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Leptin stimulates prostaglandin E_2 and $F_{2\alpha}$, but not nitric oxide production in neonatal rat hypothalamus

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Abstract

Leptin, an adipocyte-derived 16 kDa polypeptide hormone, has been found to regulate food intake and thermogenesis by modulating stimulatory and inhibitory pathways in the feeding circuitry of the hypothalamus, among which corticotropin releasing hormone (CRH). Nitric oxide (NO) and prostaglandins have been shown to be involved in both CRH neurosecretion and feeding regulation. We have investigated the role of NO, prostaglandin E_2 and prostaglandin $F_{2\alpha}$ as mediators of the hypothalamic effects of leptin and their possible involvement in leptin-stimulated CRH secretion. Using primary cultures of neonatal (5- to 6-day-old) rat hypothalamic cells, we confirmed that leptin (0.1–10 nM) stimulates CRH secretion. This effect was not blocked by L-N^G-nitro-methyl-arginine (L-NAME, 100 μM), a NO-synthase competitive inhibitor; and leptin did not stimulate NO production. Cyclooxygenase inhibition by indomethacin (10 μM) did not modify leptin-induced CRH secretion, while leptin stimulated prostaglandin E_2, and prostaglandin $F_{2\alpha}$ secretion. In conclusion, leptin-induced hypothalamic CRH secretion is not modulated by NO-synthase- or cyclooxygenase-mediated mechanisms; leptin does not stimulate NO production, but it stimulates prostaglandin E_2 and $F_{2\alpha}$ production, which could add to the growing list of mediators of leptin signaling in the hypothalamus.

Keywords: Leptin; CRH (corticotropin releasing hormone); Nitric oxide (NO); Prostaglandin E_2; Prostaglandin $F_{2\alpha}$

1. Introduction

Leptin is a 16 kDa polypeptide hormone coded by the *ob* gene (Zhang et al., 1994) and secreted by adipocytes in concentrations that are proportional to total adipose stores (Frederich et al., 1995). In *ob/ob* genetically obese mice, leptin has been found to be deficient (Zhang et al., 1994) and its exogenous administration restores normal weight (Halaas et al., 1995; Pelleymounter et al., 1995), while obesity in humans seems to be associated with elevated leptin levels and resistance to its effects (Considine et al., 1996). This resistance could be due either to reduction in leptin transit through the blood–brain barrier, as peripherally produced leptin is delivered to the central nervous system (CNS) through a saturable transport system localized on endothelial cells (Banks et al., 1996; Caro et al., 1996), and/or to defective leptin signaling in the CNS. Leptin receptors have been localized in several brain areas, including the hypothalamus (Tartaglia et al., 1995; Schwartz et al., 1996; Couce et al., 1997; Håkansson et al., 1998). Regarding its possible CNS mediators, leptin has been hypothesized to regulate food intake and thermogenesis by modulating stimulatory and inhibitory pathways in the feeding circuitry. Hypothalamic corticotropin releasing hormone (CRH) could represent such a mediator, as its release is stimulated by leptin (Costa et al., 1997; Raber et al., 1997), it decreases food intake (Krahn et al., 1988) and increases sympathetically mediated energy expenditures (Rothwell, 1990). Nitric oxide (NO), a gaseous free radical produced by the enzyme NO-synthase from L-arginine, generating equimolar amounts of citrulline and NO (Moncada et al., 1991), has been shown to be positively correlated to increased food intake and body weight (Morley and Flood, 1991, 1992; Squadrito et al., 1993; Choi et al., 1994; De Luca et al., 1995). Recent data point to a role

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for NO in neuroendocrine secretion, particularly in the modulation of hypothalamic CRH release (Brunetti et al., 1993, 1996; Brann et al., 1997). Prostaglandins have been shown to play a role in regulating feeding behavior (Scaramuzzi et al., 1971; Martin and Baile, 1973), particularly in anorexia induced by interleukins (Plata-Salaman, 1996); prostaglandins also play a modulatory role in hypothalamic CRH secretion (Bernardini et al., 1989, 1990; Navarra et al., 1991).

