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The Integrative relationship between insulin and insulin-like growth factor 1 induced cardiovascular responses and sympathetic nervous responses

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THE INTEGRATIVE RELATIONSHIP BETWEEN INSULIN AND INSULIN-LIKE GROWTH FACTOR 1 INDUCED CARDIOVASCULAR RESPONSES AND SYMPATHETIC NERVOUS RESPONSES

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INTRODUCTION

Insulin is a crucial hormonal regulator of energy metabolism. Together with counterregulatory hormones such as glucagon, cortisol, growth hormone and epinephrine, insulin modulates the flux of nutrient-derived metabolites into nutrient-storage versus nutrient-mobilizing pathways. When the action of insulin or the secretion of insulin is altered, the disorder diabetes mellitus occurs. Diabetes mellitus is a complex disorder engendered by either absolute insulin deficiency (type I, or insulin-dependent diabetes mellitus, IDDM), or relative insulin deficiency (type II, or non-insulin-dependent diabetes mellitus, NIDDM). It is characterized by alterations in the ability to utilize, store and effectively metabolize carbohydrates, lipids, and proteins. In addition to these metabolic derangements, diabetes also lead to the development of chronic degenerative change in all of the body and in particular the eyes, kidneys, nerves, and blood vessels. The alterations in vascular dynamics result in complications ranging from microvascular disease such as retinopathy, to macrovascular disease including ischemic heart disease.

Insulin resistance is a metabolic state in which a normal or higher concentration of insulin produces a less than normal biological response. Target tissue insulin resistance is a characteristic feature of NIDDM. Insulin resistance has also been found to be a common feature in essential hypertension and obesity. The coexistence of diabetes and hypertension is accompanied by increased risk of cardiovascular disease (Kannel et al., 1970 and 1979). In diabetes, one of many therapeutic challenges is to replace the physiological profile of insulin's action either by strategies to increase insulin sensitivity or in profound insulin deficiency by replacement of the hormone.

Insulin's role in the regulation of cardiovascular dynamics did not receive much early attention, however, it has become clear that insulin has important effects on cardiovascular regulation (Liang et al., 1982, Rowe et al., 1981), such as increasing vascular flow and increasing cardiac contractility. Insulin can act as a cardiotonic depressor, causing vasodilation, and this action plays a role in offsetting pressor actions which includes sympathetic activation and antinatriuretic action in regulating arterial pressure.

The insulin-like growth factors (IGF-1 and IGF-2), are insulin-like both in terms of structural homology and some biological effects (Blundell et al., 1980, Zapf et al., 1986). Although the notion that IGF-1 is critical for normal growth, relatively little attention has been focused on its influence on cardiovascular dynamics in terms of either structure or function. IGF-1 recently has been postulated to initiate, mediate, or modulate cardiac hypertrophy (Ito et al., 1993). And observations from our laboratory and others also suggest links between IGF-1 and the cardiovascular system (Isgaard et al., 1994; Pete et al., 1996).

The focus of this study is to determine the regulatory influence of both insulin and IGF-1 on cardiovascular system and sympathetic nervous system dynamics, and the comparative correlation between these responses.

PART I

Insulin and Insulin Receptors

Insulin was first isolated from pancreatic tissue in 1921 (Banting and Best, 1922). It is a polypeptide (M. W. ~ 5,800) containing two chains (A chain and B chain) of amino acids linked by disulfide bridges. It is secreted by B cells in the islets of Langerhans in the pancreas. The gene for insulin has two introns and three exons. Transcription of this gene gives rise to preproinsulin, which is cleaved by protease activity as it enters the endoplasmic reticulum, and the resultant is proinsulin. Then proinsulin is transported to the Golgi apparatus, and packaged in granules. The subsequent removal of C peptide, which is the peptide segment connecting the A chain and B chain, converts proinsulin to insulin. Finally, insulin is released by the process of exocytosis, in which the secretary granules first move close to and then fuse with the cell membrane, expelling their contents to exterior. Insulin travels through the blood stream to its primary target tissues: fat, muscle, and liver, and promotes the influx of nutrients and antagonize the release of storage forms of energy producing molecules (Birnbaum, 1993). Major target tissues are the liver, where insulin promotes glycogenesis and decreases gluconeogenesis, muscle tissue is a target where insulin also induces glycogen and protein synthesis and adipose tissue where insulin stimulates triglycerides storage.

The physiological effects of insulin are far-reaching and complex. Conveniently, it can be divided into short, intermediate, and long-term processes. Insulin is the principal hormone responsible for metabolic fuel disposal and storage into tissues (DeFronzo et al., 1992). Glucose transport, glycogen breakdown, and glycolysis are the prime example of

short-term actions of insulin. Besides, ion transport (Moore et al., 1983), membrane proliferation, antilipolysis, etc., are also included. In addition, insulin has major actions on cell growth and development (Rosen et al., 1987). Events such as cell growth and differentiation, translational control of protein synthesis are long-term actions of insulin. Intermediate effects of insulin include control of protein turnover and lipid metabolism. Several early reports suggested that insulin also has actions on more integrated systems especially the cardiovascular system (Liang et al., 1982, James et al., 1986, Milley et al., 1987). This more systemic effect of insulin has been a focus of our laboratory (Schltz-Klarr et al., 1994a and 1994b; Wright-Richey et al., 1994).

Like all peptide hormones, insulin initiates its action by binding to a cell surface receptor. Insulin receptors are found on various cells in the body, including both the classical target tissues of insulin (liver, muscle and fat) and nonclassical targets such as circulating blood cells, and brain (McCain, 1991; Yamaguchi et al., 1991). Insulin receptor consists of two alpha (MW, ~ 130 kDa) and two beta (MW, ~ 95 kDa) glycoprotein subunits. The alpha subunits bind insulin and are extracellular, whereas the beta subunits span the cell membrane. The intracellular ends of the beta subunits have tyrosine kinase activity. Binding of insulin to the alpha subunits activates the tyrosine kinase, producing autophosphorylation of the beta subunits on tyrosine residues (White et al., 1988). This autophosphorylation is necessary for insulin to exert its biological effects. Naturally occurring mutations of the insulin receptor that inhibit kinase activity are associated with severe insulin resistance (Odawara et al., 1989).

Insulin Action on Cardiovascular System

Early clinical reports indicated that insulin administration caused hypotension in non-diabetic subjects (Page et al., 1976a, Page et al., 1976b), suggesting a vasodilator effect of insulin. However, since these episodes were associated with hypoglycemia, the effect of insulin per se, could not be distinguished from those produced by counterrgulatory hormone release (Schltz-Klarr et al., 1994b; Wright-Richey et al., 1994). By using the hyperinsulinemic euglycemic clamp (DeFronzo et al., 1979), it has been confirmed that insulin, when administered in physiological concentrations, causes vasodilation and this effect is independent of significant changes in glycemia (Liang et al., 1982; Porcellati et al., 1993).

Baron et al. have conducted studies and confirmed insulin's abilities to increase skeletal muscle blood flow (Laakso et al., 1990, Laakso et al., 1992), and several other groups have also confirmed this vascular action of insulin (Steinberg, et al., 1994; Baron et al., 1994; Lembo et al., 1995). Physiological insulin concentrations also lead to significant increments in cardiac output (Baron and Brechtel, 1993). But both of the insulin's abilities to dilate vasculature and elevate cardiac output are impaired in insulin-resistance states, such as obesity and NIDDM (Laakso et al., 1992, Baron et al., 1991). Defective vasodilation may potentially contribute to insulin resistance and the increased prevalence of hypertension observed in states of insulin resistance.

Recent investigations has been conducted to evaluate the cardiac and regional blood flow in response to insulin. These studies demonstrated that insulin increased blood flow in the iliac bed but was not very effective in the superior mesenteric and renal vascular beds in normal rats (Pete, 1996). This indicated that insulin has differential

effects on different vessels, and can selectively dilate vascular beds and thus decrease blood pressure.

Experiments also showed that acute insulin or insulin induced hypoglycemia decreases cardiovascular tone in normal rats (Schltz-Klarr et al., 1994a and 1994b). Glucose-induced hyperglycemia also decreases cardiovascular tone. In contrast, 2-DG-induced glucopenia increases cardiovascular tone.

The mechanism of the alteration of vascular tone by insulin is still not completely known. Some potential mechanisms include i) the release of endothelium-derived nitric oxide (Steinberg et al., 1993), ii) sympathetic neural vasodilation (Davisson et al., 1996), iii) an indirect effect coupled to metabolic activity such as oxygen consumption (McKay and Hester, 1996), and glucose transport (Sowers, 1997), iv) stimulation of the Na^+-K^+-ATPase with hyperpolarization of vascular muscle (McKay and Hester, 1996, Nichols and Lederer, 1991; Baron and Steinberg, 1996).

The observation that acetylcholine induced vasodilation occurred only when an intact endothelium was present, suggested that endothelium may plays a role in the control of vascular tone. Many studies have provided evidence that acute increases in limb blood flow caused by hyperinsulinemia are mediated by endothelium-derived nitric oxide (EDNO) (Steinberg et al., 1993; McKay and Hester, 1996; Dunbar et al., 1996). These studies reported that the nitric oxide synthase (NOS) inhibitor N^w-nitro-L-arginine methyl ester (L-NAME) attenuated or abolished hyperinsulinemia-induced increases of blood flows or arteriolar diameters in skeletal muscles. EDNO vasodilates by diffusion to the vascular smooth muscle cells where it stimulates guanylate cyclase and generates cGMP, which leads to reduction of intracellular calcium (Moncada and Higgs, 1993).

Okamura et al. has demonstrated that perivascular nerves contain NOS and these nitroxidergic nerves innervate peripheral small arteries and arterioles, causing vasodilation. NO acts as a neurotransmitter in the vasodilator nerves. Their study suggested that NO derived from both perivascular nerves and endothelium mediates reduced vascular resistance (Okamura et al., 1996).

Insulin has been shown to increase sympathetic nerve activity. Hyperinsulinemia increases both plasma norepinephrine and muscle sympathetic nerve activity in the absence of hypoglycemia (Rowe et al., 1981, Anderson et al., 1991). Morgan et al. (1993) have reported that hyperinsulinemia with euglycemic clamp produced nonuniform increases in sympathetic nerve activity to different regions. Hyperinsulinemia increased lumber sympathetic nerve activity but not renal and adrenal sympathetic nerve activity in normotensive rats (Morgan et al., 1993). In humans, there were observations that hyperinsulinemic clamp causes increase in sympathetic nerve activity to muscle but not to skin (Berne et al., 1992). It has been suggested that the fall in hindquarter resistance may result from activation of a sympathetic neurogenic vasodilator system. And this sympathetic neurogenic vasodilation may be mediated by the release of preformed stores of NO-containing factors from the vascular endothelium or postganglionic sympathetic nerves (Davisson et al., 1996).

Insulin increases skeletal muscle oxygen consumption, which could produce metabolic vasodilation. Adenosine, one of the metabolic by-products, is a potent vasodilator and has been suggested as a likely mediator of metabolic vasodilation. Insulin stimulates glucose transport in cardiovascular tissue. Glucose transport in vascular smooth muscle cells is involved in vascular tone regulation. The decrease of insulin-

induced glucose uptake in vascular smooth muscle cells may cause the decrease in insulin's ability to stimulate endothelium nitric oxide production.

It is thought that a major cellular system mediating smooth muscle relaxation is the regulation of Na^+-K^+-ATPase. The activation of Na^+-K^+-ATPase causes cellular hyperpolarization and a decrease in intracellular calcium concentration, leading to vasodilation (McKay and Hester, 1996). Besides, insulin itself is known to stimulate Na^+-K^+-ATPase activity (Prakash et al, 1992), thus insulin could vasodilate directly by hyperpolarizing vascular smooth muscle cells, reducing calcium influx. Recent studies have shown that insulin enhances the gene expression and activity of the vascular smooth muscle cell Na^+-K^+-ATPase, and the insulin-induced EDNO may also stimulate Na^+-K^+-ATPase activity (Tirupattur et al., 1993; Sowers and Eptstein, 1995).

Studies in our laboratory have investigated the interrelationships between glucose, insulin, blood pressure and vasculature in normal rats. To determine the possibility that insulin and/or glucose may directly or indirectly modulate cardiovascular tone, plasma glucose and cardiovascular responses were measured after intracerebroventricular (ICV) or systemic administration of insulin, 2-deoxyglucose (2-DG) and glucose (Schultz-Klarr et al., 1994). Insulin decreased mean arterial pressure (MAP) after both systemic and central administration and ICV insulin decreased heart rate. 2-DG, a competitive inhibitor of glucose utilization, was used to differentiate the effects of insulin from those of insulin-induced glucopenia. The peripheral administration of 2-DG slightly increased MAP and heart rate, while the ICV administration decreased MAP and heart rate. Glucose decreased MAP and heart rate in both ICV and peripheral administration. Investigations by our laboratory as well as others demonstrated similar results when insulin was infused

peripherally in rats and dogs (Wright-Richey et al., 1994; Biggers et al., 1988). In addition, our observations indicated that increases in insulin and glucose concentration in the central nervous system (CNS) may have a direct effect on autonomic outflow, causing decreases in MAP and heart rate. Also CNS glucopenia induced by 2-DG can modify the autonomic control of cardiovascular tone by suppressed sympathetic or increased parasympathetic outflow. These results are supportive to the previous proposal that insulin may directly affect central regulation of cardiovascular responses.

To further investigate the role of insulin on CNS control of cardiovascular system, insulin was infused into normal and ventral medial hypothalamus (VMH) lesioned rats. The VMH is a sympathetic activating center known to regulate blood glucose concentration. Insulin significantly decreased the plasma glucose and the blood pressure in control studies, while in 1-week-old VMH-lesioned rats, the cardiovascular responses did not differ from the controls, but 6-week-old lesion of the VMH attenuated the insulin-induced depression in cardiovascular activity and enhanced the sympathetic response. This lead the conclusion that the VMH is not the primary regulator of insulin-induced cardiovascular responses (Wright-Richey et al., 1994).

Effects of Insulin on Sympathetic Nervous System

There is compelling evidence that insulin exerts powerful excitatory effects on the sympathetic nervous system in both humans (Anderson et at., 1991, Rowe et al., 1981, Berne et al., 1992, Lembo et al., 1992, Vollenweider et al., 1993) and experimental animals (Tomiyama et al., 1992, Liang et al., 1982, Morgan et al., 1993; Muntzel et al., 1994). Early in 1977, Young and Landsberg reported that both insulin and sympathetic

nerve activity decreased during fasting, but overfeeding stimulated peripheral sympathetic activity, implying a specific role of insulin in this response. Since then, additional studies have demonstrated that insulin infusion during a euglycemic clamp increased plasma norepinepherine levels or norepinepherine turnover rates (Liang et al., 1982, Anderson et at., 1991, Rowe et al., 1981, Berne et al., 1992). Later on, insulin's sympathoexcitatory effects were confirmed in experiments directly recording of regional sympathetic neural outflow (Berne et al., 1992, Morgan et al., 1993). Microelectrode nerve recordings in healthy humans have indicated that hyperinsulinemia produced dose-dependent sympathetic activation to muscle but not to skin (Berne et al., 1992). Morgan et al. (1993) have reported that hyperinsulinemic euglycemic clamp promoted nonuniform sympathetic activation in rats, only lumbar sympathetic nerve activity which is directed mainly to hindlimb muscle was increased, neither renal sympathetic nerve nor adrenal sympathetic nerve activity significant increased in normotensive rats.

It is still uncertain what the mechanism(s) are for the insulin-induced activation of the sympathetic nervous system. It has been hypothesized that insulin may increase sympathetic nerve activity by activation of carbohydrate metabolism (Vollenweider et al., 1993), release of norepinepherine from sympathetic nerve endings (Tomiyama et al., 1992, Edwards and Tipton, 1989), or by baroreflex (Anderson and Mark, 1993, Berne et al., 1992, Lembo et al., 1992). Besides, increasing evidence suggests that most likely insulin increases sympathetic outflow by altering neuronal activity in the central nervous system. Insulin infusion in the third cerebral ventricle suppressed norepinephrine transporter mRNA in rat locus coeruleus (Figlewicz et al., 1993). Central insulin administration has also been shown to alter catecholamine turnover and autonomic nervous system function

(McCaleb et al., 1979, Chowers et al., 1966, Taborsky and Bergman, 1980). Young and Landsberg (1980) have demonstrated that changes in cardiac norepinephrine turnover during feeding and caloric restriction were abolished by lesions of the ventromedial hypothalamus. Furthermore, it has been observed that insulin infusion into the third cerebral ventricle in normotensive rats, resulted in increased lumbar sympathetic nerve activity, but no change in renal and adrenal sympathetic nerve activities (Muntzel et al., 1994 b). This sympathoexcitatory pattern is similar to what was observed when insulin was administered systemically. In contrast, Porter (1994) reported that in conscious rats, intrahypothalamic insulin administration with euglycemia produced renal sympathoinhibition, while the renal sympathetic nerve activity of urethan-anesthesized rats increased. In follow-up studies, tissues surrounding the third cerebral ventricle (AV3V) were destroyed, and elevations in sympathetic nerve activity to systemic insulin were markedly diminished (Muntzel et al., 1995), strongly supporting insulin's central stimulatory effect. AV3V is a region involving in blood pressure regulation and sympathetic neural control (Johnson and Gross, 1993). To further specify the sites through which insulin acts, sympathetic nervous responses to insulin were investigated in subfornical organ (SFO) - lesioned rats. SFO, a chemoreceptive circumventricular organ, contains high concentrations of insulin-specific binding sites (van Houten et al., 1979, Unger et al., 1991), sends efferent fibers through the AV3V (Lind and Johnson, 1982), and functions to mediate the regulation of body fluid balance and arterial blood pressure (Johnson and Gross, 1993). It was found that SFO lesions had no effect on sympathetic nervous responses to insulin, indicating that SFO area and fibers derived from the SFO are not essential in mediating increases in sympathetic activity to euglycemic hyperinsulinemia

(Muntzel et al., 1997). Taken together, these studies suggest a modulatory role of insulin in central nervous system autonomic outflow, and hypothalamic regions is involved in the insulin-mediated sympathetic nervous system activity.

Insulin resistance and hyperinsulinemia are frequently associated with hypertension. Some experimental data suggested that insulin could have contradictory cardiovascular effects. In rats, a dose-dependent relationship between increase in insulin and increase in arterial pressure was observed (Edwards and Tipton, 1989). Chronic insulin infusions elevated blood pressure in rats (Hwang et al., 1987, Kaufman et al., 1991, Brands et al., 1991, Moreau et al., 1995), but failed to increase blood pressure in dogs (Hall et al., 1990). In addition, considerable evidence indicated significant increases in blood pressure with weight gain, and the pressure is lowered with subsequent weight loss (Reisen et al. 1978, Sowers et al. 1982, Rocchini et al. 1989a, Rocchini et al. 1989b, Hall et al. 1993). Obesity is closely associated with insulin resistance and hyperinsulinemia, which ied to speculation that insulin may link obesity and hypertension (Chiang et al. 1969, Dustan 1983, Rocchini et al. 1989b, Hall et al. 1993). One proposed mechanism of obese hypertension is the decrease in renal sodium excretory capability and increased tubular sodium reabsorption. Sympathetic activation has been suggested as another cause of arterial pressure elevation (Brands et al. 1995). Recently, in the studies using N^{w}-nitro-L-arginine methyl ester induced hypertensive rats, Sander et al (1997) reported that the sympathetic nervous system was important in the maintenance but not initiation of the hypertension. Sympathetic nervous stimulation has now been unequivocally demonstrated in a range of cardiovascular disorders. However, a large amount of accumulated evidence has shown that hyperinsulinemia per se does not result in elevation of blood pressure

(Porcellati et al., 1993, Anderson et al., 1991, Schultz-Klarr et al., 1994, Brands et al. 1995). Therefore, in insulin resistant conditions, such as essential hypertension, the impairment of insulin's ability to cause vasodilation, or decrease of peripheral resistance may attribute to the elevation of blood pressure.

