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Sex-associated differences in the leptin and ghrelin systems related with the induction of hyperphagia under high-fat diet exposure in rats

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ABSTRACT

Leptin and ghrelin are known to be main hormones involved in the control of food intake, with opposing effects. Here we have explored whether changes in the leptin and ghrelin system are involved in the long-term effects of high-fat (HF) diet feeding in rats and whether sex-associated differences exist. Male and female Wistar rats were fed until the age of 6 months with a normal-fat (NF) or an HF-diet. Food intake and body weight were followed. Gastric and serum levels of leptin and ghrelin, and mRNA levels of leptin (in stomach and adipose tissue), ghrelin (in stomach), and NPY, POMC, and leptin and ghrelin receptors (OB-Rb and GHS-R) (in the hypothalamus) were measured. In both males and females, total caloric intake and body weight were greater under the HF-diet feeding. In females, circulating ghrelin levels and leptin mRNA expression in the stomach were higher under HF-diet. HF-diet feeding also resulted in higher hypothalamic NPY/POMC mRNA levels, more marked in females, and in lower OB-Rb mRNA levels, more marked in males. In addition, in females, serum ghrelin levels correlated positively with hypothalamic NPY mRNA levels, and these with caloric intake. In males, hypothalamic OB-Rb mRNA levels correlated positively with POMC mRNA levels and these correlated negatively with caloric intake and with body weight. These data reflect differences between sexes in the effects of HF-diet feeding on food intake control systems, suggesting an impairment of the anorexigenic leptin–POMC system in males and an over-stimulation of the orexigenic ghrelin–NPY system in females.

Introduction

Appetite in mammals is regulated by a well designed homeostatic network made up of central and peripheral components that maintain the balance between energy intake and energy expenditure reviewed in (Stanley et al., 2005). Accumulating evidence supports the fact that two peripheral hormones, the adipocyte hormone leptin and the gastric hormone ghrelin, are major afferent signals for daily management of energy intake and expenditure (Bagnasco et al., 2002; Kalra et al., 2003), without ruling out the involvement of other signals. On the one hand, leptin produced by the adipose tissue works as a long-term indicator of the quantity of fat stores and functions to decrease food intake and regulate body weight and energy balance (Kalra et al., 2005). Besides the adipose tissue, leptin is also produced by the stomach (Bado et al., 1998; Cinti et al., 2000). The secretion of leptin by the stomach is stimulated by feeding (Pico et al., 2002) and there is evidence that gastric leptin is involved in the short-term regulation of food intake, acting as a satiety hormone (Cinti et al., 2001; Pico et al., 2003). On the other hand, ghrelin, which is the only known orexigenic hormone, acts as a counterpart to leptin in the regulation of food intake (Nakazato et al., 2001). In rats, a peak of circulating ghrelin, occurring just before the onset of the dark-phase, has been associated with increased food intake during this period (Sanchez et al., 2004b). In humans, ghrelin has been proposed to have a main role in contributing to the initiation of individual meals (Cummings et al., 2001). In addition, ghrelin also fulfils established criteria for an adiposity-related hormone involved in long-term regulation of body weight (Cummings, 2006).

The hypothalamus is considered the main integrator and processor of peripheral metabolic information, and it contains many neurotransmitters that stimulate or inhibit appetite (Wilding, 2002). The two key neuropeptides of the two main appetite-regulatory pathways are neuropeptide Y (NPY) and melanocyte-stimulating hormone (α-MSH). NPY stimulates feeding, decreases energy expenditure and increases fat deposition, thereby promoting weight gain and obesity (Raposinho et al., 2001), whereas α-MSH is a strong anorexigenic peptide (Hillebrand et al., 2002). Circulating hormones, such as leptin and insulin, can inhibit the anabolic NPY system and activate the melanocortin one (Ahima et al., 2000; Air et al., 2002; Hillebrand et al., 2002), while ghrelin can stimulate the activity of hypothalamic neurons expressing NPY (Wang et al., 2002).

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Feeding dysregulation is a major cause of concern as significant disturbances in feeding may result in disorders ranging from anorexia/cachexia to obesity. In particular, an increased orexigenic drive, which leads to overeating, is regarded as a primary causal factor in weight gain and obesity (Jequier, 2002). Interestingly, differences between sexes in food-intake regulation and eating behaviour have been described (Schneider, 2006), and several studies have concluded that women are more prone to develop eating disorders than men (Uher et al., 2006). In addition, epidemiologic studies have shown that the prevalence of obesity is greater in women compared to men (Ogden et al., 2003). Studies in rats have also shown that females gain more weight and have a different metabolic response from males under HF-diet feeding (Priego et al., 2008). When hyperphagia is stimulated by the availability of a palatable diet, such as the so called cafeteria diet (Llado et al., 1995; Pico et al., 1991) or other fat-or-sugar-enriched diets (Eckel and Moore, 2004), female rats turned out to be more vulnerable than male rats (Eckel and Moore, 2004; Roca et al., 1999). In the same way, females show stronger orexigenic responses (greater food intake and higher plasma ghrelin) to the orexigenic stimulus of fasting (Gayle et al., 2006).