We have investigated the role of NO, prostaglandin E_2 and prostaglandin $F_{2\alpha}$ in the hypothalamic effects of leptin and their possible involvement in leptin-stimulated CRH secretion.

2. Materials and methods

2.1. Hypothalamic cell cultures

Rat hypothalamic cell cultures were obtained as previously described (Brunetti et al., 1994). Rat pups, 5- to 6-day-old, were killed by decapitation and the hypothalami were minced with a blade into small fragments and sequentially incubated in Dulbecco's Modified Eagle's Medium (DMEM) at 37°C in a Dubnoff shaking bath, with 0.5% trypsin (type XII S) for 15 min, 0.02% deoxyribonuclease I for 1 min, 0.1% soybean trypsin inhibitor for 4 min, 2 mM and 1 mM EDTA [in Ca^{2+}- and Mg^{2+}-free Earle's Balanced Salt Solution (EBSS)] for 4 and 15 min, respectively. The remaining tissue fragments were mechanically dispersed into single cells by gentle suction and extrusion through a narrow tip Pasteur pipette; the cell suspension was then filtered through a 100-μm nylon mesh and centrifuged at $1000 \times g$ for 10 min. Finally, the cells were checked for viability with the Trypan blue exclusion test, counted by a haemocytometer (approximately 0.8–1 million cells/hypothalamus), plated into six-well dishes (1 million cells/well, for L-citrulline and prostaglandin production experiments, and 2 million cells/well, for CRH release experiments), and incubated with DMEM supplemented with 10% foetal calf serum, at 37°C in air/CO_2 95/5%. After 7–8 days in culture, with medium changes every 2–3 days, release experiments were performed as 4-h incubations with graded concentrations of test substances in DMEM (supplemented with 0.1% bovine serum albumin, 0.006% ascorbic acid and 40 IU/ml aprotinin), for CRH and prostaglandin radioimmunoassays (RIA) or Krebs buffer, for L-citrulline high-performance liquid chromatography (HPLC) assay; release aliquots were stored at −20°C until assay.

Cell culture chemicals and drugs were purchased from Sigma, except DMEM and serum from Gibco. Leptin (RBI) 1 mg was dissolved in phosphate buffered saline (PBS), separated into aliquots, 10 μM 0.1 ml, and stored at −20°C.

2.2. CRH radioimmunoassay

Tyr-rat-CRH was iodinated with Na ^{125}I by the chloramine T method. Rat CRH antibody, raised in the rabbit, was a kind gift from Dr. Philip W. Gold, NIH, Bethesda. Rat CRH standard or sample, 200 μl, was incubated at 5°C with the CRH antibody at a final dilution of 1:40 000 in assay buffer for 24 h; thereafter, 3000 cpm ^{125}I-CRH were added to each tube and incubated for 48 h. Finally, antirabbit goat serum (1:360) and 3% polyethylene glycol were added to separate bound and free fractions. After 4 h the tubes were centrifuged for 30 min at $4000 \times g$, the supernatants were aspirated, and the pellets were counted in a γ-counter. Sensitivity of the assay was 10 pg/tube; ED_{50} was 116 pg/tube.

2.3. Prostaglandin E_2 and $F_{2\alpha}$ radioimmunoassay

Prostaglandin RIA was performed according to Ciabattoni et al. (1979): 100 μl of prostaglandin E_2 or prostaglandin $F_{2\alpha}$ standard or sample was incubated overnight at 5°C with the respective ^{3}H-prostaglandin (3.000 cpm/tube; NEN) and antibody (final dilution: 1:80 000 for prostaglandin E_2; 1:120 000 for prostaglandin $F_{2\alpha}$; gift from Prof. G. Ciabattoni), in a volume of 1.5 ml of 0.025 M phosphate buffer. Free and antibody-bound prostaglandins were separated by the addition of 100 μl 5% bovine serum albumin and 100 μl 3% charcoal suspension, followed by centrifuging for 10 min at $4000 \times g$ at 5°C and decanting off of supernatants into scintillation fluid (Instagel Plus) for β emission counting. Sensitivity of the assay was 2 pg/ml; ED_{50} was 20 pg/ml.

