Anteroven tral Third Ventricle Lesions Abolish Lumbar Sympathetic Responses to Insulin

Martin Muntzel, Terry Beltz, Allyn L. Mark, Alan Kim Johnson

Abstract Insulin has been shown to increase sympathetic nerve activity. Because evidence shows that insulin acts within the central nervous system, we hypothesized that lesions of the anteroven tral third ventricle region, an area rich in insulin receptors, would abolish sympathetic responses to hyperinsulinemia. We measured mean arterial pressure and lumbar sympathetic nerve activity in fasted, anesthetized sham-lesioned (n=8) and lesioned (n=8) rats before and after intravenous insulin infusion at 0.13 U/h during euglycemic clamp. Additional sham-lesioned (n=10) and lesioned (n=5) rats received vehicle infusion. Insulin-infused sham-lesioned rats had substantially greater increases in lumbar sympathetic nerve activity (+83±18%) than vehicle-infused sham-lesioned rats (+27±4%). Most importantly, insulin-infused lesioned rats had increases in sympathetic activity (+32±11%) that were no greater than lesioned rats receiving vehicle (+23±16%). Blood pressure was not altered by insulin or vehicle. To test the possibility that lesions of the anteroven tral third ventricle region nonspecifically suppress sympathetic excitatory responses, we evaluated reflex increases in lumbar sympathetic activity to nitroglycerin in sham-lesioned (n=5) and lesioned (n=8) rats. Rats with lesions and sham lesions showed comparable increases in lumbar nerve activity during nitroglycerin-induced hypotension. In summary, increases in sympathetic nerve activity to intravenous insulin infusion are abolished by anteroven tral third ventricle lesions. These data indicate that the integrity of this brain region is necessary for activation of lumbar sympathetic nerve activity by systemic administration of insulin. (Hypertension. 1994;23[part 2]:1059-1062.)

Key Words • insulin • euglycemic clamp • AV3V lesion • lumbar

Although it is clear that insulin produces marked increases in sympathetic nerve activity (SNA), the mechanisms of sympathetic activation are not well understood. Because insulin stimulates vasodilation, it has been suggested that increases in SNA may be secondary to baroreceptor control of sympathetic neural outflow. The sympathetic actions of insulin have also been attributed to a direct central neural effect of the hormone. Supporting a central mechanism, Pereda et al found that administering low-dose insulin into the carotid arteries of dogs increased blood pressure before a fall in blood glucose. In addition, we recently demonstrated that intracerebroventricular administration of insulin increases lumbar SNA in the absence of changes in blood glucose (BG) or plasma insulin (PI) levels. Although the mechanisms of sympathoexcitation to central insulin are unclear, changes in central nervous system (CNS) insulin have been shown to alter hypothalamic electrical activity and catecholamine turnover.

Changes in blood-borne insulin may be rapidly perceived by the brain in the circumventricular organs, which lack a blood-brain barrier. Among these structures, the subfornical organ (SFO) and the organum vasculosum of the lamina terminalis (OVLT) have been identified as having specific binding sites for blood-borne insulin. The SFO and OVLT additionally function to control body fluid balance and regulate arterial blood pressure. For example, destruction of the OVLT and fibers of passage from the SFO by lesioning tissues surrounding the anteroven tral third ventricle (AV3V) abolishes blood pressure responses to intracarotid administration of angiotensin II and impairs drinking responses to hypertonic solutions. AV3V lesions also prevent the development of several forms of experimental hypertension. Given these observations, we hypothesized that AV3V-related structures activate elevations in SNA in response to hyperinsulinemia. We initiated the present studies to determine whether lesions of the AV3V region abolish increases in SNA to intravenous infusion of insulin.

Methods

Effects of Hyperinsulinemia in AV3V-Lesioned and Sham-Lesioned Rats During Euglycemic Clamp

Animals

Experiments were performed in male Sprague-Dawley rats weighing 280 to 300 g (Harlan Sprague Dawley Inc). All procedures were performed in accordance with the University of Iowa and National Institutes of Health guidelines for the care and use of experimental animals.

