ELSEVIER

Pharmacology, Biochemistry and Behavior 71 (2002) 425–430

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

# Leptin suppression of hypothalamic NPY expression and feeding, but not amygdala NPY expression and experimental anxiety

A. Thorsell*, L. Caberlotto, R. Rimondini, M. Heilig

*NEUROTEC, Karolinska Institutet, SE-141 86 Stockholm, Sweden*

Received 21 March 2001; received in revised form 24 June 2001; accepted 1 July 2001

**Abstract**

Leptin decreases food intake through actions in the hypothalamus, partly through interactions with neuropeptide Y (NPY). However, NPY also produces behavioral antistress effects mediated inter alia through the amygdala. If leptin generally suppresses NPY function, the utility of leptin-mimics for treatment of obesity might be limited. Here, we therefore compared the effects of intracerebroventricular leptin on hypothalamic and amygdala NPY expression, as well as the respective related behaviors, i.e., feeding and experimental anxiety. Rats were injected intracerebroventricularly with leptin once daily for 6 days. Leptin-treated subjects consumed significantly less chow and had reduced body weight at the end of the treatment period compared to saline-treated controls. This was accompanied by a significant suppression of hypothalamic NPY expression. In contrast, the expression of NPY within the amygdala was unaffected by leptin. In parallel, in an established animal model of anxiety, the elevated plus-maze, no effect of leptin on anxiety-related behaviors was observed. In conclusion, leptin selectively affects the hypothalamic NPY system and its functional outflow, i.e., feeding and endocrine stress responses. Despite modifying endocrine responses, leptin treatment does not affect behavioral measures of experimental anxiety. 

*Keywords:* Leptin; Feeding; Intracerebroventricular administration; Experimental anxiety; Plus-maze

## 1. Introduction

Extensive evidence links hypothalamic neuropeptide Y (NPY) to food intake regulation. For instance, increased NPY expression has been demonstrated in this area following fasting (Calza et al., 1989), and acute NPY injections into the ventricles or locally into the paraventricular nucleus of the hypothalamus induce food intake (Clark et al., 1984; Levine and Morley, 1984; Stanley and Leibowitz, 1985) When NPY is administered chronically, a state mimicking the hormonal and metabolic changes seen in obesity is induced (Vettor et al., 1994; Zarjevski et al., 1993). It is presently not clear whether the profound effects of NPY on feeding is mediated via Y5 receptors, Y1 receptors, or both (Gerald et al., 1996; Kanatani et al., 1999). Within the hypothalamus, NPY-expressing cell bodies are located in the arcuate nucleus with projections to the paraventricular nucleus (Gehlert et al., 1987; Morris, 1989).

In addition to its effects on feeding, NPY is involved in behavioral responses to stress through extrahypothalamic mechanisms. Thus, amygdala expression of NPY is strongly influenced by stress (Thorsell et al., 1998, 1999), while intra-amygdala administration of NPY leads to a marked behavioral antistress effect. This antistress action of NPY appears to be mediated through Y1 receptors (Heilig et al., 1993; Wahlestedt et al., 1993; Sajdyk et al., 1999).

The *obese* (*ob*) gene was first identified in 1994 (Zhang et al., 1994). Its protein product, leptin, is synthesized in adipose tissue and circulating levels of the protein reflect the size of the body's fat mass (Maffei et al., 1995). Leptin deficiency or leptin resistance cause overeating and obesity (Chua et al., 1996). The obesity in *ob*-deficient mice can be reversed by systemic leptin administration. Leptin receptors have been found in both the arcuate and the paraventricular nucleus (Håkansson et al., 1998; Schwartz et al., 1996), and are colocalized with NPY in the arcuate nucleus (Håkansson et al., 1996). Intracerebroventricular administration of leptin inhibits NPY-induced feeding in

* Corresponding author. Addiction Center South, Huddinge University Hospital, M46, SE-141 86 Stockholm, Sweden. Tel.: +46-8-5858-6594; fax: +46-8-5858-5760.

*E-mail address*: annika.thorsell@neurotec.ki.se (A. Thorsell).