2.4. HPLC determination of L-citrulline

We evaluated the production of NO by measuring the concentration of L-citrulline in the incubation medium. L-Citrulline, generated in equimolar amounts with NO by the enzyme NO-synthase from the substrate L-arginine, is stable in the sample, while NO has a very short half-life. To obtain the citrulline concentration, the sample was derivatized with *o*-phthaldialdehyde: 50- to 100-μl aliquots of standard or samples were mixed with 50 μl of the *o*-phthaldialdehyde reagent (50 mg *o*-phthaldialdehyde in 1.25 ml of methanol + 50 μl of 2-mercaptoethanol + 11.2 ml 0.4 M sodium borate, pH 9.5) and, after 1 min, 850 μl of 0.05 M potassium phosphate buffer, pH 6.3. Then, the L-citrulline derivative was analysed by HPLC with a Vydac 201 TPB RP-18 column (5 μm, 3 mm i.d. × 200 mm, Chrompack). Chromatography was performed using a linear gradient (flow rate: 0.6 ml/min), starting with 0.05 M aqueous potassium phosphate buffer, pH 6.3, and ending after 30 min with acetonitrile:H_2O:methanol (4:2:4). The latter solvent was continued for 10 min and the column

was then re-equilibrated for 20 min with buffer before the next sample injection. The HPLC system (Kontron) consisted of 420 pump, 490 mixer for gradient programming, Reodyne injection valve with a 200-μl filling loop and SFM 25 spectrofluorimeter. Excitation/emission wavelengths were maintained at 330/450 nm. The detector was coupled to a Data System 450 (Kontron).

2.5. Statistics

The results are expressed as group means ± S.E.M. ($n = 4–5$ in each group) and each experiment shown is representative of at least three similar ones; the significance of differences between means was determined with the Student–Newman–Keuls test, and accepted at $P < 0.05$.

3. Results

Leptin was tested at concentrations 0.1–10 nM, in the range of its circulating levels (1 nM) in vivo (Frederich et al., 1995).

3.1. Effect of leptin on CRH release

Graded concentrations of leptin (0.1–10 nM) were able to stimulate CRH release with respect to the controls, in a dose-dependent manner (Fig. 1).

3.2. Role of NO-synthase in leptin effects

NO-synthase activity was blocked by incubating cells with L-N^G-nitro-methyl-arginine (L-NAME, a NO-synthase competitive inhibitor) 100 μM, 30 min before and simultaneously with leptin. This concentration maximally inhibit NO production in the hypothalamus, as we have previously shown for L-N^G-nitro-arginine (L-NOArg), another NO-synthase inhibitor (Brunetti et al., 1996). L-NAME (100 μM) did not modify basal or leptin (10 nM)-induced CRH release (Table 1). In another set of experiments, cells were incubated with leptin in Krebs buffer instead of DMEM (to avoid interference with the HPLC assay of L-citrulline): NO production, evaluated as L-citrulline levels in the incubation buffer, was not modified by leptin (0.1–10 nM) treatment, after 4-h incubation in 1 ml of Krebs buffer (L-citrulline nM/ml: control, 7.14 ± 0.70; leptin 0.1 nM, 8.56 ± 0.78; 1 nM, 7.24 ± 0.83; 10 nM, 6.45 ± 0.76; group means ± S.E.M.; $n = 4–5$).

Table 1
CRH release after leptin, leptin + L-NAME (NO-synthase inhibitor), and leptin + indomethacin (cyclooxygenase inhibitor)

Treatment	CRH (pg/ml)
control	198 ± 18
leptin 0.1 nM	395 ± 12[a]
L-NAME 100 μM	190 ± 19
leptin 0.1 nM + L-NAME 100 μM	409 ± 15[a,b]
indomethacin 10 μM	173 ± 11
leptin 0.1 nM + indomethacin 10 μM	378 ± 14[a,b]

Values are group means ± S.E.M.; $n = 4–5$; [a] $P < 0.05$ vs. control; [b] not significantly different with respect to leptin alone. Cells (2 millions/well) were incubated for 4 h in 1 ml of DMEM. NO-synthase and cyclooxygenase were blocked by incubating cells with L-N^G-nitro-methyl-arginine (L-NAME) and indomethacin, respectively, 30 min before and simultaneously with leptin.

