Interplay Between Galanin and Leptin in the Hypothalamic Control of Feeding via Corticotropin-Releasing Hormone and Neuropeptide Y

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Over long periods, feeding and metabolism are tightly regulated at the central level. The total amount of nutrients ingested is thought to result from a delicate balance between orexigenic and anorexigenic factors expressed and secreted by specialized hypothalamic neuronal populations. We have developed a system of perifused hypothalamic neurons to characterize the relationships existing between the orexigenic peptide galanin and two other physiological modulators of feeding: neuropeptide Y (NPY) and corticotropin-releasing hormone (CRH). We demonstrated that galanin stimulates CRH and NPY secretion from hypothalamic neurons in a dose-dependent manner. Exposure to leptin for 24 h before galanin stimulation decreased NPY secretion by 30%, leaving the responsiveness of CRH neurons intact. These results suggest that CRH and NPY neurons participate to the intrahypothalamic signaling pathway of galanin, an observation that can explain the lower potency of galanin to stimulate food intake in vivo compared with NPY. The differential effects exerted by leptin on CRH and NPY suggest that there exists a subset of NPY neurons that are exquisitely sensitive to marked variations in leptin levels, and that the CRH neurons are less responsive to increases in leptin concentrations. Diabetes 50:2666–2672, 2001

Recent evidence suggests that the central nervous system plays a key role in controlling food intake and metabolic adaptations (1). A delicate interplay seems to exist between the activities of highly specialized neuronal subpopulations involved in the regulation of feeding and energy storage, or of satiety and energy expenditure. Overall, these regulations, operating at the level of the central nervous system, achieve the tight control of energy balance observed in mammals (2). The neurons involved are mainly located within the hypothalamus, where they serve as central integrators of peripheral metabolic signals. One important function of leptin (3) seems to be informing these neurons about levels of energy stored in the body (4). Changes in circulating leptin levels can therefore trigger an appropriate response of the central nervous system to modulate food intake and metabolism according to the total amount of body fat (5) as well as the variations of energy balance (6).

However, the relations between the different hypothalamic neuronal subpopulations seem to be extremely complex (1). The systems involved are redundant, and thus they are most difficult to individualize in vivo. Neuropeptide Y (NPY) and galanin can both elicit strong feeding behavior when injected centrally (7–10) and are expressed in hypothalamic areas associated with feeding and metabolic regulations (10–12). NPY is one of the most abundant peptides of the hypothalamus (11) and remains among the most potent orexigenic factors (13). In addition, it has also been implicated in the control of neuroendocrine adaptations to fasting or unfavorable metabolic conditions (14,15). Like NPY, galanin plays a physiologically important role in the regulation of neuroendocrine functions affected by metabolic changes, such as reproduction and growth (16).

The hypothalamic corticotrophin-releasing hormone (CRH) system was initially recognized for its central role as the primary regulator of the stress response (17). In addition, CRH neurons located in the paraventricular nucleus of the hypothalamus are involved in the regulation of energy balance (18), independent of the stress-regulated production of adrenal glucocorticoids. Like NPY and galanin, CRH and its closely related peptide urocortin (19) can alter feeding behavior when administered centrally. However, CRH and urocortin are potent inhibitors of food intake, while also stimulating metabolism and energy expenditure (20,21). In addition to sharing a role in the control of feeding, NPY, galanin, and CRH are closely related anatomically. Galanin is present in the hypothalamic paraventricular nucleus, and some galanin-positive neurons also express CRH (10,22). Furthermore, these cells receive a dense innervation from NPY-ergic neurons located in the hypothalamic arcuate nucleus (23). Therefore, it can be hypothesized that these neurotransmitters...
functionally are related in their modulation of feeding and metabolism.

We used cultures of fetal rat hypothalamic neurons in dynamic perfusion experiments to demonstrate that galanin can directly stimulate hypothalamic CRH and NPY secretion. Leptin was found to downregulate this galanin-stimulated NPY secretion, whereas CRH release was not affected. Our results provide a novel mechanism of action for galanin, implicating CRH as well as NPY neurons downstream of its feeding effects. Furthermore, the different effect of leptin on NPY and CRH secretion confirms in vivo observations suggesting that NPY neurons represent an important target of leptin’s effects (24–26), but that CRH neurons do not respond to elevations of leptin levels above normal physiological values (27). Overall, these results demonstrate that the use of primary neurons in culture can be a useful adjunct to in vivo studies of hypothalamic function.

