

CLIN. AND EXPER. HYPERTENSION, 17(1&2), 39-50 (1995)

IN-52-002  
6/1/95

MECHANISMS OF INSULIN ACTION ON SYMPATHETIC NERVE  
ACTIVITY

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Key Words:

insulin, sympathetic nervous system, central nervous system

ABSTRACT

Insulin resistance and hyperinsulinemia may contribute to the development of arterial hypertension. Although insulin may elevate arterial pressure, in part, through activation of the sympathetic nervous system, the sites and mechanisms of insulin-induced sympathetic excitation remain uncertain. While sympathoexcitation during insulin may be mediated by the baroreflex, or by modulation of norepinephrine release from sympathetic nerve endings, it has been shown repeatedly that insulin increases sympathetic outflow by actions on the central nervous system. Previous studies employing norepinephrine turnover have suggested that insulin causes sympathoexcitation by acting in the hypothalamus. Recent experiments from our laboratory involving direct measurements of regional sympathetic nerve activity have provided further evidence that insulin acts in the central nervous system. For example, administration of insulin into the third cerebralventricle increased lumbar but not renal or adrenal sympathetic nerve

activity in normotensive rats. Interestingly, this pattern of regional sympathetic nerve responses to central neural administration of insulin is similar to that seen with systemic administration of insulin. Further, lesions of the anteroventral third ventricle hypothalamic (AV3V) region abolished increases in sympathetic activity to systemic administration of insulin with euglycemic clamp, suggesting that AV3V-related structures are critical for insulin-induced elevations in sympathetic outflow.

### Introduction

Insulin resistance and hyperinsulinemia are frequently associated with elevated arterial pressure. The coexistence of hyperinsulinemia and hypertension in obesity (1, 2) and essential hypertension (3, 4) has led to the hypothesis that insulin plays a major role in the development of arterial hypertension (5, 6). The prohypertensive actions of insulin include antinatriuresis, trophic effects on vascular smooth muscle, and activation of muscle sympathetic nerve activity (7, 8).

Insulin-induced sympathetic activation has been suggested as a cause of arterial pressure elevation. Infusion of insulin during euglycemic clamp increased plasma norepinephrine levels in experimental animals (9, 10) and in humans (11-14). Sympathoexcitatory effects of insulin were later confirmed in investigations directly recording muscle sympathetic nerve activity. Insulin infusion augmented sympathetic outflow to skeletal muscle in humans (12, 13, 15) and to the hindlimb in normotensive rats (16, 17). The increase in activity appeared to be directed to muscle, since no alterations were observed in skin sympathetic fibers in humans (13), or in renal or adrenal sympathetic activity in normotensive rats (16).

In rats, a pressor role for insulin-induced sympathetic activation has been suggested by several studies. For example, insulin-generated elevations in heart rate and arterial pressure were prevented by ganglionic blockade (18). In addition, Tomiyama and colleagues (9) chronically infused insulin into Dahl salt-sensitive and salt-resistant rats. Insulin increased plasma norepinephrine and arterial pressure in Dahl salt-sensitive rats without affecting either parameter in Dahl salt-resistant animals. Finally, Kaufman et al. (19) reported that a high fat

diet elevated insulin levels, urinary norepinephrine, and arterial pressure in Sprague-Dawley rats.

In contrast to responses in rats, studies in humans have not been able to establish a clear link between sympathoexcitation, increases in vascular resistance and arterial pressure. Acute administration of physiologic concentrations of insulin in normotensive humans elevated sympathetic activity without increasing arterial pressure. In fact, short-term insulin administration typically produced a complex pattern of responses, consisting of increased sympathetic nerve activity, elevated heart rate, skeletal muscle vasodilation, and no change or even slight decrements in blood pressure (12-15, 20).

The failure of acute insulin-induced sympathoexcitation to elevate arterial pressure in humans does not exclude the possibility of long-term sympathetic effects on pressure regulation. For example, both sympathetic stimulation and norepinephrine treatment accelerate growth and polyploidy of vascular smooth muscle cells (21-23). These effects could combine with the vascular trophic influences of insulin (24) to promote structural alterations of arterial vessels, thereby reducing the lumen, amplifying vasoconstrictor responses to contractile agents, and promoting the development of hypertension.

This review will briefly examine the mechanisms of insulin-induced activation of the sympathetic nervous system. While it has been hypothesized that sympathoexcitation to hyperinsulinemia is mediated through stimulation of carbohydrate metabolism, release of norepinephrine from sympathetic fibers, or by the baroreflex, it seems more likely that insulin alters neuronal activity in the central nervous system which causes increases in sympathetic neural outflow.

#### Indirect Mechanisms of Sympathetic Neural Activation

Insulin may influence sympathetic outflow through indirect actions of the hormone on metabolic and cardiovascular regulation. Vollenweider and colleagues (15) proposed that insulin elevates sympathetic activity through stimulation of carbohydrate metabolism and oxidation. To test this hypothesis, Vollenweider et al. infused insulin and fructose into normotensive humans. Whereas insulin and fructose produced comparable increases in carbohydrate

metabolism, only insulin elevated sympathetic nerve activity. Thus, hyperinsulinemia per se and not increased carbohydrate oxidation appears to be the main mechanism triggering sympathetic excitation.

