, body temperature was maintained at 37±1°C using a rectal thermistor, heat lamp, and heating pad.

A tracheal tube was placed for artificial ventilation, and then femoral arterial (1) and venous (2) catheters were implanted for the measurement of MAP and drug infusions, respectively. After a midline abdominal incision, a bipolar stainless steel electrode was positioned and secured around a lumbar nerve using lightweight silicone material (Kwik-Sil, WPI, Inc) and procedures published previously.11,12 Rats were placed in a stereotaxic apparatus (David Kopf) with the incisor bar at −11 mm for LV infusions or at −3 mm for 4V infusions. A midline incision was made on the top of the skull, all of the tissue was cleared, and a small hole was drilled through the skull to allow for placement of the ventricular cannula. After completion of surgery, urethane (1.1g/kg in 1 mL of saline) was administered intravenously over 30 minutes while isoflurane was slowly withdrawn. Artificial ventilation with 100% oxygen was maintained throughout the experiment, and respiratory rate and tidal volume were adjusted to maintain expired CO2 at 30 to 35 mm Hg.

LV and 4V Infusions
Single-barreled glass pipettes drawn to a small tip were used for both LV and 4V infusions. Coordinates for positioning the LV cannulae were as follows (millimeters from bregma): 1.0 caudal, 1.4 lateral, and 4.2 dorsal, with the pipette angled 10° caudal so that it entered perpendicularly to the surface of the skull. A pipette was placed into the 4V using the following coordinates (millimeters): 2.0 caudal to interaural line, on the midline, and 7.3 ventral to the skull surface. Correct pipette placement was confirmed at the end of the experiment by infusing ~100 nL of 2.5% Alcian blue in 0.5 mol/L of sodium acetate via the same pipette, removing the brains and verifying the presence of dye in the cerebroventricles.

Data Acquisition
Pulsatile and MAPs were continuously recorded on a Grass polygraph (7D) throughout the experiment by connecting the arterial catheter to a Grass bridge amplifier (7P1). The pulsatile signal was directed to a Grass tachograph amplifier (7P4) for determination and recording of HR. In addition, before and during baroreflex curve generation, MAP, HR, and raw LSNA were sampled at 2000 Hz (Biopac); LSNA was band-pass filtered to transmit frequencies between 100 and 3000 Hz and amplified. After data collection, the LSNA signal was rectified and integrated over 1-second intervals. At the end of the experiment, background noise was quantified after ganglionic blockade with hexamethonium (30 mg/kg IV) and increases in MAP by IV infusion of phenylephrine. This background level was subtracted from values of LSNA recorded during the experiment. LSNA was normalized to baseline nerve activity before the experimental infusions were initiated (percentage of baseline).

Baroreflex Curve Generation
Baroreflex curves were generated by first quickly lowering MAP to ~50 mm Hg via nitroprusside infusion (1 mg/mL, in 5% dextrose in water; 20 μL/min), and then by steadily and smoothly raising MAP to ~175 mm Hg over 3 to 5 minutes both by decreasing the rate of nitroprusside infusion and by slowly increasing the rate of phenylephrine infusion (1 mg/mL, in normal saline; 1 to 75 μL/min); sigmoidal curves were constructed from 1-second averages of MAP, HR, and LSNA obtained during the pressure upswing from 50 to 175 mm Hg. The sigmoidal baroreflex relationships between MAP and HR or LSNA generated in each experiment were fitted and compared using the Boltzmann equation: HR or LSNA=(P1−P2)/(1+exp(MAP−P3)/P4)+P3, P1 is the maximum HR, P2 is the minimum HR, P3 is the MAP associated with the HR/LSNA value midway between the maximal and minimal HR/LSNA (BP50; denotes position of the curve on the x axis), and P4 (width) is the coefficient used to calculate maximum gain, −(P3−P2)/4P4, which is
an index of the slope of the linear part of the sigmoidal baroreflex curve.