**RESEARCH DESIGN AND METHODS**

**Isolation of embryonic hypothalamic neurons.** Hypothalamicos were obtained from E15 or E19 rat fetuses as follows: whole brains were rapidly removed from the skull, and the hypothalamus was extracted with ophthalmic scissors as previously described (28). Two coronal cuts (anteriorly at the level of the optic chiasma and posteriorly at the level of the mammillary bodies) were followed by two parasagittal cuts along the hypothalamic sulci and a final dorsal cut at depths of ~2 mm from the ventral surface of the tissue block. Enzymatic digestion of the tissue leads to poor survival of the neurons in vitro. Therefore, dispersion was performed mechanically in phosphate-buffered saline (PBS) buffer (without calcium or magnesium; Gibco) supplemented with 0.06% glucose (Fluka) and 100 units/100 μl penicillin streptomycin (Seromed). Freshly excised whole hypothalamicos were gently passed several times through Pasteur pipettes flamed in the middle of the procedure to decrease the diameter of their opening. Nondispersed tissue was allowed to settle for 5 min, and supernatant was transferred to a clean tube. The remaining pellet was resuspended in 4 ml PBS buffer, and mechanical dispersion was repeated. Supernatants from the first and second dispersion were mixed, centrifuged for 5 min at 100, and the cellular pellet was gently resuspended in 5 ml neurobasal medium (Gibco) with 0.04% B27 supplement (Gibco) containing 500 μmol/l glutamine and 25 μmol/l glutamate (Sigma). This whole process, which was always performed by the same person, is extremely delicate because cellular yield and cell death both increase with the strength of pipetting. Viability of hypothalamic neurons is low compared with other neuronal cell types (30–40% viable cells immediately after the dispersion and before plating, as assayed by Trypan blue exclusion), with a yield of ~2 × 10^5 live cells/hypothalamus.

Cultures were then prepared as previously described (29,30): cells were seeded at a density of 500 live cells/mm² in 60-mm diameter Petri dishes (Falcon) or in 6- or 12-well plates (Costar) containing round coverslips (Assisted) coated with 5 μg/ml poly-d-lysine (Sigma). After 48 h, 2 μmol/l cytosine β-D-arabinofuranoside (araC; Sigma) was added to prevent proliferation of nonneuronal cells. At 48 h after araC addition, half of the medium was replaced by neurobasal medium with 0.04% B27 supplement containing 500 μmol/l glutamine but without glutamate to avoid toxicity. Cells were maintained in culture for up to 6 weeks by renewing half of the medium every 4th day.

**Immunocytochemistry.** Immunocytochemistry was performed at room temperature using an avidin biotinylated horseradish peroxidase macromolecular complex kit (ABC Elite; Vector, Burlingame, CA). Cells were fixed for 40 min in 4% paraformaldehyde (Merck) and rinsed three times in PBS and one time in TNT (0.05 mol/l Tris [Sigma], pH 7.4, 0.6% NaCl, and 0.3% Triton X-100 [Merck] with 10% goat serum [Gibco]). After the quenching of endogenous peroxidase activity, nonspecific binding was blocked by a 1-h incubation in TNT. Incubations with the primary antibodies were performed overnight, and then cells were washed and further incubated for 1 h with anti-mouse or anti-rabbit biotinylated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA). After three washes in 1 mol/l Tris-HCl, pH 7.7, cells were incubated in the ABC reagent, washed again, and finally incubated in DAB peroxidase substrate solution (50 mg/100 ml 3’3’-diaminobenzidine [Sigma] in 0.1 mol/l Tris-HCl and 0.018% H₂O₂, pH 7.7). Coverslips were counterstained with hematoxylin (Vector). Glial cells were identified with a human monoclonal anti-glial fibrillary acid protein (GFAP) antibody (Dako) at a final concentration of 2.5 μg/ml. Neurons were identified using a rat monoclonal antibody raised against the microtubule-associated protein mitogen-activated protein 2 (MAP2; a gift of Dr. Beat Riederer) (31) at a final dilution of 1:75. CRH-positive neurons were identified using a polyclonal antibody (a-hCRH; a gift of Dr. Robert Benoit) at a final dilution of 1:5000, whereas NPY-positive neurons were identified using a monoclonal antibody at a final dilution of 1:1,000 (32). Negative controls were obtained by incubating the primary antisera with the corresponding horseradish peroxidase before staining.