0091-3057/02/$ – see front matter 
PII: S0091-3057(01)00678-5

the rat and suppresses NPY mRNA expression in the arcuate nucleus (Schwartz et al., 1998). Leptin inhibits both release and synthesis of NPY, thus giving rise to both short- and long-term effects (Schwartz et al., 1996; Stephens et al., 1995; Glaum et al., 1996). In addition to NPY, the anorexigenic effect is in part mediated by corticotropin-releasing hormone (CRH) (Uehara et al., 1998), a principal regulator of the hypothalamic–pituitary–adrenal axis (HPA axis).

In addition to its effects on feeding, leptin is involved in endocrine stress responses. Its synthesis and secretion is regulated by glucocorticoids (De Vos et al., 1901). Also, leptin has been shown to blunt the corticosterone response to stress when administered intraperitoneally to mice (Heiman et al., 1997) and to have a direct inhibitory effect on glucocorticoid secretion by human and rat adrenal gland in vitro (Pralong et al., 1998). However, contradicting evidence exist as to the effect of leptin on corticosterone levels and, thus, leptin has been demonstrated to elevate serum corticosterone both in vitro and in vivo (Malendowicz et al., 1997, 1998). Also, when administered centrally to rats, leptin has been shown to increase plasma levels of ACTH and corticosterone in a dose-dependent manner (Morimoto et al., 2000; van Dijk et al., 1997).

Thus, NPY and leptin interact at a neuronal level within the hypothalamus, having opposing roles in regulation of feeding behavior. Little is known about possible NPY–leptin interactions in the extrahypothalamic systems and within the amygdala in particular. This issue is important if leptin-mimics are to be developed for clinical use, since suppression of NPY expression within the amygdala might limit their utility, due to side effects such as increased anxiety. Here, we therefore compared the effects of repeated intracerebroventricular leptin injections in the rat on hypothalamic and amygdala NPY expression, and on NPY-related functional outflows of these two structures, feeding and experimental anxiety, respectively.

## 2. Material and methods

### 2.1. Subjects

Male Sprague–Dawley rats (body weight 220–250 g at time of surgery) were anaesthetized with ketamine/xylazine, placed in a Kopf stereotactic apparatus, and equipped with unilateral intracerebroventricular guides (toothbar: 3.3 mm below the interaural line, coordinates: 0.8 mm posterior and 1.4 mm lateral to bregma). The guide projected 3.3 mm below the skull surface and the injector used projected 1.0 mm further. Animals were single-caged and kept according to Animal Committee guidelines and under permission S81-85 (Stockholm South Ethical Committee). Food and water were available ad libitum and animals were kept in a controlled environment with a 12:12-h light/dark cycle (lights on at 7 a.m.).

### 2.2. Leptin treatment

Leptin (a kind gift from Amgen, Thousand Oaks, CA, USA) was diluted in sterile Ringer solution to a concentration of 1 mg/ml. Ten microliters of the leptin solution or vehicle were injected over 2 min and the injector left in place for an additional minute to prevent backflow into the guide cannula.

### 2.3. Food intake and body weight development

A preweighed amount of food pellets was provided daily to each cage. In conjunction with intracerebroventricular injections, remaining amount of chow was measured, and the amount of chow consumed in grams per day was calculated. Body weight was determined daily.

### 2.4. Locomotor activity

Exploratory locomotor activity was determined by placing subjects in locomotor activity cages equipped with infrared beam detection (Med Associates, St. Albans, VT, USA). Interbeam distance was 8.5 cm horizontally and 6.5 cm vertically, and activity was recorded for 30 min in intervals of 10 min.

### 2.5. The elevated plus-maze

The plus-maze was carried out as previously described (Möller et al., 1997). Briefly, the apparatus consisted of two open and two closed arms ($50 \times 10$ cm, wall height 50 cm) and was made of black plastic with a rubber floor. The maze was elevated 50 cm above the floor and testing was done under dimmed red light. Rats were placed on the central area of the maze facing one open arm and were allowed to explore for 5 min. Automatic scoring was used (EthoVision, Noldus, Wageningen, the Netherlands).

### 2.6. Corticosterone determination

Serum corticosterone levels were determined using the Coat-A-Count assay (DPC Scandinavia, Mölndal, Sweden) according to the manufacturers instructions. A 45-min restraint stress was used to elevate corticosterone levels in the subjects and leptin was injected 20 min prior to the restraint.

