Insulin-Like Growth Factor–I Decreases Sympathetic Nerve Activity: The Effect Is Modulated by Glycemic Status (44161)

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Abstract. **Insulin-like growth factor–I (IGF-I) has been demonstrated to exert metabolic as well as cardiovascular actions similar to those of insulin. In previous studies, we have demonstrated that IGF-I acts both peripherally and centrally to increase muscle and renal blood flow and to decrease mean arterial pressure (MAP). Insulin has similar cardiovascular actions and has also been demonstrated to increase sympathetic nerve activity that may or may not play a role in blood flow or arterial pressure regulation. In this study, we evaluated the effect of IGF-I on lumbar sympathetic nerve activity (LSNA) and renal sympathetic nerve activity (RSNA), and correlated these responses with MAP and glycemic status. Normal rats were anesthetized with chloralose/urethane, the femoral artery and vein were cannulated to monitor MAP or heart rate (HR), or for infusions. The lumbar sympathetic nerve or renal nerve was isolated and placed on electrodes embedded in silicone gel for recording of nerve activity. Cardiovascular and nerve activity responses to IGF-I were recorded using a Dasy Lab Acquisition System. The infusion of IGF-I resulted in a decrease in plasma glucose that was accompanied by a decrease in MAP and an increased LSNA. However, when IGF-I was infused and euglycemia was maintained by glucose replacement, the LSNA was decreased. Renal nerve recording demonstrated that the RSNA was decreased both when hypoglycemia was allowed to occur and when euglycemia was maintained. We conclude that IGF-I acts to decrease LSNA and RSNA, and that the activity of the LSNA to skeletal muscle can be increased by hypoglycemia.** [P.S.E.B.M. 1997, Vol 216]

Insulin-like growth factor–I (IGF-I) is critical for growth and development of both somatic and sympathetic nervous tissue, as well as having well-defined metabolic actions that include enhancing glucose and amino acid utilization (1–3). IGF-I and its receptor share similar structural homology with insulin and with its receptor (3–5). Studies of the biological responses to IGF-I and insulin have been focused on their metabolic actions to increase glucose utilization, maintain plasma glucose, and regulate protein and fat metabolism (3, 6, 7). IGF-I, because of these similarities, has been proposed as a possible beneficial addition in the treatment of diabetes (8). Recently, we demonstrated that IGF-I like insulin, plays a role in modulating cardiovascular dynamics (9, 10). We have observed that the administration of IGF-I peripherally enhances blood flow dynamics especially in the kidney and also in skeletal muscle (9). We also observed that central (CNS) administration of IGF-I increased skeletal muscle blood flow (10). The net effect of these responses on the cardiovascular system is associated with decreased mean arterial pressure.

It has been proposed that insulin exerts its effects on cardiovascular dynamics by acting both directly at the vasculature and on the nervous system (11, 12). Insulin has been demonstrated to increase sympathetic nerve activity, especially to skeletal muscle (13). It has also been proposed that the increased sympathetic nerve activity stimulated by insulin could counter the direct action of insulin and lead to hypertension (14). This could then be a possible mechanism for the association of hypertension with hyperinsulinemia of NIDDM. Although another possibility could be that the increased sympathetic activity could mediate a dilatory response by β_2 mechanisms (15).

This work was supported by National Institutes of Health Grants GM 08167 and MH 47181.
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Received Febrary 24, 1997. [P.S.E.B.M. 1997, Vol 216]
Accepted May 20, 1997.

0037-9727/97/2161-0093$10.50/0

In light of these functional similarities of IGF-I and insulin, in this study we investigated the effect of IGF-I on sympathetic nerve activity to skeletal muscle, lumbar sympathetic nerve activity (LSNA), and kidney, renal sympathetic nerve activity (RSNA), and correlated these responses with its effect on blood pressure. We also evaluated the role of the glycemic status in this process.

