Hypothalamic leptin receptor and signaling molecules expressions in cafeteria diet-fed rats

Charles Plut, Catherine Ribière, Yves Giudicelli and Jean-Pierre Dausse

Department of Biochemistry and Molecular Biology, Faculté de Médecine de Paris-Ile de France-Ouest, Université Versailles-St Quentin en Yvelines, France

Running titled : Leptin signaling in cafeteria-diet

Author for correspondence :

Catherine Ribière, Dept of Biochemistry and Molecular Biology, 45 rue des Saints-Pères, 75270 Paris Cedex 06, France.

Fax : 01-42-86-21-85

E-mail : catherine.ribiere@paris-ouest.univ-paris5.fr

Number of pages : 24

Number of tables : 2

Number of figures : 3

Number of references : 40

Number of words :
Abstract : 248
Introduction : 403
Discussion : 1061

Recommanded section assignement : Cardiovascular

Abbreviations :

Ob-Ra	leptin receptor-short isoform
Ob-Rb	leptin receptor-long isoform
SOCS	suppressors of cytokine signaling
CIS	cytokine inhibitor signaling
NPY	neuropeptide Y
POMC	pro-opiomelanocortin
PCR	polymerase chain reaction
RT-PCR	reverse transcription-polymerase chain reaction
T-treated	*testosterone-treated*

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 hyperinsulinemia in both male and female rats with hypertension occurring only in male rats. However, when females 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. However, alhough 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 CIS were unaltered, whereas in T-treated females SOCS-3 was over-expressed. Finally SOCS-1 mRNA level was increased only in male rats. Since 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 over-expression 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 and stimulates sympathetic nerve activity (Dunbar et al., 1997; Haynes et al., 1997; Satoh et al., 1998; Satoh et al., 1999) *via* specific receptors (Ob-R) which 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 (CART) 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 to that produced by chronic leptin 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, as 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 appears (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 as renal Ob-R were downregulated 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 could also appeared. Therefore, the aim of this study was to measure hypothalamic gene 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.

Methods

Animal Procedures. Nulliparous time-mated Sprague-Dawley female rats were purchased from Centre d'Elevage 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 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", allowed free access to standard laboratory chow (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France, 25% protein, 6% fat and 69% carbohydrate). 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 already described in our previous study (Coatmellec-Taglioni et al., 2003).

RNA Isolation and PCR experiments. Total hypothalamic RNA was isolated and RNA-directed complementary DNA (cDNA) synthesis were performed with 3µg RNA as previously described (Plut et al., 2002). Then, mixture containing the synthesized cDNAs were 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 [^{3}H]-dCTP ($1.92x10^{12}$ Bq/mmol, Amersham Pharmacia Biotech., 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 Taq DNA 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 summarised in Table 1. Primers used for β-actin amplification were chosen to span two introns in order 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) 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. As 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-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 already described (Plut et al., 2002; Coatmellec-Taglioni et al., 2003) and are summarised 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 show 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 isoforms expression in intact and testosterone-treated female rats (Fig. 1 and 2A-B). Leptin receptors belong to the cytokine receptor family (Tartaglia et al., 1995) and cytokine-inducible inhibitors of signaling (CIS), in particular suppressors of cytokine signaling (SOCS), have been identified as potential mediators of central leptin resistance (Bjorbaek et al., 1998a; Bjorbaek et al., 1999; Emilsson et al., 1999). These findings led us to study the hypothalamic expression of SOCS-1, 2, 3 and of another

potential inhibitor of leptin signaling CIS (Emilsson et al., 1999) (Fig. 1). As shown in Fig. 3A, B, C and D, gene expression of all these inhibitors were 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).

Neuropeptides

The most promising candidates for leptin-sensitive cells in the hypothalamus are arcuate nucleus (Arc) neurones that express NPY and those that express POMC (Baskin et al., 1999). Both of these cell types express leptin receptors. As 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). While 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 appears 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 appeared 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 male 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. As 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 activation 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 Arc of non-obese 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 icv leptin injection (Dunbar and Lu, 1999). As 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 occuring 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 rat. Interestingly, similar decreases in POMC were reported in the obese Koletsky (fa^f/fa^f) 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 the consequence to the development of obesity. Nevertheless, such a leptin resistance appears to be rather selective, since in the agouti obese mice which 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; Bjorbaek et al., 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 over-expressed in testosterone-treated females. Cross-talk between insulin and leptin signal transduction have been observed and selective interaction between these both hormones could appeared (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 following association of SOCS-1 or SOCS-3 with the insulin receptor (Mooney et al., 2001; Rui et al., 2002). As 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 hyperinsulinaemia and particularly the negative action of insulin towards the leptin induced sympathetic activation. If SOCS-3 could suppressed insulin signaling, it also antagonize signaling pathway activated by leptin (Bjorbaek et al., 1998a; Bjorbaek et al., 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. Adding further 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 which 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 follow : in the absence of any SOCS-1 or SOCS-3 induction, the hyperinsulinemia could

