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Region-specific diet-induced and leptin-induced cellular leptin resistance includes the ventral tegmental area in rats

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ABSTRACT

Diet-induced obesity (DIO) results in region-specific cellular leptin resistance in the arcuate nucleus (ARC) of the hypothalamus in one strain of mice and in several medial basal hypothalamic regions in another. We hypothesized that the ventral tegmental area (VTA) is also likely susceptible to diet-induced and leptin-induced leptin resistance in parallel to that in hypothalamic areas. We examined two forms of leptin resistance in F344xBN rats, that induced by 6-months of high fat (HF) feeding and that induced by 15-months of central leptin overexpression by use of recombinant adeno-associated viral (rAAV)-mediated gene delivery of rat leptin. Cellular leptin resistance was assessed by leptin-stimulated phosphorylation of signal transducers and activators of transcription 3 (STAT3) in medial basal hypothalamic areas and the VTA. The regional pattern and degree of leptin resistance with HF was distinctly different than that with leptin overexpression. Chronic HF feeding induced a cellular leptin resistance that was identified in the ARC and VTA, but absent in the lateral hypothalamus (LH), ventromedial hypothalamus (VMH), and dorsomedial hypothalamus (DMH). In contrast, chronic central leptin overexpression induced cellular leptin resistance in all areas examined. The identification of leptin resistance in the VTA, in addition to the leptin resistance in the hypothalamus, provides one potential mechanism, underlying the increased susceptibility of leptin resistant rats to HF-induced obesity.

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1. Introduction

The adipocyte-derived hormone, leptin, through its action in the hypothalamus and other brain sites, is a potent regulator of appetite and energy expenditure (Ahima and Flier, 2000). Therapeutic interest in this hormone has waned due to the reduced efficacy and sensitivity of leptin in humans or animals with common obesity (Proietto and Thorburn, 2003; Scarpace and Zhang, 2009). Serum leptin levels increase proportionally with adiposity, and leptin levels are high in rodent and human models of diet-induced obesity (DIO), yet, the increased leptin fails to curb the progression of obesity (Halaas et al., 1997; Widdowson et al., 1997; Levin and Dunn-Meynell, 2002). This apparent leptin ineffectiveness is identified as leptin resistance.

Although the exact mechanism underlying leptin resistance is not fully understood, leptin resistance in DIO rodents is associated with diminished leptin receptor signaling to centrally administered leptin (El-Haschimi et al., 2000), and this form of leptin resistance is often referred to as cellular leptin resistance (Morrison et al., 2005; Myers et al., 2008). One of the major leptin receptor signaling pathways involves activation of Janus kinase 2 (JAK2) and the subsequent phosphorylation of signal transducers and activators of transcription (STAT), in particular STAT3 (Ahima and Flier, 2000). Receptors occupied by leptin undergo phosphorylation by JAK2, promoting binding of STAT proteins that are then subject to tyrosine phosphorylation by JAK2. Phosphorylated STAT3 dimerizes and can serve as a transcription activator. Thus, the degree of leptin-mediated STAT3 phosphorylation serves as one marker of cellular leptin responsiveness.

Although leptin receptors within the arcuate nucleus (ARC) of the hypothalamus have received the most attention, leptin receptors have been found in other areas in the hypothalamus including the lateral hypothalamus (LH), ventromedial hypothalamus (VMH), and dorsomedial hypothalamus (DMH) (Ahima and Flier, 2000), as well in regions outside the hypothalamus, most notably the ventral tegmental area (VTA) (Hommel et al., 2006). Identification of the specific site or sites of cellular leptin resistance within the brain has been largely ignored. Munzberg et al. first reported that leptin

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resistance, as identified by diminished leptin-mediated STAT3 phosphorylation, was limited to the ARC in high-fat (HF) fed mice (Munzberg et al., 2004). A subsequent study, in a different strain of HF-fed mice, found evidence for leptin resistance in several regions in the medial basal hypothalamus including the ARC, VMH, DMH, and ventral premammillary area (Metlakunta et al., 2008). The only study in rats examined pregnancy-related leptin resistance and reported impaired leptin-induced STAT3 phosphorylation in two hypothalamic regions, the ARC and VMH with no evidence of diminished signaling in DMH or LH (Ladyman and Grattan, 2004).

