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Intracerebroventricular insulin produces nonuniform regional increases in sympathetic nerve activity

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Muntzel, Martin S., Donald A. Morgan, Allyn L. Mark, and Alan Kim Johnson. Intracerebroventricular insulin produces nonuniform regional increases in sympathetic nerve activity. *Am. J. Physiol.* 267 (*Regulatory Integrative Comp. Physiol.* 36): R1350–R1355, 1994.—Insulin has been shown to increase sympathetic nerve activity (SNA). Although it has been proposed that insulin acts within the central nervous system (CNS) to increase sympathetic neural outflow, there is little evidence for direct central neural sympathoexcitatory effects of insulin. To determine whether intracerebroventricular insulin elicits increases in peripheral SNA, we infused insulin (0.1 μU/min, low; 10 μU/min, medium; and 100 μU/min, high doses) or artificial cerebrospinal fluid (aCSF) into the third cerebral ventricle of chloralose-anesthetized Wistar rats while recording lumbar SNA. In separate animals, 10 μU/min of insulin were infused while recording adrenal SNA and renal SNA. Blood glucose and plasma insulin levels did not significantly change during intracerebroventricular infusion of insulin. Lumbar SNA, expressed as percentage of baseline, did not change in rats infused with aCSF (+13% ± 8%) but increased significantly in rats infused with low (+72 ± 17%), medium (+119 ± 30%), and high (+113 ± 25%) doses of insulin ($P < 0.05$). Intracerebroventricular insulin failed to significantly increase adrenal SNA or renal SNA. Blood pressure and heart rate did not change during insulin infusion. The results indicate that administration of insulin into the third cerebral ventricle produces regionally nonuniform increases in sympathetic neural outflow in the absence of changes in blood glucose or plasma insulin.

central nervous system; Wistar rat; lumbar, renal, adrenal sympathetic nerve activity

INSULIN HAS BEEN SHOWN to activate sympathetic neural outflow. Chronic insulin infusion in rats, for example, elevated norepinephrine turnover in heart, liver, and brown adipose tissue (37). In addition, intravenous administration of insulin during euglycemic clamp increased plasma norepinephrine levels in humans and experimental animals (2, 11, 21, 28).

More direct evidence for sympathoexcitatory effects of insulin was provided by nerve recording studies. In normotensive humans, infusion of insulin during euglycemic clamp produced dose-dependent elevations in muscle sympathetic nerve activity (SNA) but not in skin nerve activity (2, 6). Experiments in normotensive rats demonstrated insulin-induced elevations in lumbar SNA but no change in renal SNA or adrenal SNA (23).

Several lines of evidence suggest that insulin may increase sympathetic outflow by altering neuronal activity in the central nervous system (CNS). Pereda et al. (26) found that administration of a low dose of insulin into the carotid arteries of dogs could increase blood pressure before a fall in blood glucose. Furthermore, insulin treatment in the third cerebral ventricle produced a suppression of norepinephrine transport protein mRNA in rat locus ceruleus neurons, suggesting a modulatory role for insulin in CNS noradrenergic pathways (12). Central neural administration of insulin has been shown to alter hypothalamic electrical activity (1), catecholamine turnover (22), and autonomic nervous system function (9, 31). These studies, taken together, suggest that insulin acts in the CNS to modulate sympathetic activity (19).

This study was designed to determine if acute central neural infusion of insulin increases peripheral sympathetic outflow. We infused insulin into the third cerebral ventricle while directly recording multifiber unit activity from the lumbar, renal, and adrenal sympathetic nerves in normotensive rats. It was found that intracerebroventricular infusion of insulin increased lumbar SNA but failed to significantly elevate renal or adrenal nerve activities.

METHODS

Male Wistar rats (n = 50), weighing 280–300 g, were purchased from Simenson Laboratories (Harlan Sprague Dawley, Indianapolis, IN). The animals were caged in groups of three in a temperature-controlled colony room illuminated on a 12:12-h light-dark cycle and were given standard rat chow and tap water ad libitum.