AV3V Lesions

Animals were anesthetized with Equithesin (0.33 mL/100 g body wt) and secured in a Kopf 900 stereotaxic instrument with the skull leveled between bregma and lambda. A lesioning electrode (24-gauge nichrome wire insulated except at the tip) was lowered on the midline 0.3 mm caudal to bregma to a depth 7.5 mm from dura. Anodal current (2 to 3 mA) was passed for 25 to 30 seconds (rectal cathode). In sham-lesioned rats, the electrode was lowered to a point 0.5 mm above the intended target tissue and no current was passed.
Responses to Vehicle and Insulin in Fasted Sham-Lesioned and AV3V-Lesioned Rats

<table>
<thead>
<tr>
<th>Measure</th>
<th>Baseline Vehicle (8 μL/min)</th>
<th>Baseline Insulin (0.13 U/h)</th>
<th>Baseline Vehicle (8 μL/min)</th>
<th>Baseline Insulin (0.13 U/h)</th>
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</thead>
<tbody>
<tr>
<td>BG, mg/dL</td>
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<td>68±3</td>
<td>76±7</td>
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<tr>
<td>Pi, μU/mL</td>
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<td>18±3</td>
<td>12±2</td>
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<td>GIR, mg/kg·min</td>
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<td>127±4</td>
<td>119±5</td>
<td>118±4</td>
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<tr>
<td>HR, bpm</td>
<td>381±10</td>
<td>383±7</td>
<td>385±16</td>
<td>379±7</td>
</tr>
</tbody>
</table>

AV3V indicates anteroventral third ventricle; BG, blood glucose; Pi, plasma insulin; GIR, glucose infusion rate; MAP, mean arterial pressure; HR, heart rate; and bpm, beats per minute. *P<.05 vs baseline.

Surgical Procedure for Nerve Recording

After 4 weeks of recovery, overnight fasted rats were prepared for nerve recording during euglycemic insulin infusion. Anesthesia was induced with methohexital sodium (Brevital, Eli Lilly & Co, 40 mg/kg IP) and sustained with chloralose (50 mg/kg IV initially, followed by 25 mg/kg per hour IV infusion). The trachea was cannulated, and each rat was allowed to breathe oxygen-enriched air spontaneously. Body temperature was kept near 37.5°C. Arterial pressure was monitored with a low-volume pressure transducer (CP-01, Century Technology Co). Heart rate was recorded from a cardiotachometer (Beckman 9857B). Multifiber recordings of lumbar SNA were obtained as previously described. Briefly, a midline abdominal incision was made and a lumbar sympathetic nerve placed on a bipolar platinum electrode (Cooner Wire Co) and covered with silicone gel (Sil-Gel 604, Wacker-Chemie). Nerve signals were amplified 20 to 100×10^3 and filtered at low- and high-frequency cutoffs of 100 and 1 kHz, respectively, with a preamplifier (model PS11, Grass Instrument Co). The amplified and filtered neurograms were routed to a nerve traffic analyzer (model 700C, University of Iowa Bioengineering, Iowa City) which counted the action potentials that exceeded a threshold voltage set just above the noise level. A counter time bin was set at 1 second so that the impulse frequency for SNA was displayed on a Beckman type RM dynograph eight-channel recorder as the number of spikes collected each second (in hertz) as a time-frequency histogram.

Hyperinsulinemia With Euglycemic Clamp

Regular insulin (Iletin, Eli Lilly; 0.25 U/mL) in 50% rat plasma in isotonic saline was administered through the femoral vein with an infusion pump (model 255, Sage Instruments) at rates of 4.3 and 8.5 μU/min to obtain doses of approximately 0.06 and 0.13 U/h, respectively. Arterial BG levels were measured every 5 minutes before and during insulin infusion with a portable glucometer (Glucometer II, model 5625, Miles Laboratory) that had been calibrated against a YSI Glucose Analyzer (model 27, Yellow Springs Instrument Co). For maintenance of baseline BG, or euglycemia, 50% glucose in sterile water was infused at variable rates through the jugular vein with an adjustable peristaltic pump (Rabbit Peristaltic Pump, Rainin Instrument Co). BG was determined with the YSI Glucose Analyzer, and PI levels were measured with a radioimmunoassay.

Experimental Protocols

The goal of the protocol was to determine the effects of hyperinsulinemia on lumbar SNA in sham-lesioned (sham-insulin, n=8) and AV3V-lesioned (AV3V-insulin, n=8) rats. In control experiments, the vehicle for insulin was infused in sham-lesioned (sham-vehicle, n=10) and AV3V-lesioned (AV3V-vehicle, n=5) rats. In all rats, basal levels of mean arterial pressure (MAP), heart rate, lumbar SNA, BG, and PI were obtained during a 15-minute control period. These parameters were then monitored during 60 minutes of 0.06 U/h followed by 60 minutes of 0.13 U/h insulin in the two insulin-infused groups or by identical volume infusion of vehicle in the two vehicle-infused groups. PI and BG were obtained at the end of the 120-minute infusion period.