Materials and Methods

Male Wistar rats (Harlan, Indianapolis, IN) weighing 250–350 g were housed two to a cage in a temperature-controlled environment (23°C) with a 12-hr cycle of light and darkness and were given rodent chow and water *ad libitum.*

Fasted (18–24 hr) animals were anesthetized with urethane (500 mg/kg) and α-chloralose (80 mg/kg), and placed on a heating pad; a rectal thermometer was inserted, and body temperature was maintained at 37° ± 1.0°C. A tracheotomy was performed on all animals to minimize respiratory difficulties. Catheters filled with heparinized saline (2000 U/ml) were inserted into the left femoral artery for monitoring the cardiovascular parameters and into the left and right femoral vein for infusions and blood sample collections. The level of anesthesia was maintained by continuous intravenous infusion of 33 mg/ml/hr of α-chloralose and 5.33 mg/ml/hr of urethane.

When indicated, saline or IGF-I (40 μg/animal) was slowly injected into the femoral vein. The injection started after the establishment of stable control recordings (Time 0) and lasted 1.5 min. The total volume of each injection was 0.1 ml/100 g body wt. Mean arterial pressure (MAP), heart rate (HR), and LSNA or RSNA were monitored continuously for 60 min. Blood samples were collected at selected time intervals during the recording period. In other studies, hypoglycemia was prevented (maintenance of euglycemia) by glucose infusion. Glucose was infused first as a bolus (45 mg) followed by a glucose infusion of 150 mg/ml/hr starting at 15 min before IGF-I administration MAP and HR were determined using an acquisition processor and software (Dasy Lab Data Acquisition System Laboratory).

Recording of Sympathetic Nerve Activity. The lumbar sympathetic chain (L3–L5) was exposed through a midline incision. The nerve was dissected and cut peripherally. And the rostral end of the nerve was placed on stainless steel electrodes for recording of efferent nerve activity. Electrodes were constructed of two-stranded stainless steel Tefloncoated wire. Silicone gel (Wacker Sil-Gel 601A and 601B mixture) was used to embed the nerve bundle and electrodes, and allowed 1 hr to dry and harden. Finally, the abdomen was closed to protect evaporation. LSNA was amplified (5,000–10,000 times) and filtered (low at 30 Hz, high at 1000 Hz) using a Grass RPS 107 amplifier and a Grass IR Z probe. The amplified and filtered signal was channeled to an oscilloscope HM205. An audio amplifier-loudspeaker (Grass model AM8 audio monitor) was used for auditory evaluation. Whole nerve activity was obtained by rectifying and integrating the action potentials in 1-sec intervals using data acquisition system. At the end of each experiment, hexamethonium chloride (20 mg/kg), a ganglionic blockade, was used to determine the relative contribution of pre- and postganglionic fibers to LSNA. Approximately 95% of the LSNA is postganglionic activity. Finally, the animal was sacrificed and any residual output from the nerve was subtracted as noise when nerve activity was calculated. In other experiments, the renal sympathetic nerve was exposed through a lateral incision over the kidney. The nerve was dissected out, placed on electrodes, and the subsequent preparation and the recording were carried out as described above.

Since the absolute value of the nerve activity is dependent on the recording conditions (i.e., size of nerve bundle, amount of tissue fluid around the nerve) and these non-physiological factors vary in different preparations, nerve activity data were normalized as a percentage of the baseline nerve activity.

Data are reported as the mean ± SEM. The raw data was analyzed to determine whether several mean values changed as a function of time in the same group using one-way analysis of variance (ANOVA), and two-way ANOVA was used when different groups were compared. Dunnett's test of repeated measures was used to compare data obtained at different time periods with baseline values. Student's *t* test was used to compare pairs of means at selected time points.