Cannula Surgery

For implantation of a cannula into the third brain ventricle, rats were anesthetized with Equithesin (0.33 ml/100 g body wt) and secured in a Kopf 900 stereotaxic instrument. A 23-gauge stainless steel guide cannula was lowered 10° from the vertical into the third ventricle according to standard stereotaxic procedures. The coordinates with respect to bregma were −1.0 mm anteroposterior, −9.0 mm dorsoventral, and +1.5 mm lateral from the midline. Rats were allowed 1 wk of recovery before testing for cannula patency and position. Cannulas were considered patent in rats that drank >4.0 ml of water within 15 min after a 50-ng (2 μl) injection of carbachol (carbamylcholine chloride, Sigma, St. Louis, MO), a cholinergic receptor agonist.

Surgical Procedure For Nerve Recording

After 1 wk of recovery, rats were prepared for nerve recording during intracerebroventricular insulin. Anesthesia was induced with methohexital sodium (Brevital, Eli Lilly, Indianapolis, IN, 40 mg/kg ip) and sustained with chloralose (50 mg/kg iv initially, followed by 25 $mg \cdot kg^{-1} \cdot h^{-1}$ iv infusion). Polyethylene catheters were inserted into the caudal artery for arterial pressure recording and into the left femoral artery for

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collection of blood samples. The trachea was cannulated, and each rat was allowed to breathe oxygen-enriched air spontaneously. Body temperature was kept near 37.5°C using a temperature-controlled surgical table.

Arterial pressure was monitored using a low-volume pressure transducer (CP-01, Century Technology, Inglewood, CA). The pressure signal was directed to a coupler (Beckman 9853A; Beckman Instruments, Schiller Park, IL) for measurement of mean arterial pressure (MAP) and to a cardiotachometer (Beckman 9857B) for recording of heart rate (HR). Both MAP and HR were continuously displayed on an eight-channel Dynograph recorder (Beckman, type RM).

Multifiber recordings of lumbar SNA were obtained as previously described (23). Briefly, a midline abdominal incision was made and a lumbar sympathetic nerve was located and separated from connective tissue. The nerve was then placed on a bipolar platinum electrode (Cooner Wire, Chatsworth, CA) and covered with silicone gel (Sil-Gel 604, Wacker-Chemic, Munich, Germany). In a separate group of rats, a retroperitoneal incision was made, and sympathetic branches to the left adrenal were isolated and placed on a bipolar platinum electrode and covered with silicone gel for recording of adrenal SNA. With the same retroperitoneal incision, a branch of the sympathetic nerves leading to the left kidney was placed on a second bipolar platinum electrode and covered with silicone gel for recording of renal SNA. Nerve signals were amplified $20–100 \times 10^3$ and filtered at low- and high-frequency cutoffs of 100 and 1,000 Hz, respectively, with a Grass preamplifier (model P511, Quincy, MA). The amplified and filtered neurograms were routed to a nerve traffic analyzer (model 706C, Univ. of Iowa Bioengineering, Iowa City, IA), which counted the action potentials that exceeded a threshold voltage set just above the noise level. A counter time bin was set at 1 s 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 (Hz) as a time-frequency histogram. For each experiment, baseline SNA was set between 40 and 80 Hz.

Experimental Procedure

After surgery, chloralose-anesthetized rats were allowed to stabilize for 30 min before the experimental protocol. Rats to be used for lumbar recording were randomly divided into groups receiving third ventricular infusions of insulin or artificial cerebrospinal fluid (aCSF, $n = 11$). The aCSF was composed of (in mg/100 ml) 752.0 NaCl, 31.0 $KHCO_3$, 20.3 $MgCl_2 \cdot 6H_2O$, 180.0 $NaHCO_3$, 50.0 glucose, and 13.0 $CaCl_2$. Crystalline porcine insulin (100 U/ml, Eli Lilly) was dissolved and diluted in aCSF. This solution was continuously administered using an infusion pump (model 355, Sage Instruments, Cambridge, MA), delivering insulin at a rate of 1 µl/min. Dilutions were made to deliver insulin as 0.1 µU/min ($n = 7$), 10 µU/min ($n = 7$), and 100 µU/min ($n = 6$), in three separate groups of rats.