**Experiment Protocols**

After all of the surgical procedures, the venous catheters were filled with phenylephrine or nitroprusside, and the rats were allowed to stabilize for ~60 minutes. In most rats, a blood sample was then collected for the measurement of glucose levels (using a Freestyle Flash handheld blood glucose monitor). Basal baroreflex function was confirmed by producing 2 curves with similar gains; the final control curve was used for data analysis. After reestablishment of basal values, ICV infusion of either insulin (100 µU/min at 0.6 µL/min; Novolin R) or the artificial cerebrospinal fluid (aCSF) vehicle (0.6 µL/min) then commenced. aCSF contained (in mmol/L): 128 NaCl, 2.6 KCl, 1.3 CaCl₂, 0.9 MgCl₂, 20 NaCHO₃, and 1.3 Na₂HPO₄; pH was corrected to 7.4. In the first experiment, insulin was infused via the LV, and HR baroreflex curves were produced again 1 to 2 hours later in male (after 109 ± 8 minutes) and female (after 86 ± 8 minutes) rats. In the second experiment, both HR and LSNA (Table 2). MAP fell in female rats receiving either insulin or aCSF; however, differences between the responses were not observed.

Insulin infusion increased gain of both baroreflex control of HR and LSNA compared with initial control values and with values obtained in rats receiving LV aCSF (Figures 3 and 4). Maximum baroreflex-mediated increases in HR and LSNA were also significantly increased (Figures 3 and 4). However, no other baroreflex parameter was significantly changed during either insulin or aCSF infusion (Figures 3 and 4).

**Fourth Ventricular Infusion of Insulin**

In contrast to the effects of LV insulin infusion, neither basal HR nor LSNA nor their baroreflex control were altered during 4V insulin infusion (Figure 5 and Table 3).

**Table 2. Time Course of Effects of Lateral ICV Insulin or aCSF Infusion on MAP, HR, and LSNA**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>30 Minutes</th>
<th>90 Minutes</th>
<th>120 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin infusion (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>111 ± 1</td>
<td>105 ± 2</td>
<td>100 ± 2</td>
<td>95 ± 5*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>353 ± 4</td>
<td>374 ± 9</td>
<td>380 ± 8</td>
<td>395 ± 10†</td>
</tr>
<tr>
<td>LSNA, % control</td>
<td>100 ± 0</td>
<td>136 ± 11</td>
<td>159 ± 17†</td>
<td>169 ± 17†</td>
</tr>
<tr>
<td>aCSF infusion (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>107 ± 4</td>
<td>95 ± 4*</td>
<td>96 ± 5</td>
<td>95 ± 5*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>357 ± 8</td>
<td>353 ± 7</td>
<td>360 ± 8</td>
<td>360 ± 7</td>
</tr>
<tr>
<td>LSNA, % control</td>
<td>100 ± 0</td>
<td>97 ± 8</td>
<td>102 ± 8</td>
<td>100 ± 8</td>
</tr>
</tbody>
</table>

*P < 0.05 vs control.
†P < 0.05 vs same time, aCSF.

LSNA curves were generated in female rats 30, 90, and 120 minutes after beginning the LV insulin infusion. In the final experiment, also in females, insulin was infused via the LV, and baroreflex curves (HR and LSNA) were constructed after 30, 90, and 120 minutes. Five to 10 minutes after completing the final baroreflex curve, a second blood sample was collected for glucose measurements in most rats.

**Statistical Analysis**

Between-group differences were evaluated using a 2-way ANOVA for repeated measures and posthoc Tukey-Kramer test. P values < 0.05 were considered statistically significant.

**Results**

**Lateral Intracerebroventricular Insulin Infusion Increases Baroreflex Gain in Male and Female Rats**

Basal levels of MAP and HR were not significantly altered by 1 to 2 hours of insulin infusion (Table 1). In both males and females, ICV insulin infusion increased baroreflex gain (Figures 1 and 2); however, the increase was not different between genders. Insulin also increased maximum baroreflex-induced HR in females but not males, but these values were not different between groups (Figure 2). No other baroreflex parameters were significantly altered by insulin in males or females (Figure 2).