Baroreceptor Responses to Nitroglycerin in AV3V-Lesioned and Sham-Lesioned Rats

Protocol

AV3V-lesioned (n=8) and sham-lesioned (n=5) rats were prepared for lumbar nerve recording as described above. Rats were then paralyzed with pancuronium (0.1 mg/100 g body wt; Astra Pharmaceutical Products, Inc) and ventilated at 70 mL/100 g·min. MAP and lumbar SNA were recorded during a single 0.8-mg injection of nitroglycerin (Parke-Davis). Changes in MAP and lumbar SNA were calculated from the difference between baseline activity and the maximum depressor response to nitroglycerin.

Histology

At the end of recording, deeply anesthetized rats were perfused transcardially with physiological saline followed by 10% formalin. Brains were removed and stored in the fixative until frozen sections were taken and stained for Nissl substance with cresyl violet.

Statistical Analysis

Data were analyzed with appropriate single or repeated-measures ANOVA and are presented as mean±SEM. Post hoc comparisons were made with Fisher's least significant difference tests. Differences between groups were considered significant at a value of P<.05.

Results

Verification of Lesion

Lesion placements were verified as previously described. Lesions shared a common area of damage to the periventricular tissue surrounding the optic recess. The lesion consistently destroyed the preoptic-anterior hypothalamic nucleus, the medial preoptic nucleus, and the OVLT. Some bilateral damage was usually present in the medial portion of the medial preoptic nucleus.

Baseline Values

Baseline BG, PI, MAP, and heart rate were equivalent between the two sham-lesioned groups (sham-vehicle and sham-insulin) and the two AV3V-lesioned groups (AV3V-vehicle and AV3V-insulin) (Table).

Responses to Insulin

In the sham-insulin and AV3V-insulin groups, insulin administration produced significant increases in PI, which did not differ between the two groups. BG did not
change significantly. MAP and heart rate were not altered by insulin (Table). Whereas insulin elicited substantial increases in lumbar SNA in sham-insulin rats, the AV3V-insulin group exhibited increases in lumbar SNA that were no greater than increases with vehicle (Figs 1 and 2).

Responses to Vehicle

In the sham-vehicle and AV3V-vehicle groups, vehicle infusion produced small but significant increases in BG, PI, and lumbar SNA, which were similar between the two groups (Table and Fig 2). MAP and HR were not affected by vehicle infusion.

Lumbar SNA Responses to Nitroglycerin

Intravenous administration of nitroglycerin produced comparable maximum depressor responses in sham-lesioned (−82±2 mm Hg) and AV3V-lesioned (−74±7 mm Hg) rats. Because baroreceptor-induced increases in lumbar SNA were also similar between sham-lesioned (+198±42%) and AV3V-lesioned (+169±60%) animals, the increase in lumbar SNA for a given decrease in arterial pressure was not different between the two groups (Fig 3).

Discussion

Stimulatory actions of insulin on plasma norepinephrine levels1,4,5 and SNA have been reported in several experiments.13,20 In agreement with Morgan et al, we demonstrated insulin-induced elevations of lumbar SNA in intact normotensive rats. Although the mechanisms of sympathoexcitation are unclear, several lines of evidence suggest a central neural site of activation.2,8 The current study extends previous findings by demonstrating that increases in SNA to hyperinsulinemia are dependent on the integrity of the AV3V region.

AV3V lesions may abolish sympathetic responses to insulin by several mechanisms. This region, known to be involved in multiple processes that regulate body fluid and electrolyte homeostasis, is also implicated in blood pressure regulation and sympathetic neural outflow.14 An important part of these integrative functions includes chemoreceptive capacities of certain AV3V-related structures. One such structure, the OVLT, monitors blood-borne signals and transmits this information into the CNS.14 Because the OVLT contains specific binding sites for blood-borne insulin,13 this structure may be necessary for CNS responses to insulin. These observations suggest that the OVLT may be involved in activating increases in sympathetic activity to hyperinsulinemia.