prevent the leptin-induced sympathetic activity, a mechanism which 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 over-expressed in the hypertensive cafeteria-fed rats, could preserve the leptin induced sympathetic tone activation, thus allowing leptin to exert its pressor effect.

References

Aizawa-Abe M, Ogawa Y, Masuzaki H, Ebihara K, Satoh N, Iwai H, Matsuoka N, Hayashi T, Hosoda K, Inoue G, Yoshimasa Y and Nakao K (2000) Pathophysiological role of leptin in obesity-related hypertension. *J Clin Invest* **105**:1243-1252.

Baskin DG, Hahn TM and Schwartz MW (1999) Leptin sensitive neurons in the hypothalamus. *Horm Metab Res* **31**:345-350.

Baylis C, Samsell L, Racusen L and Gladfelter W (1996) Hypothalamic lesions induce obesity and sex-dependent glomerular damage and increases in blood pressure in rats. *Hypertension* **27**:926-932.

Bjorbaek C, El-Haschimi K, Frantz JD and Flier JS (1999) The role of SOCS-3 in leptin signaling and leptin resistance. *J Biol Chem* **274**:30059-30065.

Bjorbaek C, Elmquist JK, Frantz JD, Shoelson SE and Flier JS (1998a) Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol Cell* **1**:619-625.

Bjorbaek C, Elmquist JK, Michl P, Ahima RS, van Bueren A, McCall AL and Flier JS (1998b) Expression of leptin receptor isoforms in rat brain microvessels. *Endocrinology* **139**:3485-3491.

Clegg DJ, Riedy CA, Smith KA, Benoit SC and Woods SC (2003) Differential sensitivity to central leptin and insulin in male and female rats. *Diabetes* **52**:682-687.

Coatmellec-Taglioni G, Dausse JP, Giudicelli Y and Ribiere C (2002) Gender difference in diet-induced obesity hypertension: implication of renal alpha2-adrenergic receptors. *Am J Hypertens* **15**:143-149.

Coatmellec-Taglioni G, Dausse JP, Giudicelli Y and Ribiere C (2003) Sexual dimorphism in cafeteria diet-induced hypertension is associated with gender-related difference in renal leptin receptor down-regulation. *J Pharmacol Exp Ther* **305**:362-367.

Correia ML, Haynes WG, Rahmouni K, Morgan DA, Sivitz WI and Mark AL (2002) The concept of selective leptin resistance: evidence from agouti yellow obese mice. *Diabetes* **51**:439-442.

Dunbar JC, Hu Y and Lu H (1997) Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. *Diabetes* **46**:2040-2043.

Dunbar JC and Lu H (1999) Leptin-induced increase in sympathetic nervous and cardiovascular tone is mediated by proopiomelanocortin (POMC) products. *Brain Res Bull* **50**:215-221.

Dunbar JC and Lu H (2000) Chronic intracerebroventricular insulin attenuates the leptin-mediated but not alpha melanocyte stimulating hormone increase in sympathetic and cardiovascular responses. *Brain Res Bull* **52**:123-126.

Emilsson V, Arch JR, de Groot RP, Lister CA and Cawthorne MA (1999) Leptin treatment increases suppressors of cytokine signaling in central and peripheral tissues. *FEBS Lett* **455**:170-174.

Flier JS and Maratos-Flier E (1998) Obesity and the hypothalamus: novel peptides for new pathways. *Cell* **92**:437-440.

Hall JE, Hildebrandt DA and Kuo J (2001) Obesity hypertension: role of leptin and sympathetic nervous system. *Am J Hypertens* **14**:103S-115S.

Haynes WG, Sivitz WI, Morgan DA, Walsh SA and Mark AL (1997) Sympathetic and cardiorenal actions of leptin. *Hypertension* **30**:619-623.