3.3. Role of cyclooxygenase in leptin effects

Cyclooxygenase was inhibited by incubating cells with indomethacin (10 μM), 30 min before and simultaneously with leptin. Indomethacin treatment did not modify basal or leptin (10 nM)-induced CRH secretion (Table 1). Leptin

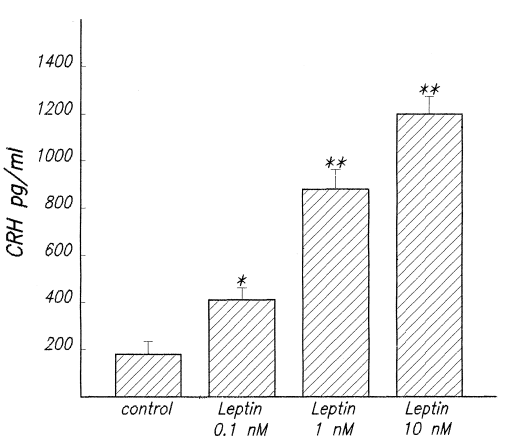

Fig. 1. Effect of leptin on hypothalamic CRH release. Neonatal rat hypothalamic cells (2 million cells/well) were incubated for 4 h with graded concentrations of leptin in 1 ml of DMEM; group means ± S.E.M.; $n = 4–5$; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

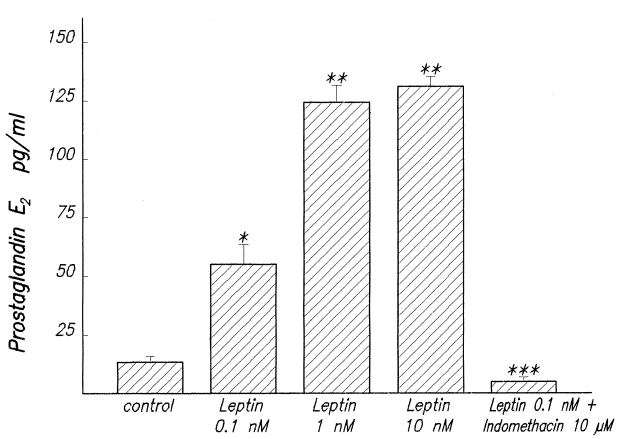

Fig. 2. Effect of leptin (0.1–10 nM) and leptin + indomethacin (10 μM) on hypothalamic prostaglandin E_2 release; neonatal rat hypothalamic cells (1 million cells/well) were incubated for 4 h with graded concentrations of leptin in 1 ml of DMEM; group means ± S.E.M.; $n = 4–5$; $^{*}P < 0.05$, $^{**}P < 0.001$ vs. control, $^{***}P < 0.05$ vs. control and leptin alone.

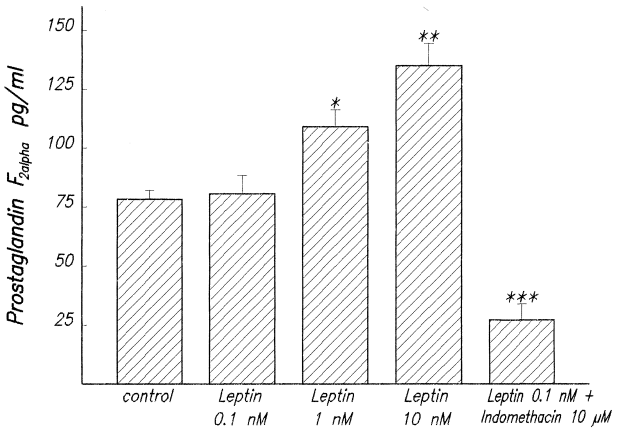

Fig. 3. Effect of leptin (0.1–10 nM) and leptin + indomethacin (10 μM) on hypothalamic prostaglandin $F_{2\alpha}$ release; neonatal rat hypothalamic cells (1 million cells/well) were incubated for 4 h with graded concentrations of leptin in 1 ml of DMEM; group means ± S.E.M.; $n = 4–5$; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control, $^{***}P < 0.05$ vs. control and leptin alone.