### 2.7. Solution hybridization RNase protection assay (SH-RPA)

The SH-RPA was performed as previously described (Thorsell et al., 1998). In brief, probes were prepared using the MAXIscript kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. The template used for in vitro transcription of NPY antisense was a pGEM-2 vector with a 290-bp insert of the rat preproNPY genomic DNA sequence (a kind gift from Prof. D. Larhammar, Uppsala,

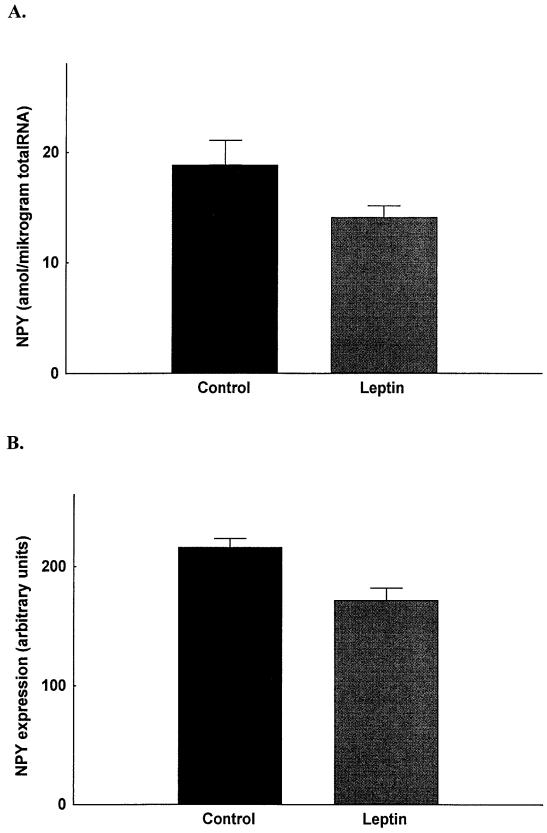


Fig. 1. (A) Analysis of NPY mRNA in whole-hypothalamus homogenates following 6 days of leptin treatment (10 μg icv) suggested suppression of NPY expression by leptin ($P = .056$). (B) This was confirmed when in situ hybridization was used to estimate NPY mRNA expression in the arcuate nucleus of the hypothalamus ($P = .007$).

Sweden). The template for the β-actin antisense riboprobe and the β-actin sense external standard was a pBluescript SK II vector with a 150-bp insert of the rat β-actin cDNA (a kind gift from Dr. M. Bader, Max-Delbróck Centrum, Berlin, Germany). In vitro transcription was performed in the presence of [α-$^{32}$P]-radiolabeled UTP.

The RPA II kit (Ambion) was used for the RPA according to the manufacturer's instructions. For construction of a standard curve, radiolabeled antisense β-actin probe was hybridized with increasing amounts of unlabeled sense β-actin. For sample analysis, total RNA (2–4 μg) was hybridized with radiolabeled NPY and β-actin antisense RNA probes (90,000 and 45,000 cpm, respectively). Following RNase treatment, samples were separated on a 5% nondenaturing polyacrylamide gel and detected on a Fuji BAS 5000 Phosphor-Imager.

### 2.8. In situ hybridization

In situ hybridization was performed as previously described (Caberlotto et al., 1998). In brief, brain sections were fixed in 4% paraformaldehyde/1 × PBS, dehydrated in graded series of ethanol and delipidated in chloroform. The slides were then air dried and stored at −70 °C until use. Labeled riboprobe was added to the hybridization cocktail in a concentration of $20 \times 10^3$ cpm/μl, and 0.1 ml of the solution was applied to each slide. The slides were coverslipped and hybridization was carried out at 55 °C overnight in a humidified chamber. The sections were washed in graded solutions of SSC, dehydrated in ethanol, allowed to dry, and exposed to Hyperfilm. The NPY riboprobe was made from a 508-bp cDNA subcloned into a pGEM4 vector (Hanze et al., 1991).