Kim EM, O'Hare E, Grace MK, Welch CC, Billington CJ and Levine AS (2000) ARC POMC mRNA and PVN alpha-MSH are lower in obese relative to lean zucker rats. *Brain Res* **862**:11-16.

Korner J, Chua SC, Jr., Williams JA, Leibel RL and Wardlaw SL (1999) Regulation of hypothalamic proopiomelanocortin by leptin in lean and obese rats. *Neuroendocrinology* **70**:377-383.

Lin S, Storlien LH and Huang XF (2000) Leptin receptor, NPY, POMC mRNA expression in the diet-induced obese mouse brain. *Brain Res* **875**:89-95.

Mark AL, Correia M, Morgan DA, Shaffer RA and Haynes WG (1999) State-of-the-art-lecture: Obesity-induced hypertension: new concepts from the emerging biology of obesity. *Hypertension* **33**:537-541.

Mark AL, Correia ML, Rahmouni K and Haynes WG (2002) Selective leptin resistance: a new concept in leptin physiology with cardiovascular implications. *J Hypertens* **20**:1245-1250.

Martin RL, Perez E, He YJ, Dawson R, Jr. and Millard WJ (2000) Leptin resistance is associated with hypothalamic leptin receptor mRNA and protein downregulation. *Metabolism* **49**:1479-1484.

Mooney RA, Senn J, Cameron S, Inamdar N, Boivin LM, Shang Y and Furlanetto RW (2001) Suppressors of cytokine signaling-1 and -6 associate with and inhibit the insulin receptor. A potential mechanism for cytokine-mediated insulin resistance. *J Biol Chem* **276**:25889-25893.

Niswender KD and Schwartz MW (2003) Insulin and leptin revisited: adiposity signals with overlapping physiological and intracellular signaling capabilities. *Frontiers in Neuroendocrinology* **24**:1-10.

Ogawa Y, Masuzaki H, Ebihara K, Shintani M, Aizawa-Abe M, Miyanaga F and Nakao K (2002) Pathophysiogical role of leptin in lifestyle-related diseases. Studies with transgenic skinny mice overexpressing leptin. *J Diabetes Complications* **16**:119-122.

Pedrazzi P, Cattaneo L, Valeriani L, Boschi S, Cocchi D and Zoli M (1998) Hypothalamic neuropeptide Y and galanin in overweight rats fed a cafeteria diet. *Peptides* **19**:157-165.

Plut C, Ribiere C, Giudicelli Y and Dausse JP (2002) Gender differences in hypothalamic tyrosine hydroxylase and alpha(2)- adrenoceptor subtype gene expression in cafeteria diet-induced hypertension and consequences of neonatal androgenization. *J Pharmacol Exp Ther* **302**:525-531.

Rizk NM, Liu LS and Eckel J (1998) Hypothalamic expression of neuropeptide-Y in the New Zealand obese mouse. *Int J Obes Relat Metab Disord* **22**:1172-1177.

Rui L, Yuan M, Frantz D, Shoelson S and White MF (2002) SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. *J Biol Chem* **277**:42394-42398.

Sahu A, Nguyen L and O'Doherty RM (2002) Nutritional regulation of hypothalamic leptin receptor gene expression is defective in diet-induced obesity. *J Neuroendocrinol* **14**:887-893.

Satoh N, Ogawa Y, Katsuura G, Numata Y, Masuzaki H, Yoshimasa Y and Nakao K (1998) Satiety effect and sympathetic activation of leptin are mediated by hypothalamic melanocortin system. *Neurosci Lett* **249**:107-110.

Satoh N, Ogawa Y, Katsuura G, Numata Y, Tsuji T, Hayase M, Ebihara K, Masuzaki H, Hosoda K, Yoshimasa Y and Nakao K (1999) Sympathetic activation of leptin via the ventromedial hypothalamus: leptin-induced increase in catecholamine secretion. *Diabetes* **48**:1787-1793.

Schwartz MW, Seeley RJ, Campfield LA, Burn P and Baskin DG (1996) Identification of targets of leptin action in rat hypothalamus. *J Clin Invest* **98**:1101-1106.

Schwartz MW, Seeley RJ, Woods SC, Weigle DS, Campfield LA, Burn P and Baskin DG (1997) Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. *Diabetes* **46**:2119-2123.