Results

The infusion of IGF-I in rats progressively decreased the blood glucose approximately 45% from a basal level of 114 ± 2 mg/dl to a nadir of 61 ± 5 mg/dl after 40 min, and this persisted during the recording period (Table I). IGF-I significantly decreased the mean arterial pressure, by approximately 10%, after 15 min and was significantly lower

Table I. The Effect of IGF-I Infusion on Plasma Glucose with and without Glucose Infusion in Normal Rats

	Plasma glucose			
	0 min	20 min	40 min	60 min
IGF-I (40 μg/animal)	114 ± 6 (6)[a]	65 ± 5 (6)	61 ± 5 (6)	68 ± 7 (6)
IGF-I + glucose infusion (150 mg/ml/hr)	117 ± 3 (6)	112 ± 13[b] (6)	140 ± 7[b] (6)	136 ± 8[b] (6)

[a] Numbers in parentheses = *n*.
[b] $P < 0.01$ versus IGF-I.

than controls for the recording period (Fig. 1A). IGF-I did not significantly alter the heart rate (Fig. 1B). IGF-I significantly increased the lumbar sympathetic nerve activity, to approximately 10% above control levels (Fig. 1C). When IGF-I was infused and the blood glucose was maintained at euglycemic levels, it resulted in a decrease in mean arterial pressure (Fig. 2A) with the heart rate being unchanged (Fig. 2B). However, the LSNA was, in contrast, significantly decreased by IGF-I under euglycemic conditions.

We repeated these same studies and recorded the RSNA. However, unlike what we observed for the LSNA, IGF-I decreased the RSNA when hypoglycemia was allowed to occur (Fig. 3A) and to a lesser degree under euglycemic conditions (Fig. 3B).

Discussion

We have observed that the infusion of IGF-I acts to increase lumbar sympathetic nerve activity, but only when hypoglycemia is allowed to occur, and it actually decreases lumbar sympathetic nerve activity when glycemia is maintained. This action of IGF-I contrasts with that for insulin where insulin acts to increase sympathetic nerve activity to skeletal muscle that appears to be independent of glycemic status (16, 17). The fact that the decreased blood glucose resulting from IGF-I infusion is associated with increased sympathetic nerve activity is consistent with previous observations that demonstrate that glucopenia induced by hypoglycemia or 2-deoxyglucose is associated with increased sympathetic nerve activity (18, 19).

The observation that IGF-I decreases lumbar sympathetic nerve activity when glycemia is maintained may be best explained by a direct effect of IGF-I on sympathetic nerves. It would be expected that the accompanying decrease in MAP would increase the sympathetic activity by the baroreceptor-mediated reflex (20). However, this was not evident, or the response was overshadowed by a direct effect of IGF-I. The proposed mechanism that IGF-I has a direct effect is also supported by the decreased RSNA under both normal and hypoglycemic conditions. The lack of effect of IGF-I on heart rate may be due to the competing effects of IGF-I to decrease heart rate versus an effect of decreased MAP and hypoglycemia to increase it. This