(0.1–10 nM) was able to stimulate prostaglandin E_2 and prostaglandin $F_{2\alpha}$ secretion, and this effect was blocked by indomethacin (Figs. 2 and 3).

4. Discussion

Leptin is believed to play its role by signaling adipose store repletion to the hypothalamic centers controlling energy expenditures and caloric intake. It has been suggested that this activity could be mediated by a reduction of excitatory and an increase in inhibitory transmitters in the feeding circuitry of the hypothalamus: leptin administration decreases food intake and body weight, in association with a reduction in neuropeptide Y, galanin and melanin-concentrating hormone gene expression in the hypothalamus (Sahu, 1998). Leptin also stimulates mRNAs for proopiomelanocortin (POMC, the precursor of melanocortins, which display anorexic activities in the hypothalamus) in the arcuate nucleus (Schwartz et al., 1997; Thornton et al., 1997). Our findings which show leptin stimulation of CRH release from neonatal (5 to 6 days old) rat hypothalamic cells in primary culture (Fig. 1), confirm previous reports on works with superfused (Raber et al., 1997), or statically incubated (Costa et al., 1997) adult rat hypothalamus in vitro, as well as leptin stimulation of mRNA for CRH in the paraventricular nucleus of the hypothalamus (Schwartz et al., 1996). CRH plays a dual role in the hypothalamus: as a neurohormone, after release into the pituitary portal vessels, it stimulates an increase in pituitary–adrenal axis secretion; as a neurotransmitter, in the feeding center of the hypothalamus, it effects a reduction in food intake (Krahn et al., 1988) and an increase in energy expenditures, via stimulated sympathetic outflow (Rothwell, 1990).

NO is a highly reactive gaseous free radical, produced by NO-synthase enzymes from the substrate, L-arginine, generating stoichiometric amounts of NO and L-citrulline (Moncada et al., 1991). Different NO-synthase isoenzymes have been characterized: *constitutive* NO-synthases, Ca^{2+}-dependent, typical of endothelial and neuronal cells, and *inducible* NO-synthases, characteristic of immune and glial cells and activated by interleukins. NO displays a wide range of activities, including neuroendocrine regulation (Brann et al., 1997). We have previously found that interleukin-1β-induced CRH secretion is blocked by NO-synthase inhibitors (Brunetti et al., 1993), and interleukin-1β is able to stimulate NO production in the hypothalamus (Brunetti et al., 1996). In the present work, we have explored the hypothesis that, in analogy with the interleukin effect, leptin-induced CRH secretion could be mediated by NO. The findings that NO-synthase inhibition by L-NAME did not block leptin effects on CRH (Table 1) and that leptin did not stimulate NO production (evaluated by measuring L-citrulline production) rule out any involvement of NO in the hypothalamic effects of leptin. In vitro, incubation of adult rat hypothalamic median eminence/arcuate nucleus fragments, gonadotropin releasing hormone was found to be stimulated by leptin via a NO-dependent pathway, as demonstrated by L-NAME blockade of this effect (Yu et al., 1997). The conflicting results concerning a role for NO in leptin activity in the hypothalamus could be explained by a different involvement of the NO pathway in leptin signaling in the neonatal and in the adult rat hypothalamus. In addition, tissue fragments include endothelial NO-synthases, lacking in our cultured cells, which could affect hormone release. Alternatively, extremely small amounts of NO produced in median eminence/arcuate nucleus neurons or glia do not produce sufficient citrulline release into the incubation medium to be detectable with the HPLC assay.

Besides its neuroendocrine effects, NO has been reported to play a facilitatory role in feeding behavior: NO-synthase inhibitors, administered either peripherally or intracerebroventricularly, reduce food intake in mice (Morley and Flood, 1991), in normal and hyperphagic obese Zucker rats (Squadrito et al., 1993), as well as in chickens (Choi et al., 1994). NO-synthase inhibitors also blocked feeding induced by NPY (Morley and Flood, 1992). Moreover, impaired brain production of NO (inhibiting NO-synthase by L-NAME) increased oxygen consumption by stimulating sympathetic nerve outflow (De Luca et al., 1995). Therefore, our results showing that leptin stimulates CRH release without any involvement of NO are consistent with a role of NO in the stimulation of feeding behavior and adipose store repletion, which is opposed to CRH, the former favoring and the latter inhibiting energy accumulation.