Rats used for simultaneous renal and adrenal recording were divided into two groups receiving aCSF ($n = 8$) or 10 µU/min insulin ($n = 11$). To test renal and adrenal SNA responses to reflex physiological activation, we gave a subset of the aCSF ($n = 6$)- and insulin ($n = 5$)-treated rats nitroglycerin shortly after the end of the infusion procedure. Nitroglycerin was given to lower arterial pressure and to provoke baroreceptor-mediated sympathetic activation. To do this, we continued chloralose anesthesia, and the animals were paralyzed with pancuronium (0.1 mg/100g body wt; Astra Pharmaceutical Products, Westborough, MA) and ventilated at 70 $ml \cdot 100\ g^{-1} \cdot min^{-1}$. MAP and lumbar SNA were recorded during a single 0.4-mg injection of nitroglycerin (Parke-Davis, Morris Plains, NJ). Maximum decreases in MAP to nitroglycerin were compared with maximum increases in lumbar SNA. To calculate the sympathetic nerve data, we took baseline values of lumbar SNA as 100%, and the following increases in nerve activity to nitroglycerin were expressed as percentage of this baseline value.

Basal levels of MAP, HR, and SNA were obtained during a 10-min baseline period. Insulin or aCSF was then infused into the third cerebroventricle for 50 min. Blood glucose and plasma insulin concentrations were determined at the end of the baseline and experimental periods. Arterial blood samples (300 µl) were tested for blood glucose using a Yellow Springs Instruments glucose analyzer (model 27, Yellow Springs, OH), and remaining blood was centrifuged (5,000 revolutions/min for 5 min). Plasma was removed and analyzed for insulin levels by the method of Yalow and Berson (36). The intra- and interassay variability of the insulin analysis to a known insulin standard was 7.0 and 8.5%, respectively. The red blood cells from the sample were suspended in isotonic saline (100–200 µl) and infused into the rat.

Statistical Analysis

In all studies, on-line acquisition and data analysis were performed using a software program and an IBM-PC. Analog values of MAP, HR, lumbar SNA, renal SNA, and adrenal SNA were digitized on-line each 1 s and then averaged over 60 s to obtain 1-min values during the control and infusion periods.

Comparisons of SNA were performed on three values obtained during the baseline period (4th, 7th, and 10th min) and on five values obtained during infusion (20th, 30th, 40th, 50th, and 60th min). Analysis of variance (ANOVA) tests comparing sympathetic activity during the 4th, 7th, and 10th min of the baseline period in the four lumbar SNA groups failed to show significant differences in baseline activity between experimental groups. Likewise, separate ANOVAs comparing baseline periods (4th, 7th, and 10th min) in the two renal SNA groups and in the two adrenal SNA groups failed to show significant differences between the vehicle- and insulin-treated conditions. Therefore, the 4th-min baseline values of lumbar, renal, and adrenal SNA were taken as 100%, and the following values were expressed as percentage of this baseline level. Comparisons of HR and MAP were performed on values obtained at the end of the baseline period (10th min) and at the end of infusion (60th min).

All data were analyzed using appropriate single or repeated-measures ANOVA and are presented as means ± SE. For the analysis of lumbar SNA, all four experimental groups were compared concurrently within the same ANOVA model. Post hoc comparisons were made using Fisher's least significant difference (LSD) tests when the global F ratio was significant. Differences between groups were considered significant at the $P < 0.05$ level.