## 3. Results

### 3.1. NPY mRNA levels

NPY mRNA levels were measured using two methods, SH-RPA and in situ hybridization. Using SH-RPA on hypothalamic tissue homogenates, a suppression of NPY mRNA expression was suggested [one-way ANOVA for treatment, $F(1,17) = 2.9$, $P = .056$; Fig. 1A]. This was confirmed using in situ hybridization to evaluate NPY mRNA levels within the arcuate nucleus of the hypothalamus, whereupon NPY expression was confirmed to be suppressed [one-way ANOVA for treatment, $F(1,9) = 11.9$, $P = .007$; Fig. 1B].

In contrast, NPY mRNA levels within the amygdala, neocortex, and striatum were not significantly affected by the leptin treatment as measured by SH-RPA (Table 1).

### 3.2. Body weight development and food intake

Leptin treatment significantly decreased body weight gain and daily food intake in treated animals as compared to saline-treated controls [two-way repeated-measures ANOVA, bodyweight: $F(12,228) = 11.47$, $P < .00001$; food intake: $F(8,152) = 3.13$, $P = .003$; Fig. 2].

### 3.3. Locomotion

No difference could be detected in locomotor behavior between the leptin-treated subjects and the controls (data not shown).

### 3.4. Plus-maze

Results are given in Table 2. Leptin treatment did not significantly affect anxiety-related behavior measured as either percentage of time spent on the open arms of the

Table 1
NPY mRNA levels within the striatum, amygdala, and neocortex determined using SH-RPA (data are given as μmol/μg total RNA)

| Region | Control | Leptin | $P$ value |
|---|---|---|---|
| Amygdala | 25.9 ± 3.5 | 33.4 ± 4.3 | .22 |
| Neocortex | 70.0 ± 5.2 | 74.4 ± 6.0 | .54 |
| Striatum | 36.9 ± 5.6 | 26.7 ± 2.2 | .09 |

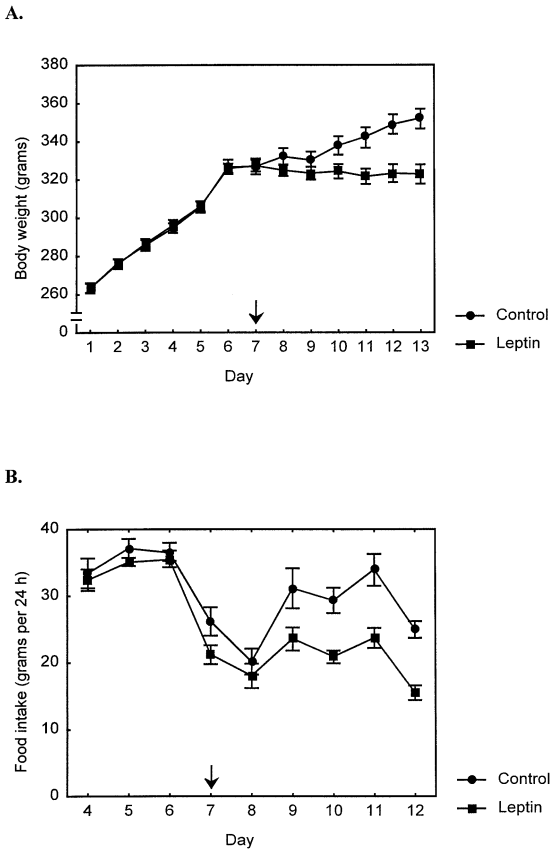


Fig. 2. Body weight development (A) and food intake (B) during leptin treatment. Leptin was given intracerebroventricularly for the last 6 days. For statistics, see Results. Arrow indicates start of leptin/saline injections.

maze or percentage of entries made onto the open arms. Total number of entries made onto any arm was also unaffected by the treatment, in agreement with the result from the locomotor activity testing.

### 3.5. Corticosterone

Leptin treatment gave rise to significantly elevated baseline corticosterone levels and restraint stress significantly elevated serum corticosterone levels in both controls and leptin-treated subjects [two-way ANOVA, treatment: $F(1,17)=7.5$, $P=.01$; stress: $F(1,17)=122$, $P<.000001$]. However, no significant interaction effect was seen indicating no effect of leptin treatment on corticosterone response to restraint stress [$F(1,17)=0.32$, $P=.58$]. Data are shown in Table 3.