We have further investigated the homologies between leptin and interleukins, evaluating the possible involvement of prostaglandins in the hypothalamic effects of

leptin. Prostaglandins have been shown to play a key role in interleukin-1β- and interleukin-6-induced CRH secretion, as this activity is blocked by cyclooxygenase inhibitors (Bernardini et al., 1990; Navarra et al., 1991), and these interleukins specifically stimulate prostaglandin E_2 production in the hypothalamus (Navarra et al., 1992). Exogenous prostaglandin $F_{2\alpha}$, but not prostaglandin E_2 also proved to stimulate CRH release (Bernardini et al., 1989).

Our findings that cyclooxygenase inhibition by indomethacin did not modify leptin-induced CRH release (Table 1) rule out a modulatory role for prostaglandins in leptin-induced CRH release, while leptin-stimulated prostaglandin E_2 and $F_{2\alpha}$ production (Figs. 2 and 3) points to a possible role of these prostaglandins as mediators of leptin effects in the hypothalamus. Actually, prostaglandins, which are cyclooxygenase-metabolised fatty acids, had been hypothesized to be the link between adipose tissue and the hypothalamus: prostaglandin E_2 and prostaglandin $F_{2\alpha}$ inhibited food intake in rats when injected peripherally (Scaramuzzi et al., 1971), while prostaglandin E_1 but not prostaglandin E_2 elicited feeding after intrahypothalamic administration in sheep (Martin and Baile, 1973). More recently, the anorexia induced by interleukins, as well as other CNS effects of these cytokines, such as fever and drowsiness, have been proposed to be mediated by prostaglandin-dependent mechanisms (Jhonson et al., 1993; Plata-Salaman, 1996). Increased production of prostaglandin E_2 and prostaglandin $F_{2\alpha}$, independently from the modulation of CRH release, could be involved in the signaling events that follow leptin binding to its receptor. Indeed the homologies between leptin and some interleukins are shared by their respective receptors. Leptin and interleukin-6-type cytokine receptors have been shown to function through the Janus kinase (JAK)/signal transducer and activator of the transcription (STAT) signaling pathway, involving gene regulatory effects (Baumann et al., 1996). On the other hand, leptin has also been demonstrated to rapidly modulate hypothalamic cell transmission, modifying transmembrane calcium currents in the arcuate nucleus cells (Glaum et al., 1996) or activating ATP-sensitive potassium channels (Spanswick et al., 1997). We hypothesize that the increased intracellular calcium induced by leptin in hypothalamic neurons could trigger membrane phospholipase A_2 activation, leading to the release of free fatty acids, which enter the cyclooxygenase pathway producing prostaglandin E_2 and $F_{2\alpha}$: in turn, these prostaglandins could play the role of mediators of anorectic signaling in the CNS.

In conclusion, our findings confirmed that leptin is able to stimulate hypothalamic CRH secretion, an effect which is not modulated by NO-synthase- or cyclooxygenase-mediated mechanisms; NO production is actually not stimulated by leptin, while increased prostaglandin E_2 and prostaglandin $F_{2\alpha}$ could be added to the growing list of mediators of leptin signaling in the hypothalamus.

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References

Banks, W.A., Kastin, A.J., Huang, W., Jaspan, J.B., Maness, L.M., 1996. Leptin enters the brain by a saturable system independent of insulin. Peptides 17, 305–311.

Baumann, H., Morella, K.K., White, D.W., Dembski, M., Bailon, P.S., Kim, H., Lai, C.F., Tartaglia, L.A., 1996. The full length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. Proc. Natl. Acad. Sci. USA 93, 8374–8378.

Bernardini, R., Chiarenza, A., Calogero, A.E., Gold, P.W., Chrousos, G.P., 1989. Arachidonic acid metabolites modulate rat hypothalamic corticotrophin-releasing hormone secretion in vitro. Neuroendocrinology 50, 708–715.

