Hypothalamic Leptin Receptor and Signaling Molecule Expressions in Cafeteria Diet-Fed Rats

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ABSTRACT

Although obesity is associated with a state of leptin resistance, it has been suggested that leptin may contribute to the pathogenesis of obesity-related hypertension. In previous studies, we reported that cafeteria diet feeding induces hyperleptinaemia and hyperinsulinaemia in both male and female rats, with hypertension occurring only in male rats. However, when female rats were neonatally treated with testosterone (T), these animals develop hypertension when fed the cafeteria diet. These observations led us to investigate leptin signaling and some neuropeptides that are leptin targets in the hypothalamus of male, intact female, and T-treated female cafeteria diet-fed rats. A decrease in the hypothalamic leptin receptors (Ob-Ra and Ob-Rb) and pro-opiomelanocortin (POMC) mRNA was observed only in male hypertensive cafeteria diet-fed rats. Although no alterations in Ob-R occurred in both groups of female cafeteria diet-fed rats, the hyperleptinaemic state of these animals had no influence on POMC mRNA levels. In intact female rats, expression of the suppressors of cytokines signaling SOCS-1, SOCS-2, SOCS-3, and cytokine inhibitor signaling were unaltered, whereas in T-treated females SOCS-3 was overexpressed. Finally SOCS-1 mRNA level was increased only in male rats. Because hyperinsulinemia was reported to counteract the leptin-induced stimulation of the sympathetic tone and because SOCS-1 and -3 are potential inhibitors of insulin signaling, our results suggest that the hypothalamic overexpression of SOCS-1 or SOCS-3 found in male or T-treated female rats after cafeteria diet feeding could block the negative influence of the hyperinsulinemia on the central pressor action of leptin, thereby contributing to their hypertensive state.

Obesity is associated with profound alterations of cardiovascular functions, including an increase in blood pressure. In the central nervous system, the hypothalamus is an important structure that regulates food intake and blood pressure. Thus, hypothalamic lesions induce not only obesity, but also sex-related differences in hypertension (Baylis et al., 1996). Leptin, an adipocyte-derived hormone, regulates food intake and neuroendocrine functions through sympathetic nerve activity (Dunbar et al., 1997; Haynes et al., 1997; Satoh et al., 1998, 1999) via specific receptors (Ob-R) that are highly expressed in the hypothalamus. These leptin effects on food intake and neuroendocrine functions involve intermediate hypothalamic neuropeptides such as pro-opiomelanocortin (POMC), neuropeptide Y (NPY), orexin, cocaine- and amphetamine-regulated transcript, and galanin (Flier and Maratos-Flier, 1998; Baskin et al., 1999). Moreover, chronic leptin infusion increases mean arterial pressure (Shek et al., 1998), and transgenic mice overexpressing leptin also develop a mild hypertension comparable with that produced by chronic lepin infusions (Aizawa-Abe et al., 2000). Leptin could therefore be one of the pathophysiological candidates linking obesity to hypertension (Mark et al., 1999; Aizawa-Abe et al., 2000; Hall et al., 2001; Ogawa et al., 2002). However, because obesity is generally associated with a state of leptin resistance, the role of leptin in the onset of obesity related-hypertension seems intriguing.

In overweight induced by the cafeteria diet feeding, we have previously shown that a sexual dimorphism in hypertension occurs (Coatmellec-Taglioni et al., 2002). Indeed, male cafeteria-fed rats develop hypertension, whereas female fed the same diet are overweight but normotensive (Coatmellec-Taglioni et al., 2002). Interestingly, this sexual dimorphism of the cafeteria diet-induced hypertension was suppressed by testosterone imprinting of female rats at birth (Plut et al., 2002). Moreover, gender difference in renal leptin receptors was also observed in cafeteria diet-fed rats because renal Ob-R was down-regulated only in hypertensive rats (Coatmellec-Taglioni et al., 2003). In the sexual dimorphism in hypertension of cafeteria diet-fed rats, gender differences in hypothalamic leptin signaling also occurred. Therefore, the aim of this study was to measure hypothalamic gene