Table 2
Behavior on the elevated plus-maze

| | Control | Leptin | *P* value |
|---|---|---|---|
| Time index (%) | 49.3 ± 3.5 | 50.9 ± 2.6 | .71 |
| Entry index (%) | 54.4 ± 3.2 | 53.8 ± 2.3 | .89 |
| Total number of entries (*n*) | 33.1 ± 1.2 | 34.2 ± 1.1 | .51 |

Time index = (time on open arms/(time on open arms + time on closed arms)) × 100%. Entry index = (entries onto open arms/(entries onto open arms + entries into closed arms)) × 100%. Total number of entries = entries onto open arms + entries into closed arms.

Table 3
Corticosterone values at baseline or following a 45-min restraint stress period in subjects treated with saline (control) or leptin (data are given as ng/ml)

| | Control | Leptin | *P* value |
|---|---|---|---|
| Baseline | 69 ± 12 | 229 ± 33 | .02 |
| Stressed | 490 ± 18 | 569 ± 46 | .32 |
| *P* value | .0002 | .0002 | |

## 4. Discussion

Our present findings confirm and extend previous reports indicating an inhibitory action of centrally administered leptin on hypothalamic NPY expression as a possible mechanism that might contribute to appetite-suppressant effects of leptin. This leptin–NPY interaction seems to be restricted to the arcuate nucleus, since neither NPY expression in the amygdala, nor an NPY-dependent functional output of the amygdala, plus-maze behavior (Möller et al., 1997), were affected by repeated leptin administration.

The interaction between leptin and NPY at the level of the hypothalamic arcuate nucleus has been suggested on the basis of leptin receptors being present in NPY-ergic neurons (Håkansson et al., 1996) and of suppressed preproNPY expression following intracerebroventricular administration of two 3.5-μg leptin doses during a 40-h fasting period (Schwartz et al., 1996). However, previous studies have shown that NPY expression is markedly up-regulated within the arcuate nucleus as a result of fasting (Sahu et al., 1992; Schwartz et al., 1998), and thus the available evidence demonstrates that the fasting-induced NPY expression is inhibited by leptin. Our present findings show that leptin, when given repeatedly into the brain, can also down-regulate basal, unstimulated NPY expression in the arcuate nucleus. Furthermore, our findings indicate that tolerance does not develop to this action of leptin, since the effect is present after 6 days of repeated daily injections.

Brain leptin receptors were initially predominantly described in the hypothalamus (Mercer et al., 1996). However, recent evidence clearly demonstrates that leptin receptors, including the long form involved in transducing the leptin signal, are also present in extrahypothalamic areas, and among these within the amygdala (Burguera et al., 2000). Regulation of amygdala NPY expression is important in adaptive responses to stress (Thorsell et al., 1999), and activation of amydala NPY receptors predominantly of the Y1 subtype attenuates behavioral stress responses in several animal models of anxiety (Heilig et al., 1993; Sajdyk et al., 1999).

The present study therefore examined the possibility that amygdala NPY expression, and the related function, plus-maze behavior, might also be affected by leptin. Our

results do not support this notion. A differential action of leptin on hypothalamic and amygdala NPY might be related to differential coexpression of NPY and leptin receptors in these two structures, with hypothalamic (Håkansson et al., 1996), but not amygdala NPY neurons coexpressing these receptors. To our knowledge, coexpression of leptin and NPY has not been demonstrated within the amygdala.

Finally, in the present study, subchronic central administration of leptin increased basal levels of corticosterone. This is in agreement with previously reported effects of centrally administered leptin (Morimoto et al., 2000; van Dijk et al., 1997). In contrast, the stress-induced corticosterone response was not affected in the present experiments. Although a blunted corticosterone response to stress following leptin treatment has been reported in mice (Heiman et al., 1997), this was after peripheral rather than central administration. Thus, leptin appears to affect the HPA axis in a complex manner, and at different levels.

In summary, we present data indicating that subchronic presence of elevated central leptin levels affects hypothalamic, but not extrahypothalamic NPY expression and function. Feeding regulation by leptin might therefore be possible to target with small molecular leptin-mimicking ligands without side effects of decreased amygdala NPY signalling, i.e., elevated anxiety.