RESULTS

Effects of Intracerebroventricular Insulin on Lumbar SNA

Effects of vehicle. In rats assigned to lumbar nerve recording, infusion of the vehicle for insulin (aCSF) had no effects on MAP, HR, lumbar SNA, blood glucose, or plasma insulin concentration (Table 1 and Figs. 1–3).

Responses to insulin. After 50 min of intracerebroventricular infusion, the low, medium, and high doses of insulin failed to increase plasma insulin concentrations (Table 1). The ANOVA for plasma insulin revealed a

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Table 1. *Effects of intracerebroventricular infusion of insulin or aCSF in Wistar rats*

Group	n	Blood Glucose, mg/dl		Plasma Insulin, μU/ml		MAP, mmHg		HR, beats/min	
		Baseline	End infusion	Baseline	End infusion	Baseline	End infusion	Baseline	End infusion
Low-dose insulin	7	82 ± 3	83 ± 4	23 ± 8*	36 ± 5*	132 ± 3	133 ± 5	416 ± 12	414 ± 8
Medium-dose insulin	18	92 ± 5	90 ± 6	48 ± 6	47 ± 6	124 ± 3	122 ± 3	377 ± 18	390 ± 20
High-dose insulin	6	92 ± 4	82 ± 4	61 ± 7	53 ± 5	114 ± 4	115 ± 3	437 ± 15	460 ± 11
aCSF	19	92 ± 4	87 ± 5	50 ± 8	48 ± 7	120 ± 3	115 ± 4	377 ± 18	372 ± 17

Values are means ± SE; aCSF, artificial cerebrospinal fluid; MAP, mean arterial pressure; HR, heart rate. The low-insulin group received 0.1 μU/min insulin, the medium-insulin group received 10 μU/min insulin, and the high-insulin group received 100 μU/min insulin. *P < 0.05 compared with all other groups.

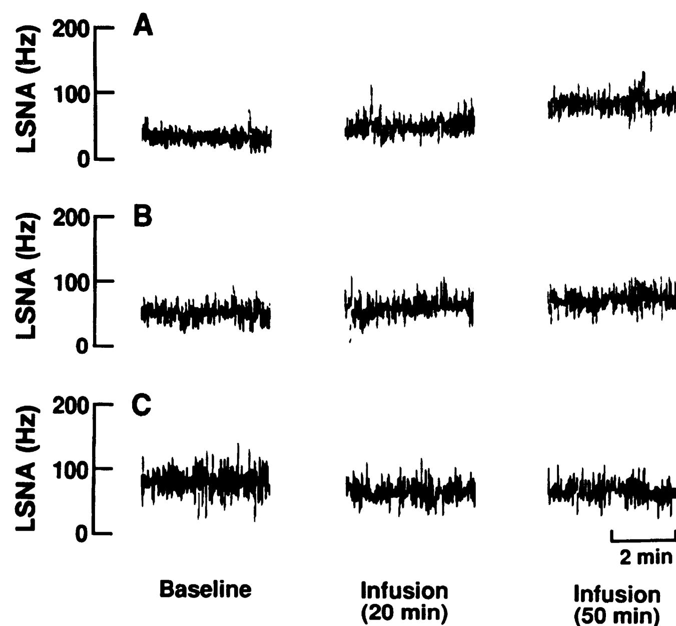

Fig. 1. Segments of original records from three Wistar rats, representing the high dose of insulin (100 μU/min; *A*), the low dose of insulin (0.1 μU/min; *B*), and the vehicle [artificial cerebrospinal fluid (aCSF)] group (*C*). These records show lumbar sympathetic nerve activity (LSNA) during the baseline period, after 20 min of infusion, and after 50 min of infusion. Intracerebroventricular infusion of insulin increased LSNA.