Shek EW, Brands MW and Hall JE (1998) Chronic leptin infusion increases arterial pressure. *Hypertension* **31**:409-414.

Takaya K, Ogawa Y, Isse N, Okazaki T, Satoh N, Masuzaki H, Mori K, Tamura N, Hosoda K and Nakao K (1996) Molecular cloning of rat leptin receptor isoform complementary DNAs-- identification of a missense mutation in Zucker fatty (fa/fa) rats. *Biochem Biophys Res Commun* **225**:75-83.

Takekoshi K, Motooka M, Isobe K, Nomura F, Manmoku T, Ishii K and Nakai T (1999) Leptin directly stimulates catecholamine secretion and synthesis in cultured porcine adrenal medullary chromaffin cells. *Biochem Biophys Res Commun* **261**:426-431.

Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Woolf EA, Monroe CA and Tepper RI (1995) Identification and expression cloning of a leptin receptor, OB-R. *Cell* **83**:1263-1271.

Xu B, Kalra PS, Farmerie WG and Kalra SP (1999) Daily changes in hypothalamic gene expression of neuropeptide Y, galanin, proopiomelanocortin, and adipocyte leptin gene expression and secretion: effects of food restriction. *Endocrinology* **140**:2868-2875.

Acknowledgements

This work was supported by grants from the Ministère de la Recherche (DGR).

JPET Fast Forward. Published on September 9, 2003 as DOI: 10.1124/jpet.103.054726
This article has not been copyedited and formatted. The final version may differ from this version.

Legends

Fig. 1 : Hypothalamic gene expression of short and long leptin receptor isoforms (Ob-Ra and Ob-Rb), NPY, POMC, CIS, SOCS-1, SOCS-2, SOCS-3 and β-actin in cafeteria diet-fed rats.

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 ± SE.M. Statistical analysis was performed using Student's t-test. * $p < 0.05$; ** $p < 0.01$ *vs*. control

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 ± SE.M. Statistical analysis was performed using Student's t-test. ** $p < 0.01$ *vs*. Control

Table 1. Nucleotide sequences of primers used for PCR amplification

Locus	**Reference of Primers**	**Size**	**Annealing**	**Cycles**
β-actin	(Plut et al., 2002)	280bp	53°C	26
Ob-Ra	(Takaya et al., 1996)	347bp	53°C	35
Ob-Rb	(Bjorbaek et al., 1998b)	400bp	55°C	35
SOCS-1	(Bjorbaek et al., 1998a)	350bp	60°C	40
SOCS-2	(Bjorbaek et al., 1998a)	300bp	60°C	40
SOCS-3	(Bjorbaek et al., 1998a)	450bp	60°C	39
CIS	(Bjorbaek et al., 1998a)	400bp	60°C	35
POMC	(Xu et al., 1999)	478bp	60°C	33
NPY	(Rizk et al., 1998)	146bp	55°C	27

JPET Fast Forward. Published on September 9, 2003 as DOI: 10.1124/jpet.103.054726
This article has not been copyedited and formatted. The final version may differ from this version.

Table 2. Physiological parameters of cafeteria-fed rats

		Blood pressure (mm Hg)	**Body weight** (g)	**Fat mass**[1] (g)	**Plasma leptin** (ng/ml)	**Plasma insulin** (ng/ml)
Males	Control	127 ± 1.2	561 ± 13	32.7 ± 2.6	6.4 ± 0.5	2.8 ± 0.3
	Cafeteria	156 ± 1.7***	628 ± 23*	54.7 ± 6.4*	12.9 ± 1.3***	4.8 ± 0.5**
Females	Control	132 ± 1	318 ± 6	22.2 ± 1.4	5.7 ± 0.8	1.6 ± 0.1
	Cafeteria	131 ± 2	386 ± 37	42.8 ± 8.7*	16.4 ± 4*	5.4 ± 1**
Testosterone-females	Control	128 ± 3.7	308 ± 7	16.6 ± 0.9	3.3 ± 0.4	1.6 ± 0.2
	Cafeteria	169 ± 5.3***	400 ± 6**	35.8 ± 3.0*	14.2 ± 2.5**	5.3 ± 0.8**

Data are means ± SEM

[1]Fat mass : mesenteric, perirenal, epididymal/parametrial and subcutaneous white adipose tissue

* P<.05 *vs* control; ** P<0.01 vs control; *** P<.001 *vs* control

JPET Fast Forward. Published on September 9, 2003 as DOI: 10.1124/jpet.103.054726
This article has not been copyedited and formatted. The final version may differ from this version.