**Time Course of Effects of LV Insulin**

LV infusion of insulin, but not aCSF, increased HR and LSNA (Table 2). MAP fell in female rats receiving either insulin or aCSF; however, differences between the responses were not observed.

Insulin infusion increased gain of both baroreflex control of HR and LSNA compared with initial control values and with values obtained in rats receiving LV aCSF (Figures 3 and 4). Maximum baroreflex-mediated increases in HR and LSNA were also significantly increased (Figures 3 and 4). However, no other baroreflex parameter was significantly changed during either insulin or aCSF infusion (Figures 3 and 4).
Blood Glucose Concentration

Blood glucose concentration (in mg/dL) was not significantly altered \((P>0.05, \text{ANOVA})\) after LV aCSF (149±7 to 145±15; \(n=5\)), LV insulin (166±20 to 124±13; \(n=5\)), or 4V insulin (139±14 to 125±11; \(n=5\)).

Discussion

The major new findings are as follows: (1) LV insulin infusion increases HR baroreflex gain similarly in both male and female rats; (2) LV insulin infusion increases the gain and maximum of baroreflex control of LSNA; (3) insulin alters the baroreflex within 60 to 90 minutes; and (4) 4V insulin infusion does not influence the baroreflex. Therefore, we conclude that acute increases in brain insulin improve baroreflex control of HR and LSNA via an action in the forebrain.

It has long been appreciated that euglycemic increases in the circulating levels of insulin increase sympathetic activity (for reviews, see References 13 and 14) and that the lumbar nerve is particularly sensitive to the sympathoexcitatory action of insulin. In addition, acute ICV insulin infusion increases HR and activates the sympathetic nervous system,
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In agreement, we found that plasma glucose did not change significantly in rats receiving insulin ICV. The acute effect on HR may be sustained for at least several days, because Okada and Bunag\(^5\) reported previously that ICV infusion of insulin (200 \text{mU/d}) for 10 days increases the tachycardic response to decreases in arterial pressure. Whether increments in brain insulin above normal can chronically improve gain of baroreflex control of the sympathetic nervous system is currently unknown. However, in pregnant and obese individuals, reductions in brain insulin may contribute to reduced baroreflex control of both HR and sympathetic activity.\(^1\) These data suggest that normal brain insulin levels may be required to chronically support optimal baroreflex function.

Insulin exerts many effects centrally, not only on neural control of the circulation, but also on the regulation of energy balance, cognition, and reproduction.\(^1\) Indeed, insulin’s first reported central action was its ability to inhibit food intake.\(^1\) Interestingly, Clegg et al\(^9\) have reported recently that males and females exhibit differential sensitivity to the appetite-suppressing effects of insulin; females are less sensitive than males. Female sensitivity was improved after ovariotomy and was hindered after central administration of estrogen, implicating estrogen in this differential effect. Therefore, we investigated whether gender differences would also be apparent in insulin’s action on the baroreflex. However, using insulin doses that are similar to those used by Clegg et al\(^9\), we found that gain increases were not significantly different between sexes. On the other hand, ICV insulin infusion increased the HR baroreflex maximum only in female rats. Thus, subtle gender differences may underlie insulin’s effect on baroreflex function.