**Northern blot.** Total RNA was prepared with the TriPure isolation reagent containing 1 μl of 20 μg/ml glycogen (Boehringer Mannheim). Northern blot analysis of 10 μg total RNA was performed as previously described (33). Relative changes in mRNA levels were determined by hybridization to specific (α²³S)²DTT-labeled cDNA probes. Exposure time of all membranes to X-ray films (Amersham) were chosen to optimize the signals in a linear range. Densitometric analysis of mRNA signals on autoradiograms was performed with a Molecular Dynamics scanner (Sunnyvale, CA). To correct for variations in the amount of RNA loaded, all membranes were rehybridized with a probe for the ubiquitously expressed gene glyceraldehyde phosphate dehydrogenase (GAPDH). The ratio of the specific and the corresponding GAPDH signal was determined.

**DNA probes.** The probes used were: the 770-bp BamHI fragment of the rat CRH cDNA (kindly supplied by Dr. L. Bain, University of Michigan) (34); a PstI fragment of brain cDNA of Dr. J. A. Haefelfiger (Lausanne, Switzer- 31000ngame) in a Perkin Elmer Gene Amp PCR System 9600 thermocycler. The intracellular region of the long isoform of the leptin receptor OB-Rb was amplified as previously described (36) using the following oligomers: 5′-TATTCTATT TTAAGGGGTCACC-3′ (sense) and 5′-ACCCCACAATACATATTTACACGAC-3′ (antisense). The amplification of the 88–497 fragment of orexin cDNA was performed at an annealing temperature of 50°C, using 5′-GGAATTCCTCG ACCATGACCTTCCTCTCACAAGG-3′ (Stull) as sense and 5′-GAAT TCACACAGTGTACAGGGCTG-3′ (HindIII) as antisense primers. The amplification of the 36–538 fragment of MCH cDNA was performed at an annealing temperature of 55°C using 5′-GGAATTCGCAG AGGTAGGGCGAAGATGAGCCTC-3′ (Stull) as sense and 5′-GAAT CACGTGAGATAGGACGCCC-3′ (HindIII) as antisense primers. Both hybridization and PCR procedures were performed according to the manufacturer’s instructions.

**Perfusion of hypothalamic neurons and experimental design.** Two Petri dishes were placed, one face up and one face down, in a chamber maintained at 37°C and perfused with Krebs-Ringer buffer (15 mmol/l HEPES, 111 mmol/l NaCl, 4.7 mmol/l KCl, 2.5 mmol/l CaCl₂, 1.2 MgSO₄, 24.8 mmol/l NaHCO₃, 1.2 mmol/l KHPO₄, and 11.1 mmol/l t-Glucose [Merck] containing 0.5% human serum albumin [ZLB Zentrrallaboratorium]), which was kept at pH 7.4 by constant gassing with 95% O₂ and 5% CO₂. The flow rate was set at 100 μl/min, and fractions were collected every 5 min and assayed for CRH and NPY. CRH was quantified by immunoradiometric assay (IRMA) (37) and NPY was quantified by enzyme-linked immunosorbent assay (32). Effluent was collected in Nunc-Gibco tubes containing 1 μl of 10% TWEEN-20 (Pierce) and was stored at −20°C until peptide measurements.

**Effects of galanin on CRH and NPY secretion.** To evaluate the effects of galanin on peptide secretion, neurons were stimulated for 30 min with graded concentrations of galanin (2 ×, 4 ×, and 8 × 10⁻⁵ mol/l) applied in random order. Galanin stimulations were followed by 30 min of recovery. In further experiments aimed at studying the potential for leptin to modulate the responsivity of CRH and NPY neurons to galanin, cells were stimulated with 4 × 10⁻⁵ mol/l galanin.

**Effects of leptin on CRH and NPY secretion.** Two different experimental paradigms were applied to test both the acute and chronic effects of leptin on basal as well as galanin-stimulated peptide secretion. Acute effects of leptin were investigated using the stimulation of perfused neurons with galanin alone, followed by a similar galanin stimulation performed 30 min after the start of a 10⁻⁷ mol/l leptin perfusion. The chronic effect of leptin on galanin-stimulated secretion was tested on our model of adrenal cell cultures (36,38). In the second paradigm, neurons were preincubated for 24 h in leptin-containing medium and then stimulated with galanin in the presence of leptin. The two paradigms were always applied in parallel columns. This allowed us to compare the...
effects of galanin alone with those of galanin applied after leptin preincuba-
tions (between-column comparisons).