A second indirect mechanism of sympathetic stimulation is related to the vasodilatory effects of infused insulin (7, 20). Insulin-induced vasodilation in skeletal muscle can lead to small decreases in arterial pressure, thereby inducing baroreceptor-mediated increases in sympathetic activity (7, 13-15). However, in several human studies, euglycemic hyperinsulinemia elevated sympathetic activity without decreasing arterial pressure (11, 13, 14, 25). Nevertheless, in such studies, there may have been transient, undetectable decreases in arterial pressure which were sufficient to engage the baroreflex. In contrast to these experiments, we observed small but significant decreases in diastolic blood pressure during infusion of insulin (12). However, the increase in muscle sympathetic nerve activity was larger than would be expected for such a small decrease in diastolic pressure. In addition, the rapid decrease in diastolic pressure did not temporally coincide with the slower increase in muscle sympathetic nerve activity (12). Further disfavoring a baroreceptor mechanism, we recently demonstrated in rats that direct central neural administration of insulin elevated lumbar sympathetic nerve activity in the absence of changes in plasma insulin (26). Because central neural insulin increased sympathetic outflow without altering systemic insulin levels, and presumably in the absence of vasodilation, it appears that insulin can activate sympathetic outflow independently of vasodilation and engagement of the baroreflex.

### Direct Neural Mechanisms

Insulin may evoke sympathetic activation through a number of direct mechanisms. For instance, insulin may affect norepinephrine release from adrenergic nerve endings (9, 18). Countering this possibility, Lembo et al. (14) failed to detect increases in forearm norepinephrine levels during local insulin infusion in humans. The fact that only systemic insulin administration increased norepinephrine release suggested that the sympathoexcitatory effects were mediated by a central neural mechanism (14).

Landsberg (6) and others (11) suggested a sympathoexcitatory role of insulin in the central nervous system. In an early study, a low dose of insulin injected into the carotid arteries of dogs increased blood pressure before a fall in blood glucose (27). A relationship between insulin sensitive regions in the hypothalamus and sympathetic activity was later established in experiments using gold thioglucose in mice (28). In these studies, changes in cardiac norepinephrine turnover during feeding and caloric restriction were abolished by gold thioglucose lesions of the ventromedial hypothalamus, suggesting that hypothalamic regions provide an important link between insulin-mediated glucose metabolism and sympathetic nervous system activity.

Results from euglycemic clamp studies in humans point to a mechanism of insulin delivery into the central nervous system. In these experiments, plasma norepinephrine and sympathetic nerve activity continued to rise even after plasma insulin levels were at a steady state level or declining (11, 12, 15). These data suggest that insulin may have to reach the interstitial space or a nonvascular third compartment to exert its excitatory effects (11, 12, 15). In this regard, Schwartz and colleagues (29) observed a 30 min delay before euglycemic hyperinsulinemia produced a rise in cerebrospinal (CSF) levels of insulin in dogs. The delayed entry of blood-borne insulin into the CSF is best explained by the passage of insulin into the brain interstitial fluid via a saturable, insulin receptor-mediated transport process (30, 31).

Once delivered into central neural tissues, insulin may bind with receptors located in several brain areas. For example, autoradiographic studies have identified insulin-specific binding sites in the median eminence (32, 33), the dorsomedial hypothalamus, the arcuate nucleus, and in the ventromedial hypothalamus (34).

We have been evaluating the sympathetic effects of intracerebroventricular infusion of insulin. In normotensive Sprague-Dawley rats, administration of insulin into the third cerebral ventricle increased lumbar sympathetic nerve activity (26). Interestingly, central neural administration of insulin failed to significantly increase renal or adrenal sympathetic nerve activity (35). This pattern of sympathetic excitation, that is, increased lumbar activity with no change in renal or adrenal outflow, is precisely the same type of activation