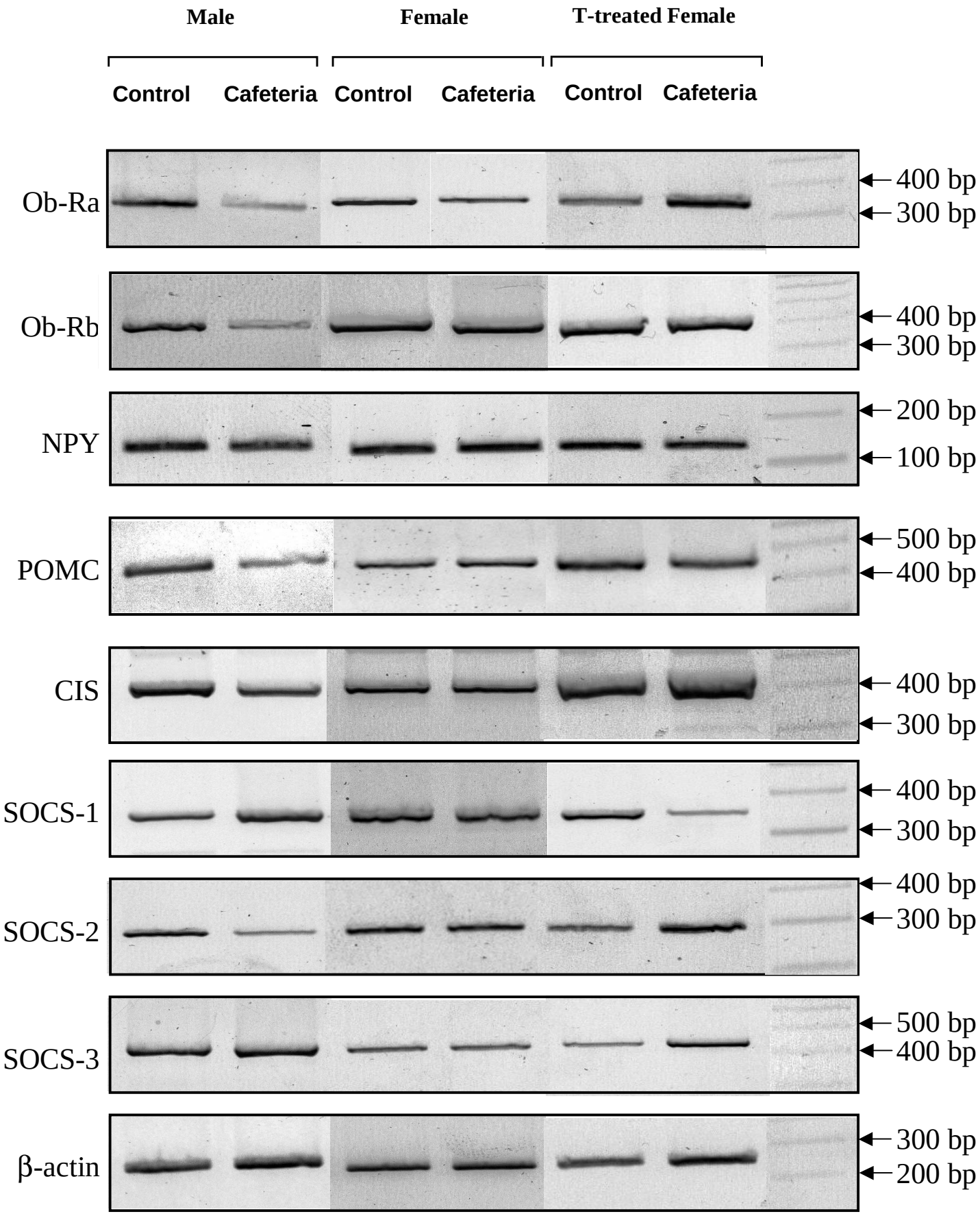
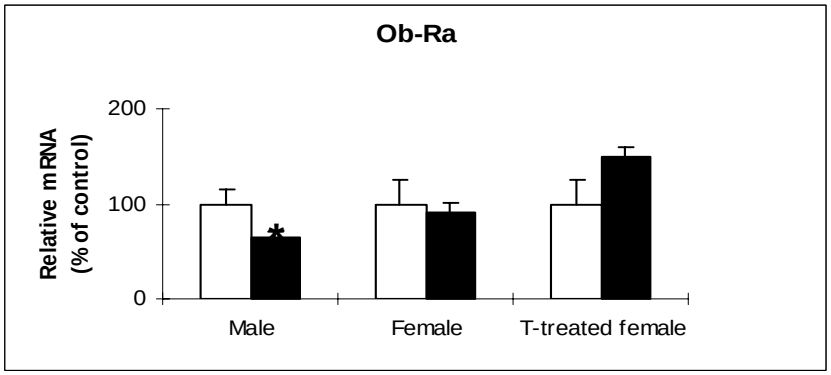