**NPY immunoneutralization.** Because of the stimulation of NPY secretion
exerted by galanin, the potential role of endogenously produced NPY in the
galanin-stimulated secretion of CRH was also evaluated. In another series of
experiments, neurons were pretreated for 1 h with a neutralizing anti-NPY
antibody (39) used at a blocking concentration of 10^{-8} mol/l. This was
followed by galanin stimulation (4 \times 10^{-8} mol/l) for an additional hour in the
presence of the same concentration of neutralizing antibody, and CRH was
measured in the effluent.

**Data analysis.** Cells positive for MAP2, GFAP, CRH, and NPY were directly
counted under a light microscope. The length of neurites was also directly
estimated under the light microscope. For dynamic experiments, a minimum
of three independent perifusions were run on different days (\( n = 4 \) for all
leptin experiments). Integrated secretion was estimated by calculating the
areas under the secretory curve (AUCs) after galanin stimulation, using the
trapezoidal method. All results were expressed as the means ± SE, and
comparisons were performed using Student’s \( t \) test or nonparametric tests
(Van der Waerden test), when appropriate.

**RESULTS**

**Characterization of primary hypothalamic neurons in culture.** Within 48 h of plating, neuronal cells were
recognizable by their growing neurites, often subdividing and reaching lengths of 5- to 10-fold the diameter of the
cell body. After 1 week, cells appeared clearly differenti-
ated: axonal prolongations were intertwining in a complex
net, attaining a length of 20- to 40-fold the diameter of the
cell body. Mechanical dispersion of tissue was limited to
insure acceptable viability of cells. Therefore, there were
remaining cellular clumps in the culture plates. Remark-
ably, some of the clump-forming cells remained alive in
culture, because numerous axonal connections were ob-
served between neurons in the clumps and neurons in the
monolayer.

After 3 weeks, immunocytochemical analysis showed
80–90% MAP2-positive neurons and a remaining 10–20%
GFAP-positive glial cells. Strong, positive MAP2 staining
was retained for up to 6 weeks, but axonal degradation
became evident by that time. Expression of CRH, NPY,
POMC, MCH, orexins, and the long isoform of the leptin
receptor was demonstrated by Northern blot or RT-PCR
analysis. In addition, the expression of CRH, NPY, gona-
drophin-releasing hormone, and arginine vasopressin at
the peptide level was confirmed by immunocytochemistry.

The relative expression of CRH and NPY by these
Secretion (0.6 fmol/ml) induced an approximately eightfold increase in mean CRH secretion in independent experiments and expressed as the mean of the stimulation. AUCs (insert panels) are calculated from three concentrations of galanin, followed by a return to baseline at the end of the stimulation. 

A FIG. 2. Dose-dependent effects of galanin to stimulate CRH (A) and NPY (B) secretion by hypothalamic neurons placed in a dynamic perifusion apparatus. Each graph displays a representative experiment, demonstrating the significant stimulation induced by various concentrations of galanin, followed by a return to baseline at the end of the stimulation. AUCs (insert panels) are calculated from three independent experiments and expressed as the mean ± SE. *P < 0.05; **P < 0.01. Shaded areas represent the duration of galanin stimulation.

neurons is illustrated in Fig. 1. CRH immunoreactivity was present in relatively scattered neurons, principally observed in cell bodies, with a slight staining of the proximal portion of the dendrites that faded distally (Fig. 1A). In contrast, NPY immunoreactivity was found in a larger number of cells and also evidenced primarily in neuronal bodies (Fig. 1C). In addition, a fine network of axons and axon terminals was strongly stained with NPY.

CRH and NPY mRNA expression was quantified weekly between weeks 2 and 5. Very strong NPY expression was present at all time points, but a trend toward a decrease was observed with time. In contrast, CRH mRNA levels remained stable throughout the study period. After these experiments, perifusions were always performed between weeks 3 and 4.