Bernardini, R., Calogero, A.E., Mauceri, G., Chrousos, G.P., 1990. Rat hypothalamic corticotropin releasing hormone secretion in vitro is stimulated by interleukin-1 in an eicosanoid-dependent manner. Life Sci. 47, 1601–1607.

Brann, D.W., Bhat, G.K., Lamar, C.A., Mahesh, V.B., 1997. Gaseous transmitters and neuroendocrine regulation. Neuroendocrinology 65, 385–395.

Brunetti, L., Preziosi, P., Ragazzoni, E., Vacca, M., 1993. Involvement of nitric oxide in basal and interleukin-1-beta-induced CRH and ACTH release in vitro. Life Sci. 53, PL219–PL222.

Brunetti, L., Preziosi, P., Ragazzoni, E., Vacca, M., 1994. Effects of lipopolysaccharide on hypothalamic–pituitary–adrenal axis in vitro. Life Sci. 54, PL165–PL171.

Brunetti, L., Volpe, A.R., Ragazzoni, E., Preziosi, P., Vacca, M., 1996. Interleukin 1-beta specifically stimulates nitric oxide production in the hypothalamus. Life Sci. 58, PL373–PL377.

Caro, J.F., Kolaczynski, J.W., Nyce, M.R., Ohannesian, J.P., Opentanova, I., Goldman, W.H., Lynn, R.B., Zhang, P.L., Sinha, M.K., Considine, R.V., 1996. Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. Lancet 348, 159–161.

Choi, Y.H., Furuse, M., Okumura, J., Denbow, D.M., 1994. Nitric oxide controls feeding behavior in the chicken. Brain Res. 654, 163–166.

Ciabattoni, G., Pugliese, F., Spaldi, M., Cinotti, G.A., Patrono, C., 1979. Radioimmunoassay measurement of prostaglandin E_2 and $F_{2\alpha}$ in human urine. J. Endocrinol. Invest. 2, 173–181.

Considine, R.V., Sinha, M.K., Heiman, M.L., Kriauciunas, A., Stephens, T.W., Nyce, M.R., Ohannesian, J.P., Marco, C.C., McKee, L.J., Bauer, T.L., Caro, J.F., 1996. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334, 292–295.

Costa, A., Poma, A., Martignoni, E., Nappi, G., Ur, E., Grossman, A., 1997. Stimulation of corticotrophin-releasing hormone release by the obese (*ob*) gene product, leptin, from hypothalamic explants. NeuroReport 8, 1131–1134.

Couce, M.E., Burguera, B., Parisi, J.E., Jensen, M.D., Lloyd, R.V., 1997. Localization of leptin receptor in the human brain. Neuroendocrinology 66, 145–150.

De Luca, B., Monda, M., Sullo, A., 1995. Changes in eating behavior and thermogenic activity following inhibition of nitric oxide formation. Am. J. Physiol. 268, R1533–R1538.

Frederich, R.C., Lollmann, B., Hamann, A., Napolitano-Rosen, A., Kahn, B.B., Lowell, B.B., Flier, J.S., 1995. Expression of ob mRNA and its

encoded protein in rodents. Impact of nutrition and obesity. J. Clin. Invest. 96, 1658–1663.

Glaum, S.R., Hara, M., Bindokas, V.P., Lee, C.C., Polonsky, K.S., Bell, G.I., Miller, R.J., 1996. Leptin, the *obese* gene product, rapidly modulates synaptic transmission in the hypothalamus. Mol. Pharmacol. 50, 230–235.

Håkansson, M.L., Brown, H., Ghilardi, N., Skoda, R.C., Meister, B., 1998. Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. J. Neurosci. 18, 559–572.

Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K., Friedman, J.M., 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269, 543–546.

Jhonson, R.W., Curtis, S.E., Dantzer, R., Kelley, K.W., 1993. Central and peripheral prostaglandins are involved in sickness behavior in birds. Physiol. Behav. 53, 127–131.

Krahn, D.D., Gosnell, B.A., Levine, A.S., Morley, J.E., 1988. Behavioral effects of corticotropin-releasing factor: localization and characterization of central effects. Brain Res. 443, 63–69.

Martin, F.H., Baile, C.A., 1973. Feeding elicited in sheep by intrahypothalamic injections of PGE_1. Experientia 29, 306–307.