Although sex-associated differences in appetite and feeding behaviour are documented, there are few studies concerning the molecular basis of the feeding-dysregulation induced by HF-diet exposure. Understanding the mechanisms or knowing the factors involved in the increased orexigenic drive under particular feeding conditions, as well as the differences between sexes, may help to develop sex-specific approaches in obesity treatment. Thus, the aim of this study was to assess whether sex-associated differences exist in the mechanisms responsible for hyperphagia in rats chronically exposed to an HF-diet from weaning to the age of 6 months.

Materials and methods

Animals and experimental design

The study was performed using 28 male and female Wistar rats fed from day 21 (after weaning) until the age of 6 months with a normal-fat (NF) diet (3.8 kcal/g; 20% calories from protein, 70% from carbohydrate and 10% from fat) or an HF-diet (4.7 kcal/g; 20% calories from protein, 35% from carbohydrate and 45% from fat). The NF-diet contained 5.5% calories from soybean oil and 4.5% from lard; the HF-diet contained 5.5% calories from soybean oil and 39.5% from lard. (Ref# D12450B and D12451 respectively, from Research Diets, Inc, NJ, USA). Rats were single-caged and kept in a room with controlled temperature (22 °C) and a 12 h light–dark cycle (light on from 08.00 to 20.00 h). Body weights and food intake were recorded 3 times a week from weaning until the age of 6 months.

At the age of 6 months, animals were killed by decapitation under fed conditions, during the first 2 h of the beginning of the light cycle and in different consecutive days (including every day animals from each group). The hypothalamus, different white adipose tissue depots (inguinal, mesenteric and retroperitoneal), and stomach were rapidly removed. The hypothalamus was harvested by using the following landmarks, i.e., frontal edge of the optical chiasm, lateral sulci, caudal edge of the mammary bodies, and a depth of 2 mm. The stomach was opened and rinsed with saline containing 0.1% Diethyl Pyrocarbonate (Sigma, Madrid, Spain) and the epithelium was scraped off using a glass slide. All samples were immediately frozen in liquid nitrogen and stored at −70 °C until RNA analysis. Blood was also collected and centrifuged at 1000 *g* for 10 min to collect the serum, which was stored at −20 °C until analysis. The animal protocol followed in this study was reviewed and approved by the Bioethical Committee of our University and guidelines for the use and care of laboratory animals of the University were followed.

Quantification of gastric and serum leptin levels

Stomach samples were homogenised at 4 °C in 1:3 (w/v) phosphate buffer saline (mM: 137 NaCl, 2.7 KCl, 10 phosphate buffer, pH 7.4) in a Teflon/glass homogeniser. The homogenate was centrifuged at 7000 *g* for 2 min at 4 °C and the supernatant used for leptin quantification. Leptin concentration in the stomach homogenates and in serum was measured with a mouse leptin enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, Minn., USA).

Quantification of gastric and serum ghrelin levels

For ghrelin determination in stomach samples, ghrelin peptide was extracted according to (Sanchez et al., 2004a). Stomach samples were homogenised in PBS, as for leptin determination, and the supernatant mixed with 10 vol 1 M acetic acid containing 20 mM HCl. Homogenates were boiled for 20 min and centrifuged at 7000 *g* for

Table 1
Nucleotide sequences of primers and cycling conditions used for PCR amplification

Gene	Forward Primer (5′ to 3′)	Reverse primer (5′ to 3′)	Cycling conditions	Amplicon size (pb)
NPY	TGGACTGACCCTCGCTCTAT	GTGTCTCAGGGCTGGATCTC	95 °C for 2 s 62 °C for 6 s 72 °C for 12 s	188
POMC	CCTGTGAAGGTGTACCCCAATGTC	CACGTTCTTGATGATGGCGTTC	95 °C for 2 s 62 °C for 6 s 72 °C for 12 s	266
OB-Rb	TGAAACATTTGAGCATCTTT	CGATGCACTGGCTGACAGAA	95 °C for 2 s 58 °C for 6 s 72 °C for 16 s	368
GSH-R	TCAGCCAGTACTGCAACCTG	GGAGAGATGGGATGTGCTGT	95 °C for 2 s 64 °C for 6 s 72 °C for 8 s	222
Leptin	CCAGGATGACACCAAAACCCTC	ATCCAGGCTCTCTGGCTTCTG	95 °C for 2 s 58 °C for 8 s 72 °C for 18 s	316
Ghrelin	CAGAAAGCCCAGCAGAGAAA	GAAGGGAGCATTGAACCTGA	95 °C for 2 s 60 °C for 6 s 72 °C for 6 s	144
18 s	CGCGGTTCTATTTTGTTGGT	AGTCGGCATCGTTTATGGTC	95 °C for 2 s 60 °C for 6 s 72 °C for 10 s	219