PART II

Insulin-like Growth Factors and Receptors

IGF-1 and IGF-2 are two polypeptide growth factors that have insulin-like activities and play a major role in the growth and development of humans and other vertebrates (Sara and Hall, 1990, Daughaday and Rotwein, 1989). They were initially discovered as somatomedins which mediated growth-like effects of growth hormone by McConaghey and Sledge in 1970. The complete structures of IGFs were first elucidated by Rinderknecht and Humbel (1978a and 1978b). Both IGF-1 and IGF-2 are single-chain molecules (MW, ~ 7.6 kDa) with three disulfide bridges. Comparison of the primary sequences of the IGFs allows recognition of A, B, and C domains, as in proinsulin. In addition the IGFs have a D domain, not present in proinsulin. IGF-1 shares approximately 45% sequence homology with the A and B chains of insulin, suggesting that IGFs and insulin may have evolved from a common precursor gene. In response to growth hormone, IGF-1 is produced by liver, and plays a key mediator in its physiological responses. IGF-1 synthesis is supported by insulin and reduced by fasting. The liver, although the major site, is not the only site of IGF-1 synthesis and secretion. In fact, a number of peripheral tissues as well as the central nervous system can locally synthesize this hormone. And locally produced IGF-1 may exert physiological effects in an autocrine and paracrine fashion. IGF-1 has been implicated in a wide variety of trophic and metabolic actions in tissues.

Only very small amounts of the IGFs are present in free form. In circulation, as well as in tissues extracts and extracellular fluids, the IGFs are complexed to carrier

proteins, the so called IGF binding proteins (IGFBPs). At least six classes of IGFBPs have been fully characterized with respect to their cDNA sequences, biochemical properties and structures, designated IGFBP1-6.

IGFBP-1, a low molecular weight IGFBP present in limited amounts in plasma, is the predominant IGFBP in amniotic fluid and fetal plasma (Nonoshita et al., 1994). Its regulation is often associated with metabolism and reproduction (Cotterill et al., 1988, Brismar et al., 1988, Lee et al., 1993, Adashi, 1994). IGFBP-1 is strongly expressed in liver and kidney, while IGFBP-2 is strongly expressed in the central nervous system and is the major IGFBP in cerebrospinal fluid (Lee et al., 1993, Lamson et al., 1989). IGFBP-3 has the most complex distribution. In the plasma of adult humans and higher vertebrates, the most abundant IGFBP is IGFBP-3. An acid-labile subunit (ASL) binds IGFBP-3-IGF complexes to form the 150 kDa ternary complex. The predominant 150 kDa complex carries 75% of the IGF-1 and IGF-2 in normal adult human and rat plasma (Daughaday et al., 1982, Zapf et al., 1990). The hepatic synthesis is the most important contributor to circulating levels of IGFBP-3 (Chin et al., 1994). In human, IGFBP-3 increased rapidly after birth, and threefold from birth to puberty. It was positively correlated with growth hormone levels (Baxter and Martin, 1986, 1989; Blum and Ranke, 1990). There is a smaller complex in plasma (40-50 kDa) contains IGF-1 or IGF-2 bound to IGFBP-1, IGFBP-2, or IGFBP-4. It may also contain some IGFBP-3. IGFBP-4 is the smallest of the six IGFBPs. IGFBP4 mRNA is expressed in a large variety of tissues, with the liver having the highest level of expression (Ceda et al., 1991). IGFBP-5 mRNA is also expressed in a number of tissues. The most abundantly expressed IGFBP in the kidney is IGFBP-5 (Shimasaki and Ling, 1991). IGFBP-5 is the predominant IGFBP in bone

extracts and it unique among the IGFBPs in that it binds to bone cells via its strong affinity for hydroxyapatite (Mohan et al., 1994). The more recently isolated and cloned IGFBP, IGFBP-6, has the highest expression in lung and heart, and a very low expression in liver (Shimasaki et al., 1991). Differently from the other IGFBPs, IGFBP-6 has a markedly high affinity for IGF-2 than for IGF-1 (Roghani et al., 1991).

The IGFBPs are thought to be important modulators of IGF action on target cells. Under appropriate experimental conditions, particular IGFBPs may inhibit or potentiate IGF actions. One function of IGFBPs is to buffer the effective concentrations of IGFs, thus prolonging the biological half-life of the IGFs. For example, the 150 kDa complex retains IGF-1 and IGF-2 within the vascular compartment, buffering their insulin-like activities. And the half-life of infused radio-labeled IGF-1 is substantially prolonged in human plasma (12-15 hours), contrasted with 10-12 minutes for free IGF-1 (Guler et al., 1989). On the other hand, IGFBPs delay and blunt the biological effects of IGFs by slowly releasing only small amounts of free IGFs (Rechler and Nissy, 1990, Sara and Hall, 1990). The IGFBPs may limit efflux of growth factors from the vascular space, limit access to receptors on cell surfaces, or target directly the growth factor to surface sites of action (Robert and Clemmons, 1992). The predominant effect of adding purified IGFBP 1-6 to cells *in ivtro* is inhibition of the biological activity of exogenous or endogenous IGFs. However, the cellular actions of IGFBP on *in vivo* IGF-mediated functions have been reported to be both inhibitory and stimulatory. All IGFBPs except for IGFBP-4 have also been reported to potentiate IGF action in some cell systems (Koistinen et al., 1990; De Mellow and Baxter, 1988; Conover et al., 1990; Bautista et al., 1991; Andress and Birnbaum, 1991). Although the mechanism by which IGFBPs potentiates IGFs action has

not been precisely defined, cell association, multimerization, phosphorylation, proteolysis, proteoglycans, etc., may be involved (Busby et al., 1988; Clemmons 1989; Frost and Tseng, 1991; Baxter, 1990; Conover 1991). In addition, there are new notions of IGFBPs that they may indeed have actions independent of IGFs (Oh et al., 1993).

The cellular actions of IGFs are mediated by two types of receptors: the IGF-1 receptor and the IGF-2 (mannose-6-phosphate) receptor. The IGF-1 receptor is a heterotetrameric transmembrane glycoprotein made up of two α-subunits and two β-subunits. The α-subunits (MW, 135 kDa) contain a N-terminal cystein rich domain thought to be involved in the high affinity ligand binding activity (Waugh et al., 1989). The β-subunits (MW, 95 kDa) contain the membrane spanning regions and exert tyrosine kinase activity, showing a high degree of similarity to insulin receptor (White et al., 1988). Overall sequence identity between insulin and IGF-1 receptor varies from 50% to 60% and 84% in the tyrosine kinase domains (Czech, 1989).

Due to the similarities in ligand and receptor structures, it is not surprising that both insulin and IGF-1 can cross-react with each other's receptor. The IGFs are capable of binding to the insulin receptor but with lower affinities (10^{-8} mol/l). Most of the well-known biological effects of the IGFs are mediated via the IGF-1 receptor or alternatively use the insulin receptor as the signal-transducing molecule. The IGF-1 receptor binds IGF-1 with high affinity (10^{-10} mol/l) and IGF-2 and insulin with considerably lower affinities (approximately 10-fold and 100-fold lower affinities, respectively). By contrast, the insulin receptor binds insulin with high affinity (10^{-10} mol/l) and a 100- to 500-fold lower affinity for IGF-1 (Anderson et al., 1990; Kjeldsen et al., 1991).

The distribution of insulin and IGF-1 receptors are considerably different in rodent

and human tissue. Skeletal muscle carries both insulin and IGF-1 receptors. But hepatocytes and adipocytes are the only classical target tissues for insulin, they lack functional receptors for IGF (Froesch et al., 1995).

IGF-2 receptor is a large glycoprotein, structurally unrelated to insulin receptor and does not contain tyrosine kinase activity. It is a bifunctional binding protein that binds IGF-2 and lysosomal enzymes bearing the mannose-6-phosphate recognition marker at distinct binding sites. It plays a role in targeting lysosomal enzymes to lysosomes, but the physiological role of IGF-2 binding to their receptors remains a mystery (Nissley and Lopaczynski, 1991; Kiess et al., 1994).

IGF-1 Action on Cardiovascular System

The IGFs, more specifically IGF-1, were originally characterized based on their ability to mediate the growth stimulating action of growth hormone. More recently, additional roles for IGF-1 have been proposed. These include both acute metabolic effects and more long-term growth promoting effects. In addition, IGF-1 may be involved in tissue repair, regeneration, and wound healing (Van Wyk, 1984; Zapf et al., 1984; Jennische et al., 1987a and 1987b). Acute metabolic effect which IGF-1 stimulates via an interaction with its receptor are glucose and amino acid uptake. IGF-1 also has the ability to stimulate RNA and protein synthesis (Hill et al., 1986; Janicot and Lane, 1989). The long term growth promoting effects of IGF-1 include stimulation of DNA synthesis, cell proliferation and differentiation of certain cell types (Van Wyk, 1984; Zapf et al., 1984).

Due to the availability of recombinant human (rh) IGF-1, IGF-1 has been considered as a potential therapeutic agent for treatment of a variety of diseases, such as

diabetes mellitus and growth hormone resistant short stature, because of its effects on growth and metabolism. An acute intravenous bolus administration of IGF-1 to rats and humans leads to hypoglycemia similar to that seen after insulin injection (Zapf et al., 1986, Guler et al., 1987). It has been demonstrated that IGF-1 suppresses insulin secretion, but insulin response to a glucose challenge is prompt (Hussain et al., 1993).

The heart is a target organ of IGF-1. There are both IGF-1 and IGF-2 receptors in ventricular tissue. The IGF-1 receptor is expressed at low levels in normal rat heart and mainly in the media in aorta (Donohue et al., 1994; Sidawy et al., 1990). Han et al. (1987) has demonstrated expression of IGF-1 in the heart was localizes predominantly to the epicardium and in coronary vessel walls. IGF-1 mRNA is expressed at low level in neonatal rat cardiomyocytes and in the left ventricle of adult rat, but at significant higher level in resistance (muscular) arteries (Wahlander et al., 1992; Donohue et al., 1994; Hanson et al., 1993).

Evidence suggested that IGF-1 has direct trophic effect on the myocardium. Chronic exogenous administration of IGF-1 in normal rat causes enhancement of cardiac performance, while total peripheral resistance is reduced (Cittadini et al., 1996). In human, a slight elevation of heart rate, tachycardia with palpitations, and orthostatic hypotension were observed after IGF-1 infusion (Jabri et al., 1994). Furthermore, infusion of IGF-1 in rat following myocardial infarction enhanced ventricular hypertrophy and showed potentially beneficial effects on hemodynamic function (Duerr et al., 1995). Recent studies have reported that IGF-1 and IGF-1 receptor plays a important role in cardiac myocytes growth. IGF-1 induces hypertrophy and stimulates DNA synthesis in neonatal rat cardiomyocytes (Ito H et al., 1993; Kajstura et al., 1994). In addition, in

adult rat cardiac myocytes, IGF-1 enhanced myofibril development and potently stimulated protein synthesis (Donath et al., 1994; Fuller et al., 1992).

Myocardial growth stimulated by IGF-1 may result from the effects of both systemic IGF-1 that crosses the endothelium, and of locally synthesized peptides which includes IGF-1 from myocytes, endothelium, vascular smooth muscle cell, and fibroblasts (Gajdusek CM et al., 1993; Donohue et al., 1994). The vasculature is an IGF-sensitive tissue. IGF-1 regulates both vascular tone and metabolic processes. Like insulin, IGF-1 has potentially important effects on endothelial cells. Endothelial cells represent the initial cellular component of the blood vessel wall with which plasma components of blood come into contact, thus they are directly exposed to circulating IGF-1. IGF-1 can attenuate vasoconstrictive responses, increase blood flow in certain vascular beds and lower blood pressure in healthy individuals (Copeland and Sreekuran, 1994, Kerr et al., 1993). In both normal and fasting rats, IGF-1 infusion increases glomerular blood flow and filtration rate (Hirschberg et al., 1991; Hirschberg and Kopple, 1989). Infusion of IGF-1 into the brachial artery in humans has been shown to increase forearm blood flow (Copeland and Nair, 1994). Both *in vivo* and *in vitro* experiments have shown that IGF-1 diminishes vascular contractility (Walsh et al., 1996, Pete et al., 1996). Recent studies conducted in our laboratory confirmed previous observations that in both normals and diabetics, IGF-1 infusion decreased MAP (Pete, 1996). IGF-1 also increased blood flow in skeletal muscle and in the splanchnic vasculature in normals, but the iliac and renal flow response to IGF-1 was attenuated in diabetic groups. The increase in blood flow caused by IGF-1 was inhibited by preinfusion with L-NAME. Therefore, similar to insulin, IGF-1 can selectively dilate vascular beds and thus decrease blood pressure and nitric oxide may be the mediator

of their action on vasculature. Our previous observation that IGF-1 stimulates NO production in intact vessels supports this conclusion (Walsh et al., 1996). But unlike insulin, which needs to pass across the endothelium to reach the vascular smooth muscle cells, IGF-1 is synthesized by vascular smooth muscle cells (Sarzani et al., 1989; Sowers et al., 1994) and probably acts in an autocrine and paracrine fashion.

The effect of IGF-1 and the role of IGF binding protein in modulating IGF-1 effects on cardiovascular system are largely unexplored.

Effects of IGF-1 on Sympathetic Nervous System

There is evidence supporting the hypothesis that IGFs, as well as insulin, function not only as growth factors, but also as neuroactive substances. They play a role in neuronal development, maintenance and regulation. Ishii and Recio-Pinto (1987) have reported that IGF-1 and IGF-2 increase both the proportion of cells with neurites and the average neurite length in cultured human neuroblastoma SH-SY5Y cells which are most likely of sympathetic origin. Subsequent studies found that IGFs are able to increase neurite outgrowth and support neuron survival. Zackenfels et al. (1995) found a stimulation of [^{3}H] thymidine incorporation into immature chick sympathetic neurons in vivo in response to IGF-1. Anti-IGF-1 antibody treatment to embryos resulted in a reduction in the sympathetic neuron proliferation and neuron number, and administration of IGF-1 caused increase in sympathetic neuron proliferation in vivo. These results suggest an important role of IGF-1 on sympathetic neurons development. Although investigations have yielded exciting hypotheses for IGF action in the developing nervous system, a major challenge remains in elucidating the function of the IGFs in the mature

nervous system. There is limited information about how sympathetic nervous system is physiologically regulated or affected by IGF-1.

The purpose of our study is to evaluate the integrative relationship between insulin or IGF-1 induced cardiovascular responses and sympathetic nervous response in normal rats. The integrative interaction of endocrine, cardiovascular and neurophysiological actions of insulin and IGF-1 will allow us to understand more clearly the full significance of their effects.

It is paradoxical that intravenous insulin, which causes sympathoexitation, a rise in plasma epinephrine, and a rise in heart rate, induces vasodilation and decreases in arterial blood pressure. Changes in arterial pressure are mainly due to a disproportion between cardiac output, the volume of blood pumped into the arteries, and the total peripheral resistance. Previous studies in our laboratory have already confirmed that insulin increases blood flow and decrease the vascular resistance in skeletal muscles. Now we are hoping by selectively choosing lumbar sympathetic nerve and monitoring its activity, that we can see the correlation between the sympathetic nerve activity and the altered blood flow in response to insulin. Based on other studies, the fall in hindlimb resistance may result from the activation of sympathetic neurogenic vasodilation and nitric oxide may be a neurotransmitter of the vasodilator nerves (Okamura et al., 1996; Davisson et al., 1996). By removing the lumbar sympathetic innervation to the hindlimb and comparing the cardiac and flow change in response to insulin infusion, we hope that we can determine whether lumbar sympathetic nerves mostly consist of vasodilatory fibers or vasoconstrictory fibers, and what their functional role is following insulin infusion. However, the increased sympathetic nerve activity to muscle during insulin is at least

partly sympathetic vasoconstrictor because increased nerve activity is accompanied by increases in plasma norepinephrine (Anderson et al., 1991).

We propose that insulin increases lumbar sympathetic nerve activity which reflects sympathetic nerve activity to skeletal muscle. Since insulin selectively increases regional blood flow, we are interested to explore whether the lumbar sympathetic nervous response to insulin is a general or selective response. Therefore, we also evaluated the renal sympathetic nerve activity in response to insulin infusion.

Previous studies have shown that IGF-1 mimics insulin's effect on cardiovascular parameters, decreasing in MAP and increasing in heart rate. Since IGF-1 has its own receptor and IGF-1 receptors localize differently from insulin receptor, we speculate that IGF-1 will induce either similar or distinct cardiovascular and sympathetic responses when compared to insulin, but perhaps via different mechanisms.

MATERIAL AND METHODS

General Animal Care

All experiments were performed on male Wistar rats (Harlan, Indianapolis, Indiana) weighing between 250 to 400 grams. The animals were housed individually in a cage and maintained in a temperature-controlled (23°C) room with a 12-hour light/dark cycle. The animals were fed laboratory rodent chow and water *ad libitum*.

Monitoring of Cardiovascular Parameters

Surgical procedures

Following an 18-hour fast, rats were anesthetized by a intraperitoneal injection of a mixture of α-chloralose (80 mg/kg) and urethane (500 mg/kg) (Sigma Chemical Co., St. Louis, Missouri). The level of anesthesia was maintained by a continuous intravenous infusion (5.33 $mg \cdot ml^{-1}h^{-1}$ of α-chloralose and 33.33$mg \cdot ml^{-1}h^{-1}$ of urethane). Once the animals were anesthetized, a midline 1.0 cm incision was made on the anterior cervical surface and the trachea was exposed and cannulated. The 3.0 cm trachea tube with a beveled end was made of polyethylene 200 tubing (Becton Dickinson and Company, Parsippany, New Jersey). Throughout the experiment, the body temperature was monitored with a rectal probe and maintained in the range 36.5°C~37.5°C with a heating pat or heating lamp.

Instrumentation

Rats were instrumented with arterial and venous catheters. The left common carotid artery was cannulated with polyethylene catheter (PE50) filled with heparinized saline (22 U/ml) for mean arterial pressure (MAP) and heart rate (HR) recording. MAP and HR were determined using a Spectra-Med pressure transducer. The left femoral vein was cannulated for infusion of anesthesia and administration of drugs. The right femoral vein was cannulated for glucose replacement. In insulin infusion experiment, a 90 mg bolus of glucose was administered followed by variable infusion ($\cong$ 300 $mg \cdot ml^{-1}h^{-1}$). In IGF-1 infusion experiment, a 45 mg bolus of glucose was administered followed by variable infusion ($\cong$ 150 $mg \cdot ml^{-1}h^{-1}$).

Placement of Pulsed Doppler Flow Probes

After a midline incision on the ventral surface of the rat's abdomen, both left and right iliac arteries were isolated by blunt dissection. The iliac arteries were dissected free of surrounding tissues. Two 1.0 mm pulsed-Doppler flow probes (Crystal Biotech Co., Hopkinton, MA) were placed around the left and right iliac arteries, respectively. These flow probes were connected to a plused-Doppler flowmeter (Baylor Electronics) for measuring of blood flows in both limbs.

Unilateral Sympathetic Denervation of Iliac Vessels

Under a surgical microscope, the left lumbar sympathetic chain was identified and carefully isolated. It was extirpated from the kidney vein level to the bifurcation of the left common iliac artery and vein. The right sympathetic chain remained intact.

Recording of Sympathetic Nerve Activity

Arterial and Venous Catheterization

Catheter (PE 50) filled with heparinized saline was inserted to the left femoral artery for mean arterial pressure and heart rate recording. MAP and HR were determined using an acquisition processor and software (Dasy Lab Data Acquisition System Laboratory). The left femoral vein was cannulated for infusion of anesthesia and administration of drugs. The right femoral vein was cannulated for glucose replacement. Blood samples (0.5 ml) were obtained from left femoral vein 20 minutes before the injection of drugs and 60 minutes after the injection of drugs for plasma glucose determination. An equal volume of heparinized saline (100U/ml) was infused immediately to replace the blood volume.

Recording of lumbar sympathetic nerve activity (LSNA)

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 Teflon-coated wire. Silicone gel (Wacker Sil-Gel 601A and 601B mixture) was used to embed the nerve bundle and electrodes and allowed 1 hour to dry and harden. Finally, the abdomen was closed to reduce evaporation. LSNA was amplified (5,000 ~ 10,000 times) and filtered (low at 30 Hz, high at 1,000 Hz) using a Grass RPS 107 amplifier and a Grass HI 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 with a root mean square integrator. At the end of each experiment, hexamethonium chloride (20 mg/kg), a ganglionic blocker, 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 killed and any residual output from the nerve was subtracted as noise when nerve activity was calculated.

