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Central infusion of leptin improves insulin resistance and suppresses β-cell function, but not β-cell mass, primarily through the sympathetic nervous system in a type 2 diabetic rat model

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ABSTRACT

Aims: We investigated whether hypothalamic leptin alters β-cell function and mass directly via the sympathetic nervous system (SNS) or indirectly as the result of altered insulin resistant states.
Main methods: The 90% pancreatectomized male Sprague Dawley rats had sympathectomy into the pancreas by applying phenol into the descending aorta (SNSX) or its sham operation (Sham). Each group was divided into two sections, receiving either leptin at 300 ng/kg bw/h or artificial cerebrospinal fluid (aCSF) via intracerebroventricular (ICV) infusion for 3 h as a short-term study. After finishing the infusion study, ICV leptin (3 μg/kg bw/day) or ICV aCSF (control) was infused in rats fed 30 energy % fat diets by osmotic pump for 4 weeks. At the end of the long-term study, glucose-stimulated insulin secretion and islet morphometry were analyzed.
Key findings: Acute ICV leptin administration in Sham rats, but not in SNSX rats, suppressed the first- and second-phase insulin secretion at hyperglycemic clamp by about 48% compared to the control. Regardless of SNSX, the 4-week administration of ICV leptin improved glucose tolerance during oral glucose tolerance tests and insulin sensitivity at hyperglycemic clamp, compared to the control, while it suppressed second-phase insulin secretion in Sham rats but not in SNSX rats. However, the pancreatic β-cell area and mass were not affected by leptin and SNSX, though ICV leptin decreased individual β-cell size and concomitantly increased β-cell apoptosis in Sham rats.
Significance: Leptin directly decreases insulin secretion capacity mainly through the activation of SNS without modulating pancreatic β-cell mass.

Introduction

Leptin is a secretory hormone primarily produced by adipocytes that regulates energy and glucose homeostasis through the hypothalamus (Sahu 2004). Increased adiposity elevates circulating leptin levels, which activate leptin signaling in the hypothalamus through potentiating phosphorylation of signal transducer and activator of transcription-3 (STAT3). This activation decreases adiposity by reducing appetite and increasing energy expenditure in a normal condition (Sahu 2004; Sandoval and Davis 2003). However, excessive gain of body fat suppresses the phosphorylation of STAT3, which attenuates hypothalamic leptin signaling, inducing leptin resistance and disturbing energy homeostasis so that increased body fat accumulation and increased food intake occurs. This condition is also accompanied by hyperinsulinemia (Bjorbaek and Kahn 2004).

The importance of leptin signaling in energy and glucose homeostasis is shown in rodents with leptin deficiency and/or leptin receptor mutation (Lee et al. 1996; Friedman and Halaas 1998). These rats exhibit obesity and glucose intolerance. In addition, leptin injected into leptin-deficient *ob/ob* mice dramatically reduces food intake, increases body temperature, and results in a loss of body weight (Minokoshi et al. 1999; Kamohara et al. 1997). Hypothalamic leptin injection also enhances glucose uptake in skeletal muscles, brown adipose tissues and the heart, through mediation of a β-adrenergic mechanism (Haque et al. 1999). Leptin injection reversed hyperinsulinemia by a synergistic interaction of central leptin and peripheral insulin in augmenting tissue glucose utilization (Haque et al. 1999). Thus, these results suggest that serum leptin levels are correlated with the body mass index, serum insulin levels, and insulin resistance in humans and experimental animals. However, patients with lipoatropic diabetes lose this correlation (Pardini et al. 1998). Although these patients have lower serum leptin levels due to lipoatropy, their insulin resistance is increased. These data indicate that insulin c in lipoatropic patients may be associated with impairment of β-cell function due to islet amyloidosis and β-cell atrophy rather than directly related to decreased glucose utilization in adipocytes (Garg et al. 1996).

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Leptin may also directly suppress insulin secretion. Acute physiological increase in serum leptin levels *in vivo* significantly reduces glucose-mediated insulin secretion in rats in a dose-dependent fashion (Cases et al. 2001). Demonstration of acute glucose intolerance by intracerebroventricular (ICV) administration of pharmacological doses of leptin in mice suggests that the effects of leptin in decreasing insulin secretion could be through its actions in the central nervous system (Fan et al. 2000). Muzumdar et al. (2003) and Haynes et al. (1999) showed that leptin decreases insulin levels by a predominantly central mechanism, probably via increasing sympathetic nervous system (SNS) activity through the melanocortin receptors. However, the effects of leptin in direct suppression of SNS on β-cell function and mass have not been studied yet.

SNS activity is associated with both energy balance and metabolic syndrome. Sympathomimetic medications decrease food intake, increase resting metabolic rate and thermogenic responses, whereas blockage of the SNS exerts opposite effects (Spraul et al. 1993). In addition, activation of the sympathetic nervous system inhibits insulin secretion by inhibiting Ca^{2+} influx through the voltage-dependent Ca^{2+} channel through alpha 2-adrenergic receptor (Hsu et al. 1991). However, the direct SNS effect on insulin secretion by its denervation into the pancreas and the modulation of β-cell mass by SNS remains unknown. Most studies have been performed in rats that had broad SNS denervation through the destruction of all the peripheral sympathetic nerve terminals by repeated injections of guanethidine or 6-hydroxydopamine (Dobbins et al. 2003; Haque et al. 1999). Central leptin improves insulin resistance through brown adipose tissues, skeletal muscles and the heart through activating SNS (Park et al. 2008). The improvement of insulin resistance is known to influence insulin secretion by increasing glucose utilization in those tissues to normalize serum glucose levels with less serum insulin levels (DeFronzo 2004). Thus, the injections of guanethidine or 6-hydroxydopamine cannot determine the specific effect of hypothalamic leptin on β-cell function and morphometry through SNS. Sympathetic innervations of the pancreas originate from preganglionic perikarya located in the thoracic and upper lumbar segments of the spinal cord, which receives the stimulus from the ventromedial hypothalamus (Kiba 2004). Sympathetic fibers appear to reach pancreatic islets by following blood vessels and nerve growth factor signaling via guided remodeling of blood vessels (Cabrera-Vásquez et al. 2009). Phenol (a neural toxic agent) application in the descending aorta between kidney and pancreas abolished all the postganglionic sympathetic fibers and part of the vagus nerve into the pancreas. The absence of the SNS into the pancreas means that any signal sent into the pancreas as a result of leptin infusion into the lateral ventricle has to have gone through the autonomic nervous system.