## References


Burguera B, Couce ME, Long J, Lamsam J, Laakso K, Jensen MD, Parisi JE, Lloyd RV. The long form of the leptin receptor (OB-Rb) is widely expressed in the human brain. Neuroendocrinology 2000;71(3):187–95 (March).

Caberlotto L, Fuxe K, Overstreet DH, Gerrard P, Hurd YL. Alterations in neuropeptide Y and Y1 receptor mRNA expression in brains from an animal model of depression: region specific adaptation after fluoxetine treatment. Brain Res, Mol Brain Res 1998;59(1):58–65.

Calza L, Giardino L, Battistini N, Zanni M, Galetti S, Protopapa F, Velardo A. Increase of neuropeptide Y-like immunoreactivity in the paraventricular nucleus of fasting rats. Neurosci Lett 1989;104(1–2): 99–104.

Chua SCJ, Chung WK, Wu-Peng XS, Zhang Y, Liu SM, Tartaglia L, Leibel RL. Phenotypes of mouse diabetes and rat fatty due to mutations in the *OB* (leptin) receptor (see comments)Science 1996;271(5251): 994–6.

Clark JT, Kalra PS, Crowley WR, Kalra SP. Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. Endocrinology 1984;115(1):427–9.

De Vos P, Saladin R, Auwerx J, Staels B. Induction of *ob* gene expression by corticosteroids is accompanied by body weight loss and reduced food intake. J Biol Chem 1901;270(27):15958–61.

Gehlert DR, Chronwall BM, Schafer MP, O'Donohue TL. Localization of neuropeptide Y messenger ribonucleic acid in rat and mouse brain by in situ hybridization. Synapse 1987;1:25–31.

Gerald C, Walker MW, Criscione L, Gustafson EL, Batzlhartmann C, Smith KE, Vaysse P, Durkin MM, Laz TM, Linemeyer DL, Schaffhauser AO, Whitebread S, Hofbauer KG, Taber RI, Branchek TA, Weinshank RL. A receptor subtype involved in neuropeptide-Y-induced food intake. Nature 1996;382(6587):168–71.

Glaum SR, Hara M, Bindokas VP, Lee CC, Polonsky KS, Bell GI, Miller RJ. Leptin, the obese gene product, rapidly modulates synaptic transmission in the hypothalamus. Mol Pharmacol 1996;50(2):230–5.

Håkansson ML, Hulting AL, Meister B. Expression of leptin receptor mRNA in the hypothalamic arcuate nucleus — relationship with NPY neurones. NeuroReport 1996;7(18):3087–92.

Håkansson ML, Brown H, Ghilardi N, Skoda RC, Meister B. Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. J Neurosci 1998;18(1):559–72.

Hanze J, Kummer W, Haass M, Lang RE. Neuropeptide Y mRNA regulation in rat sympathetic ganglia: effect of reserpine. Neurosci Lett 1991;124(1):119–21.

Heilig M, McLeod S, Brot M, Heinrichs SC, Menzaghi F, Koob GF, Britton KT. Anxiolytic-like action of neuropeptide Y: mediation by Y1 receptors in amygdala, and dissociation from food intake effects. Neuropsychopharmacology 1993;8:357–63.

Heiman ML, Ahima RS, Craft LS, Schoner B, Stephens TW, Flier JS. Leptin inhibition of the hypothalamic–pituitary–adrenal axis in response to stress. Endocrinology 1997;138(9):3859–63.

Kanatani A, Kanno T, Ishihara A, Hata M, Sakuraba A, Tanaka T, Tsuchiya Y, Mase T, Fukuroda T, Fukami T, Ihara M. The novel neuropeptide Y Y(1) receptor antagonist J-104870: a potent feeding suppressant with oral bioavailability. Biochem Biophys Res Commun 1999;266(1): 88–91.

Levine AS, Morley JE. Neuropeptide Y: a potent inducer of consummatory behavior in rats. Peptides 1984;5(6):1025–9.

Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM. Leptin levels in human and orodent — measurement of plasma leptin and *Ob* mRNA in obese and weight-reduced subjects. Nat Med 1995;1(11): 1155–61.

Malendowicz LK, Nussdorfer GG, Markowska A. Effects of recombinant murine leptin on steroid secretion of dispersed rat adrenocortical cells. J Steroid Biochem Mol Biol 1997;63(1–3):123–5.