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 nonphysiological factors vary in different preparations, nerve activity data were normalized as percent of the baseline nerve activity.

Recording of renal sympathetic nerve activity (RSNA)

The left kidney was exposed retroperitoneally and, with the aid of an dissection microscope, a branch of the renal sympathetic nerve bundles was isolated from the surrounding connective tissue and artery. The renal nerve was cut distally and the central cut end was placed on a stainless steel wire bipolar electrode. After the condition for optimal nerve recording had been established, both the nerve and the electrode were covered with silicone gel. During surgical procedures, animals were artificially ventilated (volume-regulated ventilator SAR-830, CWE, Ardmore, PA). All further preparations for recording were similar to those described above for recording lumbar sympathetic nerve.

Sinoaortic Denervation (SAD)

This procedure entails denervation of aortic baroreceptor by bilateral section of the aortic depressor and superior laryngeal nerves at their junction with the vagus nerve. The cervical sympathetic nerve trunks were also cut bilaterally below the superior cervical ganglia. Carotid sinus baroreceptors were denervated by stripping of all nerve fibers and connective tissues from the carotid bifurcation region for a distance of 6-10 mm from carotid sinus. The effectiveness of SAD was confirmed before experiments by injection of phenylephrine (2.5-5 μg/kg iv) to increase of MAP 30 mmHg. If the decrease of LSNA and bradycardia normally associated with the increase in MAP were abolished, the SAD was considered successful.

After sino-aortic nerves were denervated, rats were artificially ventilated with 30% oxygen. In order to minimize inspiratory drive, animals were slightly hyperventilated.

Experimental Protocols

Protocol 1. Anesthetized rats were instrumented with one venous and one arterial femoral catheter and lumbar sympathetic nerve electrodes. They were divided into four groups. After 15 minutes of stabilization and equilibration, the rats in each group were given a bolus injection of insulin (5U/animal), 2-DG (200 mg/kg), saline or IGF 1 (40μg/animal), respectively. The rats were terminated approximately one hour after injection.

Protocol 2. In addition to the procedures described in protocol 1, another femoral vein was cannulated and glucose replacement was employed, starting at 20 minutes before

injection. In the IGF-1 infusion group, a bolus of glucose (45 mg) was administered followed by constant infusion (150 $mg \cdot ml^{-1}h^{-1}$). In the insulin infusion group, a bolus of glucose (90 mg) was administered followed by constant infusion (300 $mg \cdot ml^{-1}h^{-1}$).

Protocol 3. After the anesthetized rats were instrumented with lumbar sympathetic nerve electrodes and one arterial and two venous femoral catheters, they underwent SAD. And then, a bolus injection of insulin (5u/animal) was administered with glucose replacement.

Protocol 4. Protocols 1 to 3 were reperformed with recording of renal sympathetic nerve activity (RSNA) instead of lumbar sympathetic nerve activity (LSNA). Insulin was administered without glucose replacement.

Protocol 5. A femoral vein and left common carotid artery of anesthetized rats were catheterized and the left lumbar sympathetic nerves were selectively removed. Those animals were divided into two groups. After 15 minutes of stabilization and equilibration from surgery, one group of rats were given a bolus injection of insulin (5U/kg), and the other group received a bolus of IGF 1 (40μg/animal). The rats were terminated approximately one hour after injection.

Protocol 6. In addition to the procedures described in protocol 5, another femoral vein was cannulated and glucose replacement was employed, starting at 20 minutes before injection. In the IGF-1 infusion group, a bolus of glucose (45 mg) was administered followed by constant infusion (150 $mg \cdot ml^{-1}h^{-1}$). In the insulin infusion group, a bolus of

glucose (90 mg) was administered followed by constant infusion (300 $mg \cdot ml^{-1}h^{-1}$).

Plasma glucose assays

After collection, the blood samples were immediately stored in a 4°C refrigerator until centrifuged. Following centrifugation, the plasma was collected and stored at -30°C for subsequent assays. Plasma glucose was measured by a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, Ohio).

Data collection and analysis

For the set of experiments measuring iliac blood flow and cardiovascular parameters with unilateral lumbar sympathectomy, a Micro 5000 signal processing system (Modular Instruments, Melven, PA) and a BioWindows Software Program (Modular Instruments) were used to continuously record those parameters. For the set of experiments recording sympathetic nerve activity and cardiovascular parameters, an acquisition processor and software (Dasy Lab Data Acquisition System Laboratory) were used to monitor the responses.

The arterial conductance was determined by dividing blood flows by the mean arterial pressure. Because of the limitations of comparing values from multifiber sympathetic nerves between animals, the sympathetic nerve activities are expressed as percentage change from control values.

The data were statistically analyzed by analyses of variance (ANOVA) and post

hoc test. Student's t-test was used to compare pairs of means. When comparing values in which each animal served as its own control, the paired T-test was applied, whereas the unpaired T-test was used for comparison of different groups. A 0.05 level of significance was used for statistical test. Data are presented as mean $\pm$ SE.

RESULTS

PART I

In this series of experiment, we studied cardiovascular responses and sympathetic nervous responses to systemic insulin infusion under various conditions. The basal mean arterial pressure (MAP) and heart rate (HR) for controls, with glucose replacement or following sino-aortic denervation are presented in table 1. The basal MAP and HR were not significantly different between the groups.

Table 1

Basal mean arterial pressure (MAP) and heart rate (HR) in rats at time 0, for controls, glucose infusion, and sino-aortic denervation (SAD) studies. (Mean $\pm$ SE)

	Controls	Glucose Infusion	SAD
MAP (mmHg)	77$\pm$2.1	74$\pm$3.2	79$\pm$2.0
	(6)	(6)	(6)
HR (bpm)	400$\pm$19	414$\pm$16	419$\pm$10
	(6)	(6)	(6)

Numbers in parentheses = n.

The plasma glucose levels achieved after intravenous bolus infusion of insulin with or without glucose replacement at each time point are shown in Table 2. Insulin infusion

rapidly and markedly decreased plasma glucose from approximately 105 mg/dl to an average 44 mg/dl for 60 minutes. When glucose replacement was applied, euglycemia was maintained during the experiment period.

Table 2

The effect of systemic insulin on plasma glucose in the presence and in the absence of glucose replacement. In the group with glucose replacement, animals were given a glucose bolus of 90 mg at -20 minutes, followed by a constant infusion of glucose ≅ 300 mg/hour. (Mean ± SE)

Time (minutes)		Insulin 5U/animal (N=8)	Insulin 5U/animal + glucose (N=6)
Basal Period	-20	104±5.4	101±1.5
	0	107±4.5	146±5.9*
Treatment Period	20	50±6.8	107±5.0*
	30	51±5.7	116±14.4*
	45	36±4.4	85±2.9*
	60	40±9.0	82±4.2*

* $P<0.01$ vs. insulin alone.

I. The Effect of Systemic Insulin Infusion on Cardiovascular and Sympathetic Nervous System.

A. The effect of systemic infusion of insulin (5U/animal) on cardiovascular system and lumbar sympathetic nervous activities (LSNA) with or without glucose replacement.

Following the injection of an intravenous bolus of insulin, the mean arterial pressure (MAP) decreased promptly (approximately 20%, Figure 1A). The heart rate (HR) decreased slightly after 15 minutes and subsequently was elevated significantly to an average 10% above basal levels (Figure 1B). The LSNA increased 15 minutes after insulin infusion and continued to a peak of 15% above control level at 25 minutes and then slowly recovered toward baseline (Figure 1C). When insulin infusion was repeated with glucose replacement, it again resulted in significant decrease in MAP and HR. The decrease of MAP was comparable to insulin without glucose maintenance. However, the decrease of HR (approximately 5% below control levels) was in contrast to the response when hypoglycemia was allowed to occur. Again the LSNA gradually increased to 10% above the basal level after a bolus of insulin with glucose replacement and did not recover (Figure 2).

B. The effect of 2-DG (200 mg/kg) on cardiovascular system and LSNA.

2-DG infusion also induced progressively increase in LSNA, which was similar to insulin induced increase in LSNA (Figure 3C). In the mean time, the MAP and HR showed small but significant decreases (Figure 3A, 3B).

C. The effect of systemic insulin infusion (5 U/animal) on cardiovascular system and renal sympathetic nervous activities (RSNA) with or without glucose replacement in normal rats.

As was observed before, systemic insulin infusion resulted in significant decrease in MAP and increase in heart rate. However, the RSNA decreased (the maximum change was about 15% below basal values) during the first 15 minutes following insulin infusion and was then followed by a dramatic elevation. The RSNA increased approximately 60% compared to basal values (Figure 4).

When insulin infusion was repeated and meanwhile hypoglycemia was prevented by glucose replacement, again, we observed slight decrease in MAP and HR. But in contrast to what was observed in figure 4, the RSNA was reduced approximately 12% below baseline after insulin infusion and recovered after 60 minutes of the experiments (Figure 5).

II. The Effect of Systemic Insulin Infusion on Cardiovascular and Sympathetic Nervous System after Sino-Aortic Denervation.

A. The effect of insulin (5U/animal) on cardiovascular system and LSNA with glucose replacement and sino-aortic nerve denervation (SAD) in normal rats.

After SAD, the animals were given 30 minutes to recover and stabilize. The basal MAP and HR of the SAD rats were not different from normals (Table 1). As shown in Figure 6A, the average decrease in MAP caused by insulin infusion with glucose replacement was 7%. The HR did not respond significantly (Figure 6B), and the LSNA

increased 7% above control level (Figure 6C). When compared to insulin infusion in the absence of SAD, the MAP and LSNA responses were attenuated and the insulin-induced suppression of the heart rate was blunted.

B. The effect of insulin (5U/animal) on cardiovascular system and RSNA with sino-aortic nerve denervation (SAD) in normal rats.

A intravenous bolus of insulin resulted in a decrease in MAP, increase in HR and RSNA in SAD rats. Those responses were similar to those of normal rats, but were all slightly attenuated (Figure 7).

III. The Effect of Iliac Artery Denervation on Iliac Blood Flow in Response to Systemic Insulin.

In this group of experiments, we investigated the influence of iliac artery denervation on the iliac vasculature (expressed as iliac conductance: flow/MAP) in response to intravenous insulin with or without glucose replacement. As described in Protocol 5, left side lumbar sympathetic nerves were removed while right side lumbar sympathetic nerve were kept intact. The right intact iliac blood flow served as control. After surgery, animals were allowed to recover and stabilize.

In Figure 8, it can be observed that after denervation of left iliac artery, its conductance elevated immediately (approximately 25% above basal level) and the corresponding conductance of right iliac artery went down. Insulin infusion with hypoglycemia resulted in prompt increase in the conductance of both left and right iliac

arteries, but the vascular conductance of sympathetic denervated iliac artery had a greater average increase than that of the intact iliac artery(Figure 10).

A repeat of this same study under euglycemic condition, insulin infusion caused a gradually elevation of the conductance of denervated iliac artery and a delayed increase of the intact iliac conductance. Forty-five minutes after insulin infusion, the conductances of both side iliac arteries were not different (Figure 9). Again, the vascular conductance of sympathetic denervated iliac artery had a greater average increase in response to insulin than that of the intact iliac artery (Figure 10).

PART II

In Part II of this study, we conducted studies with a protocol similar to that described for insulin in Part I. IGF-1 decreased plasma glucose significantly from a basal level 114 $\pm$ 6 mg/dl to a nadir of 61 $\pm$ 5 mg/dl at 40 minutes after injection. The plasma glucose was reduced approximately 45% and the decrease persisted all through the recording period (Table 3).

I. The Effect of Systemic IGF-1 Infusion on Cardiovascular and Sympathetic Nervous System.

A. The effect of systemic infusion of IGF-1 (40 ug/animal) on cardiovascular system and lumbar sympathetic nervous activities (LSNA) with or without glucose replacement.

The systemic infusion of IGF-1 in normal rats resulted in a slight but significant

decrease in MAP (approximately 10%) compared to controls (Figure 11A). The maximal decrease in blood pressure occurred at 15 minutes post IGF-1 infusion. The heart rate response did not differ from saline controls (Figure 11B). A significant increase in LSNA after IGF-1 infusion was observed (Figure 11C). The LSNA gradually increased to approximately 10% above basal at 15 minutes after IGF-1 infusion, and kept at this level until the end of the experiment (one hour after IGF-1 administration).

Table 3

The effect of systemic insulin-like growth factor 1 (IGF-1) 40 ug/animal on plasma glucose in the presence and in the absence of glucose replacement. In the group with glucose replacement, animals were given a glucose bolus of 45 mg at -20 minutes, followed by a constant infusion of glucose ≅ 150 mg/hour. (Mean ± SE)

	Plasma Glucose			
Time (minutes)	0	20	40	60
IGF-1	114±6	65±5	61±5	68±7
	(6)	(6)	(6)	(6)
IGF-1 + Glucose Infusion	117±3	112±13*	140±7*	136±8*
	(6)	(6)	(6)	(6)

* $P<0.01$ vs. IGF-1 alone; numbers in parentheses = n.

The response to intravenous infusion of IGF-1 with accompanying glucose infusion demonstrated that the MAP was also slightly decreased, and heart rate did not significantly change (Figure 12A, 12B). The changes in LSNA were significantly different

from controls. As indicated in Figure 12C, with glucose replacement, IGF-1 caused a sustained lowering of LSNA (the maximum decrease is about 10% compared to saline controls).

B. The effect of systemic IGF-1 (40 ug/animal) infusion on renal sympathetic nervous system.

When we repeated IGF-1 infusion in the presence or in the absence of glucose maintenance and recorded RSNA, we found that unlike LSNA, the RSNA decreased gradually after a bolus intravenous IGF-1. The maximum decrease of RSNA, which was approximately 20% below basal level, occurred 30 minutes after IGF-1 administration and was maintained at this low level until the end of the experiment (Figure 13A). When blood glucose was controlled at euglycemic levels, IGF-1 again decreased RSNA but to a lesser degree (approximately 10% below basal level). Twenty minutes after IGF-1 infusion, RSNA gradually recovered towards baseline and reached to the level 10% above the baseline (Figure 13B).

II. The Effect of Iliac Artery Denervation on Iliac Blood Flow in Response to Systemic IGF-1 Infusion.

The denervation of left iliac artery resulted in increase in left iliac conductance (approximately 10% above baseline) while the conductance of right iliac artery which had intact innervation remained almost the same as basal level. In response to IGF-1 administration in the absence of glucose replacement, iliac conductance in both the

innervated and denervated sides increased to approximately 40% above baseline at 20 minutes post IGF-1 injection and then declined towards basal level, but the denervated iliac conductance decreased to a lesser extent (Figure 14).

When glucose replacement was used to prevent hypoglycemia, the responses were different from those under hypoglycemia. The conductance of right iliac artery with intact innervation increased rapidly in response to IGF-1 and reached the peak of 37% above baseline at 25 minutes after injection. This increase was much greater than that in the denervated side (Figure 15).

Under hypoglycemia condition, the average increase in iliac conductance of sympathetic denervated iliac artery was greater than that in sympathetic intact iliac artery, but when hypoglycemia was prevented, the average increase was less (Figure 16).

Figure 1

Mean arterial pressure (MAP, panel A), heart rate (HR, panel B), and lumbar sympathetic nerve activity (LSNA, panel C) response to intravenous infusion of insulin (5U/animal) compared to saline controls. The arrows are the time of insulin or control saline infusion. Values are mean $\pm$ SE expressed as percent change from basal at time 0. MAP $p< 0.001$, insulin vs. control; HR $p< 0.001$ insulin vs. control; LSNA $p<0.001$ insulin vs. control.
* $p<0.05$ at selected time points.

THE EFFECT OF SYSTEMIC INSULIN ON BLOOD PRESSURE, HEART RATE, AND LUMBAR SYMPATHETIC NERVE ACTIVITY (MEAN ± SE)

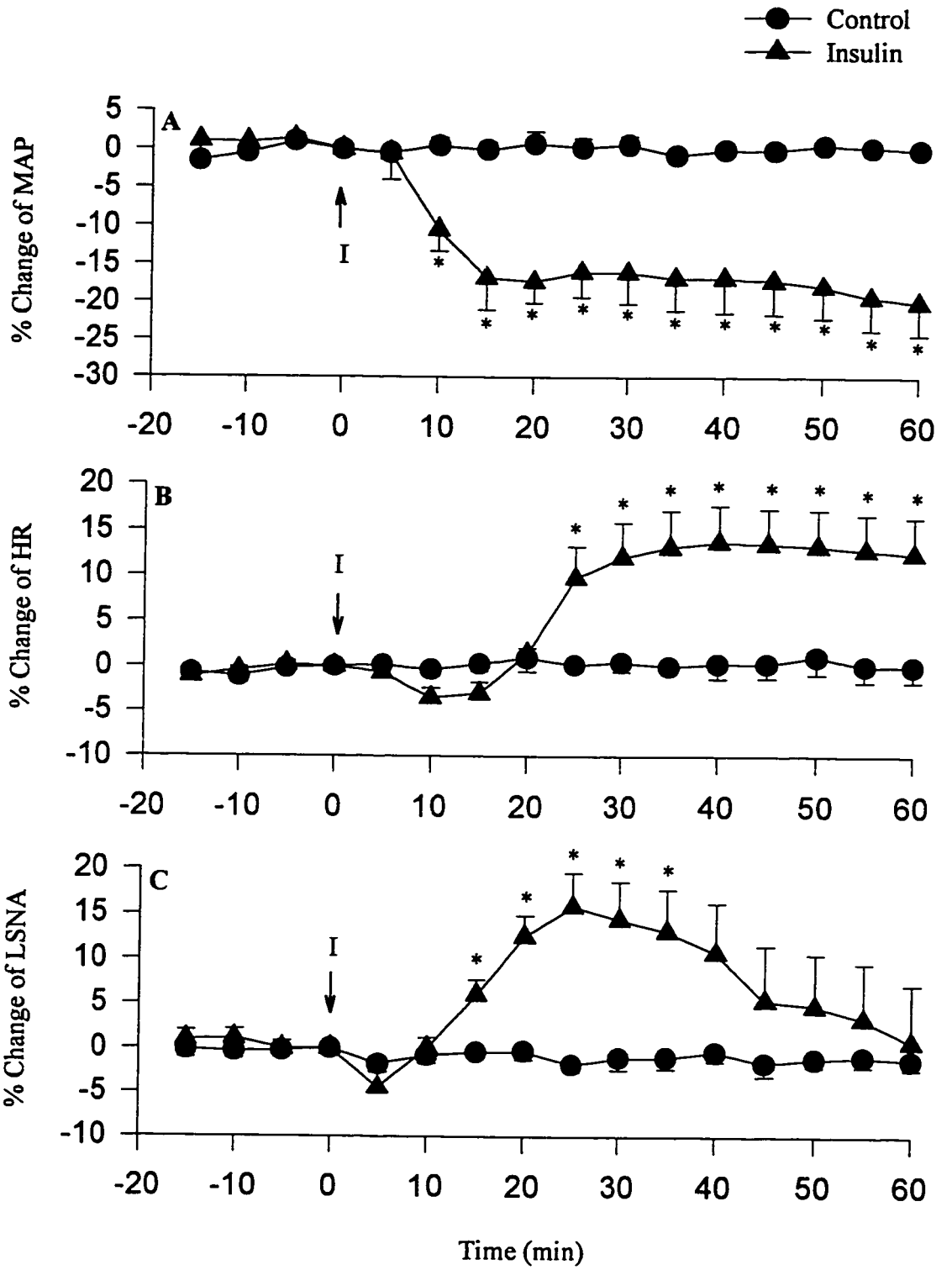


Figure 2

Mean arterial pressure (MAP, panel A), heart rate (HR, panel B), and lumbar sympathetic nerve activity (LSNA, panel C) response to intravenous infusion of insulin (5U/animal) compared to saline controls. Animals were given a glucose bolus 90 mg at time -20 minutes, followed by a constant infusion of glucose ≅ 300 mg/hour. The arrows are the time of insulin or control saline infusion. Values are mean ± SE expressed as percent change from basal at time 0. MAP $p<0.001$, insulin vs. control; HR $p<0.001$, insulin vs. control; LSNA $p< 0.001$ insulin vs. control. * $p<0.05$ at selected time points.