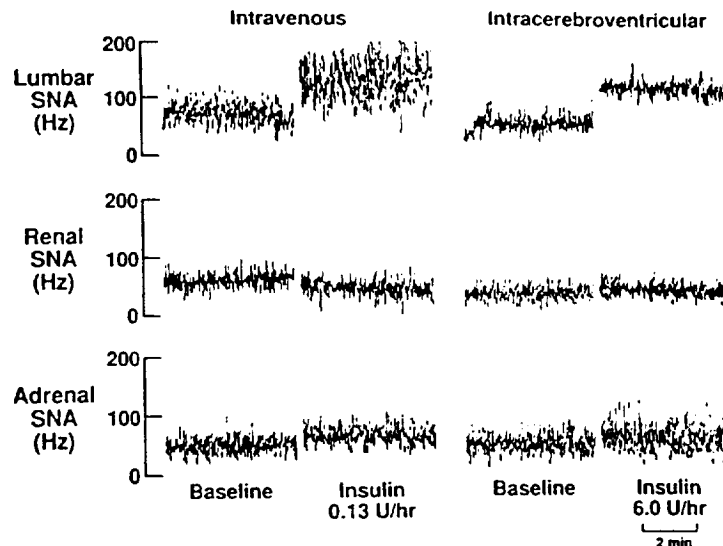


FIG. 1. Segments of original records from normotensive rats showing responses of lumbar, renal, and adrenal sympathetic nerve activity (SNA) to intravenous and intracerebroventricular insulin. The two left columns show SNA during baseline and the last 3 min of 120 min infusions of intravenous insulin (60 min at 0.06 U/hr followed by 60 min at 0.13 U/hr). The two right columns show SNA during baseline and the last 3 min of 50 min infusion of insulin into the third cerebralventricle. Administration of insulin either systemically or into the brain increased lumbar SNA (top) but failed to affect renal SNA (middle) or adrenal SNA (bottom).

observed when insulin is administered systemically during euglycemic clamp (see Figure 1). Thus, direct central neural administration of insulin generates a pattern of sympathetic activation similar to that produced by systemic insulin. These data strongly suggest that hyperinsulinemia produces sympathetic excitation through effects on the central nervous system.

We reasoned that if infusion of insulin into the third cerebralventricle increases lumbar nerve activity, destruction of third ventricular structures should abolish increases in sympathetic outflow to systemic euglycemic hyperinsulinemia (17). To test this hypothesis, we subsequently lesioned tissues surrounding the anteroventral portion of the third ventricle (AV3V), a region clearly implicated in

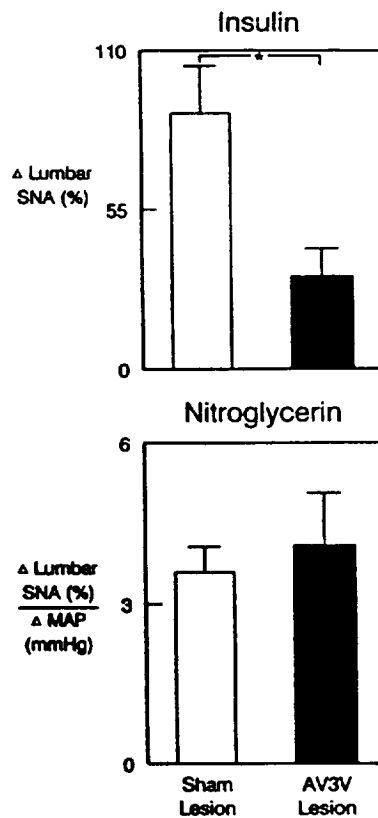


FIG. 2. The top bar graph shows percentage increases in lumbar SNA after 120 min infusions of intravenous insulin (60 min at 0.06 U/hr followed by 60 min at 0.13 U/hr) in sham-lesioned rats and in rats that had received lesions of the anteroventral third ventricle (AV3V) region. During hyperinsulinemia, lumbar SNA increased substantially in sham-lesioned rats but only slightly in AV3V-lesioned rats (\*  $p < 0.01$ , sham lesion vs. AV3V lesion). In fact, sympathetic elevations in AV3V-lesioned rats were no greater than increases observed in vehicle-infused rats (data not shown). The graph on the bottom shows the percent increase in lumbar SNA per maximum decreases in mean arterial pressure (MAP) induced by nitroglycerin. AV3V lesions did not affect baroreceptor-mediated increases in lumbar SNA.

arterial pressure regulation and sympathetic neural control (36). Intravenously administered insulin produced a typical increase in lumbar sympathetic nerve activity in sham-lesioned rats (Figure 2, top). In contrast, AV3V-lesioned rats showed markedly diminished elevations in sympathetic activity to systemic insulin. In fact, the sympathetic increases in the lesioned group were no greater than increases observed in vehicle-infused rats (data not shown). AV3V lesions, therefore, abolished elevations in sympathetic activity to systemic hyperinsulinemia.

We considered that systemic insulin treatment may have produced vasodilation and activation of the baroreceptor reflex. Therefore, AV3V lesions may have reduced sympathetic responses to insulin simply by interrupting part of the baroreceptor reflex arc. To test this possibility, sham-lesioned and AV3V-lesioned animals were analyzed for lumbar responses to nitroglycerin-induced hypotension (Figure 2, bottom). AV3V lesions did not affect increases in sympathetic activity to a given fall in blood pressure. Therefore, destruction of the AV3V region does not produce non-specific depression to sympathoexcitatory stimuli.

In summary, these studies provide important evidence supporting the concept that insulin acts within the central nervous system to increase sympathetic activity. Further, they suggest that AV3V-related structures are critical for insulin-induced elevations in sympathetic outflow. In light of these findings, it should be remembered that AV3V lesions protect against several forms of experimental hypertension (37). Our demonstration of decreased sympathetic activation to insulin in AV3V-lesioned rats suggests a role for the AV3V region in hypertension characterized by insulin resistance and hyperinsulinemia.

**Acknowledgments:** This research was supported in part by a National Research Service Award HL-08704 and National Institutes of Health Grants HL-14338 and HL-44546 from the National Heart, Lung, and Blood Institute, and by research funds from the Department of Veterans Affairs.



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