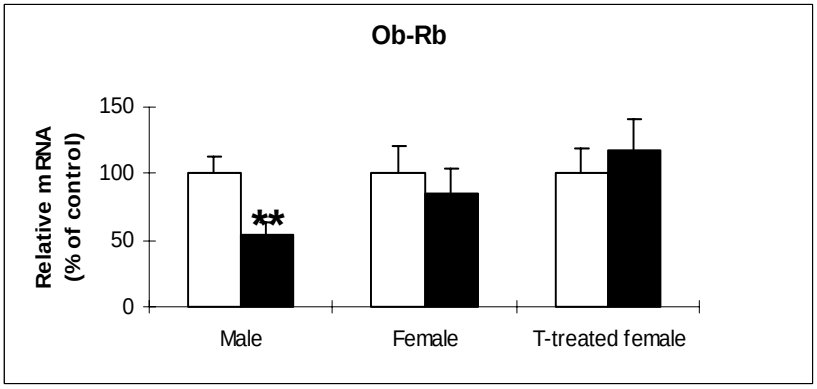
Fig. 1 Plut et al.

JPET Fast Forward. Published on September 9, 2003 as DOI: 10.1124/jpet.103.054726
This article has not been copyedited and formatted. The final version may differ from this version.

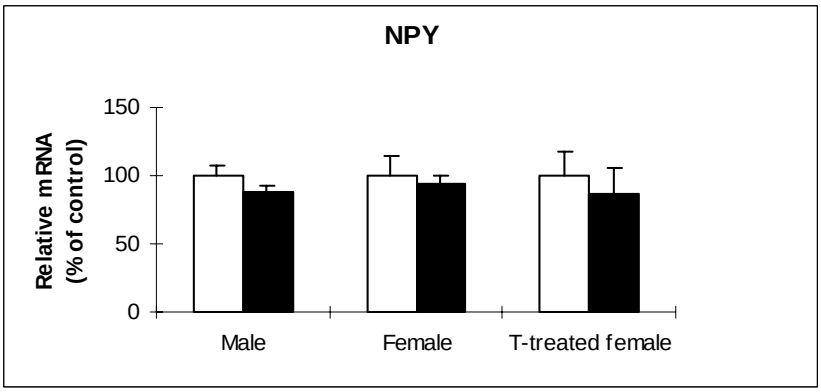
A.



B.



C.



D.

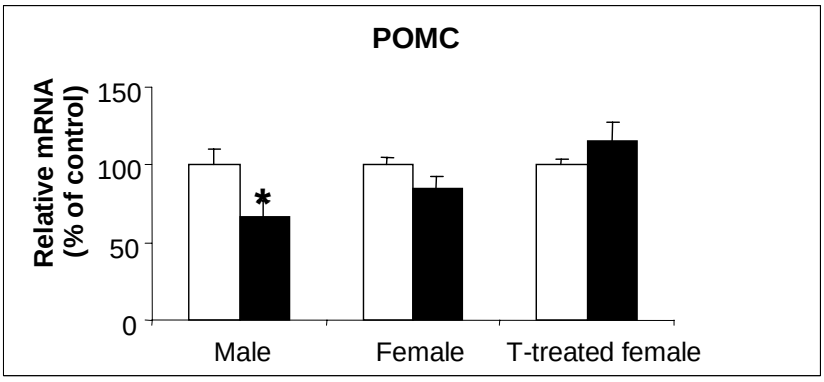
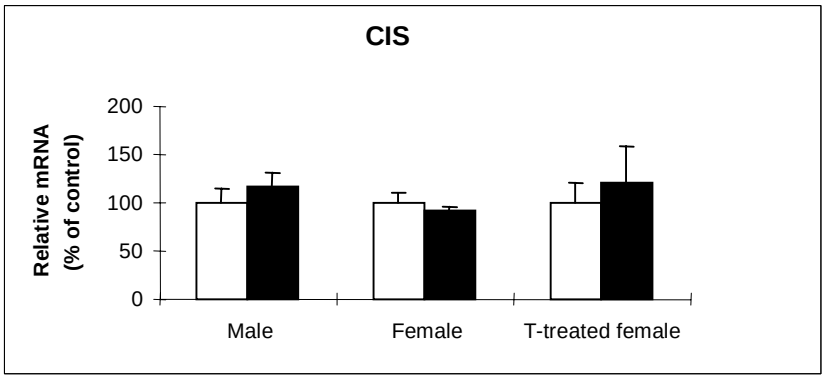