THE EFFECT OF SYSTEMIC INSULIN WITH GLUCOSE REPLACEMENT ON BLOOD PRESSURE, HEART RATE, AND LUMBAR SYMPATHETIC NERVE ACTIVITY (MEAN ± SE)

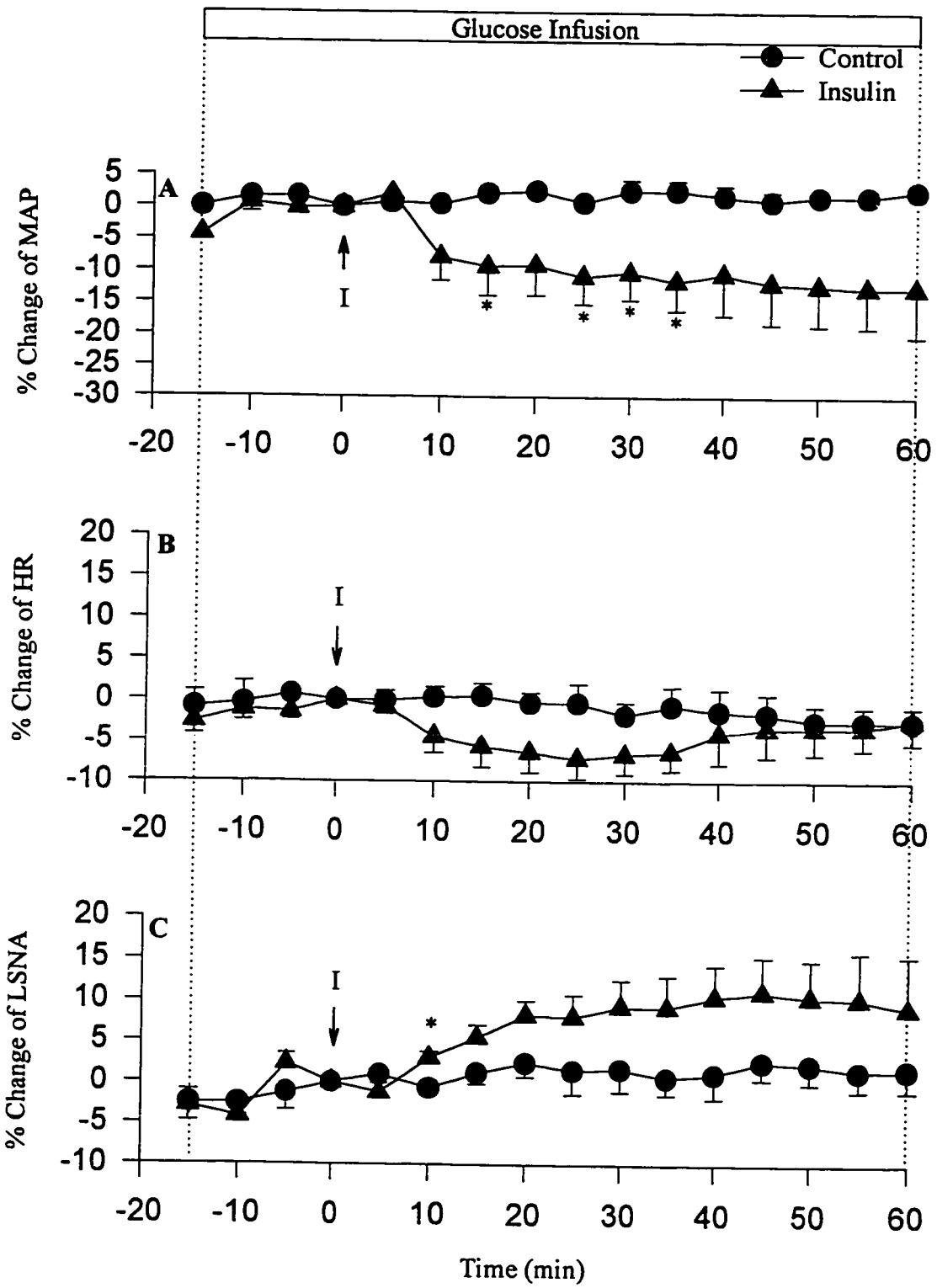


Figure 3

Mean arterial pressure (MAP, panel A), heart rate (HR, panel B), and lumbar sympathetic nerve activity (LSNA, panel C) response to intravenous infusion of 2-deoxyglucose (2-DG) 200 mg/kg compared to saline controls. The arrows are the time of 2-DG infusion or control saline infusion. Values are mean $\pm$ SE expressed as percent change from basal time 0. MAP $p<0.05$, 2-DG vs. control; HR $p<0.05$, 2-DG vs. control; LSNA $p<0.001$, 2-DG vs. control. * $p<0.05$ at selected time points.

THE EFFECT OF SYSTEMIC 2-DG ON BLOOD PRESSURE, HEART RATE, AND LUMBAR SYMPATHETIC NERVE ACTIVITY (MEAN±SE)

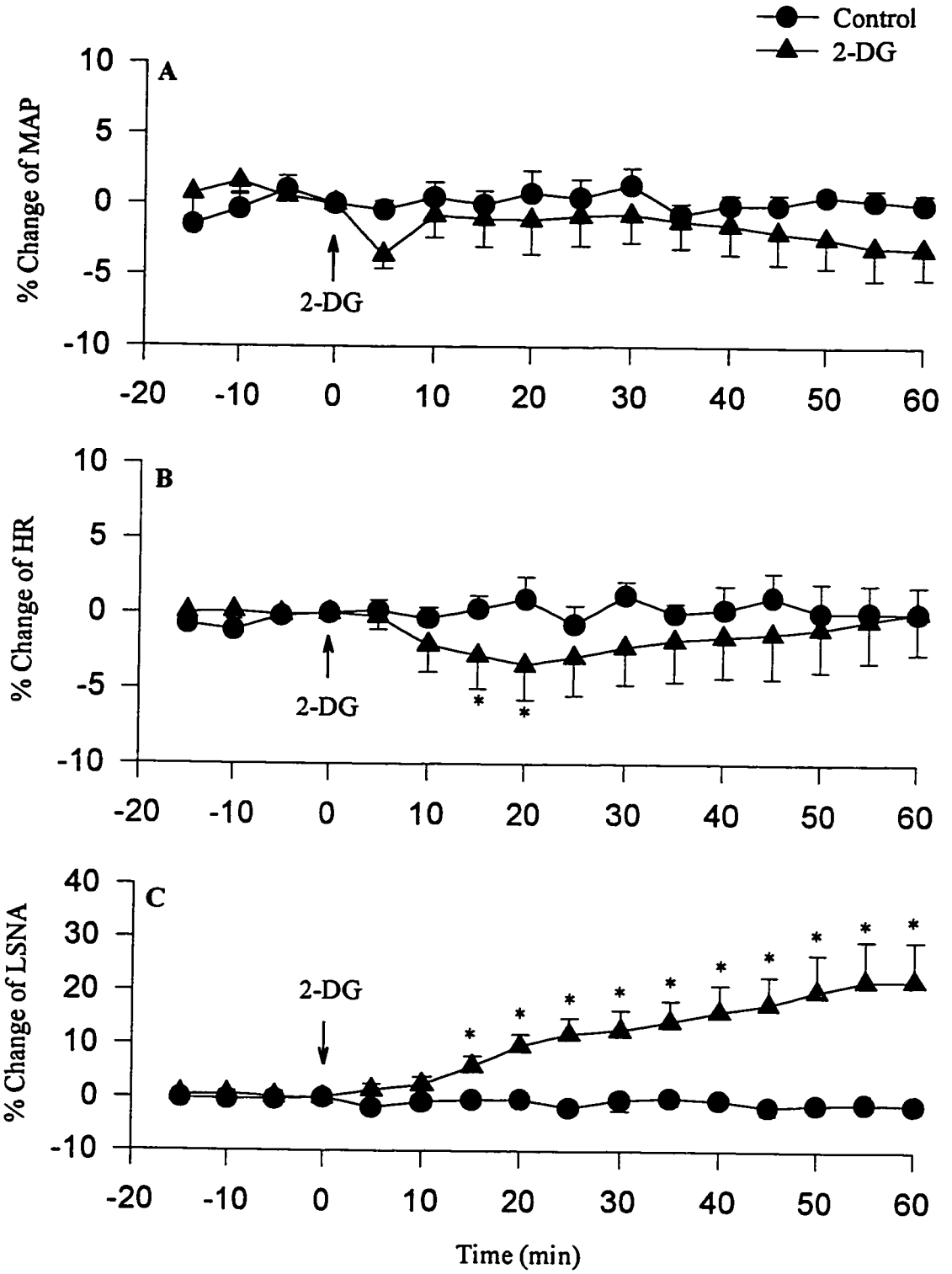


Figure 4

Mean arterial pressure (MAP, panel A), heart rate (HR, panel B), and renal sympathetic nerve activity (RSNA, panel C) response to intravenous infusion of insulin 5U/animal compared to saline controls. Values are mean $\pm$ SE expressed as percent change from basal at time 0. The arrows represent the time of insulin or saline infusions. MAP $p<0.001$, insulin vs. control; HR $p<0.001$, insulin vs. control; RSNA $p< 0.001$, insulin vs. control. * $p<0.05$ at selected time points.

THE EFFECT OF SYSTEMIC INSULIN ON BLOOD PRESSURE, HEART RATE, AND RENAL SYMPATHETIC NERVE ACTIVITY (MEAN ± SE)

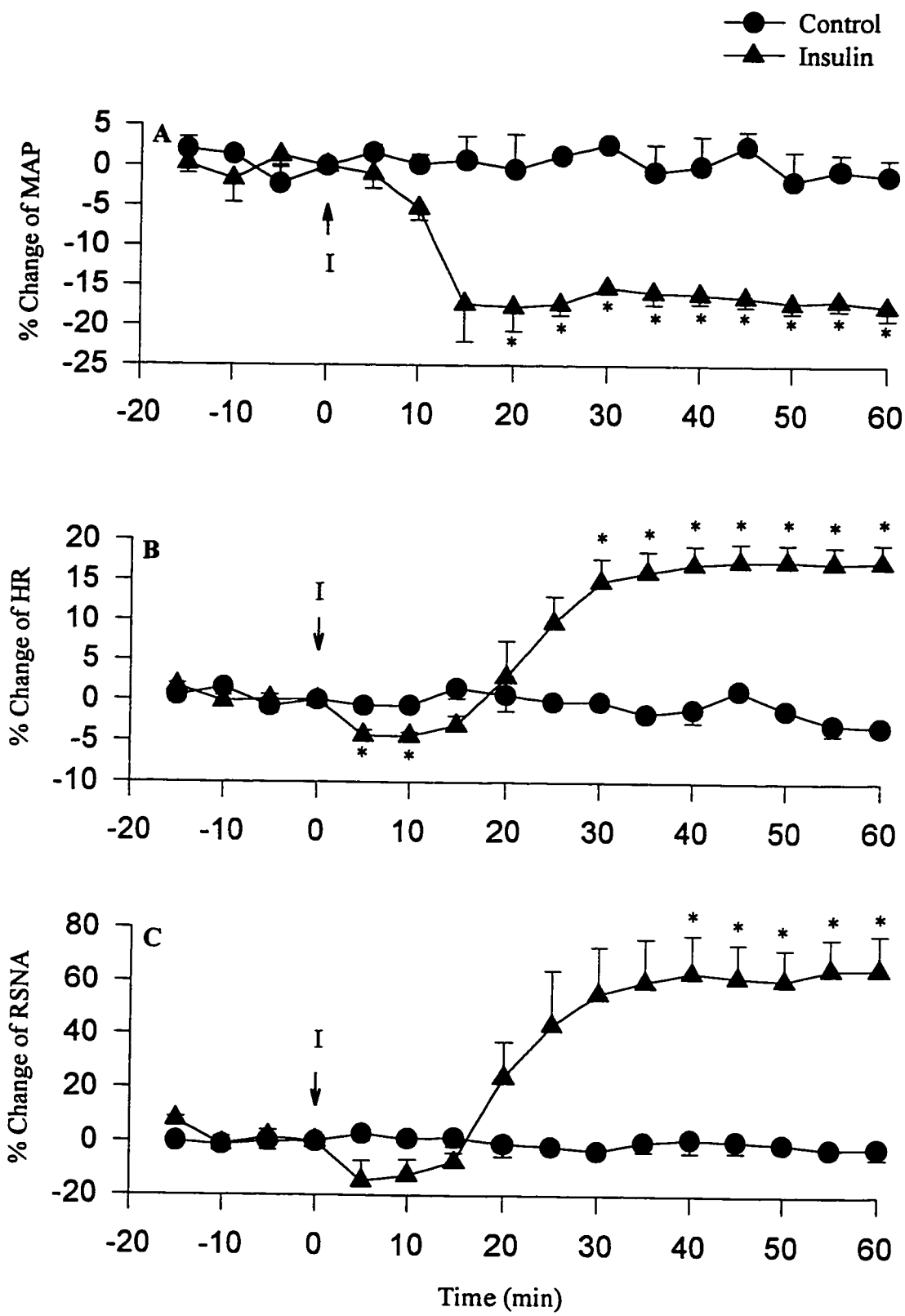


Figure 5

Mean arterial pressure (MAP, panel A), heart rate (HR, panel B), and renal sympathetic nerve activity (RSNA, panel C) response to intravenous infusion of insulin (5U/animal) compared to saline controls. Animals were given a glucose bolus 90 mg at time -20 minutes, followed by a constant infusion of glucose ≅ 300 mg/hour. The arrows are the time of insulin or control saline infusion. Values are mean ± SE expressed as percent change from basal at time 0. MAP $p<0.001$, insulin vs. control; HR $p<0.001$, insulin vs. control; RSNA $p>0.05$, insulin vs. control. * $p<0.05$ at selected time points.

THE EFFECT OF INSULIN WITH GLUCOSE REPLACEMENT ON BLOOD PRESSURE, HEART RATE, AND RENAL SYMPATHETIC NERVE ACTIVITY (MEAN ± SE)

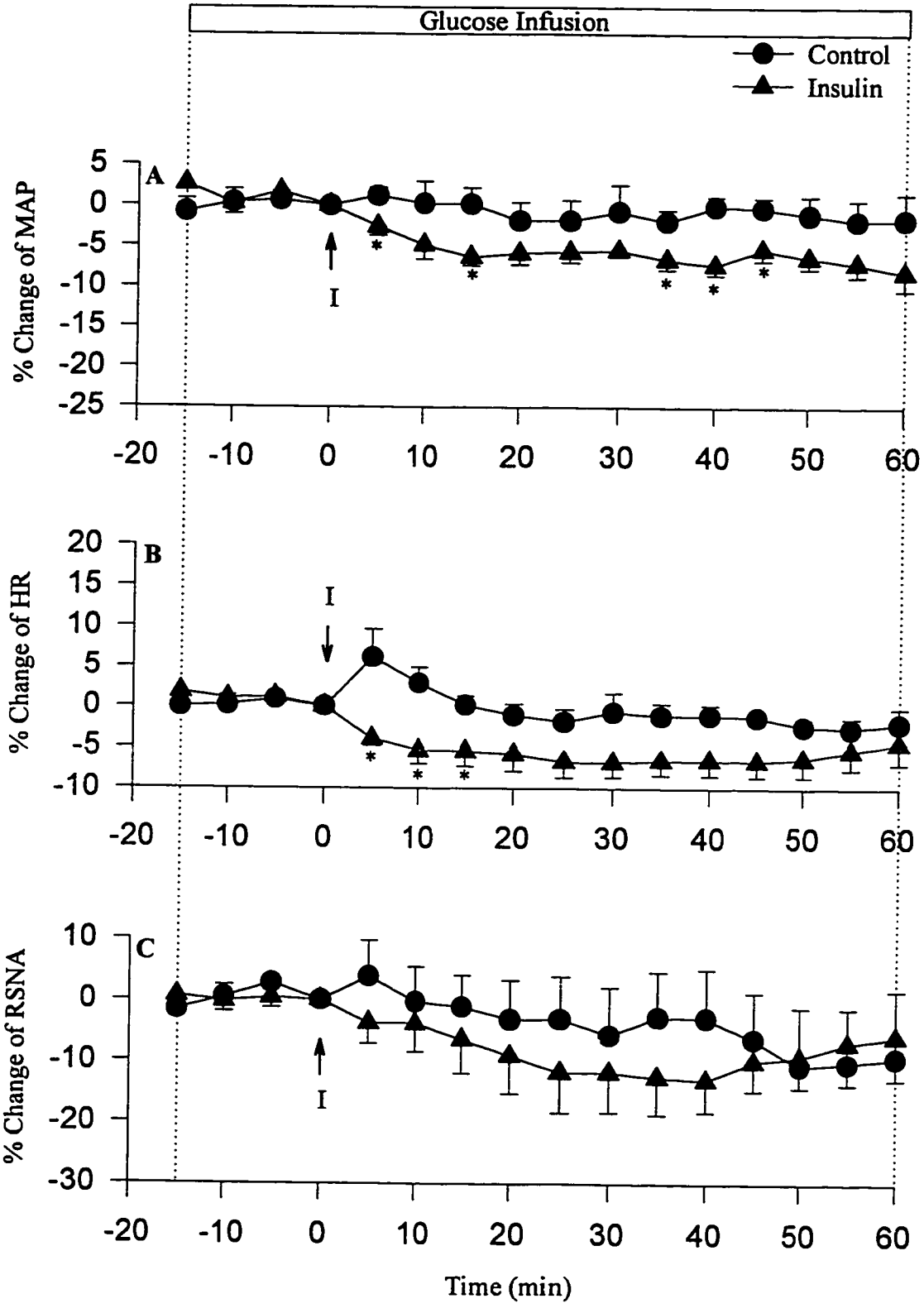


Figure 6

Mean arterial pressure (MAP, panel A), heart rate (HR, panel B), and lumbar sympathetic nerve activity (LSNA, panel C) response to intravenous infusion of insulin 5U/animal in normal or sino-aortic denervation (SAD) rats. Animals were given a glucose bolus 90 mg at -20 minutes, followed by a constant infusion of glucose ≅ 300 mg/hour. The arrows are the time of insulin infusion. Values are mean ± SE expressed as percent change from basal at time 0. MAP $p<0.05$, insulin + SAD vs. insulin; HR $p< 0.001$ insulin + SAD vs. insulin; LSNA $p<0.01$, insulin + SAD vs. insulin. * $p<0.05$ at selected time points.