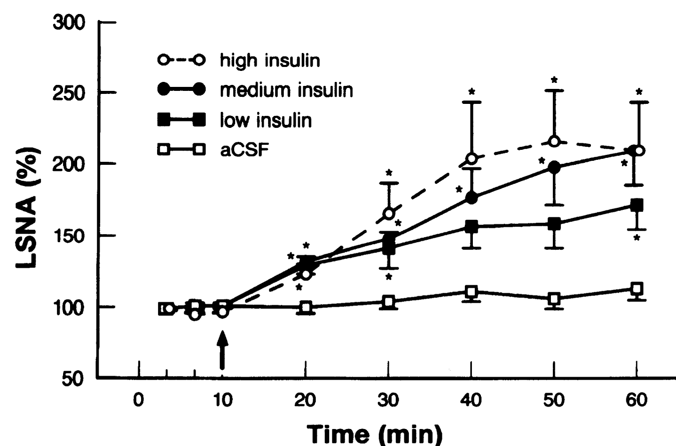

Fig. 2. Lumbar sympathetic nerve responses to intracerebroventricular infusion of vehicle or insulin in Wistar rats. Arrow indicates onset of infusion. Values are means ± SE. *P < 0.05, insulin-infused rats vs. controls.

main effect for group, indicating lower baseline and lower infusion levels in the low-dose group compared with the other experimental groups (P < 0.05). Blood glucose was not significantly altered by insulin infusion.

Whereas infusion of the vehicle had no effect on SNA, intracerebroventricular insulin caused significant increases in lumbar SNA (Figs. 1 and 2). A four (group) by seven (repeated-measures) ANOVA of these data revealed significant main effects for group (P < 0.01) and for repeated measures (P < 0.001), which were explained by a significant interaction between the two factors (P < 0.001). Subsequent analysis of the interaction at each of the seven time points using Fisher's LSD

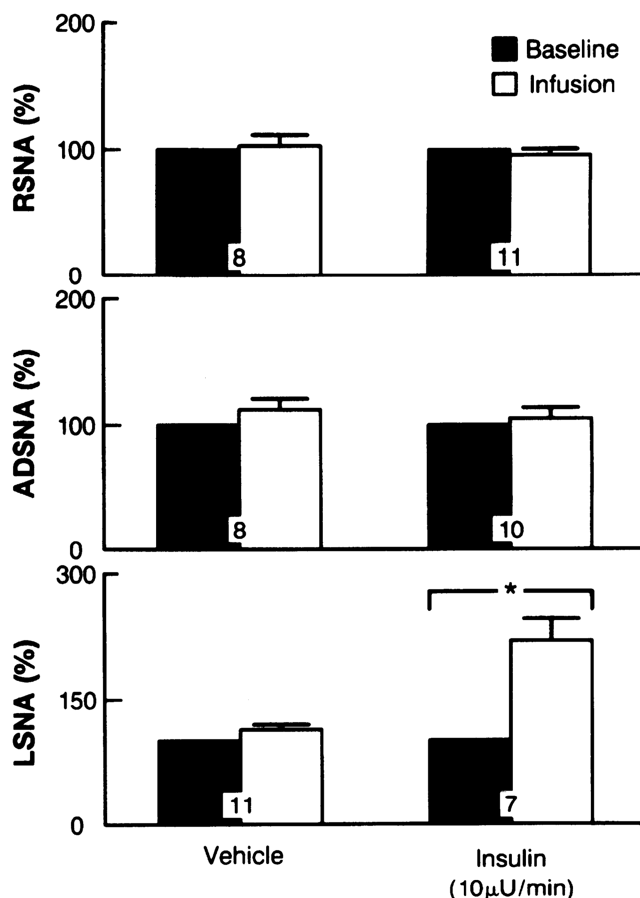

Fig. 3. Renal (RSNA), adrenal (ADSNA), and lumbar sympathetic nerve (LSNA) responses during baseline and after 50 min of intracerebroventricular infusion of vehicle (aCSF) or insulin (10 μU/min). Nos. within bars indicate no. of animals for each group. Entries are means ± SE. *P < 0.01 vs. baseline.

