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In vivo leptin infusion impairs insulin and leptin signalling in liver and hypothalamus

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Abstract

Leptin resistance contributes to the pathogenesis of common obesity related metabolic diseases, including insulin resistance. However, the relationship between leptin and insulin resistance is not clearly established. Here, we show that induced hyperleptinemia by leptin infusion alters insulin signalling in rat liver. Leptin infusion clearly reduced the insulin or leptin dependent IRS-1/IRS-2 association to p85 regulatory subunit of PI 3-kinase. Leptin infusion also abolished STAT-3 phosphorylation in response to insulin or leptin and similar results were obtained for MAP-kinase phosphorylation. Hypothalamic leptin resistance was also induced by leptin infusion since leptin was unable to induce STAT-3 phosphorylation. These results provide evidence that induced hyperleptinemia can contribute to the onset of insulin resistance at least at the hepatic level.

Keywords: Leptin and insulin resistance; Signal transduction; Hypothalamus; Liver

1. Introduction

Leptin, a hormone secreted by white adipose tissue, is involved in the regulation of energy balance and glucose homeostasis; and leptin plasma levels are well correlated with adipose tissue mass (Perez et al., 2004). In obese humans, plasma leptin concentrations are high leading to leptin-resistance which is believed to contribute to metabolic diseases, including insulin resistance and type 2 diabetes (Ceddia et al., 2002). Since the discovery of leptin gene, the relationship between hyperleptinemia and insulin resistance has been suggested (Sivitz et al., 1997). However, the link between hyperleptinemia and insulin resistance remains matter of controversy where diabetogenic or antidiabetogenic effects were attributed to leptin (Sivitz et al., 1997; Chinookoswong et al., 1999). Furthermore, weight loss is associated with reduction in leptinemia and increased insulin sensitivity (Sivitz et al., 1997; Chinookoswong et al., 1999). Leptin treatment increases insulin sensitivity in normal, hyperinsulinemic or diabetic rats and corrects the diabetic phenotype of ob/ob mice (Muzzin et al., 1996). Other studies have showed insulin sensitivity was not affected by leptin (Sivitz et al., 1997; Widdowson et al., 1998; Cases et al., 2001; Zierath et al., 1998).

At the cellular level, several evidences support an interaction between leptin and insulin signaling networks. Both leptin and insulin receptors signal through common key intracellular signalling pathways such as JAK2/STAT-3, MAP-kinase and IRS/PI 3-kinase. It has been reported that leptin affects insulin signalling in insulin sensitive tissues (Kim et al., 2000) and also that insulin is able to modulate leptin signalling through JAK2/STAT-3 signalling cascade in rat hypothalamus (Carvalheira et al., 2001). Other studies have showed an alteration of insulin action following leptin treatment in isolated rat adipocytes (Muller et al., 1997), human hepatic cell line (Cohen et al., 1996) and in skeletal

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muscle (Sweeney et al., 2001). In HepG2 human hepatoma cell line, leptin inhibits the insulin-induced IRS-1 phosphorylation. In the same cells transfected with leptin receptor cDNA, leptin increased IRS-2 association with p85 subunit, regulatory subunit of PI 3-kinase (Wang et al., 1997). In FAO hepatoma cells, leptin pre-treatment transiently activated IRS/PI 3-kinase pathway (Szanto and Kahn, 2000). In rat adipocytes, leptin impairs insulin signalling at the level of MAP-kinase activity, insulin receptor phosphorylation and glycogen synthase-kinase phosphorylation (Perez et al., 2004).

In vivo, leptin alters skeletal muscle insulin stimulated PI 3-kinase activity and glucose transport (Singh et al., 2003). However, in liver, leptin seems to enhance insulin's action, such as reversing insulin resistance and hepatic steatosis in patients with severe lipodystrophy (Petersen et al., 2002). This is in good agreement with reports showing that leptin reduces hepatic glucose production by decreasing the synthesis of the key enzyme of gluconeogenesis PEPCK (Rossetti et al., 1997; Liu et al., 1998; Anderwald et al., 2002).