THE EFFECT OF SYSTEMIC INSULIN WITH GLUCOSE REPLACEMENT AND WITH SINO-AORTIC DENERVATION ON BLOOD PRESSURE, HEART RATE, AND LUMBAR SYMPATHETIC NERVE ACTIVITY (MEAN ± SE)

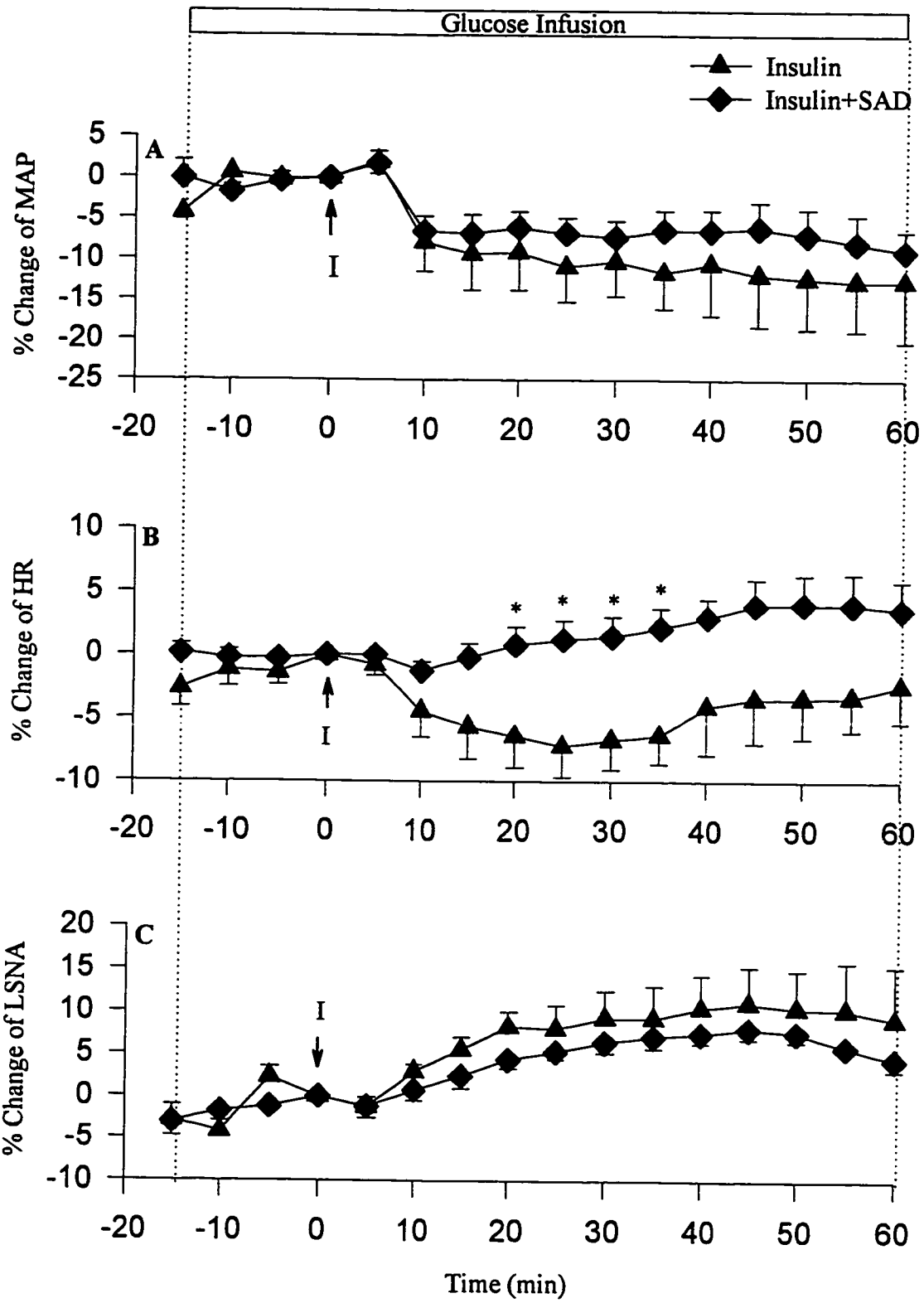


Figure 7

Mean arterial pressure (MAP, panel A), heart rate (HR, panel B), and renal sympathetic nerve activity (RSNA, panel C) response to intravenous infusion of insulin 5U/animal in normal or sino-aortic denervation (SAD) rats. The arrows are the time of insulin infusion. Values are mean $\pm$ SE expressed as percent change from basal at time 0. MAP $p>0.05$, insulin + SAD vs. insulin; HR $p<0.05$, insulin + SAD vs. insulin; RSNA $p>0.05$, insulin + SAD vs. insulin.

THE EFFECT OF SYSTEMIC INSULIN WITH SINO-AORTIC DENERVATION ON BLOOD PRESSURE, HEART RATE, AND RENAL SYMPATHETIC NERVE ACTIVITY (MEAN ± SE)

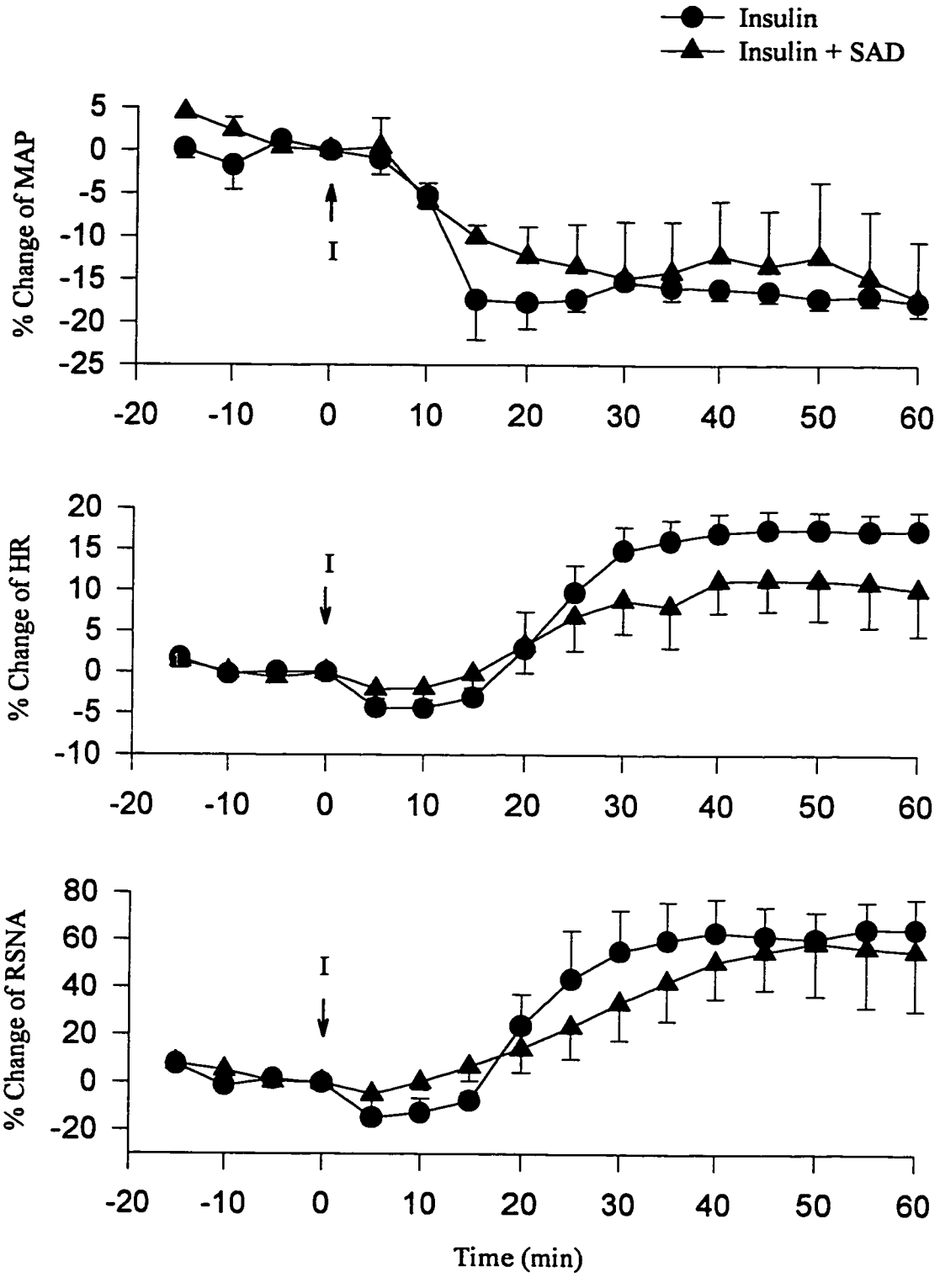


Figure 8

Left iliac conductance (the circles) response to intravenous insulin infusion 5U/kg compared to right iliac conductance (the triangles) after the denervation of left iliac artery. The arrows are the time of denervation and insulin infusion. Values are mean $\pm$ SE expressed as percent change from baseline at the time of denervation. Iliac conductance, $p<0.001$, sympathetic denervated iliac vs. sympathetic intact iliac. * $p<0.05$ at selected time points.

THE EFFECT OF DENERVATION OF THE LEFT ILIAC ARTERY ON VASCULAR FLOW (EXPRESSED AS CONDUCTANCE) RESPONSE TO SYSTEMIC INSULIN (MEAN ± SE)

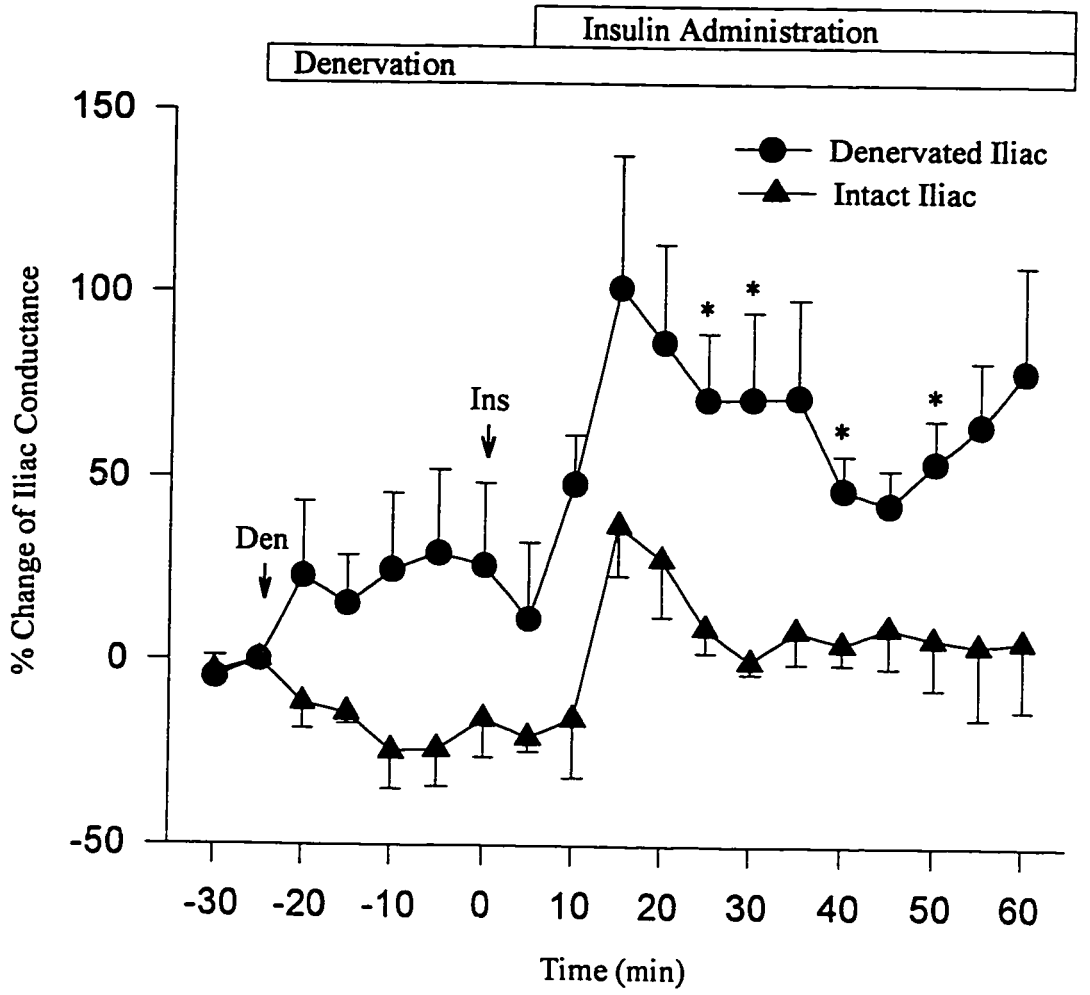


Figure 9

Left iliac conductance (the circles) response to intravenous insulin infusion 5U/kg compared to right iliac conductance (the triangles) after the denervation of left iliac artery. Animals were given a glucose bolus 90 mg at 20 minutes before insulin infusion, followed by a constant infusion of glucose ≅ 300 mg/hour. Values are mean ± SE expressed as percent change from baseline at time 0, which is the time of insulin infusion. $p>0.05$ sympathetic denervated iliac conductance vs. sympathetic intact iliac conductance.
* $p<0.05$ at selected time points.

THE EFFECT OF ILIAC DENERVATION ON VASCULAR FLOW (EXPRESSED AS CONDUCTANCE) RESPONSE TO SYSTEMIC INSULIN WITH GLUCOSE REPLACEMENT (MEAN ± SE)

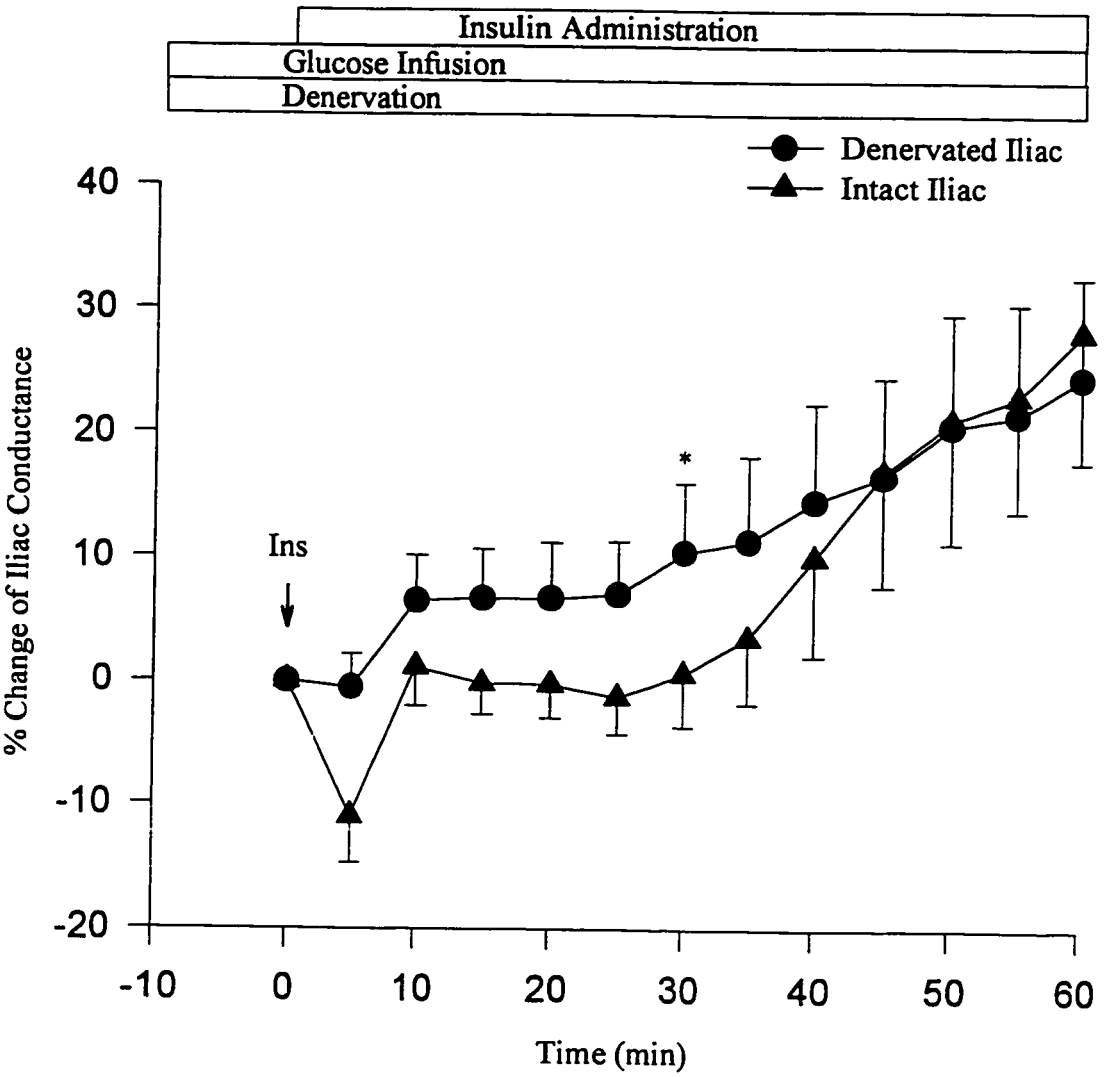


Figure 10

A comparison of sympathetic denervation on average iliac conductance response to intravenous infusion of insulin 5U/kg in the absence or presence of glucose infusion. Values are mean $\pm$ SE, expressed as percent change from baseline, which is the basal level before insulin infusion. Open bars, sympathetic intact iliac conductance; hatched bars, sympathetic denervated iliac conductance. *$p<0.05$ sympathetic denervated iliac conductance vs. sympathetic intact iliac conductance.

A COMPARISON OF THE EFFECT OF ILIAC DENERVATION ON VASCULAR FLOW (EXPRESSED AS CONDUCTANCE) RESPONSE TO INSULIN IN THE PRESENCE OR ABSENCE OF GLUCOSE REPLACEMENT (MEAN ± SE)

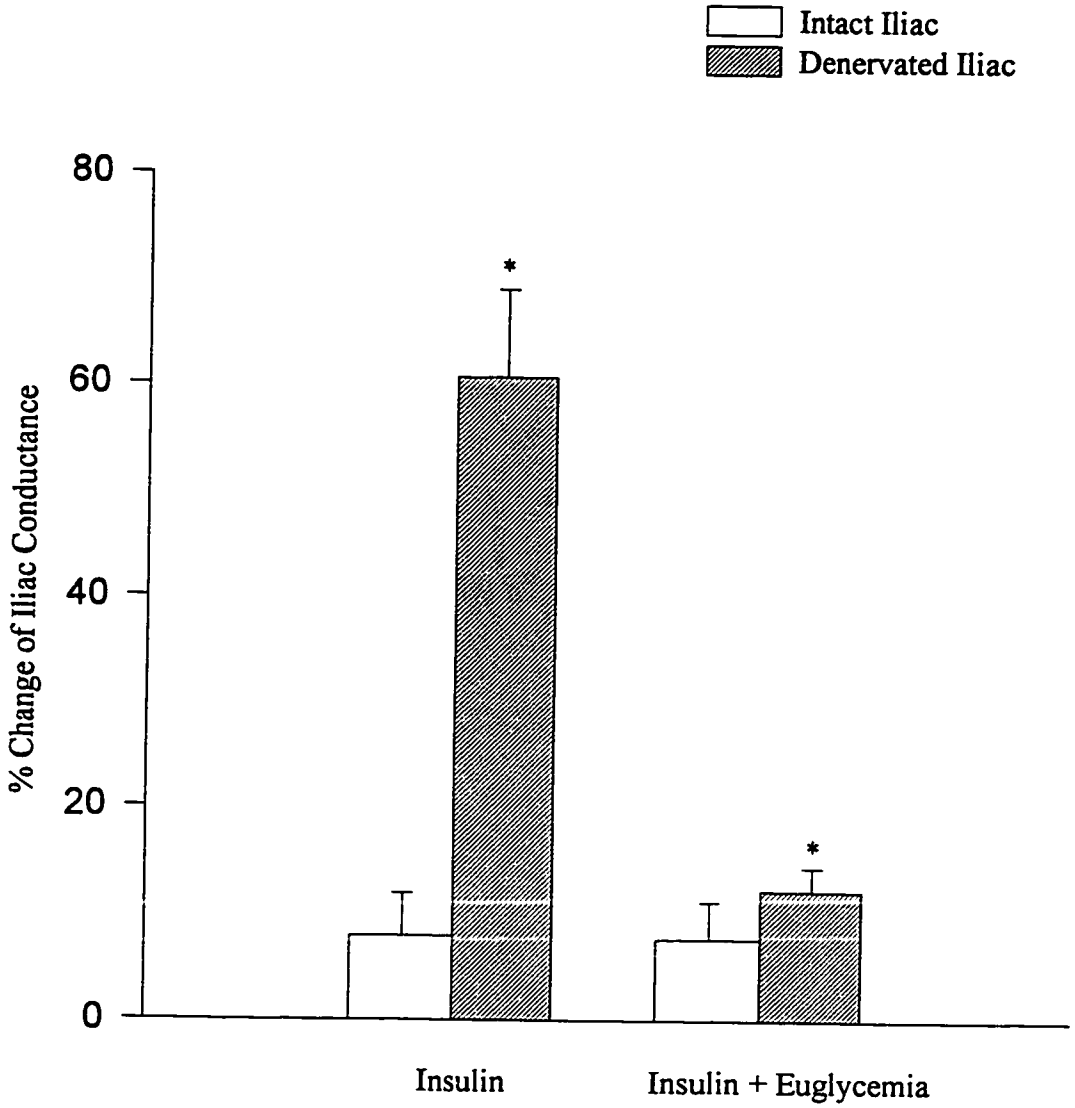


Figure 11

Mean arterial pressure (MAP, panel A), heart rate (HR, panel B), and lumbar sympathetic nerve activity (LSNA, panel C) response to intravenous infusion of insulin-like growth factor 1 (IGF-1) 40ug/animal compared to saline controls. The arrows are the time of IGF-1 or control saline infusion. Values are mean $\pm$ SE expressed as percent change from basal at time 0. MAP $p< 0.001$, IGF-1 vs. control; HR $p>0.05$, IGF-1 vs. control; LSNA $p<0.01$, IGF-1 vs. control. * $p<0.05$ at selected time points.