ABBREVIATIONS: Ob-Ra, leptin receptor-short isoform; Ob-Rb, leptin receptor-long isoform; POMC, pro-opiomelanocortin; NPY, neuropeptide Y; SOCS, suppressors of cytokine signaling; CIS, cytokine inhibitor signaling; PCR, polymerase chain reaction; TH, tyrosine hydroxylase.
expression of leptin receptor isoforms in cafeteria diet-fed rats. Moreover, some components regulated by leptin and involved either in the control of energy balance and blood pressure (POMC and NPY) or in leptin signaling modulation (the suppressors of cytokine signaling, SOCS, and the cytokine inhibitor signaling, CIS) were also investigated in the hypothalamus of male, intact female, and neonatal androgenized female cafeteria diet-fed rats.

Materials and Methods

Animal Procedures. Nulliparous time-mated Sprague-Dawley female rats were purchased from Centre d’Élevage de Rats Janvier (Le Genest St Isle, France), maintained at constant room temperature (24°C) on a 12-h light/dark cycle and housed in individual cages until parturition. At birth, female pups were divided into two groups receiving one subcutaneous injection of either 1 mg of testosterone propionate in olive oil (n = 8) or vehicle alone (n = 10) (Plut et al., 2002). At 22 days, male (n = 12), female, and testosterone-treated female rats were divided into two subgroups and housed in individual cages. The first group, “control”, was allowed free access to standard laboratory chow (25% protein, 6% fat, and 69% carbohydrate; Usine d’Alimentation Rationnelle, Villemoison-sur-Orge, France). The second group, “cafeteria”, was given four palatable foods for human consumption in addition to the chow (Coatmellec-Taglioni et al., 2002). The average composition of the cafeteria diet was 15% protein, 49% fat, and 36% carbohydrate (Coatmellec-Taglioni et al., 2002).

Blood Pressure Measurement and Hormonal Determinations. Systolic blood pressure, plasma leptin and insulin levels measurements were described in our previous study (Coatmellec-Taglioni et al., 2003).

RNA Isolation and PCR Experiments. Total hypothalamic RNA was isolated and RNA-directed cDNA synthesis were performed with 3 μg of RNA as described previously (Plut et al., 2002). Then, mixture containing the synthesized cDNAs was diluted in 4 volumes of sterile water and used for PCR experiments. Each PCR reaction (100-μl final volume) was carried out with 10 μl of cDNA as template, in the presence of 1 μCi of [3H]dCTP (1.92 × 10^12 Bq/mmol; Amersham Biosciences Inc., Les Ullis, France) and HotStarTaq DNA polymerase (QIAGEN S.A., Courtaboeuf, France), under the conditions recommended by the supplier. PCR amplifications were performed using a programmed temperature control system (Appligen Oncor, Illkirch, France). After initial activation of the TaqDNA polymerase at 95°C for 15 min, samples were subjected to the following amplification cycles: denaturation at 94°C for 1 min, annealing at a specific temperature for each primer for 1 min (Table 1), and elongation at 72°C for 1 min. Primers used for amplification, annealing temperatures, and number of cycles are summarized in Table 1. Primers used for β-actin amplification were chosen to span two introns to discriminate the cDNA amplification products from genomic DNA contamination. Linear ranges for each leptin receptor isoform, each SOCS, POMC, NPY, and actin were determined to ensure that all reactions were analyzed during the exponential phase of amplification in all groups. Each reaction mixture was resolved in a 1.5% low melting point agarose gel (Invitrogen, Carlsbad, CA) stained with ethidium bromide and documented on Polaroid 665 film (Polaroid UK Ltd., St Albans, UK). For quantification, respective bands of interest were excised, melted at 70°C, and the incorporated radioactivity was determined by scintillation counting in Ready Safe (Beckman Instruments France S.A., Gagny, France). The incorporated radioactivity was normalized with respect to the length of each cDNA. Because one cDNA preparation was used for all signal studied, mRNA levels of each signal were expressed versus the same β-actin mRNA content. Measurements were made twice on two different cDNA preparations with similar results between both preparations. Results reported in this work are representative of one of these both measurements and are expressed as percentages of their respective control group.