The present study aimed to investigate in normal rats the effect of chronic leptin treatment on the hypothalamic and liver sensitivity of JAK2/STAT-3, MAP-kinase and IRS/PI 3-kinase signalling pathways towards leptin and insulin. We show that after 7 days of leptin infusion (IP) in normal rats, there is a decrease in body fat weight and food intake concomitant with an augmentation of plasma leptin concentrations without significant changes in glycaemia or insulinemia. In the hypothalamus of leptin infused rats, leptin bolus completely abolished or attenuated STAT-3 or MAP-kinase phosphorylation, respectively. In liver of leptin infused rats, neither leptin nor insulin did increase IRS1 or IRS-2 association to p85. Furthermore, we show that insulin is able to activate STAT-3 and MAP-kinase phosphorylation in liver but not in the hypothalamus of untreated rats. Our data suggest that in normal rats the progressive augmentation of plasma leptin concentrations during leptin infusion induced hypothalamic resistance to leptin and importantly it induced both leptin and insulin resistance in liver.

2. Material and methods

2.1. Chemicals

Bovine serum albumin (fraction V radio immunoassay grade), leupeptin, aprotinin and protein A-agarose were purchased from Sigma Chemical Company (St. Louis, MO, USA). Pre-made polyacrylamide solution Protogel was from National Diagnostics (Prolabo, France). Antibodies directed towards IRS-1, IRS-2, total STAT-3, phospho-STAT-3, insulin receptor, were purchased from UBI (Euromedex, France); antibodies directed towards p44-42 MAP-kinase and phospho-p44-42 MAP-kinase were from cell signaling (Ozyme, France) and antibodies against p85 were from Santa Cruz Biotechnology (Tebu, France). Nitrocellulose membranes were from Euromedex (France). Ovine leptine was produced in our laboratory as previously described (Gertler et al., 1998).

2.2. Animals

Eighteen male Wistar rats (Harlan, France, 150–160 g, 7–8 weeks old at the beginning of the experiment) were used. They were housed individually and placed in a temperature-, humidity- and light-controlled room ($22 \pm 1\,^{\circ}C$) with a 12–12 h light–dark cycle (light on: 10:00 am). Food (standard chow diet) and water were available ad libitum in their cages. The standard chow diet (P14 = 14.6 kJ/g) was composed of protein energy (14.8%), fat (10.5%) and CHO supplemented with minerals and fibres. Food intake and body weight were measured daily at 10:00 am and fresh diets were provided. Although spillage was minimal, when it did occur, the food lost from the stainless holders was collected and added to the total not yet consumed.

2.3. Chronic leptin infusion (minipumps) and treatments

After habituation for 1 week to the standard diet, the 18 rats were divided in two groups, nine rats (controls) received saline and nine rats received ovine leptin, dissolved in sterile bidistillated water (9/10, v/v) and sodium dihydrogen phosphate buffer (1/10, v/v, 1 M) and diluted to appropriate concentrations, via osmotic minipumps (1 mg/kg/day) for 7 days as previously described (Wetzler et al., 2004). The minipumps (Alzet no. 2001 model, constant rate 1 μl/h, were implanted under light ether anaesthesia, in the intraperitoneal cavity. At the end of the experiment, 20 min before lethal anaesthesia, rats of each group were injected either with IP leptin (1 mg/kg) ($n = 3$), or insulin (1 IU/kg) ($n = 3$) or saline ($n = 3$) in order to test hypothalamic and liver response to those injections.

2.4. Sample collection and analysis

On the 7th day after minipump implantation, and after 6 h of food deprivation, a drop of blood was taken from the femoral vein of each rat for glycaemia evaluation. Each blood drop was applied to a blood glucose test strip and inserted in a glucose meter (Glucosetrend 2, Accu-Chek System, Roche, limit of sensitivity 0.6 mmol/l). Then rats were anesthetized with a lethal dose of pentobarbital (50 mg/kg BW by i.p injection) and heparinized (100 units heparin/100 g BW). Blood samples were collected from the inferior vena cava and centrifuged, and the plasma was frozen at $-20\,^{\circ}C$ until insulin and leptin assays were carried out. Then, the liver and hypothalamus were quickly removed and immediately frozen into liquid nitrogen for further analysis. Three depots of white adipose tissue: epididymal, retroperitoneal and subcutaneous, were carefully removed and weighed.