Malendowicz LK, Macchi C, Nussdorfer GG, Nowak KW. Acute effects of recombinant murine leptin on rat pituitary–adrenocortical function. Endocr Res 1998;24(2):235–46.

Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, Trayhurn P. Localization of leptin receptor mRNA and the long form splice variant (OB-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. FEBS Lett 1996;387(2–3):113–6.

Möller C, Wiklund L, Sommer W, Thorsell A, Heilig M. Decreased experimental anxiety and voluntary ethanol consumption in rats following central but not basolateral amygdala lesions. Brain Res 1997;760(1–2): 94–101.

Morimoto I, Yamamoto S, Kai K, Fujihira T, Morita E, Eto S. Centrally administered murine-leptin stimulates the hypothalamus–pituitary–adrenal axis through arginine–vasopressin. Neuroendocrinology 2000; 71(6):366–74 (June)

Morris BJ. Neuronal localisation of neuropeptide Y gene expression in rat brain. J Comp Neurol 1989;290(3):358–68.

Pralong FP, Roduit R, Waeber G, Castillo E, Mosimann F, Thorens B, Gaillard RC. Leptin inhibits directly glucocorticoid secretion by normal human and rat adrenal gland. Endocrinology 1998;139(10): 4264–8.

Sahu A, White JD, Kalra PS, Kalra SP. Hypothalamic neuropeptide Y gene expression in rats on scheduled feeding regimen. Brain Res, Mol Brain Res 1992;15(1–2):15–8.

Sajdyk TJ, Vandergriff MG, Gehlert DR. Amygdalar neuropeptide Y Y-1 receptors mediate the anxiolytic-like actions of neuropeptide Y in the social interaction test. Eur J Pharmacol 1999;368(2–3):143–7.

Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of leptin action in rat hypothalamus. J Clin Invest 1996;98(5):1101–6.

Schwartz MW, Erickson JC, Baskin DG, Palmiter RD. Effect of fasting and leptin deficiency on hypothalamic neuropeptide Y gene transcription in vivo revealed by expression of a *lacZ* reporter gene. Endocrinology 1998;139(5):2629–35.

Stanley BG, Leibowitz SF. Neuropeptide Y injected in the paraventricular hypothalamus: a powerful stimulant of feeding behavior. Proc Natl Acad Sci USA 1985;82(11):3940–3.

Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A, et al. The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 1995;377(6549):530–2.

Thorsell A, Svensson P, Wiklund L, Sommer W, Ekman R, Heilig M. Suppressed neuropeptide Y (NPY) mRNA in rat amygdala following restraint stress. Regul Pept 1998;75–6:247–54.

Thorsell A, Carlsson K, Ekman R, Heilig M. Behavioral and endocrine adaptation, and up-regulation of NPY expression in rat amygdala following repeated restraint stress. NeuroReport 1999;10(14):3003–7.

Uehara Y, Shimizu H, Ohtani K, Sato N, Mori M. Hypothalamic corticotropin-releasing hormone is a mediator of the anorexigenic effect of leptin. Diabetes 1998;47(6):890–3.

van Dijk G, Donahey JC, Thiele TE, Scheurink AJ, Steffens AB, Wilkinson CW, Tenenbaum R, Campfield LA, Burn P, Seeley RJ, Woods SC. Central leptin stimulates corticosterone secretion at the onset of the dark phase. Diabetes 1997;46(11):1911–4.

Vettor R, Zarjevski N, Cusin I, Rohner-Jeanrenaud F, Jeanrenaud B. Induction and reversibility of an obesity syndrome by intracerebroventricular neuropeptide Y administration to normal rats. Diabetologia 1994;37(12):1202–8.

Wahlestedt C, Pich EM, Koob GF, Yee F, Heilig M. Modulation of anxiety and neuropeptide Y-Y1 receptors by antisense oligodeoxynucleotides. Science 1993;259:528–31.

Zarjevski N, Cusin I, Vettor R, Rohner-Jeanrenaud F, Jeanrenaud B. Chronic intracerebroventricular neuropeptide-Y administration to normal rats mimics hormonal and metabolic changes of obesity. Endocrinology 1993;133(4):1753–8.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372(6505):425–32.