Statistical Analysis. All results were expressed as the means ± S.E.M. Statistical analyses were performed using the Student’s t test. p < 0.05 was considered statistically significant.

Results

Effects of Cafeteria Diet Feeding on Physiological Parameters

Physiological data obtained for male, intact female, and testosterone-treated female cafeteria diet-fed rats were described previously (Plut et al., 2002; Coatmellec-Taglioni et al., 2003) and are summarized in Table 2. Briefly, 10 weeks of cafeteria diet feeding lead to elevated body weight, increased fat pad weight, and elevated plasma leptin levels in the three groups of rats. Plasma insulin levels were also significantly increased in all cafeteria diet-fed rats. As previously observed, male cafeteria diet-fed rats had significantly higher systolic blood pressure than control rats, whereas intact female rats fed the diet were normotensive (Coatmellec-Taglioni et al., 2002). Moreover, female treated with testosterone at birth and fed a cafeteria diet had similar increase in blood pressure than male rats (Plut et al., 2002).

Effects of Cafeteria Diet on Hypothalamic Leptin Receptors and Neuropeptide mRNAs

Leptin Receptors. Fig. 1 shows the hypothalamic expression of the two major isoforms of leptin receptors after 10 weeks of cafeteria diet. In male rats, the short isoform Ob-Ra mRNA level was decreased by 35% (Fig. 2A) and the long isoform Ob-Rb mRNA by 45% (Fig. 2B). In contrast, cafeteria diet failed to alter leptin receptor isoform expression in intact and testosterone-treated female rats (Figs. 1 and 2, A and B). Leptin receptors belong to the cytokine receptor family (Tartaglia et al., 1995), and CIS, in particular, SOCS, have been identified as potential mediators of central leptin resistance (Bjorbaek et al., 1998a, 1999; Emilsson et al., 1999). These findings led us to study the hypothalamic expression of SOCS-1, -2, and -3 and of another potential inhibitor of leptin signaling, CIS (Emilsson et al., 1999) (Fig. 1). As shown in Fig. 3, A–D, gene expression of all these inhibitors was unaltered in untreated female cafeteria-diet fed rats. In contrast, a significant increase in the expression of SOCS-1 was observed only in male cafeteria diet-fed rats (Fig. 3B). Moreover, cafeteria diet feeding lead to higher hypothalamic SOCS-3 mRNA levels in testosterone-treated females rats (Fig. 3D).

### Table 1

<table>
<thead>
<tr>
<th>Locus</th>
<th>Reference of Primers</th>
<th>Size (bp)</th>
<th>Annealing (°C)</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>Plut et al., 2002</td>
<td>280</td>
<td>53</td>
<td>26</td>
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<td>Ob-Ra</td>
<td>Takaya et al., 1996</td>
<td>347</td>
<td>53</td>
<td>35</td>
</tr>
<tr>
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<td>Bjorbaek et al., 1998a</td>
<td>400</td>
<td>55</td>
<td>35</td>
</tr>
<tr>
<td>SOCS-1</td>
<td>Bjorbaek et al., 1998a</td>
<td>350</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>SOCS-2</td>
<td>Bjorbaek et al., 1998a</td>
<td>300</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
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<td>450</td>
<td>60</td>
<td>39</td>
</tr>
<tr>
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<td>Bjorbaek et al., 1998a</td>
<td>400</td>
<td>60</td>
<td>35</td>
</tr>
<tr>
<td>POMC</td>
<td>Xu et al., 1999</td>
<td>478</td>
<td>55</td>
<td>27</td>
</tr>
<tr>
<td>NPY</td>
<td>Rizzoli et al., 1998</td>
<td>146</td>
<td>55</td>
<td>32</td>
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Neuropeptides. The most promising candidates for leptin-sensitive cells in the hypothalamus are arcuate nucleus neurons that express NPY and those that express POMC (Baskin et al., 1999). Both of these cell types express leptin receptors. Because NPY and POMC are implicated in the control of energy balance and modulation of the leptin-induced sympathetic activity, we studied NPY and POMC expression in cafeteria diet-fed rats (Fig. 1). Although NPY gene expression was unchanged in the hypothalamus of all cafeteria diet-fed rats (Fig. 2C), POMC mRNA levels were decreased by 34% only in male rats (Fig. 2D).