THE EFFECT OF SYSTEMIC INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) ON BLOOD PRESSURE, HEART RATE, AND LUMBAR SYMPATHETIC NERVE ACTIVITY (MEAN ± SE)

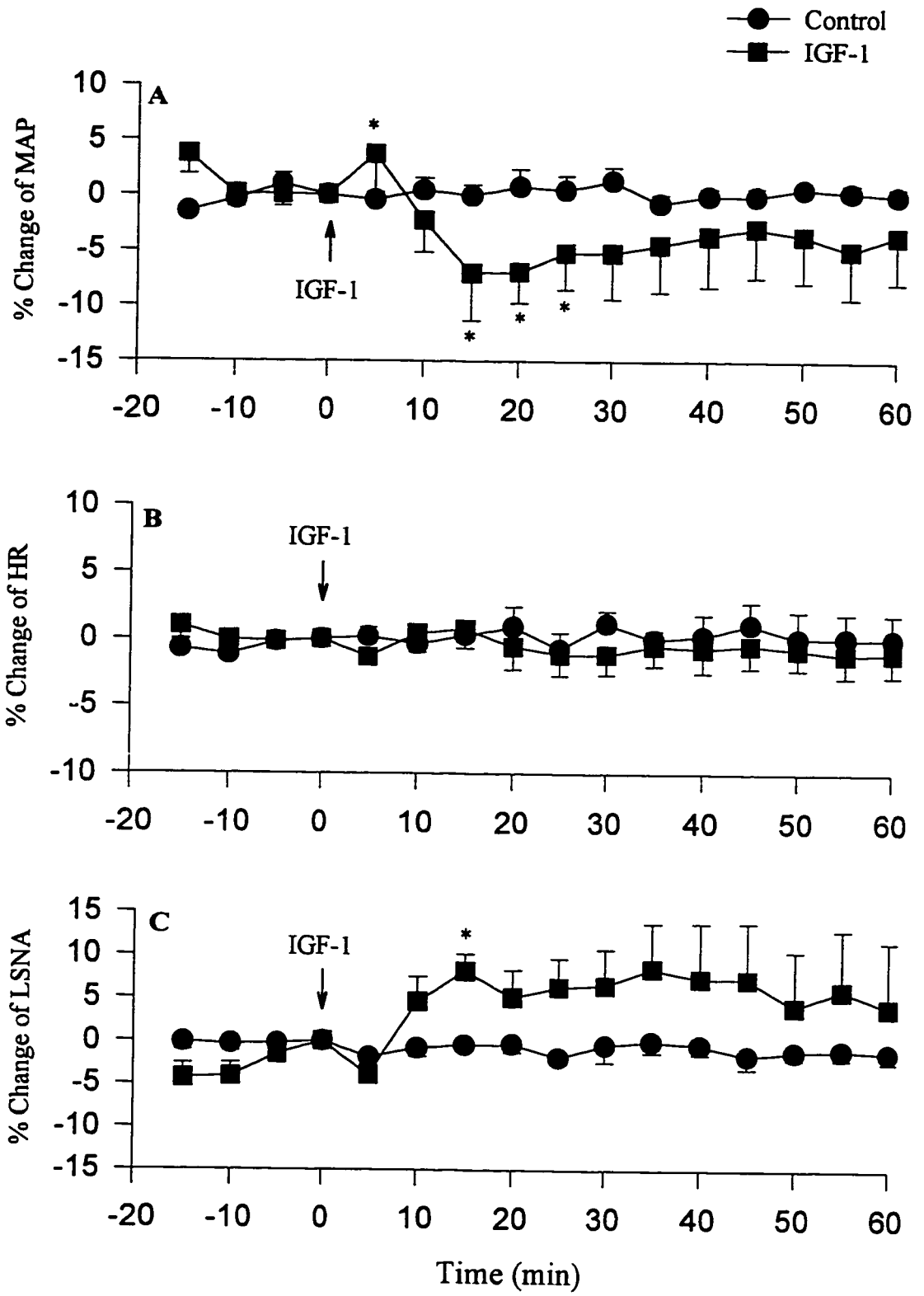


Figure 12

Mean arterial pressure (MAP, panel A), heart rate (HR, panel B), and lumbar sympathetic nerve activity (LSNA, panel C) response to intravenous infusion of insulin-like growth factor 1 (IGF-1) 40ug/animal compared to saline controls. Animals were given a glucose bolus 45 mg at time -20 minutes, followed by a constant infusion of glucose $\cong$ 150 mg/hour. The arrows are the time of IGF-1 or control saline infusion. Values are mean $\pm$ SE expressed as percent change from basal at time 0. MAP $p<0.001$, IGF-1 vs. control; HR $p<0.05$, IGF-1 vs. control; LSNA $p< 0.001$, IGF-1 vs. control. * $p<0.05$ at selected time points.

THE EFFECT OF INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) WITH GLUCOSE INFUSION ON BLOOD PRESSURE, HEART RATE, AND LUMBAR SYMPATHETIC NERVE ACTIVITY (MEAN ± SE)

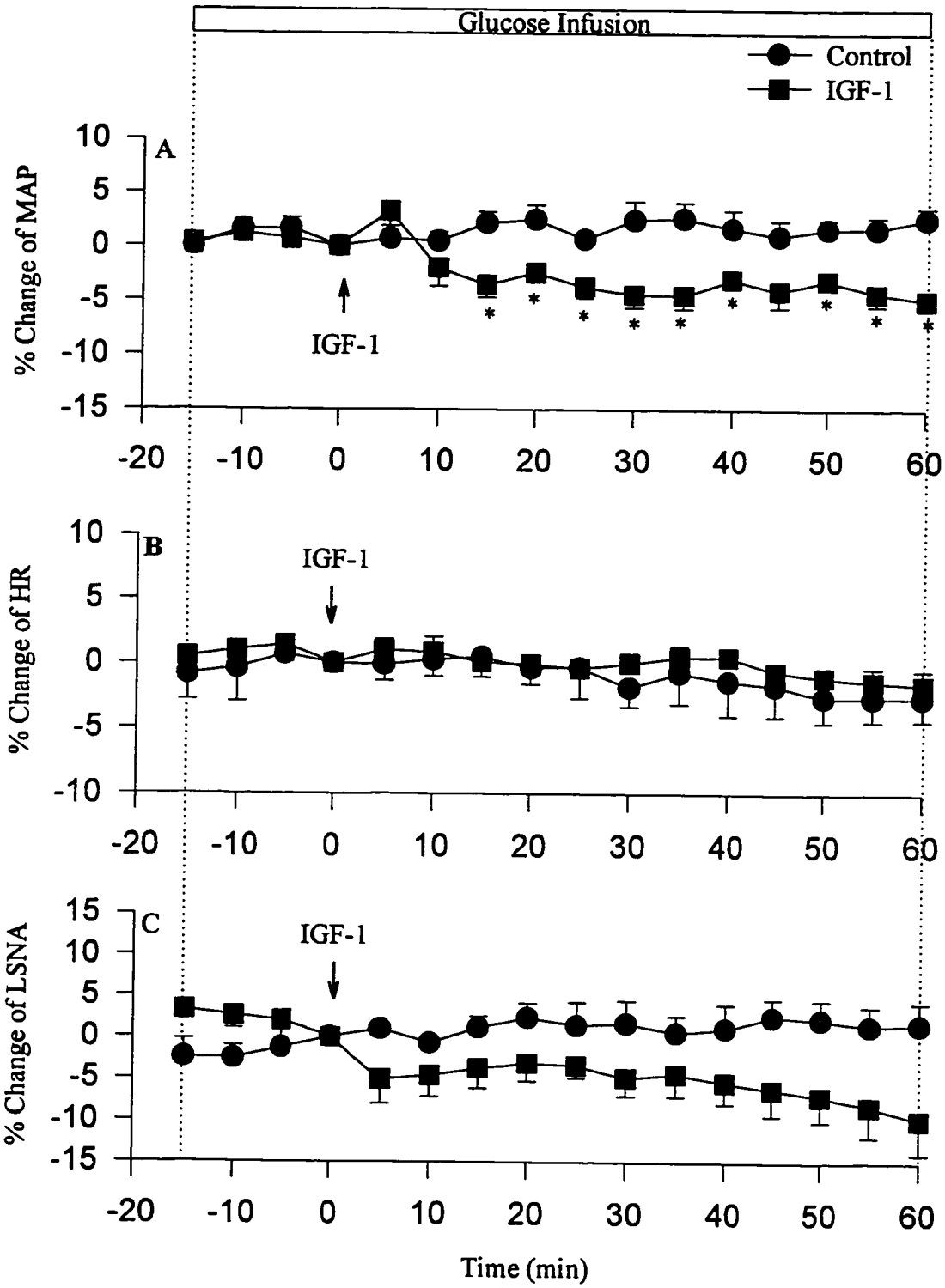


Figure 13

Renal sympathetic nerve activity (RSNA) response to intravenous infusion of insulin-like growth factor 1 (IGF-1) 40ug/animal compared to saline controls. Panel A is RSNA response in the absence of glucose infusion. In panel B, animals were given a glucose bolus 45 mg at time -20 minutes, followed by a constant infusion of glucose $\cong$ 150 mg/hour. The arrows are the time of insulin or control saline infusion. Values are mean $\pm$ SE expressed as percent change from basal at time 0. Panel A, IGF-1 vs. control, $p<0.001$; Panel B, IGF-1 vs. control, $p<0.05$. * $p<0.05$ at selected time points.

THE EFFECT OF SYSTEMIC INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) ON RENAL SYMPATHETIC NERVE ACTIVITY (RSNA) IN THE ABSENCE OR IN THE PRESENCE OF GLUCOSE REPLACEMENT (MEAN ± SE)

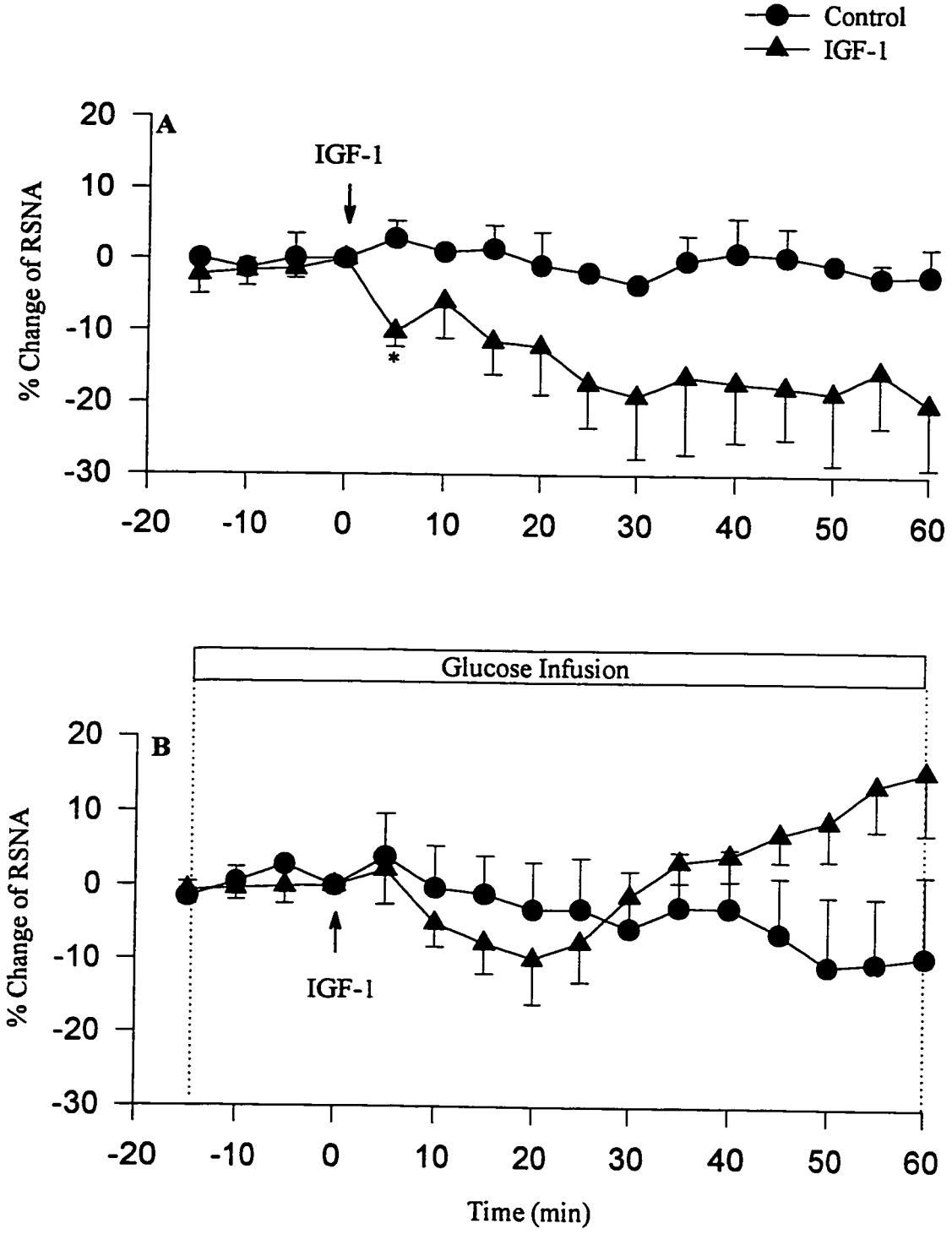


Figure 14

Left iliac conductance (the circles) response to intravenous insulin-like growth factor 1 (IGF-1) infusion 40ug/animal compared to right iliac conductance (the triangles) after the denervation of left iliac artery. The arrows are the time of denervation and insulin infusion. Values are mean $\pm$ SE expressed as percent change from baseline at the time of denervation. $p>0.05$, sympathetic denervated iliac conductance vs. sympathetic intact iliac conductance.

THE EFFECT OF DENERVATION OF THE LEFT ILIAC ARTERY ON VASCULAR FLOW (EXPRESSED AS CONDUCTANCE) RESPONSE TO SYSTEMIC INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) (MEAN ± SE)

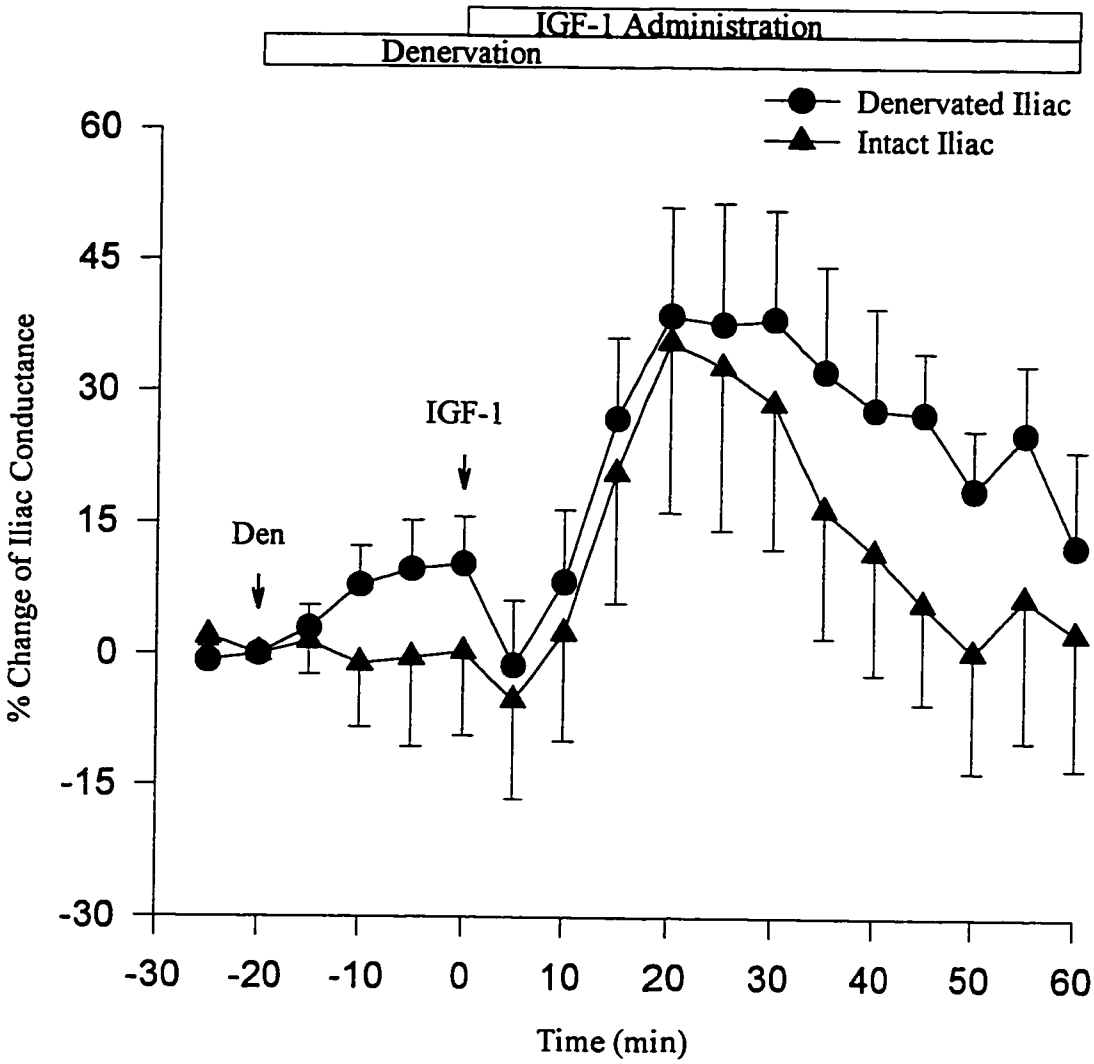


Figure 15

Left iliac conductance (the circles) response to intravenous insulin-like growth factor-1 (IGF-1) infusion 40ug/animal compared to right iliac conductance (the triangles) after the denervation of left iliac artery. Animals were given a glucose bolus 45 mg at 20 minutes before insulin infusion, followed by a constant infusion of glucose ≅ 150 mg/hour. Values are mean ± SE expressed as percent change from baseline at time 0, which is the time of IGF-1 infusion. $p<0.001$, sympathetic denervated iliac conductance vs. sympathetic intact iliac conductance.

