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Palmitic acid induces central leptin resistance and impairs hepatic glucose and lipid metabolism in male mice☆

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Abstract

The consumption of diets rich in saturated fat largely contributes to the development of obesity in modern societies. A diet high in saturated fats can induce inflammation and impair leptin signaling in the hypothalamus. However, the role of saturated fatty acids on hypothalamic leptin signaling, and hepatic glucose and lipid metabolism remains largely undiscovered. In this study, we investigated the effects of intracerebroventricular (icv) administration of a saturated fatty acid, palmitic acid (PA, C16:0), on central leptin sensitivity, hypothalamic leptin signaling, inflammatory molecules and hepatic energy metabolism in C57BL/6 J male mice. We found that the icv administration of PA led to central leptin resistance, evidenced by the inhibition of central leptin's suppression of food intake. Central leptin resistance was concomitant with impaired hypothalamic leptin signaling (JAK2-STAT3, PKB/Akt-FOXO1) and a pro-inflammatory response (TNF-α, IL1-β, IL-6 and pIκBa) in the mediobasal hypothalamus and paraventricular hypothalamic nuclei. Furthermore, the pre-administration of icv PA blunted the effect of leptin-induced decreases in mRNA expression related to gluconeogenesis (G6Pase and PEPCK), glucose transportation (GLUT2) and lipogenesis (FAS and SCD1) in the liver of mice. Therefore, elevated central PA concentrations can induce pro-inflammatory responses and leptin resistance, which are associated with disorders of energy homeostasis in the liver as a result of diet-induced obesity.

Keywords: Palmitic acid; Leptin resistance; Hypothalamus; Inflammation; Glucose metabolism; Lipid metabolism

1. Introduction

The hypothalamus is capable of sensing nutritional status [1,2], and as a result nutrients can influence brain function [3]. Understanding the mechanisms by which specific nutrients, such as fatty acids influence signaling within the brain regulating energy balance will help to prevent and even treat obesity and other metabolic disorders.

Leptin is secreted by adipocytes, and its circulating levels reflect the amount of energy stored in fat. This hormone acts centrally, particularly in the hypothalamus, to reduce food intake and body weight [4]. Leptin can bind to the hypothalamic leptin receptor and activates the JAK2 (Janus kinase-2)-STAT3 (signal transducer and activator of transcription-3) pathway that promotes negative energy balance [5]. Besides the JAK2-STAT3 pathway, leptin also acts through the serine/threonine protein kinase B/Akt signaling pathway to induce forkhead box protein O1 (FOXO1) phosphorylation and degradation, and decrease FOXO1 activity in the hypothalamus [6]. FOXO1 binds to STAT3 and prevents STAT3 from interacting with the pro-opiomelanocortin (POMC) promoter complex, and consequently, inhibits STAT3-mediated leptin action [7]. Phosphorylation of FOXO1 results in FOXO1 release from the nucleus and allows pSTAT3 to bind to neuropeptide promoters, stimulating the transcription of anorexigenic POMC and inhibiting orexigenic agouti-related protein (AgRP) expression [8].

Obesity results in resistance to the effect of leptin. For example, the administration of leptin to obese subjects failed to decrease body weight and food intake [9]. Furthermore, an intracerebroventricular (icv) injection of leptin failed to inhibit food intake and body weight in chronic high-saturated-fat diet-induced obese mice [10,11]. This suggests that resistance to leptin in the central nervous system (CNS) compromises the ability of leptin to regulate food intake and body weight in the presence of a diet high in saturated fat. However, the cause of obesity and leptin resistance in most forms of human and rodent obesity is still poorly understood. It is known that leptin binds to long-form LepRb on neurons in several regions in the hypothalamus, including the mediobasal hypothalamus (MBH) and paraventricular

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hypothalamic nuclei (PVN), to regulate food intake and energy homeostasis [12]. El-Haschimi et al. [13] showed that leptin failed to induce STAT3 activation in hypothalamic extracts from obese mice induced by a high-fat diet, suggesting hypothalamic leptin resistance.