Discussion

From the present study, it seems that cafeteria diet feeding led to a sexual dimorphic regulation of leptin receptor in rat hypothalamus. Indeed, a significant lower hypothalamic Ob-Ra and Ob-Rb expression occurred in male cafeteria-fed
rats, whereas no differences could be observed between intact, testosterone-treated female cafeteria diet-fed rats and their respective controls. The lower Ob-R mRNA levels observed in males are in agreement with other studies in male animals also reporting decreased hypothalamic Ob-R gene expression after central leptin resistance induced by high fat diet or by chronic leptin infusion (Lin et al., 2000; Martin et al., 2000). More recently, evidence was provided that the nutritional regulation of hypothalamic Ob-R gene expression is defective in obese male rats (Sahu et al., 2002). Thus, it seems likely the decreased Ob-R mRNA level observed in our fasted male cafeteria diet-fed rats is related to an insensitivity to nutritional changes of leptin receptor gene expression that could be secondary to hyperleptinemia and/or obesity. Because male and female rats also elicit different sensitivities to central leptin (Clegg et al., 2003), this could explain why Ob-R mRNAs were differently affected by the cafeteria diet in male and female rats.

On the other hand, POMC and NPY are implicated in the regulation of food intake, sympathetic nervous system acti-

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**Fig. 2.** Quantification of Ob-Ra (A), Ob-Rb (B), NPY (C), and POMC (D) PCR products. Results for cafeteria diet-fed rats (●) are expressed as the percentage of control rats (□) and are means values ± S.E.M. Statistical analysis was performed using Student’s t test. *, p < 0.05; **, p < 0.01 versus control.

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**Fig. 3.** Quantification of CIS (A), SOCS-1 (B), SOCS-2 (C), and SOCS-3 (D) PCR products, respectively in male, female and testosterone-treated female rats. Results for cafeteria diet-fed rats (●) are expressed as the percentage of control rats (□) and are means values ± S.E.M. Statistical analysis was performed using Student’s t test. **, p < 0.01 versus control.
vation, and energy expenditure (Schwartz et al., 1996; Flier and Maratos-Flier, 1998; Satoh et al., 1998). Leptin is known to modulate the hypothalamic expression of these peptides by promoting POMC mRNA and by reducing NPY mRNA expression in the arcuate nucleus of nonobese rats (Schwartz et al., 1996; Schwartz et al., 1997). In agreement with a previous report on cafeteria diet feeding (Pedrazzi et al., 1998), we failed to observe any significant changes in the hypothalamic expression of NPY after 10 weeks of this diet in both male and female rats. In contrast, we found a decrease in POMC mRNA level in the hypothalamus of male cafeteria diet-fed rats. Some POMC products mediate the increase in mean arterial pressure that follows acute i.c.v. leptin injection (Dunbar and Lu, 1999). Because male rats had a decreased POMC mRNA expression, elevated blood pressure in male cafeteria diet-fed rats seems independent of the melanocortin system activation as observed in hyperleptinemic transgenic skinny mice (Aizawa-Abe et al., 2000). However, the decreased Ob-R mRNA level occurring in male cafeteria diet-fed rats could well contribute to the decreased expression of POMC, one satiety factor (Satoh et al., 1998), thus explaining the hyperphagia observed in male cafeteria diet-fed rats. Interestingly, similar decreases in POMC were reported in the obese Koletsky (fa/fa) rats and the obese Zucker rats, two models of leptin receptor disruption (Korner et al., 1999; Kim et al., 2000). In female rats, where no modifications in leptin receptors expression occurred in spite of their hyperleptinemic state, cafeteria diet failed to increase POMC expression. Thus, the altered leptin regulation of POMC expression in cafeteria diet-fed rats may contribute to the state of resistance to the anorexigenic action of leptin and by consequence to the development of obesity. Nevertheless, such a leptin resistance seems to be rather selective, because in the agouti obese mice that have blockade of melanocortin receptors (Correia et al., 2002), leptin is still able to increase sympathetic activity. Moreover, selective leptin resistance with disruption of satiety effect and preservation of sympathetic nervous activation has been proposed as a new concept in leptin physiology with cardiovascular implications (Mark et al., 2002). Therefore, a central pressor effect of leptin cannot be excluded in the cafeteria diet-fed rat model.