To date, there have been no investigations of the occurrence of leptin resistance outside the hypothalamus. One region recently identified to be important in the leptin-mediated regulation of ingestive behavior is the VTA of the midbrain reward circuitry (Fulton et al., 2000; Hommel et al., 2006). The effects of leptin on energy intake in the VTA are mediated by STAT3 phosphorylation (Morton et al., 2009), and leptin receptor knockdown in the VTA increases the preference for sucrose consumption over water (Hommel et al., 2006). We recently demonstrated that voluntary wheel running in rodents curtails high-fat (HF)-related hyperphagia and eliminates the preference for a palatable HF diet, and that this is associated with enhanced leptin-mediated STAT3 signaling specifically in the VTA (Scarpace et al., 2010). These data suggest that leptin-mediated STAT3 signaling in the VTA plays a role in HF feeding behavior, and because HF feeding leads to leptin resistance, we hypothesized that the VTA is likely susceptible to diet-induced leptin resistance in parallel to that in hypothalamic areas.

To this end, we examined leptin-mediated STAT3 phosphorylation under two conditions known to induce leptin resistance, chronic HF feeding and chronic overexpression of leptin in the brain. For diet-induced leptin resistance, we fed rats a HF diet for 6 months, and for leptin-induced leptin resistance, we overexpressed leptin in the brain for 15 months via delivery of a recombinant adeno-associated viral (rAAV) vector encoding rat leptin. Subsequently, we assessed signaling by central leptin administration and examined STAT3 phosphorylation in multiple brain regions, including the ARC, VTA, VMH, DMH, and LH.

2. Materials and methods

2.1. Experimental animals

Three-month-old male F344 x Brown Norway (F344xBN) rats were obtained from Harlan Sprague–Dawley (Indianapolis, IN). Upon arrival, rats were examined and remained in quarantine for one week. Animals were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals and protocols were approved by the University of Florida Institutional Animal Care and Use Committee. Rats were housed individually with a 12:12 h light-dark cycle (07:00 to 19:00 h). During the experimental period, rats were fed either a standard rodent chow (17%kcal from fat, no sucrose, 3.3 kcal/g, diet 2018, Harlan Teklad; Madison, WI) or a HF diet (60% kcal from fat, 7% kcal from sucrose, 5.24 kcal/g, D12492, Research Diets, New Brunswick, NJ).

2.2. Experimental design

This study consists of two experiments. In the first experiment, rats were administered either recombinant adeno-associated virus (rAAV)-leptin or control vector by i.c.v. injection. Rats ($N = 16$/group) were allowed free access to food and water, ad libitum, and food consumption and body weight were recorded daily to weekly for 15 months. During two 4-day periods, (starting at day 75 and day 371), rats were evaluated for extent of voluntary wheel running (WR). Prior to death, at day 452, those administered control and rAAV-leptin were further divided into two groups ($N = 8$), and either artificial cerebral spinal fluid (ACSF) or leptin (1ug) were administered by i.c.v. injection to determine leptin signaling in various brain regions.

In the second experiment, rats ($N = 12$/group) were chow or HF fed for 190 days. At day 190, whole body adiposity was assessed by time domain nuclear magnetic resonance (TD-NMR) using a Minispec lean fat analyzer (Bruker Optics, Inc., The Woodlands, TX). Validation of TDNMR methodology has been provided (Tinsley et al., 2004). Prior to death, the chow and HF-fed group were further divided into two groups ($N = 6$), and either ACSF or leptin were administered by i.c.v. injection to determine leptin signaling in various brain regions.

