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Chronic central leptin infusion modulates the glycemia response to insulin administration in male rats through regulation of hepatic glucose metabolism

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ABSTRACT

Leptin and insulin use overlapping signaling mechanisms to modify hepatic glucose metabolism, which is critical in maintaining normal glycemia. We examined the effect of an increase in central leptin and insulin on hepatic glucose metabolism and its influence on serum glucose levels. Chronic leptin infusion increased serum leptin and reduced hepatic SH-phosphotyrosine phosphatase 1, the association of suppressor of cytokine signaling 3 to the insulin receptor in liver and the rise in glycemia induced by central insulin. Leptin also decreased hepatic phosphoenolpyruvate carboxykinase levels and increased insulin's ability to phosphorylate insulin receptor substrate-1, Akt and glycogen synthase kinase on Ser9 and to stimulate glucose transporter 2 and glycogen levels. Peripheral leptin treatment reproduced some of these changes, but to a lesser extent. Our data indicate that leptin increases the hepatic response to a rise in insulin, suggesting that pharmacological manipulation of leptin targets may be of interest for controlling glycemia.

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1. Introduction

The liver is a key regulator of glucose homeostasis due to its ability to store glucose as glycogen and to release it according to peripheral demands (Jiao et al., 2013). Under physiological conditions insulin regulates these fluxes by modifying transport into the hepatocyte and suppressing the expression of genes involved in gluconeogenesis. Leptin also participates in the control of glycemia, promoting glucose uptake and glycogen storage through stimulation of phosphatidylinositol (PI)-3 kinase in the liver (Kellerer et al., 1997).

Leptin and insulin regulate glycemia by acting on both central and peripheral tissues and share several signaling targets in this process (Li et al., 2011; D'Souza et al., 2014; Crépin et al., 2014). Leptin acting centrally modulates peripheral insulin signaling through several mechanisms, including changes in peripheral hormones that affect insulin sensitivity and glucose metabolism (Hidaka et al., 2002; Cettour-Rose et al., 2005; Bartell et al., 2011). In liver, insulin-dependent glucoregulatory mechanisms are modulated by phosphatases and kinases that regulate intracellular targets and their interactions. Indeed, association of insulin receptor (IR) with suppressor of cytokine signaling 3 (SOCS3) and SH-phosphotyrosine phosphatase 1 (SH-PTP1) are determining factors in hepatic glucoregulation (Oriente et al., 2011). The

Abbreviations: α-MSH, α-melanocyte stimulating hormone; Akt, protein kinase B; AQP9, aquaglyceroporin-9; DU, densitometry units; ER, endoplasmic reticulum; GSK3β, glycogen synthase kinase-3β; GLUT2, glucose transporter 2; IL, interleukin; IR, insulin receptor; IRS1, insulin receptor substrate 1; JAK2, Janus kinase 2; JNK, c-Jun N-terminal kinase; MFI, median fluorescence intensity; OB-Rb, long form of leptin receptor; PEPCK, phosphoenolpyruvate carboxykinase; PI3K, phosphatidylinositol-3 kinase; p85, regulatory subunit of PI3K; p110, catalytic subunit of PI3K; SH-PTP1, SH-phosphotyrosine phosphatase 1; SOCS3, suppressor of cytokine signaling 3; STAT3, signal transducer and activator of transcription factor 3.

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relationship between central and peripheral signaling and the actions of leptin and insulin are more evident in obesity, as in most instances these subjects exhibit insulin resistance related to an increase in proinflammatory cytokines that aggravate this pathological situation (Dali-Youcef et al., 2013). Although the contribution of adipose tissue to obesity related inflammation has been reported (Kulyté et al., 2014), the role of the liver in the generation of a pro- or anti-inflammatory profile remains unclear.

The effect of exogenous leptin on insulin's actions and metabolic outputs in peripheral tissues has been analyzed in models of obesity and diabetes (Roman et al., 2010). However, there is little information regarding the effects of an increase in central leptin and insulin bioavailability on hepatic insulin sensitivity and its relationship with interleukin levels and carbohydrate metabolism in non-obese animals. Leptin decreases food intake and could modify insulin sensitivity and hepatic glucose metabolism; hence it would be important to discriminate the direct effects of leptin from those due to decreased food intake (de Vries et al., 2014). Therefore, we asked whether chronic central leptin infusion could improve hepatic insulin signaling and glycemia in response to an insulin bolus in a non-obese experimental model and the possible involvement of systemic changes in this response. To discriminate between the direct effects of leptin and those due only to a reduction in food intake, a group of pair-fed rats were included.

2. Material and methods

2.1. Animals

This study was approved by the Ethics Committee of the Universidad de Alcalá de Henares (SAF 22277, Ministerio de Ciencia y Tecnología) and complied with Royal Decree 53/2013 pertaining to the protection of experimental animals and with the European Communities Council Directive (2010/63/EU).