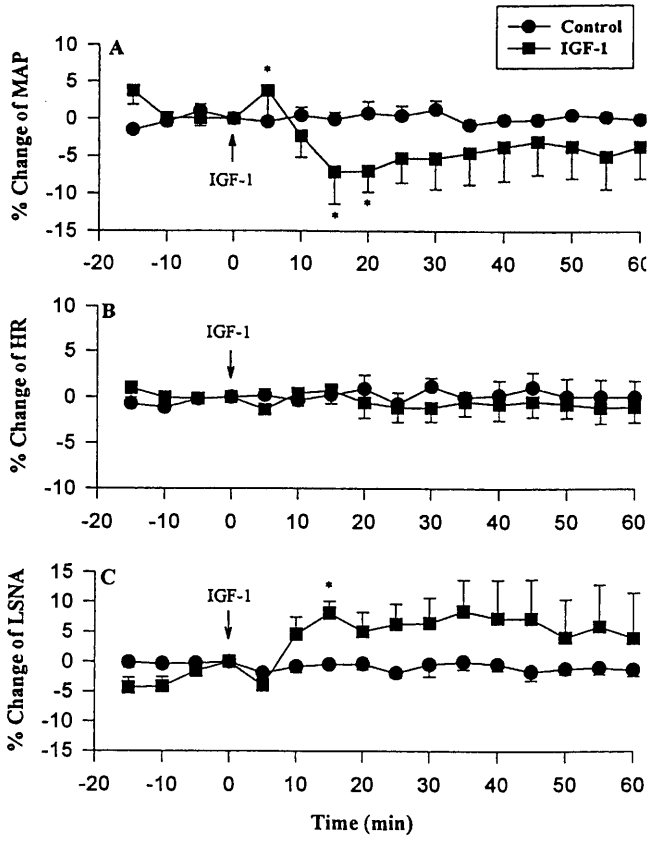

Figure 1. Effects of IGF-I (40 μg/animal) infusion on MAP (A), HR (B), and LSNA (C) in normal animals. The time points selected for evaluation were before (−10), and 0, 5, 10, 15, 30, 45, and 60 min following IGF-I infusion. The MAP was 77 ± 2.1 and 74 ± 2.3, and the HR was 400 ± 19 and 367 ± 16 for control and IGF-I at Time 0, respectively. Data are expressed as mean percent change ± SEM. The significance of the differences was as follows: (A) IGF-I versus control, $P < 0.001$; (C) IGF-I versus control, $P < 0.01$ (ANOVA). $^*P < 0.05$ at selected time points versus controls. $n = 5$ and 6 for control versus IGF-I.

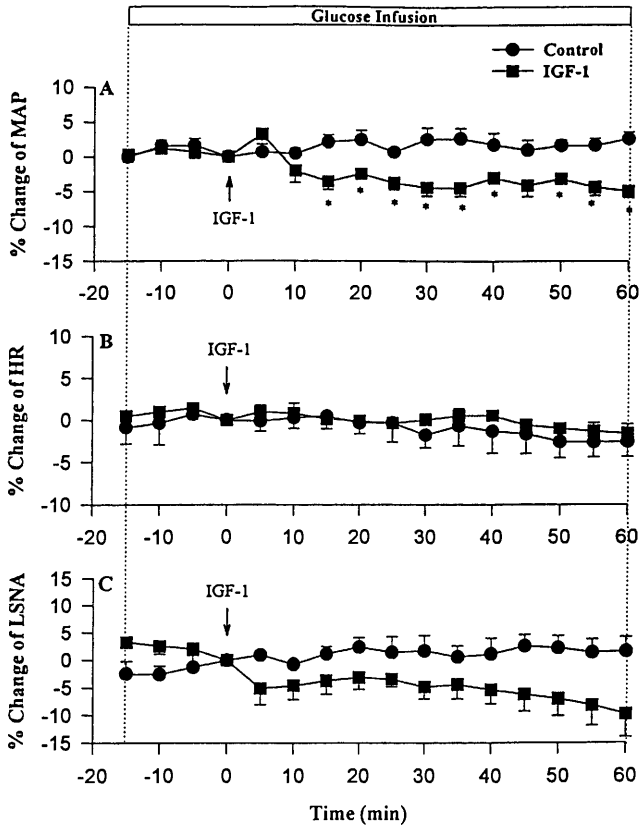

Figure 2. Effects of IGF-I (40 μg/animal) infused in the presence of euglycemia, MAP (A), HR (B), and LSNA (C) in normal animals. The time points that were selected for evaluation were before (−10), and 0, 5, 10, 15, 30, 45, and 60 min following IGF-I infusion. MAP was 74 ± 3.2 and 76 ± 3.3, and the HR was 414 ± 16 and 368 ± 18 for control and IGF-I at Time 0, respectively. Data are expressed as mean percent change ± SEM. (A) IGF-I versus control, $P < 0.001$; (C) IGF-I versus control, $P < 0.001$. $^*P < 0.05$ at selected time points versus controls. $n = 4$ and 6 for controls versus IGF-I.