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tests indicated that all insulin groups had significantly higher lumbar SNA than vehicle groups at 20 and 30 min into infusion. In addition, the increase in nerve activity to insulin followed a dose-response relationship. The medium and high doses of insulin produced significant increases compared with vehicle during all measurement periods ($P < 0.05$), whereas increases in lumbar SNA with the low dose of insulin were significant only at the beginning and end of infusion. Insulin infusion did not significantly affect MAP. Although the overall ANOVA revealed a significant main effect for group ($P < 0.05$), these variations were not related to the administration of insulin. There was no significant effect of insulin on HR during the course of the study.

Effects of Intracerebroventricular Insulin on Renal and Adrenal SNA

In separate groups of rats, we observed the effects of aCSF and the medium dose of insulin (10 μU/min) on renal and adrenal SNA. Infusion of insulin had no significant effect on either renal or adrenal SNA (Fig. 3). Because we observed no influence of aCSF or insulin on MAP, HR, blood glucose, or plasma insulin, as in the preceding experiments, these data were pooled with the previous data in Table 1.

Because insulin did not alter renal or adrenal SNA, it was necessary to test whether increases in these sympathetic nerves could be evoked under the present experimental conditions. To do this, a subset of the vehicle- and insulin-infused rats received an injection of nitroglycerin at the end of the infusion procedure. Because the vehicle- and insulin-treated animals did not differ in their responses to nitroglycerin, the data for the two groups were collapsed and are presented together. Intravenous administration of nitroglycerin produced a maximum depressor response of −77.7 ± 6 mmHg associated with baroreceptor-mediated increases in renal SNA (+116 ± 46%) and adrenal SNA (+102 ± 27%).

DISCUSSION

Several lines of evidence indicate that hyperinsulinemia produces sympathetic activation in both humans and in rats (2, 6, 21, 23, 28). Although it has been proposed that insulin acts in the CNS to increase sympathetic outflow (2, 19), a sympathoexcitatory effect of centrally administered insulin has not yet been demonstrated.

In the current studies, we infused insulin into the third cerebral ventricle while recording activity from the lumbar, renal, and adrenal sympathetic nerves. The results demonstrated that centrally administered insulin produced substantial increases in lumbar SNA in the absence of changes in blood glucose or plasma insulin. Although intracerebroventricular insulin failed to alter renal or adrenal SNA, the demonstration of baroreceptor-mediated increases in both of these nerves indicated that elevations in nerve activity could be elicited under the present experimental conditions.

In contrast to our renal sympathetic nerve findings, Nishimura et al. (24) reported decreases in renal SNA after microinjection of insulin into the third cerebral ventricle of urethan-anesthetized rats. The disparity in results may be related to the dose of insulin and the method of administration. Nishimura et al. (24) infused 20 μg of insulin, representing a 100-fold higher dose than the 100 μU/min used in the present study (1 μU = 0.04 ng). In addition, the total dose of insulin was administered over 10 s in the study of Nishimura et al. (24) compared with 50 min of infusion in the current experiments.

The mechanisms of central insulin-induced elevations in lumbar SNA are not clear. It has been shown that insulin and other peptides infused into the cerebroventricular space can reach neuronal loci after passing between ependymal cells or glial processes, to enter the interstices of the underlying cerebral neuropil (8, 10). Injection of labeled insulin into the lateral cerebral ventricles of rats produced heavy staining in regions nearest the third ventricle (5). Additional findings from the same study indicated that uptake of insulin from CSF into periventricular regions was mediated by a saturable transport system (5). Once delivered into neural tissues, insulin may bind with receptors located in several areas of the brain. In vivo and in vitro autoradiographic techniques have identified insulin-specific binding sites in the median eminence (18, 33), the dorsomedial hypothalamus, the arcuate nucleus, and the ventromedial hypothalamus (35). Although a physiological role of central insulin remains to be identified, insulin binding to axonal or synaptic receptors in the CNS influences hypothalamic norepinephrine release (22, 29) and peripheral autonomic function (9, 31). In addition, other studies have demonstrated behavioral responses to intracerebroventricular insulin, including decreases in nighttime food intake with resulting declines in body weight (27).