**Effects of galanin.** CRH secretion by unstimulated neurons was around the detection limit of our IRMA method (0.48 fmol/ml). Galanin was found to stimulate CRH secretion in a dose-dependent manner, the minimal effective concentration being 2 × 10⁻⁵ mol/l. This concentration induced an approximately eightfold increase in mean CRH secretion (0.6 ± 0.4 vs. 4.6 ± 0.6 fmol/ml; P < 0.001), followed by a return to baseline levels at the end of the stimulation (Fig. 2A). Galanin also produced a dose-dependent stimulation of NPY secretion (Fig. 2B), with mean hormone levels increasing from 1.1 ± 0.2 fmol/ml at baseline to 4.2 ± 0.6 fmol/ml (P < 0.001) at the lowest galanin dose. The dose-dependency of the effect of galanin on the secretion of both neurotransmitters was demonstrated as well by the comparison of the AUCs for CRH and NPY (Fig. 2, inserts). When the galanin concentration was increased from 2 × 10⁻⁵ to 4 × 10⁻⁵ mol/l, the AUC for CRH increased from 28.8 ± 1.8 to 46.4 ± 4.2 fmol/pulse (P < 0.05). A further doubling of the galanin concentration, from 4 × 10⁻⁵ to 8 × 10⁻⁵ mol/l, resulted in a corresponding increase in the AUC for CRH, from 46.4 ± 4.2 to 111.1 ± 15.4 fmol/pulse (P < 0.01) (Fig. 2A, insert). The increases in the AUCs of NPY following the same increments in galanin concentration were slightly less pronounced (Fig. 2B, insert), the difference being statistically significant only between the 4 × 10⁻⁵ and 8 × 10⁻⁵ mol/l concentrations (28 ± 6.6 vs. 69.2 ± 18.9 fmol/pulse; P < 0.05). Further perifusion experiments were always performed at a galanin concentration of 4 × 10⁻⁵ mol/l. Of note, NPY immunoneutralization before galanin stimulation did not alter the CRH secretory response to galanin (data not shown), strongly suggestive of a direct galanin effect on CRH neurons present in the cultures.

**Effects of leptin.** The acute and chronic effects of leptin on CRH and NPY secretion were then analyzed. Leptin administered 30 min before galanin had no effect on CRH or NPY secretion (data not shown). When administered over longer time periods (24-h preincubations), 10⁻⁷ mol/l leptin did not modify either basal or galanin-stimulated CRH and NPY (Fig. 2, inserts). When the galanin concentration was increased from 2 × 10⁻⁵ to 2 × 10⁻⁴ mol/l concentrations (28 ± 6.6 vs. 69.2 ± 18.9 fmol/pulse; P < 0.05). Further perifusion experiments were always performed at a galanin concentration of 4 × 10⁻⁵ mol/l. Of note, NPY immunoneutralization before galanin stimulation did not alter the CRH secretory response to galanin (data not shown), strongly suggestive of a direct galanin effect on CRH neurons present in the cultures.

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FIG. 3. Effect of 24 h preincubation with leptin (10⁻⁷ mol/l) on galanin-stimulated CRH (A and C) and NPY (B and D) secretion. A and B: Each graph displays a representative experiment, with shaded areas indicating the duration of galanin stimulation. Gray areas in the background represent the mean ± SE of CRH (fmol/ml) or NPY (fmol/ml) secretion stimulated by galanin in the absence of leptin. C and D: Bar graphs represent the areas under the secretory curve for CRH (C) and NPY (D) (Mean ± SE, n = 4 experiments). *P < 0.05.
Galanin is known to increase feeding when injected centrally (40). This role in feeding regulation has been ascribed to the hypothalamic paraventricular nucleus (PVN) (9,41), but little is known about the mechanism(s) involved in this effect. The PVN being an important site of NPY and CRH expression, we hypothesized that the effects of galanin to stimulate food intake in vivo could be mediated via a modulation of the activity of CRH or NPY neurons.

We have used hypothalamic neurons to explore in vitro the interplay between galanin, NPY, and CRH. These cells express many hypothalamic neurotransmitters in a differential way, suggesting that they reflect the physiological relations existing among different neuropeptides in vivo. Grown on coverslips, they develop a dense neuronal network and can be successfully used in dynamic perfusion experiments. Compared with static cultures (42), this perfusion model presents the advantage of minimizing potential autocrine or paracrine effects occurring in vitro, because the effluent is continuously washed out of the chamber. We were also able to measure neurotransmitter secretion from a much lower number of neurons than previously reported using hypothalamic neurons adsorbed onto Biogel beads (43). This difference suggests that preservation of the neuronal network upon transfer into the perfusion chambers may result in increased cellular viability and possibly better preservation of normal cellular functions.