Evidence suggests that the CNS is a critical target for leptin regulation of glucose and lipid metabolism in the peripheral tissue, such as liver, muscle and adipose tissue. For example, the icv infusion of leptin increases glucose turnover and glucose uptake, but decreases hepatic glycogen content without changing plasma glucose in wild-type mice [14]. Central treatment with leptin decreased mRNA expression of the hepatic gluconeogenic enzymes, glucose 6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) in streptozotocin-induced diabetic rats [15]. In addition, chronic icv administration of leptin caused the down-regulation of genes encoding stearoyl-CoA desaturase-1 (SCD1), acetyl-coenzyme A-carboxylase (ACC) and fatty acid synthase (FAS) in the liver when compared with vehicle-infused pair-fed rats [16]. This suggests that leptin acts in the brain to suppress liver lipogenic gene expression independent of feeding. Furthermore, the attenuation of leptin-mediated Akt signaling in LepRb neurons causes decreased sympathetic tone in the liver and increases hepatic steatosis [17].

Recent evidence shows that hypothalamic inflammation induced by dietary saturated fats is implicated in the development of obesity and its associated leptin resistance [11]. Within 1 week of consuming a high-fat diet, there is low-grade hypothalamic inflammation and an increase in pro-inflammatory cytokines, evidenced by an increase in tumor necrosis factor alpha (TNF-α), interleukin-l-beta (IL-1β) and interleukin 6 (IL-6) mRNA expression [18]. These cytokines activate the nuclear factor-κB (NF-κB) inflammatory signaling pathway by phosphorylating and degrading inhibitor kappa B alpha (IκBα) [19]. Recent studies have also revealed that hypothalamic inflammation plays an important role in mediating central leptin resistance and the interruption of leptin signaling in the hypothalamus of rodents. Constitutive activation of NF-κB inflammatory signaling in the hypothalamus of mice induced central leptin resistance and impaired leptin signaling through pSTAT3 [20]. In contrast, a genetic or pharmacological blockade of hypothalamic inflammatory signaling has improved leptin sensitivity and elevated pSTAT3 [20,21].

Palmitic acid (PA, C16:0) is the most common saturated fatty acid in human diets [22], accounting for approximately 65% of saturated fatty acids and 32% of total fatty acids in human serum [23]. Patients with metabolic syndrome have a significantly higher level of serum PA than controls [23], and also have high levels of PA, but not myristic acid or stearic acid within erythrocytes [24]. The central administration of PA significantly decreases the anorexigenic effect of leptin in mice [25]. In this study, we investigated hypothalamic leptin sensitivity, signaling and inflammation in response to PA administration, and further examined the effect of icv PA and leptin on glucose levels and the expression of hepatic genes involved in glucose and lipid metabolism.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (10 weeks old, body weight: 22.74 ± 3.22 g) were obtained from the Animal Resource Centre (Perth, WA, Australia) and housed in environmentally controlled conditions (temperature 22°C, 12 h light/dark cycle). Mice were maintained on a normal lab chow diet (LC, Vella Stock feeds, Doonside, NSW, Australia) throughout this study. All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia, and complied with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*.

2.2. Experiment protocols

After 1 week of acclimatization, mice were anesthetized by isoflurane inhalation and placed in a stereotactic device. An icv cannula was implanted into the right lateral brain ventricle (0.25 mm posterior and 1.0 mm lateral relative to bregma and 2.5 mm below the surface of the skull) as described in our previous study [10]. The accuracy of cannula implantation into the lateral ventricle was confirmed by examining the needle track in the brain sections of each animal (Fig S1).

2.3. Central leptin sensitivity test

Five days after the cannula implantation, a central leptin sensitivity test was performed as described previously [11]. The mice were randomly divided into two groups (n = 24) and received icv injections of PA (25 pmol twice a day for 2.5 days, 5 injections in total) or vehicle [26]. At the end of Day 2 of the test, the mice were fasted overnight, and each group of mice was divided into two subgroups (n = 12) that received an icv injection of either leptin (0.5 μg in 2 μl) or vehicle (2 μl saline) 1 h after the last PA injection. Food intake and animal weights were then measured 24 h after the icv leptin or vehicle injection. As described previously [27], PA (P5585, Sigma-Aldrich, Australia) was dissolved in 96% ethanol, dried using nitrogen gas and then dissolved in 40% hydroxypropyl-b-cyclodextrin (H107, Sigma-Aldrich) and stored at −20 °C. The working solution contained 25 pmol PA every injection.