Another major goal of the present study was to begin to investigate the site in the brain at which insulin initiates its action to increase baroreflex gain. Insulin receptors are present in numerous but discrete sites throughout the brain, including regions directly or indirectly involved in central pathways regulating the cardiovascular system. These sites include the paraventricular nucleus, arcuate nucleus, the dorsomedial nucleus, and the ventromedial nucleus of the hypothalamus, as well as the nucleus tractus solitarius in the brainstem.\(^1\) The present results show that, with the dose used, 4V insulin does not increase basal levels of HR or LSNA or alter baroreflex function. Therefore, we conclude that insulin can initiate effects on the baroreflex via a site in the forebrain, presumably within the hypothalamus. Nevertheless, these data do not preclude an additional action for the forebrain, presumably within the hypothalamus. Nevertheless, these data do not preclude an additional action for insulin in the hindbrain for the following reasons. First, previous brainstem or nucleus tractus solitarius microinjection studies have reported effects of insulin on nucleus tractus solitarius neurons or baroreflex function; however, high doses of insulin were administered, and both inhibitory and facilitatory effects of insulin were observed. Second, although the present dose of insulin was previously reported

Table 3. Effect of 4V Insulin Infusion on MAP, HR, and LSNA

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>30 Minutes</th>
<th>90 Minutes</th>
<th>120 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg (n=7)</td>
<td>100±4</td>
<td>95±4</td>
<td>96±5</td>
<td>95±4</td>
</tr>
<tr>
<td>HR, bpm (n=7)</td>
<td>337±11</td>
<td>354±15</td>
<td>358±22</td>
<td>362±19</td>
</tr>
<tr>
<td>LSNA, % control (n=4)</td>
<td>100±0</td>
<td>105±16</td>
<td>103±22</td>
<td>108±24</td>
</tr>
</tbody>
</table>

indicating that insulin acts centrally; again, the greatest action of insulin is on LSNA.\(^2\) The present results confirm these previous studies. Importantly, the dose of insulin chosen for this study clearly acts centrally and not secondarily after reaching the circulation. Previous reports have documented that this and significantly higher insulin doses do not increase plasma insulin concentration or lower plasma glucose concentration.\(^3\) In agreement, we found that plasma glucose did not change significantly in rats receiving insulin ICV.

A major novel finding of the present study is that acute ICV insulin influences baroreflex control of both HR and LSNA, by increasing gain and maximum baroreflex levels. The acute effect on HR may be sustained for at least several days, because Okada and Bunag\(^5\) reported previously that ICV insulin influences baroreflex control of both HR and LSNA, by increasing gain and maximum baroreflex levels. The present results confirm these previous studies. Importantly, the dose of insulin chosen for this study clearly acts centrally and not secondarily after reaching the circulation. Previous reports have documented that this and significantly higher insulin doses do not increase plasma insulin concentration or lower plasma glucose concentration.\(^3\) In agreement, we found that plasma glucose did not change significantly in rats receiving insulin ICV.
ineffective in altering other sympathetic nerves (adrenal or renal), higher doses infused for ≥6 hours activated these nerves. Therefore, higher doses and longer applications of insulin may act in the hindbrain to alter baroreflex control of HR or sympathetic activity as well.

Within the hypothalamus, several sites are potential candidates. Importantly, the effect of insulin occurred within 1 hour, implicating a periventricular site, such as the paraventricular nucleus or arcuate nucleus. Indeed, >1 site may be involved, because insulin increases the basal activity of various sympathetic nerves via differing signaling mechanisms and, therefore, neuronal pathways. In addition, leptin, of which the central effects are remarkably parallel to insulin, has been shown to increase MAP and activate various sympathetic nerves after microinjection into the arcuate nucleus, paraventricular nucleus, dorsomedial nucleus, ventromedial nucleus, and the lateral hypothalamic area.

Perspectives
The prototypical insulin-resistant disease is type 2 diabetes mellitus, but decreased insulin sensitivity is also expressed in obesity, metabolic syndrome, congestive heart failure, Alzheimer’s disease, aging, hypertension, and pregnancy. Intriguingly, insulin-resistant individuals also exhibit reduced baroreflex gain, including individuals with obesity, diabetes mellitus, heart failure, pregnancy, and aging. Moreover, rats fed a high fructose diet, a relatively specific model over, rats fed a high fructose diet, a relatively specific model

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Disclosures
None.

References


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