Given the strong inhibition on feeding exerted by CRH (21), our observation of a stimulation of CRH secretion by galanin could be somewhat surprising. However, it is fully consistent with the relative potencies of galanin and NPY to stimulate feeding in vivo: galanin was recently reported to be significantly less effective than NPY on a molar basis (13). Therefore, the present results on CRH and NPY secretion suggest that galanin expressed in the PVN may act as an integrator of feeding behavior, at least partially by balancing the respective endogenous tones of two opposite systems. However, the possibility that galanin may also modulate feeding independent of NPY or CRH neurons in vivo remains open. Finally, the requirement for relatively high doses of galanin could be inherent to this novel perfusion system, but it should be stressed that very little is known about the actual concentrations of neuropeptides present within synaptic connections in vivo.

The influence of short- and long-term treatment with leptin (3) was also analyzed. In our system, long-term leptin treatment interfered downstream of the galanin signaling pathway, at the level of NPY secretion. In contrast, leptin affected neither the basal nor the galanin-mediated induction of CRH secretion. The main physiological role of leptin is probably to function as a peripheral satiety signal to the central nervous system. As such, a considerable amount of data obtained in vivo concurs to demonstrate that hypothalamic NPY neurons represent an important, although not exclusive, target of leptin’s effects (rev. in 1). According to the prevalent hypothesis, increasing levels of circulating leptin induce a progressive decline in hypothalamic NPY expression associated with a resulting decrease in food intake. However, there is only one report demonstrating that leptin can affect hypothalamic NPY at the level of peptide secretion. In experiments using perfused hypothalamic fragments, Stephens et al. (44) showed that leptin decreases the secretion of NPY stimulated by glucocorticoids. Therefore, by providing evidence that leptin can also decrease the secretion of NPY stimulated by galanin, our results represent the second demonstration that leptin can directly modulate hypothalamic NPY secretion. Furthermore, the lack of effect of leptin on stimulated CRH secretion suggests that the anorexigenic effects of leptin observed in vivo are not mediated by a direct action on CRH neurons. Our observation that 24 h of exposure to leptin are required to modulate NPY secretion was somewhat puzzling. The possibility that stimulation with high concentrations of galanin may obliterate the acute effects of leptin could not be excluded, even though the dose of galanin used was of intermediate potency in our system. However, this time course is consistent with electrophysiological data obtained in rat hypothalamic slices (45). In this model, leptin induced a progressive hyperpolarization of hypothalamic neurons that was not reversible after leptin removal over the duration of the experiment (60 min). It could therefore indicate that the leptin-induced hyperpolarized state represents the trigger of a cascade of intracellular events, with a resulting modulation of neurotransmitter secretion occurring at later time points, as reported here. It is noteworthy that such a long time course has also been reported for the leptin modulation of adrenocortical cell function (36,38,46,47). Another similarity between the two models (adrenocortical cells and hypothalamic neurons) lies in the fact that leptin does not exert any measurable effect alone but modulates stimulated secretion. It is therefore tempting to speculate that leptin acts to alter the neuronal responsiveness to physiological stimuli, at least partly by activating ATP-sensitive K+ channels and thus depolarizing its target neurons (45). A better understanding of the leptin-induced modulation of neuronal function will probably involve the careful description of the cellular and molecular mechanisms participating in the transduction of the leptin signal (45,48,49).

Our observation of a differential modulation of NPY and CRH secretion by leptin sheds a novel light on earlier data in the literature. It has been shown that leptin can prevent food intake stimulated by galanin in vivo (50), whereas it does not seem to interfere with CRH expression at high doses (27). Our results suggest that leptin may act downstream of galanin by blocking transmission of the portion of its feeding effects that is mediated via NPY, leaving CRH...
secretion unaffected. Such a mechanism would result in a decrease of galanin-induced feeding, as has been observed in vivo (50). In addition, the present observations are also in accordance with data showing that NPY neurons respond across a broad range of variations in leptin levels (27).

In conclusion, using our model of primary neurons in culture, we could generate data suggesting that hypothalamic CRH and NPY neurons can interact in a very subtle way to mediate the stimulation of food intake induced by galanin. In addition, we could demonstrate that leptin differentially affects the galanin-stimulated secretion of NPY and CRH, decreasing NPY while leaving CRH untouched. These results on neurotransmitter secretion can then be correlated to data obtained in vivo and dealing exclusively with measures of expression, thus providing a more comprehensive knowledge of integrated hypothalamic function. Such detailed knowledge probably represents a prerequisite for the design of novel therapeutic agents aimed at treating obesity and related disorders.

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