2.1.1. Central leptin and insulin infusion

Thirty-six adult male Wistar rats (250 ± 10 g) were individually caged with a 12-h light/dark cycle and fed standard chow and water ad libitum. After an overnight fast, rats were anesthetized (4 mg of ketamine/100 g bw and 0.5 mg of xylazine/100 g bw) and positioned in a stereotaxic apparatus. A cannula attached to an osmotic minipump (Alzet, Durect Corporation, Cupertino, CA) containing either saline or leptin (Preprotech, Rocky Hill, NJ, USA; 12 μg/day with a delivery volume of 0.5 μl/h) with 1% BSA was implanted into the right cerebral ventricle (−0.3 mm anteroposterior, 1.1 mm lateral from Bregma) and maintained during 14 days, and another cannula linked to a catheter in the rat left lateral cerebral ventricle where insulin was injected. Previously, in order to verify the stereotactic coordinates, three male rats received an icv injection of 7 μl 0.4% Trypan Blue solution (Sigma−Aldrich, St. Louis, MO, USA) and were killed 2 h later (Miller et al., 2006). To discriminate the inhibitory effect of leptin on appetite, a pair-fed group was included. Food intake and body weight were measured daily. On the last day, including a fasting period of 12 h, 10 mU of insulin (Novo Nordisk Pharma, Madrid, Spain) in 7 μl PBS or PBS was injected icv and the rats sacrificed by decapitation 2 h later. Glycemia was measured before and 2 h after PBS or insulin administration (Accu-Check Sensor, Roche, Mannheim, Germany). This resulted in the following groups (n = 6 per group): chronic vehicle plus acute vehicle (control), chronic vehicle plus insulin (insulin), pair-fed group with chronic vehicle and acute vehicle (pair-fed), pair-fed group with chronic vehicle plus acute insulin (pair-fed plus insulin), chronic leptin plus acute vehicle (leptin) and chronic leptin plus acute insulin (leptin plus insulin).

A dose of leptin that might be considered supra-physiological was chosen for this study as it is known to exert an effect on food intake (Friedman and Halaas, 1998; Banks and Lebel, 2002). With respect to insulin, brain levels are 10−100 times higher than in blood and studies using higher icv insulin doses have been considered to be in the physiological range (Tanaka et al., 2000). Therefore, the dose employed in this study could be considered to be in the physiological range and compatible with variations between fed and fasting states (Havrankova et al., 1978). A two-hour post-insulin injection interval was chosen because at one hour differences in activation of some signaling targets were observed, but not in parameters related to metabolism. At longer time-periods changes in the activation of many signaling targets were no longer detectable.

2.1.2. Oral glucose tolerance test

To determine how chronic central leptin infusion affects glucose metabolism, an oral glucose tolerance test (OGTT) was performed in control, pair-fed and central leptin-treated rats (n = 4 per group). After 14 days of central saline or leptin (12 μg/day) infusion and a fasting period of 12 h, glucose (2 g/kg body weight) dissolved in water was administered orally using gavage tubes (Suresha et al., 2013). Blood samples were extracted from the tail vein before administration of a glucose bolus and at 15, 30, 60, 120 and 180 min for determination of glucose levels (Accu-Check Sensor).

2.1.3. Intraperitoneal insulin tolerance test

Insulin sensitivity was assessed after overnight fasting by performing an intraperitoneal insulin tolerance test (IPITT) in control, pair-fed and central leptin-treated rats (n = 4 per group). After the injection of a bolus of 2 U/kg of insulin, blood samples were drawn consecutively at 15, 30, 60, 90, 120 and 180 min for glucose measurement (Ndisang et al., 2010), as described above.

2.1.4. Peripheral leptin and central insulin administration

Sixteen adult male Wistar rats (250 ± 10 g) were anesthetized as stated above and received either saline (n = 8) or leptin (n = 8, 0.2 mg/kg/day) via a subcutaneously implanted Alzet osmotic minipump (Scarpace et al., 2000). After 14 days, 10 mU of insulin (in 7 μl PBS) or PBS alone was injected icv and the rats sacrificed by decapitation 2 h later. This resulted in the following groups (n = 4 per group): chronic peripheral vehicle plus acute central vehicle (control), chronic peripheral vehicle plus acute central insulin (insulin), chronic peripheral leptin plus acute central vehicle (leptin) and chronic peripheral leptin plus acute central insulin (leptin plus insulin).

2.2. Hormone measurements

Serum leptin and insulin concentrations were determined using ELISA kits from Millipore Corporate Headquarters (Billerica, MA, USA). Serum glucagon and acylated and total ghrelin were measured by using RIA kits from Millipore. Serum α-melanocyte stimulating hormone (α-MSH) levels were evaluated by an ELISA kit from Cusabio (Wuhan, China). The intra- and inter-assay variations were lower than 10% in all cases.

2.3. Immunoprecipitation

The associations between targets were studied by immunoprecipitation. Thirty mg of liver were homogenized on ice in 500 μl of lysis buffer pH 7.6 containing 50 mmol/l HEPES, 10 mM EDTA, 50 mmol/l sodium pyrophosphate, 100 mmol/l NaF, 10 mmol/l Na_3VO_4, 1% Triton X-100, 2 mmol/lphenylmethylsulfonyl fluoride, 10 μg/ml leupeptin and 10 μg/ml aprotinin. Before immunoprecipitation, liver homogenates were centrifuged twice at 12,000 g

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