THE EFFECT OF DENERVATION OF LEFT ILIAC ARTERY ON VASCULAR FLOW (EXPRESSED AS CONDUCTANCE) RESPONSE TO SYSTEMIC INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) WITH GLUCOSE REPLACEMENT (MEAN ± SE)

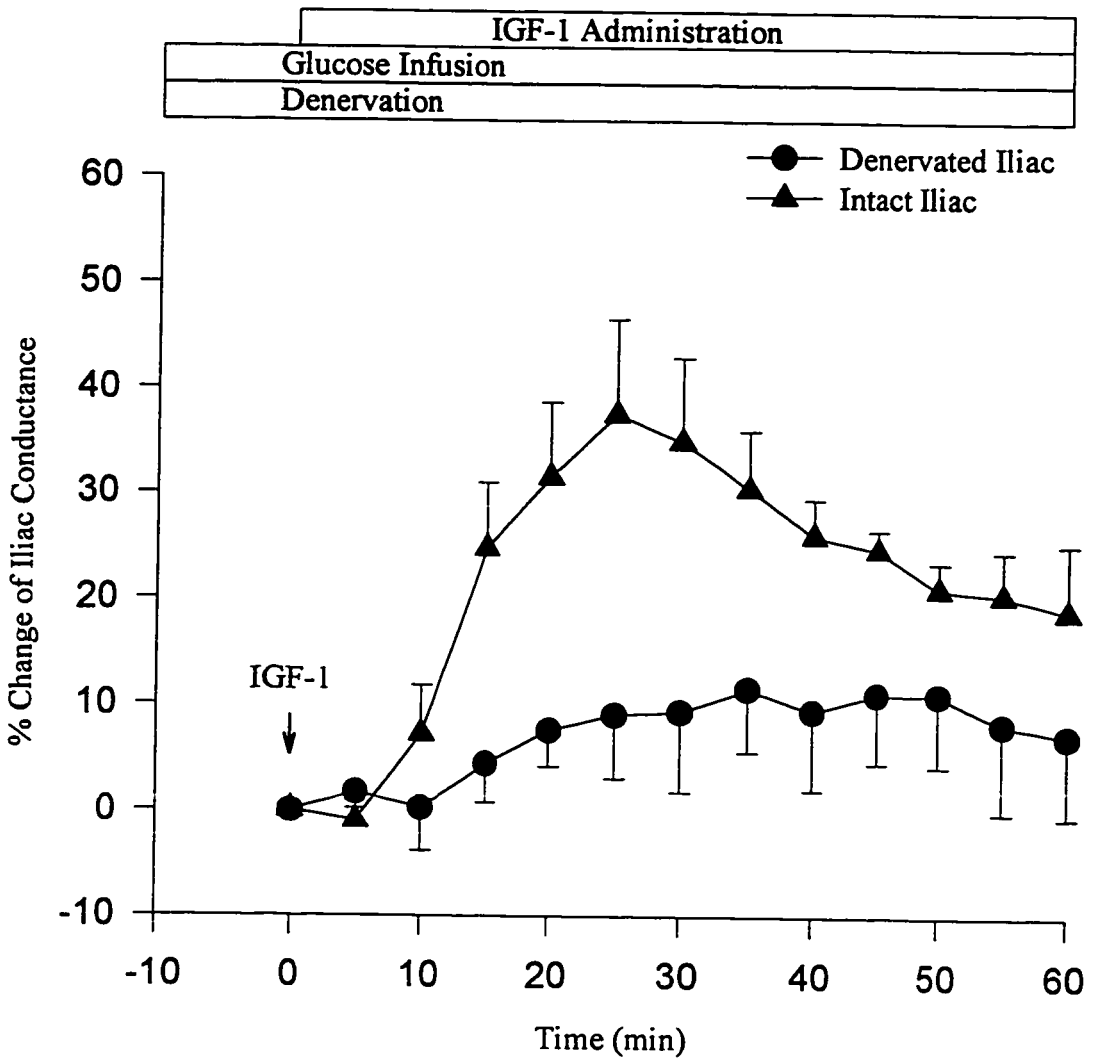
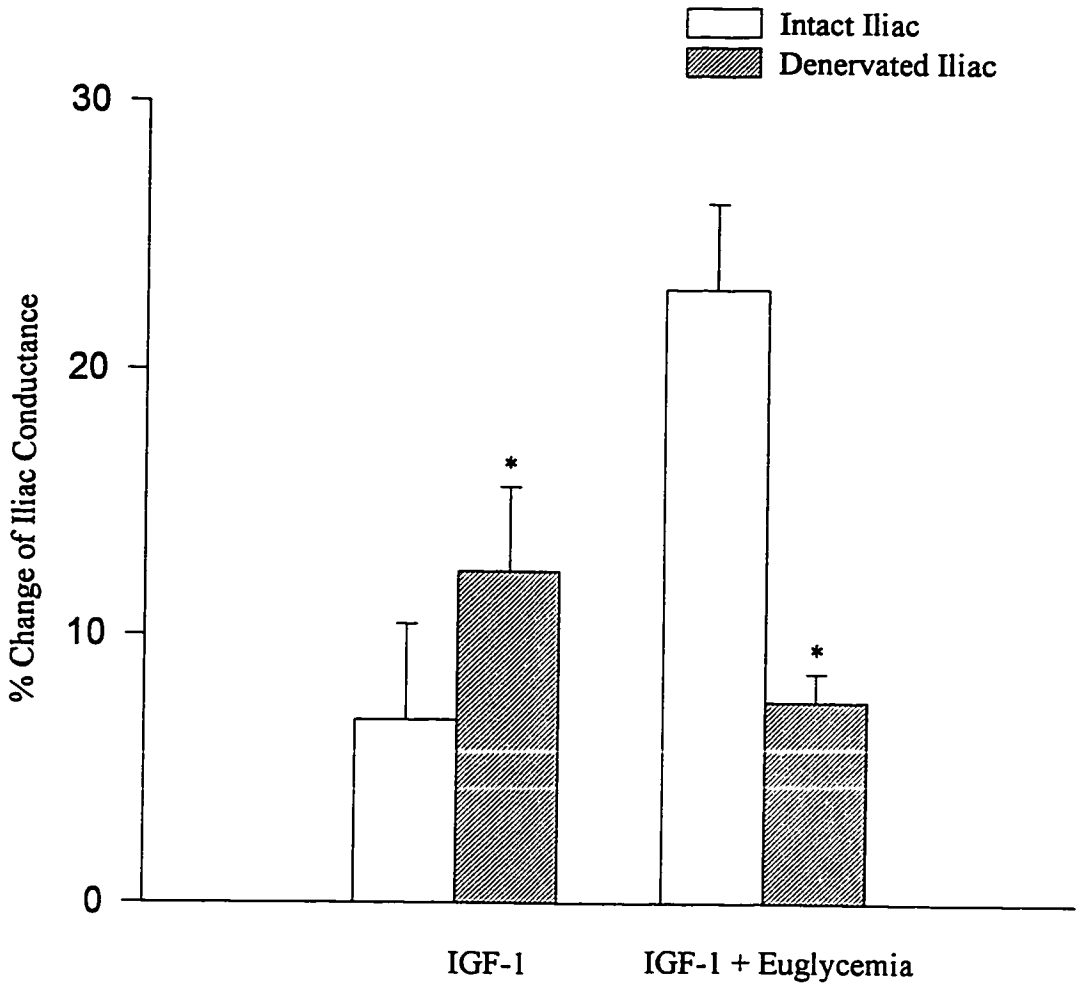


Figure 16

A comparison of sympathetic denervation on average iliac conductance response to intravenous infusion of insulin-like growth factor 1 (IGF-1) 40ug/animal in the absence or presence of glucose infusion. Values are mean $\pm$ SE, expressed as percent change from baseline, which is the basal level before IGF-1 infusion. Open bars, sympathetic intact iliac conductance; hatched bars, sympathetic denervated iliac conductance. *$p<0.05$ sympathetic denervated iliac conductance vs. sympathetic intact iliac conductance.

A COMPARISON OF THE EFFECT OF ILIAC DENERVATION ON VASCULAR FLOW (EXPRESSED AS CONDUCTANCE) RESPONSE TO IGF-1 IN THE PRESENCE OR ABSENCE OF GLUCOSE REPLACEMENT (MEAN ± SE)



DISCUSSION

PART I

I. The Effect of Systemic Insulin Infusion on Cardiovascular and Sympathetic Nervous System.

The short-term effects of insulin on carbohydrate metabolism has been most extensively studied and well-established, and our observations that acute intravenous insulin significantly decreased plasma glucose level were consistent with our previous studies and others (Schultz-Klarr et al., 1994; Liang et al., 1982). The cardiovascular responses following intravenous injections of insulin were also similar and consistent with former reports from both our laboratory and other laboratory (Schultz-Klarr et al., 1994; Wright-Richey et al., 1994; Liang et al., 1982), where we observed a prompt, significant decrease of mean arterial pressure (MAP) and a initial decrease at 10 to 15 minutes followed by a sustained increase of heart rate (HR) after 25 minutes post insulin infusion. The delayed rise of HR could have been due to baroreceptor reflex elicited by insulin-induced decrease in blood pressure, or the insulin-induced hypoglycemia that can strongly increase catecholamine release (Goldfien et al., 1958; Garber et al., 1976; Lamarche et al., 1992). Therefore, an increased plasma catecholamine level which can act directly on the heart may also attribute to the increased HR.

We observed a progressive increase in lumbar sympathetic nerve activity (LSNA) response to systemic insulin, which confirmed previous findings that insulin causes sympathetic excitation (Muntzel et al., 1995; Anderson et al., 1991). The underlying

mechanisms of insulin induced sympathoexcitation are still unknown. There are several potential mechanisms. First, a direct central effect of the hormone is likely. Supporting this central mechanism, Muntzel et al (1994) found that lesions of the anteroventral third ventricle region, an area rich in insulin receptors, abolish increases in lumbar sympathetic nerve activity in response to insulin. The fact that insulin and insulin receptors are present in the brain and insulin can cross the blood-brain barrier led to more and more investigations of insulin's central action. Most brain insulin is derived from the circulation and has a rapid access to its specific receptors in the brain (Baskin et al., 1983; Unger et al., 1991). It has been demonstrated that insulin may directly affect central regulation of cardiovascular responses and insulin affects CNS functions through multiple mechanisms, including alteration of neuronal activity in the hypothalamus, changes of catecholamine turnover and reuptake, or changes of glucose metabolism, although the precise sites of insulin action in the CNS have yet to be determined (Peinado and Myers, 1991; Oliver et al., 1989; Muntzel et al., 1994b; Kuo et al., 1993). Furthermore, acute central infusion of insulin resulted in increase in LSNA in the absence of changes in blood glucose or plasma insulin levels (Muntzel et al., 1994b). This gives direct evidence and support to the idea drawn from our results that insulin-induced elevation in LSNA may involve a CNS mechanism.

A second mechanism could be baroreflex mediated increases in sympathetic activity, since insulin induces vasodilation which leads to decreases in arterial pressure. When we looked at our results in detail, we found that the response of LSNA was slower than that of MAP to insulin. LSNA increased significantly 15 minutes after insulin infusion while the significant decrease of MAP occurred at 10 minutes, suggesting the

sympathetic nervous response might be secondary to the decrease of blood pressure or mediated by central nervous system (CNS) (Kuo et al., 1993; Nishimura et al., 1991; Muntzel et al., 1994b; Schultz-Klarr et al., 1994). Therefore, the decrease in arterial pressure following insulin administration may induce baroreceptor-mediated increases in sympathetic activity.

A third mechanism may involve hypoglycemia because when insulin is administered intravenously without glucose replacement, the insulin-induced hypoglycemia could contribute to the sympathetic activation (Fagius et al, 1986; Fagius and Berne, 1989; Hoffman et al., 1994). To eliminate the influence of insulin induced hypoglycemia on cardiovascular and sympathetic nervous response, we applied continous glucose infusion to maintain blood glucose at euglycemic levels. Again, MAP dropped and LSNA progressively increased after insulin infusion. These responses were similar to those when hypoglycemia was allowed to occur and support the notion that a co-existent decrease in MAP and increase in LSNA can be elicited by systemic insulin (Anderson et al., 1991; Frandsen et al., 1991; Berne et al., 1992; Vollenweider et al., 1993). This occurs because the sympathetic vasoconstrictor action is opposed by vasodilation. Insulin produces direct vasodilatory effect on the vasculature, leading to decreases in vascular resistance and increases in skeletal muscle blood flow. Hindlimb skeletal muscles are mostly innervated by the lumbar sympathetic nerves. Although insulin increased LSNA, this effect was overridden by the vasodilator action of insulin so that a decrease rather than increase in MAP occurred. But this observation is not consistent with some of the other studies that hyperinsulinemia significantly increased arterial pressure in normotensive and hypertension rats (Brands et al., 1991; Edwards et al., 1989; Meehan et al., 1990; Tomiyama et al.,

1992). One possible explanation for the differences is that we used anesthesia in our studies, but in contrast, conscious rats were used in the other studies.

The HR response to insulin when hypoglycemia was prevented was absolutely different from the response when blood glucose was allowed to drop. When hypoglycemia was prevented, systemic insulin resulted in a slight but significant decrease in HR. A likely explanation is that the increase in catecholamine release following hypoglycemia was prevented and thus catecholamine's positive chronotropic effect on the heart to increase HR disappeared. Instead, insulin's direct effect on heart rate to decrease it has been showed. This effect may be elicited through CNS mechanism. Supporting this, a report has demonstrated that intracerebroventricular injection of insulin decreased HR (Schultz-Klarr et al., 1994a). In both hypoglycemic and euglycemic conditions, insulin decreased MAP, but HR increased during hypoglycemia and decreased with blood glucose maintained. This latter observation does not support the baroreceptor mechanism which suggests the baroreflex-mediated increases in sympathetic tone being responsible for the increased HR.

To further differentiate the effects of insulin from those of insulin-induced hypoglycemia, we used 2-DG, a competitive inhibitor of glucose utilization, to produce glucopenia. Systemic 2-DG induced small decreases in MAP and HR, while LSNA was continuously increased to the level comparable to that followed by insulin infusion. These findings were consistent with previous studies from our laboratory as well as others which has demonstrated that 2-DG may act centrally to decrease peripheral sympathetic tone to the heart, leading to decreased MAP and HR (Schultz-Klarr et al., 1994a; Egawa et al., 1989). Previous studies also reported that 2-DG increased plasma catecholamine levels

(Carlsson et al., 1992). Our observations are supported by these studies. But there was evidence showing increased cardiovascular tone by systemic 2-DG, which is not consistent with our observation (Schultz-Klarr et al., 1994a and 1994b). However, we still can not explain why increased LSNA is accompanied by the decline in blood pressure and heart rate. It is possible that the rise of LSNA which innervates the hind limb vasculature causes neurogenic vasodilation instead of vasoconstriction (Davisson et al., 1996).

Evidence has already shown that under euglycemic clamp, systemic infusion of insulin produces non-uniform regional responses in sympathetic nerve activity in normotensive humans or rats. Elevation of muscle sympathetic nerve activity and lumbar sympathetic nerve activity cannot extend to skin, renal, and adrenal sympathetic nerve activity (Morgan et al., 1993; Anderson et al., 1991; Berne et al., 1992). To explore more closely the regional sympathetic nervous response to insulin and the role of insulin-induced hypoglycemia, renal sympathetic nerve activity (RSNA) in response to peripheral insulin was examined, either in the absence of or in the presence of glucose infusion. The cardiovascular responses to systemic insulin was similar to what we observed before when LSNA was recorded. When hypoglycemia was allowed to occur, insulin infusion resulted in a delayed but dramatic increase in RSNA which reached to the maximum value approximately 60% above basal control level at 40 minutes. The possible mechanisms for the increase in RSNA by systemic insulin include 1) alteration of blood glucose, 2) baroreflex mediated response, 3) central neuronal actions. As we mentioned before, hypoglycemic causes the elevation of plasma catecholamine levels and sympathetic nerve activity. Supporting this, we observed that when glucose replacement was applied to prevent hypoglycemia, RSNA actually went down. The increased RSNA may also

represent baroreceptor-mediated baroreflex secondary to the significant fall in MAP. But obviously, this would not be the sole mechanism, since we have already seen the important role hypoglycemia played in it. Besides, insulin may directly act on CNS to alter RSNA. Nishiura et al. (1991) have reported that intracerebroventricular infusions of insulin elicited significant decreases in blood pressure and bradycardia accompanied by decreases in RSNA. Compared to the elevation of LSNA, insulin infusion resulted three fold greater increase in RSNA.

Thus, bolus intravenous insulin when hypoglycemia was allowed to occur resulted in a significant increase in LSNA and RSNA. When glucose infusion was applied to prevent hypoglycemia, insulin infusion again elicited a decrease in MAP and a rise in LSNA, but HR and RSNA were lowered.

II. The Effect of Systemic Insulin Infusion on Cardiovascular and Sympathetic Nervous System after Sino-Aortic Denervation.

We have seen that insulin-mediated vasodilation can cause significant decreases in arterial pressure. This has raised a further possibility that the increases in sympathetic nerve activity after insulin infusion may be mediated by the baroreceptor reflex. To investigate the role of baroreceptor reflex in insulin-induced decrease in MAP and increases in LSNA or RSNA, we performed experiments using sino-aortic baroreceptor denervated rats. We have found that in SAD rats, insulin infusion in the presence of glucose maintenance still evoked significant decrease in MAP and increase in LSNA. But compared to the responses in rats with intact baroreceptors, both the decrease in MAP and the increase in LSNA was attenuated. These findings suggested that insulin-induced

lumbar sympathetic nerve activation is mostly independent of the baroreflex. But the fact that there was a small attenuation of increased LSNA in SAD rats also indicates the baroreflex may have a little contribution to the increased LSNA. As for the attenuation of insulin-induced decrease in MAP, an elevation of sympathetic-mediated vascular tone in SAD animals may be responsible for it (Scislo and DiCarlo, 1994; Sheriff et al., 1990). Probably due to the same reason, the decrease in HR after insulin was also prevented by SAD. It appears that barorecepter-denervated rats have altered sympathetic control of vasomotor tone, renin release from the kidney, and vasopressin release from the posterior pituitary. Besides SAD rats are more reactive to environmental stimuli and show exaggerated cardiovascular responses to various treatments compared with baroreceptor-intact rats (Trapani et al., 1986; Alper 1987; Jacob et al., 1991).

When hypoglycemia was allowed to occur, insulin infusion in SAD animals decreased MAP and increased RSNA to similar degrees as the responses in baroreceptor intact rats. This indicates the baroreceptor reflex does not play a major role in insulin induced elevation in RSNA. In addition, the increase of heart rate by insulin was attenuated by SAD, which suggests that baroreflex takes part in the HR response to insulin and may have some excitatory effect on it.

However, these data support our demonstration that insulin causes a selective increase in sympathetic nerve activity. The action of insulin on cardiovascular and sympathetic nervous system is contributed by its direct peripheral action as well as central mechanisms. The baroreceptor mechanism is also involved.

III. The Effect of Iliac Artery Denervation on Iliac Blood Flow in Response to Systemic Insulin.

To further evaluate the role of sympathetic nervous system in the regulation of blood flow and blood pressure, we investigated the effects of lumbar sympathectomy on hindlimb blood flow and its responses to peripheral insulin. Changes in arterial pressure are affected by altering cardiac output which is the volume of blood pumped into arteries per unit time and diameter of the resistance vessels. Peripheral resistance is the resistance against which the heart pumps. Total vascular resistance is equal to mean arterial pressure divided by the blood output of left ventricle. The small arteries and arterioles are the principal site of the peripheral resistance. We examined the vascular responses (calculated as conductance which is the reciprocal of resistance) of iliac arteries which are the main supplies of blood to hindlimb skeletal muscles.

Acute left lumbar sympathectomy caused immediate increase in blood flow in corresponding hindlimb , which suggests that at least part, if not all, of lumbar sympathetic nerve contains vasoconstrictor fibers. And these fibers have tonic activity to this vascular bed. Immediately upon cutting the lumbar sympathetic nerve, the vasoconstrictor tone decreased, vascular resistance fell and blood vessels dilated in hindlimb. Similar to previous studies in our laboratory, insulin increased iliac conductance (Pete 1996), in addition, we observed a greater elevation of iliac conductance in sympathetically denervated iliac artery than that of sympathetic intact iliac artery. This also suggests that lumbar sympathetic nerve contains tonic vasoconstrictor fibers.

All blood vessels except capillaries and venules are innervated by sympathetic nerves of the autonomic system. The motor nerve fibers to the resistance vessels regulate

tissue blood flow and arterial pressure. The fibers to the venous capacitance vessels control the volume of blood in the veins. In skeletal muscle, the resistance vessels are innervated by noradrenergic fibers which can cause vasoconstriction. There are vasodilator fibers which ending on the resistance vessels of the skeletal muscles. They are cholinergic fibers but travel with the sympathetic nerve (sympathetic vasodilator system).

