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Age-related changes in acute central leptin effects on energy balance are promoted by obesity

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ABSTRACT

Leptin is a key catabolic regulator of food intake (FI) and energy expenditure. Both aging and obesity have been shown to induce leptin-resistance. The present study aimed to analyze age-related changes in the anorexigenic and hypermetabolic responsiveness to acute intracerebroventricular leptin administration in different age-groups of normally fed male Wistar rats (adult and old rats from 3 to 24 months of age, NF3 to NF24, respectively). The expressions of the long form of the leptin receptor (Ob-Rb) and inhibitory SOCS3 genes were also assessed by quantitative RT-PCR in the arcuate nucleus (ARC). The influence of high-fat diet-induced obesity (HF) on the anorexigenic leptin effects were also tested in younger and older middle-aged groups (HF6 and HF12).

Leptin-induced anorexia varied with age: leptin suppressed re-feeding FI (following 48-h fasting) strongly in young adult (NF3), but not in younger or older middle-aged (NF6 or NF12) or in aging (NF18) rats. However, anorexigenic leptin effects reached statistical significance again in old NF24 rats. Leptin-induced hypermetabolism, on the other hand, showed monotonous age-related decline and disappeared by old age. Ob-Rb expression declined until 12 months of age followed by a partial recovery in NF18 and NF24 groups. On the other hand, SOCS3 expression was high in NF6 and NF18 and to some extent in NF24 rats. Age-related alterations of Ob-Rb and SOCS3 expression in the ARC may partly contribute to the explanation of age-related variations in anorexigenic but not hypermetabolic leptin effects. High-fat diet-induced obesity was associated with resistance to leptin-induced anorexia in HF6, similar to that seen in NF6. However, instead of the expected leptin-resistance in HF12, a strong leptin-induced suppression of re-feeding was detected in these obese middle-aged rats.

Our results suggest that acute central effects of leptin on anorexia and hypermetabolism change in disparate ways during aging, implying separate mechanisms (e.g. signal transduction pathways) of different leptin actions. The age-related pattern shown by leptin-induced anorexia may contribute to the explanation of middle-aged obesity, and partly to that of aging anorexia. Our findings concerning obese rats are in accord with previous observations on anorexigenic effects of peripherally administered cholecystokinin: diet-induced obesity appeared to accelerate the development of age-related regulatory alterations. Similarly, our present data also raise the possibility that chronic diet-induced obesity promotes responsiveness to centrally applied leptin at least concerning anorexigenic effects.

1. Introduction

Aging and obesity are two important world-wide public health burdens (WHO, 2012, 2014, 2015) that are interconnected. On the one hand, middle-aged humans and mammals tend to become obese (Scarpace et al., 2000b), on the other hand, obesity appears to accelerate aging (Balaskó et al., 2013; Carter et al., 2013). One of the common features of aging and obesity is a dysregulation of energy homeostasis. Such dysregulation involves resistance to different regulatory peptides, e.g. insulin or leptin, leading to abnormalities of body weight (BW) and/or body composition) (Ahima, 2009; Carter et al., 2013). Therefore, investigation of regulatory alterations that develop in obesity or during the course of aging is of outstanding importance.

Leptin is known as an adipokine produced mainly, though not exclusively, in the subcutaneous (and to a lesser extent in the visceral) white

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adipose tissue, proportionately with the amount of fat mass (Friedman and Halaas, 1998). Numerous other sites of leptin production have been identified in the gastric epithelium, in placental trophoblasts (Masuzaki et al., 1997; Badd et al., 1998), in skeletal muscles (Wang et al., 1998), in the heart (Purdham et al., 2004) and also in the brain of rats and humans (Morash et al., 1999; Wiesner et al., 1999).

During the past decades, leptin has emerged as one of the most important adiposity signals in the regulation of BW and energy balance. Leptin has coordinated catabolic activity: it does not only induce anorexia, but it also increases metabolic rate and body temperature (Hwa et al., 1996; Sahu, 2004; Steiner and Romanovsky, 2007). Congenital lack of leptin, or structural defects of the peptide or of its receptors are accompanied by severe obesity (for review see Rosenbaum and Leibel, 2014).

The peptide passes through the blood-brain barrier via a special saturable transport system (Zlokovic et al., 2000) and binds to its receptors. These central catabolic leptin actions are mainly mediated by alterations in the expression of neuropeptides in the arcuate nucleus of the hypothalamus. Downstream to leptin, the activity of the catabolic melanocortin system [involving pro-opiomelanocortin (POMC) derived melanocortins, primarily alpha-melanocyte stimulating hormone, alpha-MSH] and activation of the catabolic cocaine-amphetamine regulated transcript (CART) system are enhanced. On the other hand, the activation of the anabolic neuropeptide Y (NPY) and that of the endogenous melanocortin antagonist agouti-related peptide (AgRP) are suppressed (Baskin et al., 1999; Berglund et al., 2012). In the periphery, leptin also acts on different sites of the afferent vagus (Wang et al., 1997; Gaigé et al., 2002; Shiraishi et al., 1999; Buyse et al., 2001), transmitting information to the brainstem and to the nucleus of the solitary tract (NTS) (Buyse et al., 2001; Grill et al., 2002; Székely and Szelényi, 2005).