2.4. Intraperitoneal glucose tolerance test

After at least 3 day interval, the mice were repeated with PA and leptin as described for the leptin sensitivity test with individual mice assigned to the same previous treatment or vehicle group. Glucose tolerance tests were then performed 30 minutes after leptin injection. Blood glucose was measured at 0, 30, 60 and 120 min after glucose administration (0.5 g/kg glucose, ip) using a glucometer (Alameda, CA, USA).

2.5. Tissue collection

Again, after at least 3 day interval, the mice were repeated with PA and leptin as described above. Thirty minutes after leptin injection, the mice were sacrificed by CO_2 asphyxiation. The brain and liver were immediately collected, snap frozen in liquid nitrogen, and stored at −80 °C for further processing and analysis. In a cryostat at a temperature of −18 °C, 500 μm frozen brain sections were cut from bregma −0.58 mm to −2.72 mm according to a standard mouse brain atlas [28]. The MBH and PVN were dissected using a Stoelting Brain Punch (#57401, 0.5 mm diameter, Wood Dale, Stoelting Co, USA) from frozen coronal sections based on previously described coordinates [11,28].

2.6. Western blot analysis

Western blotting was performed on protein extracts from frozen tissue as described in our previous study [10]. The expression of specific proteins was determined using the following antibodies: TNF-α (sc-8301), IL-1β (sc-7884), IL-6 (sc-7920), pIκBα (sc-8404) and pJAK2 (sc-21870) from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and pSTAT3 (Tyr705) (#9145), SOCS3 (#2932), pAkt (#9271) and pFOXO1 (#9461) from Cell Signaling Technology (Beverly, MA, USA). Bands corresponding to the proteins of interest were scanned and band density analysed using the automatic imaging analysis system, Quantity One (Bio-Rad). All quantitative analyses were normalized to β-actin, based on our previous studies [29]. Due to the small amount of tissue in the MBH and PVN of the hypothalamus, we used a previously-described modified multi-strip western blot, which allows the detection of multiple proteins with a smaller sample size than in a standard Western blot [11].

2.7. RNA isolation and RT-PCR

Total RNA from the liver was extracted using the Aurum total RNA mini kit (Bio-Rad Laboratories, Hercules, CA) and reverse-transcribed to first-strand complementary DNA using the high-capacity cDNA reverse transcription kit (AB Applied Biosystems, CA, USA) according to the manufacturer's instructions. Quantitative real-time PCR was performed in a 20 μl final reaction volume using SYBR green I master on a Lightcycler 480 Real-time PCR System (F. Hoffmann-La Roche Ltd, Switzerland). Amplification was carried out with 45 cycles of 95 °C for 10 seconds, 60 °C for 30 seconds, and 72 °C for 30 s. The mRNA expression levels were normalized to GAPDH, which served as the internal control. Expression levels for each gene were calculated using the comparative threshold cycle value (Ct) method, using the formula $2^{-\Delta\Delta Ct}$ (where $\Delta\Delta Ct = \Delta Ct$ sample - ΔCt reference) as described previously [30]. The primers used are listed in supplementary Table 2.

2.8. Statistics

Data were analysed using the statistical package SPSS 19.0 (SPSS, Chicago, IL, USA). The two-tailed Student's *t* test was used to compare hypothalamic cytokine expression between the PA and vehicle groups. One-way analysis of variance and the post hoc Tukey–Kramer honestly significant difference test were used to analyse hypothalamic leptin signaling molecules, central leptin sensitivity and mRNA expression of genes regulating hepatic glucose and lipid metabolism. $P < .05$ was regarded as statistically significant. Values are expressed as mean ± S.E.M.