A possible indirect mechanism underlying the increase in nerve activity includes insulin-induced changes in CNS glucose metabolism. Supporting such a mechanism, glucose deprivation in the CNS generated by intracerebroventricular injection of 2-deoxy-D-glucose (2-DG) increased adrenal SNA in rats (16). However, experiments using cultured neurons and neural tissues labeled with radioactive 2-DG support the traditional view that the brain is not responsive to insulin with respect to glucose uptake and metabolism (13, 15). Furthermore, in a recent study, relatively large doses of intracerebroventricular insulin given to cats did not alter the measured CSF glucose levels (17). Our observation that intracerebroventricular insulin did not increase adrenal SNA supports our conclusion that the effect of insulin to increase lumbar SNA in the present study was not mediated through glucodeprivation. If CNS glucodeprivation had occurred, we would also have expected to observe increases in adrenal SNA.

Although insulin may not directly affect CNS glucose uptake, influences of centrally administered insulin on peripheral metabolism may vary depending on whether it is administered by itself or in combination with glucose. Holt and York (14), for instance, demonstrated inhibitory effects of intracerebroventricular insulin alone

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and stimulatory effects of insulin plus glucose on the activity of sympathetic nerves supplying interscapular brown adipose tissue in rats. In addition, other groups have shown differential effects of third ventricular insulin and glucose on peripheral glucose levels in rats. Steffens and colleagues (30) found that insulin alone increased blood glucose levels, whereas insulin plus glucose attenuated this rise. In apparent contrast, Ono and colleagues (25) indicated that central insulin by itself caused no change in blood glucose, while insulin plus glucose stimulated increases in blood glucose levels.

The present studies do not allow conclusions about the physiological importance of the increased lumbar SNA. For example, our results do not establish whether the increased SNA was associated with increased vascular resistance in the hindlimbs. In conscious humans, intravenous administration of insulin produced sympathetic activation to skeletal muscle (2, 6, 34). However, these increases in sympathetic activity were not accompanied by increased vascular resistance but by unexpected decreases in forearm and calf vascular resistance (2, 34). Indeed, in many of these studies, blood pressure did not change or even decreased despite increases in sympathetic activity to muscle (2, 3, 6, 20, 34). In most of these human studies, insulin infusion was associated with increases in heart rate (2, 4, 6, 34).

In contrast to human experiments, hyperinsulinemia in conscious rats produced increases in both blood pressure and heart rate (7, 11, 32). However, in the only study directly examining SNA, systemic infusion of insulin produced substantial increases in lumbar SNA without causing changes in blood pressure or heart rate (23). The lack of effect of insulin on these cardiovascular parameters may be explained by the use of anesthesia in the present study and in the study by Morgan et al. (23), compared with experiments using conscious animals in previous studies (7, 11, 32).

In summary, central neural administration of insulin increased the firing rate of the lumbar sympathetic nerves in the absence of changes in blood glucose, plasma insulin, heart rate, or mean arterial pressure. Central insulin failed to increase renal or adrenal SNA. These data support the concept that insulin increases SNA via a central neural mechanism and demonstrate that central neural administration of insulin produces regionally nonuniform increases in sympathetic activity.

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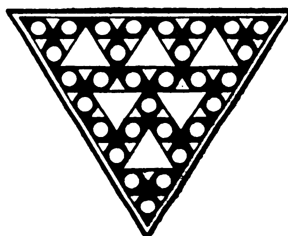

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