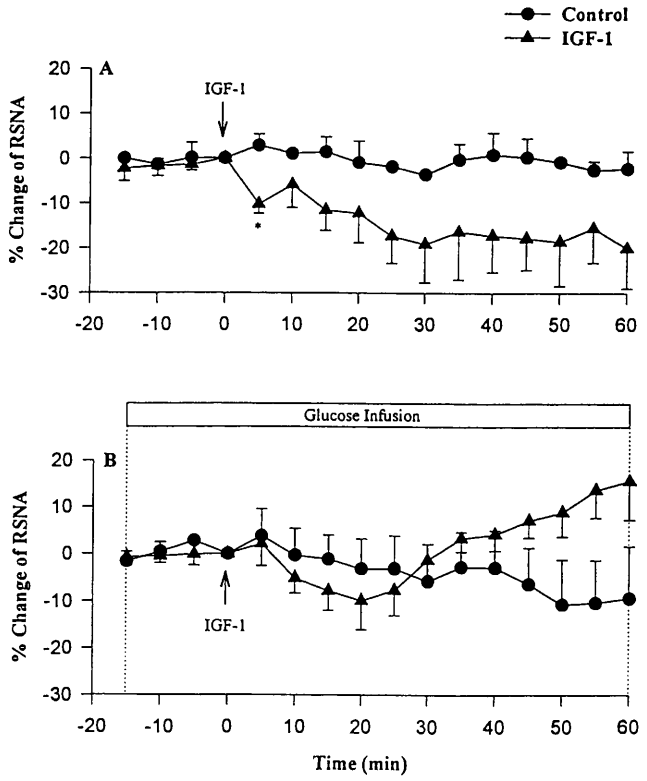

Figure 3. Effects of IGF-I (40 μg/animal) infusion on RSNA in the absence (A) and in the presence of glucose maintenance (B) in normal animals. The time points that were selected for evaluation were before (−10), and 0, 5, 10, 15, 30, 45, and 60 min following IGF-I infusion. Data are expressed as mean percent change ± SEM. (A) IGF-I versus control, $P < 0.001$; (B) IGF-I versus control, $P < 0.05$. *$P < 0.05$ at selected time points versus controls. $n = 4$ and 6 for control versus IGF-I.

would be consistent with previously reported actions of insulin on heart rate (11). We have demonstrated that IGF-I acts to increase blood flow to skeletal muscles and the kidney which leads to a decrease in MAP (9, 10). Thus, it could be that IGF-I, in addition to increasing nitric oxide (NO) production in the vasculature, could act to decrease sympathetic activity in skeletal muscle, thus augmenting its capacity to increase flow (10, 21). Our observation that IGF-I decreases RSNA when hypoglycemia is allowed to occur and when euglycemia is maintained could also support the observation of a relative greater effect of IGF-I to increase renal blood flow (10, 22). One possible explanation for the difference between the capacity of IGF-I and insulin to increase sympathetic nerve activity may be based on the ability of IGF-I to gain access to the central nervous system. Studies have demonstrated that insulin stimulation of the hypothalamic regions of the CNS is necessary for the increased sympathetic nervous as well as the cardiovascular response to insulin (11, 23, 24). Ablation studies by Mark *et al.* (24) demonstrated that areas of the third ventricle mediated this increased sympathetic nervous response. And studies from our laboratory have demonstrated that hypothalamic lesions can modulate the cardiovascular actions of insulin (11). It has been demonstrated that insulin can gain rapid access to the brain, and that it has specific transporters at the blood-brain barriers that facilitate its entry (25, 26). This has not been demonstrated for IGF-I.

IGF-I may directly alter sympathetic neuron metabolism and/or decrease its membrane activity. The action of IGF-I cannot be easily explained by an increase in the tissue metabolism. In previous studies by Vollenweider (27), it has been demonstrated that there was a minimal correlation between tissue metabolism and sympathetic nerve activity.

Nevertheless, the decreased LSNA and RSNA observed with IGF-I would be consistent with the capacity of IGF-I to decrease cardiovascular tone and increase the blood flow to the kidney and skeletal muscle (9, 28). Although much of IGF-I activity at the vasculature has been attributed to NO mechanisms in the endothelial cells, the decreased sympathetic nerve activity may augment directly or indirectly the NO-mediated process. From these studies we conclude that the major action of IGF-I is to decrease LSNA and RSNA, and that hypoglycemia can modulate this process at the skeletal muscle vascular bed.

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