According to a recent study, the fall in hindlimb resistance may result from the activation of sympathetic neurogenic vasodilation (Davisson et al., 1996). Our observation that insulin exerted co-existence of the fall of MAP and the elevation in LSNA also led us to postulate that activation of LSNA may result in vasodilation in hindlimb, and thus increase blood flow in the hindlimb and decrease arterial pressure. However, our results suggests that insulin stimulated increase in blood flow in skeletal muscle is not mediated by lumbar sympathetic nerve activation induced neurogenic vasodilation. But, so far we cannot make a definitive conclusion that lumbar sympathetic nerves only contain primarily vasoconstrictor fibers, and contain minimal vasodilator fibers. Lumbar sympathetic nerves may contain vasodilator fibers that have low tonic discharge. If they are cut, no vasodilation or elevation in vascular conductance would be observed. But if they were activated, they might still cause sympathetic vasodilation in skeletal muscle.

In addition, insulin-induced hypoglycemia causes increased norepinephrine and epinephrine secretion from adrenal medulla (Goldfien et al., 1958, Liang et al., 1982; Yamaguchi et al., 1989). We suggest that epinephrine probably reinforces the insulin-induced dilation of muscle blood vessels.

When insulin-induced hypoglycemia was prevented by glucose infusion, the iliac conductances were increased equivalently in sympathetic denervated iliac and sympathetic

intact iliac arteries. But the average increases in iliac conductances were significantly lower than that of denervated iliac artery in the absence of glucose replacement, which was supportive of our previous observation that insulin itself, despite of glycemic status, as well as insulin-induced hypoglycemia can elicit lumbar sympathetic activation, associated with vasodilation and increased muscle blood flow. Since lumbar sympathetic denervation did not affect the iliac blood flow response to systemic insulin when blood glucose was maintained, the rise of blood flow in hindlimb is probably mainly due to insulin's direct vasodilatory effect, and not to sympathetic neuronergic vasodilation.

Taken together, intravenous infusion of insulin resulted in a decrease in MAP, increase in HR, LSNA and RSNA. When glucose replacement was applied to prevent hypoglycemia, insulin again decreased MAP and increased LSNA, but now, HR and RSNA were decreased. 2-DG induced glucocytopenia also exerted a increase in LSNA. In sino-aortic baroreceptor denervated (SAD) animals, when blood glucose was maintained, the decreased MAP and increased LSNA in response to insulin were all attenuated compared to those of baroreflex intact rats. And the insulin induced decrease in HR was blunted by SAD. Under hypoglycemia, insulin elicited a similar fall in MAP, elevations in HR and RSNA in SAD rats compared to those in normal rats. Systemic insulin caused a greater increase of blood flow in sympathetic denervated iliac artery compared to that in sympathetic intact iliac artery, when hypoglycemia was allowed to occurred. When hypoglycemia was prevented, insulin still increased blood flows in both sympathetic denervated and intact iliac artery, but now the average increases were lowered. From all of these data, we conclude that insulin may decrease MAP through its direct effect on vasculature to induce endothelial derived NO and cause vasodilation.

Insulin also decreases HR and this effect could be mediated by CNS mechanism. Insulin selectively increases sympathetic nerve activity and this process is modulated by glycemic status and the baroreflex.

PART II

I. The Effect of Systemic IGF-1 Infusion on Cardiovascular and Sympathetic Nervous System.

We observed a decline in plasma glucose in response to systemic IGF-1 in normal animals as we expected. It has been established that IGF-1 exerts a hypoglycemic effect in both normal animals and humans as well as insulin, but with a potency much less than that of insulin (Jacob et al., 1989; Schmitz et al., 1991; Guler et al., 1987). The lowered plasma glucose level caused by IGF-1 is primarily due to the stimulation of peripheral glucose disposal (Laager et al., 1993). IGF-1 has no effect on hepatic glucose production or lipolysis at low doses, and this is mostly attributed to a lack of functional IGF-1 receptors in hepatocytes and adipocytes. At high doses, IGF-1 exerts its effects on hepatic and adipose tissues by cross reaction with insulin receptor (King et al., 1980; Zapf et al., 1981). IGF-1 also suppresses insulin secretion, as well as inhibiting glucagon secretion. This latter effect contributes to its hypoglycemic effect (Leahy and Vandekerhove, 1990; Guler et al., Rennert et al., 1993). IGF-1 induced hypoglycemia is accompanied by reduced counter-regulatory glucagon secretion which impairs glucose recovery, and most likely, the suppression of insulin and glucagon by IGF-1 is direct via receptor mediated effects on the respective endocrine cells. IGF-1 receptors have been

found on both pancreatic α and β cells (Van Schravendijk et al., 1987). The secretion of cortisol and norepinephrine, the other counterregulatory hormones to hypoglycemia during IGF-1 administration, remains unaffected but epinehprine secretion is reduced (Kerr et al., 1993).

Since IGF-1 has potent effects on glucose, lipid and amino acid metabolism that closely resemble those of insulin (Binoux, 1995; Fryburg, 1994; Lewitt, 1994), it has been proposed as a possible treatment for clinical disorders associated with insulin resistance. There has been a rapid expansion of interest in the physiological function of IGF-1 and the mechanisms for its effects. Recently, investigations conducted in our laboratory have demonstrated that IGF-1, similar to insulin, plays a role in modulating cardiovascular dynamics (Pete et al., 1996; Hu et al., 1996). A bolus systemic administration of IGF-1 resulted in a significant depressor effect on blood pressure and enhanced regional blood flow preferentially in the kidney and in the skeletal muscle. To define the effects of IGF-1 on cardiovascular regulation more precisely, we simultaneously measured cardiovascular parameters and sympathetic nerve activity in response to IGF-1.

Similar to insulin, IGF-1 lowered mean arterial pressure no matter hypoglycemia occurred or not. As we mentioned previously, intravenous infusion a bolus of insulin results in a fall of mean arterial pressure, which appears to be mediated by its direct effect on the vascular endothelial cells through insulin receptors. IGF-1 receptors are also present on vascular smooth muscle cells and endothelial cells in both skeletal muscle and the viscera (Pillion, 1988; Motham et al., 1989). Since insulin and IGF-1 and their receptors share structural similarities, IGF-1 could also decrease MAP by its direct action on its receptors on vascular smooth muscle cells. Numerous evidences suggest that the

endothelium-derived relaxing factor, nitric oxide (NO), is a important mediator of IGF-1 induced vascular relaxation (Tsukahara et al., 1994; Wu et al., 1994; Sowers, 1996; Walsh et al., 1996). NO is the most potent endogenous vasodilator. It induces vasodilation by stimulating soluble guanylate cyclase in vascular smooth muscle cell to produce cyclic guanosine 3'5'-monophosphate (cGMP) (Palmer et al., 1988; Rees et al., 1989; Vallance et al., 1989). IGF-1 can stimulate NO production in intact vessels. It has been reported that in the aorta, renal, and tail arteries, IGF-1 induces vasodilation and increases blood flow (Guler et al., 1989a; Hirschberg et al., 1993; Wu et al., 1994; Walsh et al., 1996). The NO synthase inhibitors N^G-nitro-l-arginine methyl ester (L-NMMA) or N^W-nitro-L-arginine methyl ester (L-NAME) inhibit the vasodilatory effects of IGF-1. In addition, IGF-1 induces a dose-dependent increase in cGMP, which is enhanced in vascular smooth muscle by NO (Baron et al., 1988).

Another possible mechanism of IGF-1 induced decrease in MAP is IGF-1's central neural action. The presence of IGF-1 and its receptors in the CNS is now firmly documented (Oomura and Plata-Salaman, 1987; Baskin et al., 1987). Previous studies in our laboratory have demonstrated that intracerebroventricular infusion of IGF-1 decreases mean arterial pressure. This response is associated with selective increases in blood flow to skeletal muscle (Hu et al, 1996). Since it has not been reported that systemic IGF-1 can gain rapid access to the brain by crossing blood brain barrier, we can not be certain whether the decrease in MAP by systemic infusion of IGF-1 is partially mediated by central mechanism or not. IGF-1 may exert its effect through either its own receptors or insulin receptors (Yagi et al., 1992; Ram et al., 1993; Pete et al., 1996).

In addition to the reduction of MAP, we have observed that IGF-1 administration

increased LSNA but only during hypoglycemia. When blood glucose was maintained so that the confounding changes followed by hypoglycemic were excluded, IGF-1 actually suppressed LSNA. This observation suggests that IGF-1 acts to decrease the sympathetic nerve activity to the skeletal muscle, which is contrary to the effects of insulin. The elevation of LSNA induced by IGF-1 in the absence of glucose replacement is caused by IGF-1 induced hypoglycemia. It is supported by previous findings that hypoglycemia and 2-DG induced glucocytopenia can exert sympathetic activation (Carlsson et al., 1992; Havel et al., 1996). IGF-1 may have direct effect on sympathetic nerves to decrease activity. This process could be mediated by IGF-1 induced alteration of sympathetic neuron metabolism and/or decrease in membrane activity.

We would expect that when glycemia was maintained, the lumbar sympathetic nerve activity would be increased by baroreceptor mediated reflex, secondary to IGF-1 induced decrease in MAP. The absence of this response in our study is probably because the direct inhibitory action of IGF-1 on sympathetic nerve activity overrode the baroreflex mediated response.

Under both hypoglycemia and normal condition, heart rate did not change in response to systemic infusion of IGF-1. Heart rate could be raised by the baroreflex, induced by the decrease in MAP and/or hypoglycemia following IGF-1 infusion. In the same time, the decreased sympathetic nerve activity induced by IGF-1 could lower heart rate. These opposite responses may result in no change in heart rate. But this result is not consistent with the previous finding that intravenous IGF-1 induced a elevation of heart rate (Pete et al., 1996). This discrepancy may be explained by the predominate effects of decreased MAP and hypoglycemia to increase sympathetic nerve activity under these

experimental condition.

When we investigated the effects of systemic infusion of IGF-1 on renal sympathetic nerve activity, it was found that IGF-1 decreased RSNA during hypoglycemia and euglycemia. This supports our proposal that IGF-1 acts directly to decrease sympathetic nerve activity. Therefore, the increased blood flow to the kidney could be mediated by not only IGF-1's effect on vasculature to cause vasodilation, but also its effect on renal sympathetic nerve to decrease RSNA. This could also explain the relative greater response of renal blood flow after IGF-1 infusion (Pete et al., 1996; Hu et al., 1996; Jaffa et al., 1994).

Therefore, insulin and IGF-1 have different effects on sympathetic nervous system, and obviously they act through different mechanisms. Studies have demonstrated that stimulation of hypothalamic regions in the CNS is critical for insulin induced cardiovascular responses and elevations in sympathetic outflow. Lesions of areas around the third ventricle abolished elevations in sympathetic nerve activity in response to systemic insulin (Muntzel et al., 1995; Schultz-Klarr et al., 1994; Wright-Richey et al., 1994). In addition, lesions of the ventro-medial hypothalamus modulated the cardiovascular actions of insulin (Wright-Richey et al., 1994). These data indicate that the effects of acute peripheral insulin on the sympathetic and cardiovascular system may be mediated by a CNS mechanisms. The fact that insulin can gain rapid access to the brain with specific transporters to facilitate its passage through the blood brain barrier supports this notion (Van Houten et al., 1979; Baura et al., 1993;). The lack of ability of IGF-1 to quickly enter the brain may attribute to the different capacity of insulin and IGF-1 to increase sympathetic nerve activity.

However, systemic IGF-1 can act directly to decrease LSNA and RSNA. This effect is modulated by glycemic status. IGF-1 also decreases MAP. This response has been attributed to IGF-1's effect on vasculature to produce NO, which causes vasodilation and increases blood flow in skeletal muscle and renal vasculature. The NO-mediated process may be enhanced directly or indirectly by IGF-1 induced decreases in sympathetic nerve activity.

II. The Effect of Iliac Artery Denervation on Iliac Blood Flow in Response to Systemic IGF-1 Infusion.

Despite the similarities of structural features and some biological effects between insulin and IGF-1 and their receptors, some differences, such as signaling pathways, receptor distribution, and regulators for synthesis and secretion, etc., do exist. Therefore it is not surprising that insulin and IGF-1 have distinct effects on sympathetic nervous system. To further evaluate the role of the lumbar sympathetic nerves on regulation of hindlimb blood flow to systemic IGF-1, we conducted lumbar sympathectomy experiments similar to what we have done for insulin.

Acute lumbar sympathectomy resulted in a immediate increase in blood flow in the corresponding hindlimb. This response is consistent with the previous observation in the insulin study. Again, it is confirmed that lumbar sympathetic nerve has tonic vasoconstrictive discharge to the vasculature in hindlimb. Under hypoglycemic conditions, systemic IGF-1 administration elicited similar increases in vascular conductance in both sympathetic denervated iliac artery and sympathetic intact iliac artery, in agreement with the observations that IGF-1, similar to insulin, can dilate vascular beds and increase blood

flow in skeletal muscle (Copeland and Nair, 1994; Kerr et al., 1993; Pete et al., 1996). Given the similarities between insulin and IGF-1 and their respective receptors, we propose that IGF-1, like insulin, causes vasodilation by its direct action on the vasculature. It stimulates endothelial cells and smooth muscle cells to produce NO, which in turn dilates blood vessels. Supporting this, it has been shown that preinfusion with the NO inhibitor, LNAME, abolished the effects of IGF-1 on flow (Pete et al., 1996). But unlike insulin, which is produced outside vascular system and needs to pass across the endothelium to reach the vascular smooth muscle sells, IGF-1 is synthesized by vascular smooth muscle cells and probably acts in an autocrine and/or paracrine fashion (Sarzani et al., 1989; Ginnella-Neto et al., 1992; Sowers, 1994; Sowers, 1997).

Another contribution for IGF-1 induced increase in vascular blood flow is hypoglycemia elicited by IGF-1. As we have mentioned, hypoglycemia can stimulate catecholamine release from adrenal medulla, the elevated epinephrine can activates β_2 receptors in skeletal muscle blood vessels, leading to their dilation.

Although there was no statistically significant difference of the increased iliac conductance between sympathetic denervated iliac artery and intact sympathetic innervated iliac artery in response to systemic IGF-1, there is a tendency that in sympathetic denervated iliac artery, the average increase of iliac conductance is greater than that in the intact iliac artery. This result also agrees with the demonstration that lumbar sympathetic nerves have vasoconstrictive effects on vascular beds in skeletal muscle.

Then, we repeated the experiments with continuous glucose infusion to eliminate the influence of IGF-1 induced hypoglycemia. Again, in sympathetic intact iliac artery,

IGF-1 increased iliac conductance to a level comparable to that under hypoglycemic condition. But, in the sympathetic denervated iliac artery, the vascular conductance was suppressed by IGF-1. According to our previous finding, when hypoglycemia was prevented, IGF-1 decreased LSNA, which could enhance its vasodilatory effect in skeletal muscle. This could explain why in sympathetic denervated iliac artery, the ability of IGF-1 to increase blood flow was diminished. This observation also supports that IGF-1 has direct effect on vascular beds to cause vasorelaxation.

In summary, a bolus intravenous infusion of IGF-1 resulted in decrease in MAP. This is mainly mediated by IGF-1's direct action on vasculature to produce endothelial-derived NO and cause vasodilation. IGF-1 decreased LSNA, which was modulated by glycemic condition. IGF-1 also lowered RSNA independent of glycemic status. IGF-1 induced decrease in sympathetic nerve activity is probably due to its direct effect on sympathetic nerves. In addition, the decreased sympathetic nerve activity may directly or indirectly augment IGF-1's vasodilatory effect.

CONCLUSION

Intravenous infusion of insulin resulted in a significant decrease in mean arterial pressure (MAP) and significant increase in heart rate (HR), lumbar sympathetic nerve activity (LSNA), and even greater increases in renal sympathetic nerve activity (RSNA). However, when glucose was infused to prevent hypoglycemia, insulin deceased MAP and increased LSNA, but HR and RSNA were deceased. 2-DG induced cyto-glucopenia elicited a increase in LSNA. When the influence of the baroreflex on cardiovascular system was removed by sino-aortic denervation (SAD) and in the absence of hypoglycemia, insulin infusion resulted in an attenuated decrease in MAP and an attenuated increase in LSNA compared to that with intact baroreflex. SAD also blunted the insulin-induced decrease in HR. Under hypoglycemia conditions, insulin elicited a decrease in MAP and increase in HR as well as RSNA in SAD rats to the similar levels to those in normal rats. Systemic insulin caused a greater increase in blood flow in sympathetic denervated iliac artery compared to that in sympathetic intact iliac artery, when hypoglycemia was allowed to occur. When hypoglycemia was prevented, insulin still increased blood flows in both sympathetic denervated and intact iliac artery, but now the average increases were lowered.

We propose that insulin decreases MAP via direct vasodilation. Insulin has selective effects on regional sympathetic nerve activities. Insulin may act directly on LSNA and RSNA to increase or decrease them respectively. In addition, the decrease of HR displayed via insulin injection with glucose replacement suggests that insulin acts directly to decrease the HR probably by a central nervous system mechanism.

Hypoglycemia can augment insulin's vasodilatory effect in skeletal muscle and also contribute to increased HR and partially contribute to increased sympathetic nerve activity after insulin infusion. The baroreflex is involved in these responses but is not responsible for them.

Systemic insulin-like growth factor 1 (IGF-1) caused a decrease in MAP and increase in LSNA similar to those observed in systemic insulin, but to a lesser extent. When glucose was infused to prevent hypoglycemia, IGF-1 decreased both MAP and LSNA. IGF-1 decreased RSNA independent of the glycemic state. Systemic IGF-1 caused similar increases in blood flow in sympathetic denervated iliac artery compared to that in sympathetic intact iliac artery, when hypoglycemia was allowed to occurred. When hypoglycemia was prevented, IGF-1 induced increases in blood flow were suppressed in sympathetic denervated iliac artery, but the blood flow in intact iliac artery increased comparable to that under hypoglycemic condition.

Therefore, we propose that IGF-1 decreases MAP by direct action on vasculature similar to insulin, but the sympathetic nervous response to IGF-1 is different from that of insulin. IGF-1 may act directly to decrease LSNA as well as RSNA, which is modulated by glycemic status. Like insulin, IGF-1 elevates blood flow in skeletal muscle by its direct effects on vascular beds to cause vasodilation and this process is augmented by IGF-1 induced decreases in sympathetic nerve activity to hindlimb blood vessels.

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ABSTRACT

THE INTEGRATIVE RELATIONSHIP BETWEEN INSULIN AND INSULIN-LIKE GROWTH FACTOR 1 INDUCED CARDIOVASCULAR RESPONSES AND SYMPATHETIC NERVOUS RESPONSES

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Insulin and insulin-like growth factor 1 (IGF-1) share some structure homologies and exert similar metabolic as well as cardiovascular actions. Insulin and IGF-1 have been demonstrated to decrease cardiovascular tone and increase blood flows in skeletal muscle. Insulin has also been showed to increase sympathetic nerve activity that may play a role in the regulation of cardiovascular dynamics. This study investigated the effects of insulin and IGF-1 on cardiovascular parameters and sympathetic nerve activity and the correlation between them. We also evaluated the role of baroreflex, plasma glucose level and regional sympathectomy in those insulin and IGF-1 induced responses. Normal wistar rats were anesthetized with chloralose/urethane. The femoral artery and vein were cannulated to monitor mean arterial pressure (MAP) and heart rate (HR) or for infusion or blood sampling. The lumbar sympathetic nerve or renal sympathetic nerve was isolated and placed on electrodes for nerve activity recording. Electromagnetic flow probes were placed around the iliac arteries for blood flow measurement. The systemic administration of insulin and IGF-1 resulted in significant decrease in MAP. Insulin increased lumbar

sympathetic nerve activity (LSNA) independent of the prevailing glucose concentration and baroreflex. It increased renal sympathetic nerve activity (RSNA) only under hypoglycemia condition. IGF-1 decreased both LSNA and RSNA, but this effect was modulated by glycemic status. Lumbar sympathectomy caused greater increase in skeletal muscle blood flow in response to both insulin and IGF-1 when hypoglycemia occurred. But when hypoglycemia was prevented, IGF-1 induced increase in blood flow was suppressed in sympathetic denervated iliac artery. We concluded that insulin and IGF-1 have both similar and distinct effects on cardiovascular system and sympathetic nervous system. They both may act directly on vasculature to elicit vasodilation thus decrease MAP. Insulin can selectively increase sympathetic nerve activity, while IGF-1 decreases sympathetic nerve activity. These processes are modulated by glycemic status. Baroreflex may be involved but is not responsible for them.

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