Moncada, S., Palmer, R.M.J., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. 43, 109–142.

Morley, J.E., Flood, J.F., 1991. Evidence that nitric oxide modulates food intake in mice. Life Sci. 49, 707–711.

Morley, J.E., Flood, J.F., 1992. Competitive antagonism of nitric oxide synthetase causes weight loss in mice. Life Sci. 51, 1285–1289.

Navarra, P., Tsagarakis, S., Faria, M.S., Rees, L.H., Besser, G.M., Grossman, A., 1991. Interleukins-1beta and -6 stimulate the release of corticotropin releasing hormone-41 from rat hypothalamus in vitro via the eicosanoid cyclooxygenase pathway. Endocrinology 128, 37–44.

Navarra, P., Pozzoli, G., Brunetti, L., Ragazzoni, E., Besser, M., Grossman, A., 1992. Interleukin-1beta and interleukin-6 specifically increase the release of prostaglandin E_2 from rat hypothalamic explants in vitro. Neuroendocrinology 56, 61–68.

Pelleymounter, M.A., Cullen, M.J., Baker, M.B., Hecht, R., Winters, D., Boone, T., Collins, F., 1995. Effects of the obese gene product on body weight regulation in *ob / ob* mice. Science 269, 540–543.

Plata-Salaman, C.R., 1996. Anorexia during acute and chronic disease. Nutrition 12, 69–78.

Raber, J., Chen, S., Mucke, L., Feng, L., 1997. Corticotropin-releasing factor and adrenocorticotrophic hormone as potential central mediators of ob effects. J. Biol. Chem. 272, 15057–15060.

Rothwell, N.J., 1990. Central effects of CRF on metabolism and energy balance. Neurosci. Biobehav. Rev. 14, 263–271.

Sahu, A., 1998. Evidence suggesting that galanin (GAL), melanin-concentrating hormone (MCH), neurotensin (NT), proopiomelanocortin (POMC) and neuropeptide Y (NPY) are targets of leptin signaling in the hypothalamus. Endocrinology 139, 795–798.

Scaramuzzi, O.E., Baile, C.A., Mayer, J., 1971. Prostaglandins and food intake of rats. Experientia 27, 256–257.

Schwartz, M.W., Seeley, R.J., Campfield, L.A., Burn, P., Baskin, D.G., 1996. Identification of targets of leptin action in the hypothalamus. J. Clin. Invest. 98, 1101–1106.

Schwartz, M.W., Seeley, R.J., Woods, S.C., Weigle, D.S., Campfield, L.A., Burn, P., Baskin, D.G., 1997. Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes 46, 2119–2123.

Spanswick, D., Smith, M.A., Groppi, V.E., Logan, S.D., Ashford, M.L.J., 1997. Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. Nature 390, 521–525.

Squadrito, F., Calapai, G., Cucinotta, D., Altavilla, D., Zingarelli, B., Ioculano, M., Urna, G., Sardella, A., Campo, G.M., Caputi, A.P., 1993. Anorectic activity of N^G-nitro-L-arginine, an inhibitor of brain nitric oxide synthase, in obese Zucker rats. Eur. J. Pharmacol. 230, 125–128.

Tartaglia, L.A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G.J., Campfield, L.A., Clark, F.T., Deeds, J., Muir, C., Sanker, S., Moriarty, A., Moore, K.J., Smutko, J.S., Mays, G.G., Woolf, E.A., Monroe, C.A., Tepper, R.I., 1995. Identification and expression cloning of a leptin receptor. OB-R. Cell 83, 1263–1271.

Thornton, J.E., Cheung, C.C., Clifton, D.K., Steiner, R.A., 1997. Regulation of hypothalamic proopiomelanocortin mRNA by leptin in *ob / ob* mice. Endocrinology 138, 5063–5066.

Yu, W.H., Walczewska, A., Karanth, S., McCann, S.M., 1997. Nitric oxide mediates leptin induced luteinizing hormone-releasing hormone (LHRH) and LHRH and leptin-induced LH release from the pituitary gland. Endocrinology 138, 5055–5058.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., Friedman, J.M., 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425–432.