The responsiveness to leptin has been shown to decline (with consequent hyperleptinemia) during the course of age-related weight gain (Scarpace et al., 2000a) and in obesity of other etiologies at any age (Lin et al., 2000; Myers et al., 2012). The combination of aging and obesity, i.e. age-related obesity is characterized by a tendency towards progressive weight gain starting at a younger age in humans and mammals reaching a peak in late middle-aged or aging groups. Such a weight gain has been associated with the development of progressive peripheral and later on also central leptin resistance (Van Heek et al., 1997; Scarpace et al., 2000a, 2000b; Shek and Scarpace, 2000; Sahu, 2004).

However, several questions remain unresolved in this field. For example, evidence is not conclusive whether age per se or rather the accompanying obesity leads to the development of leptin-resistance. Moreover, very old age-groups of humans and mammals tend to lose weight [aging anorexia (Morley, 2001)] that cannot be explained on the basis of aging-induced leptin-resistance. In addition, previous animal studies focused mainly on anorexigenic leptin effects, while the thermoregulatory changes related to the hypermetabolic actions of leptin were left largely unexplored.

Leptin-induced anorexia was strong in the young but not in old rats (Scarpace et al., 2000b). These observations support the role of aging in the development of leptin-resistance. However, resistance to the peptide did already appear at a young age in diet-induced obese rats rather supporting the primary role of obesity in this phenomenon (Soós et al., 2010). It may be assumed that caloric restriction that reduces fat mass would prevent leptin-resistance. However, some studies failed to confirm the efficacy of caloric restriction in the restoration of leptin responsiveness (Gabriely et al., 2002a). In contrast, others reported that a 3-month food deprivation successfully re-established anorexigenic leptin responsiveness in old rats (Fernández-Galaz et al., 2002). Concerning the responsiveness to centrally applied leptin, previous observations indicated the primary role of obesity in the development of age-related leptin-resistance (Pétervári et al., 2014). Surprisingly, no leptin-resistance was found in non-obese (normally fed or caloric restricted) old male Wistar rats, in case of a high-dose 7-day intracerebroventricular (ICV) leptin infusion. Based on the above findings, the question arises, whether acute central leptin actions also show a similar age-related pattern. Previously, different, even inverse leptin effects have been shown upon central chronic and acute application of the peptide (García-Cáceres et al., 2011). Moreover, diverse intracellular pathways of chronic and acute leptin actions have been identified in hypothalamic POMC neurons (Hill et al., 2008). It would be therefore important to investigate the age-related pattern of acute central leptin effects with regard to energy metabolism, as well.

Such studies injecting or infusing leptin directly into the brain may be of importance, as central leptin responsiveness appears to be maintained longer than the peripheral one during the development of obesity (Van Heek et al., 1997). Moreover, synthesis of this peptide has also been detected in the brain of humans and mammals (Wiesner et al., 1999; Morash et al., 1999; Eikelis et al., 2007). Investigation of the development of age- and obesity-related changes in central leptin-resistance may therefore implicate later therapeutic possibilities (e.g. regarding intranasal application of leptin in obesity, Schulz et al., 2012; Spetter and Hallschmid, 2015).

Based on the above analyzed data, in our present study we aimed to investigate age-related changes in acute central leptin effects on parameters of energy balance. We tested the anorexigenic and hypermetabolic responsiveness to ICV injections of the peptide in different age-groups of rats, along with expression of the long form of the leptin receptor (Ob-Rb) and that of the signal transduction inhibitor suppressor of cytokine signaling 3 (SOCS3) genes by quantitative RT-PCR in the arcuate nucleus (ARC). We also aimed to investigate the influence of high-fat diet-induced obesity on the anorexigenic actions. In addition, we carried out a detailed thermoregulatory analysis of hypermetabolic leptin effects.

2. Materials and methods

2.1. Animals

Different age-groups of male Wistar rats from the Colony of the Institute for Translational Medicine of the Medical School, University of Pécs, Hungary were used in the present study. After they reached the appropriate age, rats were maintained individually in plastic home-cages covered with steel grid, equipped with feeder and bottle container that contained wood-chip bedding at an ambient temperature of 22–25 °C (up to 27–28 °C, thermoneutral in the nest). Lights were on between 06.00 and 18.00 h. The following age- groups were established: 3 (young adult), 6 (younger middle-aged), 12 (older middle-aged), 18 (aging) and 24 (old) months old. (The maximal life-span of our colony reaches 30 months, about 50% of rats survive 26 months, but after the age of 24 months surgical interventions are difficult.) The younger and older middle-aged groups were divided into two subgroups: normally fed (NF6, NF12), and high-fat diet-induced obese (HF6, HF12) rats. NF rats were fed standard laboratory rat chow ad libitum (11 kJ/g; CRLT/N rodent chow, Szindbád Kft., Gödöllő, Hungary), HF rats received IPS TestDiet (Diet-Induced Obesity Rodent Purified Diet with 60% Energy from Fat, 21.6 kJ/kg) from age 2 months. All other groups were normally fed (NF). Table 1 shows BW of the different age-groups of treated vs. control animals. Initial BW-s of treated and those of control animals

Table 1
Primer sequences of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and those of the tested leptin receptor long form (Ob-Rb) and suppressor of cytokine signaling 3 (SOCS3) genes.

Primers	Forward	Reverse
Ob-Rb	TCTGGAGCCTGAACCAGTTT	GGAAGTGCTCCACCCGATAG
SOCS-3	GGATTCTACTGGAGTGCCGT	CTCAGTGTGAAGAAGTGGCG
GAPDH	TGCCATCACTGCCACTCAGA	GTCAGATCCACAACGGATACATTG