2.3. rAAV-vector administration

A single dose (5×10^{12}viral genomes/ml) of either control vector encoding green fluorescent protein (GFP, 3 μl) or rAAV-leptin (3 μl), was delivered by i.c.v. injection into the third cerebral ventricle as previously described (Scarpace et al., 2002b). The coordinates for injection are 1.3 mm anterior to Bregma, 9.4 mm ventral from the skull surface, at an angle of 20 degrees anterior to posterior. Leptin transgene expression was determined by RT-PCR using sense (TGACACCAAAACCCTCATCA)and antisence primers (TGAGCTATCTGCAGCACGTT) as described previously (Scarpace et al., 2002b).

2.4. Wheel running

Rats were housed in cages equipped with Nalgene Activity Wheels (1.081 meters circumference, Fisher Scientific, Pittsburgh, PA) that allowed free access to the wheel. Each wheel was equipped with a magnetic switch and counter. The number of revolutions were recorded daily for a four-day period. Average daily WR was calculated from the last 3 days of WR.

2.5. Dietary selection

Rats accustomed to standard chow, were provided simultaneous access to two novel diets, a 60% HF diet (Research Diets D12492, 60% kcal from fat, 6.7% kcal from sugar, 13.3% kcal from carbohydrates other than sugar, 20% kcal from protein, 5.24 kcal/g) and a 32% HF diet (Research Diets D12266B, 32% kcal from fat, 25.2% kcal from sugar, 26.2% kcal from carbohydrates other than sugar, 16.8% kcal from protein, 4.41 kcal/g). Food consumption of both diets were determined separately by weight of food consumed over a 4 day period. The position of the food trays containing the chow and HF food was alternated daily. Spillage of food was accounted for in calculating food consumption.

2.6. Leptin administration

A single dose of leptin (1 μg) was injected into the third cerebral ventricle as previously described (Scarpace et al., 2007). The coordinates for injection are 1.3 mm anterior to Bregma, 9.4 mm ventral from the skull surface, at an angle of 20 degrees anterior to posterior. Rats were killed one hour later.

2.7. Tissue harvesting

Rats were killed by thoracotomy under 150-mg/kg pentobarbital anesthetic. Subsequently, 40 ml of cold saline were perfused through the circulatory system. The perirenal and retroperitoneal white adipose tissues (PWAT and RTWAT, respectively) were each excised and their individual weights recorded. Additionally, 2 mm coronal sections containing the regions of the VTA and ARC were sliced using a micrometer controlled tissue slicer (Stoelting Co, Wood Dale, Il) and a punch of the respective regions were taken as subsequently described. Aligning a straight edge razor blade with the optic tract (−1.5 mm posterior bregma), a 2 mm caudal coronal section was cut. Thereafter, a 1 mm circumference brain punch (Stoelting, Wood Dale, IL) was used to excise regions centered around the arcuate nucleus, the ventromedial hypothalamic nucleus, the dorsomedial hypothalamic nucleus, and the lateral hypothalamic nucleus. Similarly, aligning a straight edge razor blade with the caudal end of the hypothalamus (−5 mm posterior to bregma) a coronal section was cut 2 mm posterior. Thereafter, a 1 mm brain punch was used to biopsy regions of the VTA. All punches were taken bilaterally.

2.8. Western analysis and radioimmunoassay

Protein homogenate (20 μg) was separated on a SDS-PAGE gel and electrotransferred to nitrocellulose membranes (Scarpace et al., 2001). Immunoreactivity was assessed with antibodies specific to phospho-tyrosine 705 of STAT3, and reprobed with antibodies specific to STAT3 regardless of phosphorylation state (Cell Signaling, Danvers, MA). The ratio of phosphorylate STAT3 to total STAT3 is reported. Immunoreactivity to total STAT3 was compared with that for beta-tubulin (Abcam, Cambridge, MA). Immunoreactivity to tyrosine phosphotase 1B (PTP1B, Calbiochem, San Diego, CA) was determined in the experiment examining leptin overexpression.

Radioimmunoassay was used to determine serum leptin (Millipore, Billerica, MA).

2.9. Statistical analysis

Data were analyzed by two-way ANOVA with repeated measures when appropriate. A post-hoc test (Bonferroni) was applied to determine individual differences between means. A P-value of less that 0.05 was considered significant.

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