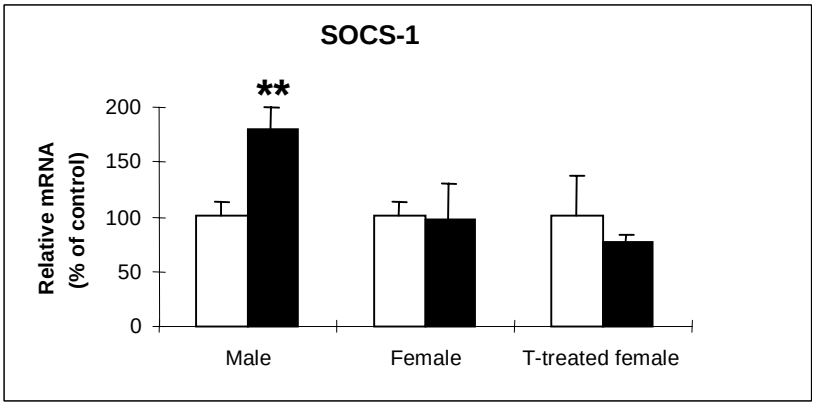
Fig. 2 Plut et al.

JPET Fast Forward. Published on September 9, 2003 as DOI: 10.1124/jpet.103.054726
This article has not been copyedited and formatted. The final version may differ from this version.

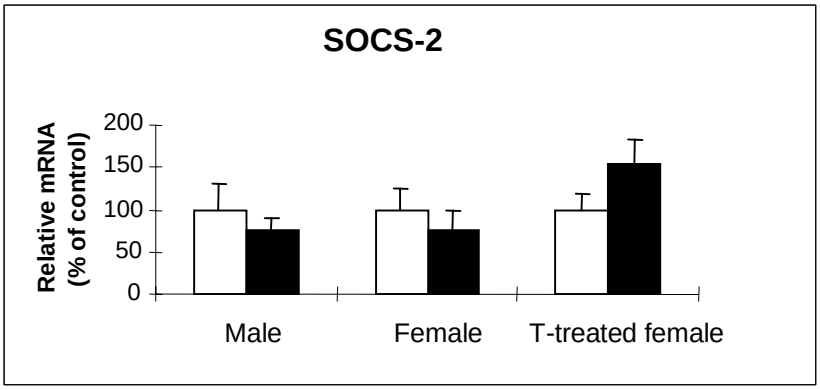
A.



B.



C.



D.

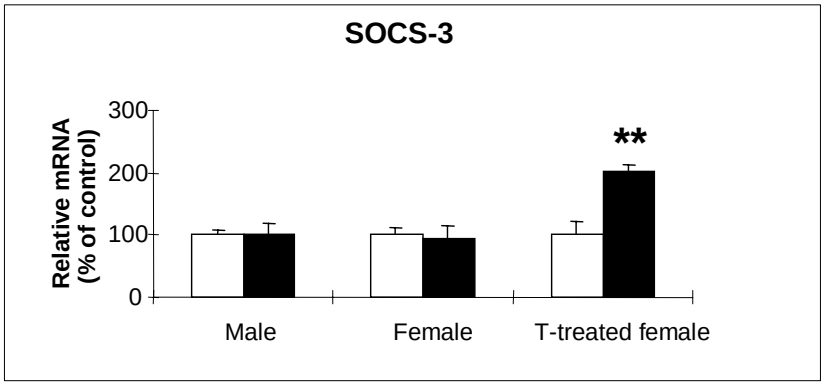


Fig. 3 Plut et al.