Electrolytic lesions of the AV3V destroy both cell bodies and fibers of passage from sites removed from the targeted area. For example, AV3V lesions are likely to destroy fibers or terminals of cells originating in the SFO.20 As with the OVLT, the SFO functions as a blood-chemoreceptive organ and contains insulin-specific binding sites.13,14 These data point to the SFO as another possible target for interactions between insulin and the CNS. In future studies, we will examine the effects of OVLT and SFO lesions on sympathetic responses to euglycemic hyperinsulinemia.

If activated by insulin, the SFO and OVLT may initiate elevations in sympathetic activity through well-defined neural pathways. Electrical stimulation of the AV3V produces vasodilation in the hindquarters and vasoconstriction in the mesenteric and renal beds.21 Neuronal projections mediating these changes descend from the AV3V to innervate the paraventricular nucleus and lateral hypothalamus.22,23 These areas in turn influence activity in the ventrolateral medulla and preganglionic neurons in the intermediolateral cell column.

Although the integrity of the AV3V region is necessary for sympathetic increases to intravenous insulin, the current findings do not rule out the possibility that other CNS control centers are involved in sympathetic responses to hyperinsulinemia. Supporting this possibility, insulin receptor autoradiography studies have demonstrated binding sites for insulin in several brain areas, including the olfactory bulb, the arcuate nucleus of the hypothalamus, and the ventromedial hypothalamus.24 Of particular interest are the hypothalamic nuclei, as Young and Landsberg demonstrated that gold thioglucone lesions of the ventromedial hypothalamus abolished sympathetic responses to feeding and caloric restriction in mice.

Systemically administered insulin produces vasodilation, which may be associated with small decrements in blood pressure.1,7 These vascular effects suggest the possibility that insulin increases sympathetic activity via baroreceptor reflex mechanisms.5,6 To examine the possibility that AV3V lesions abolished sympathetic increases to insulin simply by interrupting the barorecep-

Fig 1. Segments of original records from fasted sham-lesioned rat and fasted anteroventral third ventricle (AV3V)-lesioned rat showing lumbar sympathetic nerve activity (SNA) during baseline and last 4 minutes of 60-minute insulin infusion at 0.13 U/h. During hyperinsulinemia, lumbar SNA increased substantially in sham-lesioned rats but only slightly in AV3V-lesioned rats.

Fig 2. Line graph shows lumbar sympathetic nerve activity (SNA) in fasted rats. Baseline values were taken as 100%, and lumbar SNA responses to insulin and vehicle were expressed as a percentage of baseline level. Vehicle-infused sham-lesioned (Sham-Vehicle) and AV3V-lesioned (AV3V-Vehicle) rats are shown during baseline and after 60 and 120 minutes of vehicle infusion. Insulin-infused sham-lesioned (Sham-Insulin) and AV3V-lesioned (AV3V-Insulin) rats are shown during baseline and after 60 minutes of insulin infusion at 0.06 and 0.13 U/h during euglycemic clamp. Entries are mean±SEM. *P<.01, sham-insulin group vs other groups.
tor reflex, we analyzed lumbar nerve responses to nitroglycerin-induced hypotension in lesioned and sham-lesioned rats. We found that AV3V lesions had no effect on lumbar responses to a given fall in arterial pressure. This finding indicates that (1) the effect of AV3V lesions on SNA responses to insulin is not a nonspecific depressant effect on responses to various sympathoexcitatory stimuli, and (2) there is dissociation of the sympathetic responses to insulin and to baroreceptor perturbations.

In contrast to the present findings, other groups have demonstrated insulin-induced elevations of blood pressure in normotensive and hypertensive rats. Although insulin tended to increase blood pressure in sham-lesioned rats, this elevation did not attain statistical significance. The disparity in results may be explained by the use of anesthesia in the present study compared with conscious experiments in previous studies. Consistent with this possibility, Morgan et al. observed no change in blood pressure after insulin infusion in anesthetized Wistar-Kyoto and spontaneously hypertensive rats.

In conclusion, lesions of the AV3V region abolished increases in lumbar SNA to hyperinsulinemia. This effect was not secondary to alterations of the baroreceptor reflex because lumbar nerve responses to hypotension with nitroglycerin were not altered by the lesion. These data support the concept that insulin increases sympathetic activity via a central mechanism and suggests that structures within or associated with the AV3V region monitor blood-borne insulin levels and activate sympathetic increases in response to hyperinsulinemia.

Acknowledgments

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References


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