Some cytokine-inducible inhibitors of signaling are inducible by leptin or insulin and can then modulate the signaling of these hormones (Bjorbaek et al., 1998a; 1999; Emilsson et al., 1999; Rui et al., 2002). As shown in the present study, no modifications in CIS, SOCS-1, SOCS-2, and SOCS-3 mRNA levels were observed in the hypothalamus of intact female cafeteria diet-fed rats. In contrast, after 10 weeks of feeding the cafeteria diet, hypothalamic SOCS-1 mRNA level was increased only in male rats, whereas hypothalamic SOCS-3 was overexpressed in testosterone-treated females. Cross talk between insulin and leptin signal transduction have been observed, and selective interaction between these both hormones occurred (Niswender and Schwartz, 2003). Thus, chronic insulin treatment was reported to attenuate the leptin-induced increases in sympathetic tone and cardiovascular responses (Dunbar and Lu, 2000). Interestingly, recent studies suggest that the insulin signal transduction could be suppressed after association of SOCS-1 or SOCS-3 with the insulin receptor (Mooney et al., 2001; Rui et al., 2002).Because cafeteria diet induces hyperinsulinemia in the three groups of rats, SOCS-1 and SOCS-3 induction in male and testosterone-treated female cafeteria diet-fed rats, respectively, could thus prevent the effect of hyperinsulinemia and particularly the negative action of insulin toward the leptin-induced sympathetic activation. If SOCS-3 could suppress insulin signaling, it also could antagonize the signaling pathway activated by leptin (Bjorbaek et al., 1998a, 1999). However, the effects of an overexpression of SOCS-3 on food intake or on sympathetic tone are still unclear. It is tempting to suggest that SOCS-3 could contribute to a selective leptin resistance with alteration of food intake and preservation of sympathetic activation. Additional weight to the latter hypothesis is our previous observation that hyperleptinemia in cafeteria-fed rats is associated with an overexpression of the tyrosine hydroxylase (TH) gene (the rate-limiting enzyme of catecholamines biosynthesis) only in the hypothalamus of male and testosterone-treated female rats. Indeed, this finding strongly suggests that these rats have an increased sympathetic activity that could contribute to their hypertension (Plut et al., 2002). It is noteworthy that leptin is able to up-regulate TH mRNA expression and stimulates catecholamines secretion (Satoh et al., 1999; Takekoshi et al., 1999). Conversely, the normal blood pressure observed in intact female cafeteria diet-fed rats could best be explained as follows: in the absence of any SOCS-1 or SOCS-3 induction, the hyperinsulinemia could prevent the leptin-induced sympathetic activity, a mechanism that is strongly supported by our finding of unaltered TH expression in these rats (Plut et al., 2002).

In summary, in cafeteria diet-fed rats, alteration in leptin receptor, and/or signaling could contribute to the resistance to satiety and weight-reducing actions of leptin. Moreover, SOCS-1 or SOCS-3, which are exclusively overexpressed in the hypertensive cafeteria-fed rats, could preserve the leptin-induced sympathetic tone activation, thus allowing leptin to exert its pressor effect.

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References


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