Insulin secretion is influenced by insulin resistance and β-cell mass, especially in the case of long-term changes of insulin secretion capacity (Weir and Bonner-Weir 2004). However, no study has been done on the effects of ICV leptin on β-cell mass. Therefore, long-term study is important to observe the changes of islet morphology and metabolism, such as leptin and insulin resistance in diabetic rats. We examined the effects of hypothalamic leptin on insulin secretion and β-cell mass in diabetic rats and also determined the role of the SNS in hypothalamic leptin action in short-term and long-term studies. A short-term study showed a direct effect of leptin on β-cell function through the SNS in diabetic rats while a long-term study found the leptin action in β-cell function and mass after leptin adjusted glucose metabolism with and without SNS. In the present study we found that hypothalamic leptin modulates insulin secretion mainly through the SNS without modulation of β-cell mass.

Methods and materials

Animals and diets

Male Sprague Dawley rats, weighing 223 ± 15 g were housed individually in stainless steel cages in a controlled environment (23 °C and a 12 h light and dark cycle) with unrestricted access to food and water. All rats had a 90% pancreatectomy (Px) using the Hosokawa technique (Hosokawa et al. 1996) and Px rats were used as an animal model to investigate whether ICV leptin modulates β-cell function and islet morphology in the present study. The experimental design was given in Fig. 1. The denervation of the SNS (sympathectomy) into the pancreas (SNSX) was performed by chemical (phenol) application in the descending aorta between the kidney and the pancreas while a sham operation of the denervation (Sham) was done by applying saline. The Px rats in the leptin- or CSF-infused groups were intracerebroventricularly infused with leptin solution or artificial cerebrospinal fluid (aCSF).

All rats consumed a 30 energy percent (En%) fat diet, which was a semi-purified diet modified from the AIN-93 formulation for experimental diets (Committee of the American Institute of Nutrition 1997). The diet consisted of 48 En% carbohydrates, 22 En% protein, and 30 En% fats. The sources of carbohydrates, protein, and fats were starch, casein, and shortening, respectively. All surgical and experimental procedures were performed in accordance with the recommendations found in the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, USA and they were approved by the Institutional Animal Care and Use Committee of Hoseo University, Korea.

Implantation of cannula into the lateral ventricle

All rats were anesthetized with ketamine and xylazine (100 mg and 10 mg/kg body weight, respectively) and then placed in a stereotaxic device and given a midline incision of the scalp, exposing the periosteum. The rats were then implanted with a stainless steel cannula (22-gauge) in the right lateral ventricle stereotactically, using the following coordinates: 0.8 mm posterior, 1.6 mm lateral, 3.7 mm ventral to bregma (Park et al. 2008). After the cannula was secured with dental cement, it was connected to a 22-gauge tube filled with a designated solution (leptin solution or aCSF) and connected to an osmotic pump. The leptin solution was made of dissolving recombinant rat leptin (PeproTech Inc., Rocky Hill, NJ) into aCSF with the concentration of 6 μg/mL. The aCSF was made of 124 mM NaCl, 5 mM KCl, 1.3 mM $MgCl_2$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$ and 10 mM D-glucose.

Experimental design and metabolic analysis

In a short-term study, after 1 week of recovery from the implantation of the cannula into the lateral ventricle, the leptin solution (6 μg/mL) was infused into lateral ventricle by flow rate of approximately 0.25 μL/min with microinjection pump (Harvard Apparatus) into 10 h fasted Px rats of the Sham-leptin and SNSX-leptin groups for 3 h. The flow rates were adjusted to provide 300 ng/kg body weight/h leptin into lateral ventricle according to body weight of rats. The rats of the Sham-CSF and SNSX-CSF were provided with the same volume of aCSF as a negative control. At the end of short-term infusion, hyperglycemic clamp was performed to determine the changes of insulin secretion capacity.

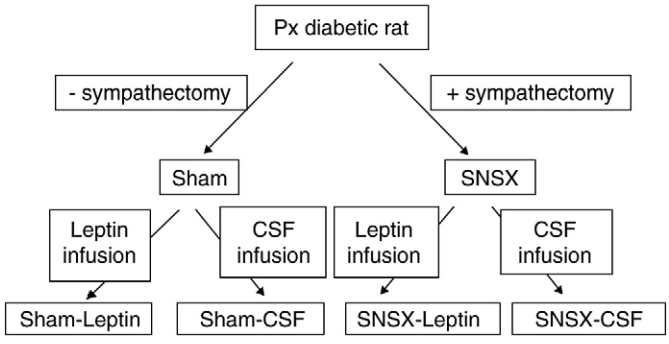

Fig. 1. Experimental design of the study.