Functional Interactions Between Melanin-Concentrating Hormone, Neuropeptide Y, and Anorectic Neuropeptides in the Rat Hypothalamus

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A growing body of evidence indicates that a number of peptides expressed in the mammalian hypothalamus are involved in the regulation of food intake and energy balance. Among these, melanin-concentrating hormone (MCH) and neuropeptide Y (NPY) are potent appetite stimulants, whereas α-melanocyte–stimulating hormone (α-MSH), neurotensin, and glucagon-like peptide (GLP)-1(7-36) amide have appetite-suppressing properties. However, the functional interactions between pathways involving these neuropeptides remain incompletely understood. In the current study, we describe the functional interactions between orexigenic (appetite-stimulating: MCH and NPY) and anorectic (appetite-suppressing: α-MSH, neurotensin, and GLP-1) peptides after intracerebroventricular (ICV) administration in the rat. The ICV administration of GLP-1 completely prevents the orexigenic effects of both MCH and NPY. However, ICV administration of α-MSH prevents only the orexigenic effect of MCH, as we have previously shown, but does not prevent the effect of NPY on food intake. Similarly, ICV administration of neurotensin prevents only the orexigenic effect of MCH, but does not prevent the appetite-stimulating effect of NPY. Thus, our study suggests that the functional interactions between these neuropeptides are specific, although the underlying mechanisms are as yet unexplored. *Diabetes* 47:1687–1692, 1998

Obesity is a common metabolic disorder that affects at least one-third of the adult population in the U.S. (1,2) and constitutes an important risk factor for type 2 diabetes (1), hypertension, coronary artery disease, and certain neoplasms (2). The pathophysiological basis of obesity is poorly understood and is likely to involve complex interactions between neurotransmitter and neuropeptide systems participating in the regulation of food intake and energy expenditure. A number of very different approaches have been used to determine the role of neuropeptides in the regulation of eating behavior. These include evaluation of neuropeptide expression at the mRNA level in different models of obese rodents (3,4), transgenic approaches leading to the generation of knockout animals (5,6), and physiological studies evaluating the response of cannulated rats to the intracerebroventricular (ICV) injection of a peptide (4,7–9). In aggregate, these studies are providing increasing insight on the role of different peptides in regulating eating. Although some single gene lesions may lead to obesity (e.g., *ob/ob* mice, MC4-R knockout mice) (3,10), the unique significance of any particular peptide is unclear. For example, both neuropeptide Y (NPY) (5) and corticotropin-releasing hormone (CRH) (6) knockout mice have a normal feeding phenotype, indicating that neither peptide plays an absolutely critical role in eating. However, knowledge about the potential interactions between different orexigenic and anorectic pathways is limited and remains fragmented.

NPY, possibly the best characterized orexigenic peptide, is expressed in the arcuate nucleus of the hypothalamus (11), projects predominantly to the paraventricular and the dorsomedial hypothalamic nuclei, and is upregulated by fasting (12,13). Moreover, NPY has potent appetite-stimulating properties after ICV or intrahypothalamic administration (7,14) and may have a key role integrating information from the periphery regarding energy store size and flux, possibly mediated through leptin (15) and insulin (16). Repeated ICV injection of NPY leads to obesity in rats (14). However, NPY cannot be the sole peptide involved in appetite stimulation, as NPY knockout mice have a normal feeding phenotype and respond normally to fasting and refeeding (5). Furthermore, while arcuate expression of NPY is significantly upregulated in *ob/ob* mice (3), crossbreeding of *ob/ob* mice with NPY knockout mice attenuates, but does not normalize, the ob phenotype (17).

Melanin-concentrating hormone (MCH) is a cyclic peptide originally isolated from teleost fish pituitaries (18) that is expressed in the mammalian brain, specifically the lateral hypothalamus and the zona incerta (19); MCH neurons project to a variety of areas throughout the brain, including the cortex, as well as the nucleus of the tractus solitarius and the parabrachial nucleus in the hindbrain (19). We have previously demonstrated that MCH expression is upregulated in the hypothalamus of *ob/ob* mice, where it is further increased by fasting (4). Furthermore, we and others have shown that MCH is an orexigenic peptide after ICV administration in the rat (4,20).

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aa, amino acid; CRH, corticotropin-releasing hormone; CSF, cerebrospinal fluid; GLP, glucagon-like peptide; ICV, intracerebroventricular; MCH, melanin-concentrating hormone; α-MSH, α-melanocyte–stimulating hormone; NEI, neuropeptide glutamic acid isoleucine amide; NGE, neuropeptide glycine glutamic acid; NPY, neuropeptide Y.

α-Melanocyte–stimulating hormone (α-MSH) (8), neurotensin (21), and glucagon-like peptide (GLP)-1(7-36) amide (9), which are expressed in distinct hypothalamic nuclei, have all been demonstrated to suppress food intake after ICV administration in rodents. Furthermore, while levels of both NPY and MCH mRNA rise with fasting, the hypothalamic levels of anorectic peptides, such as neurotensin content (22) and pro-opiomelanocortin mRNA (23), the latter being the precursor of α-MSH, decline with fasting.

The interactions between the pathways involving these appetite-stimulating and appetite-suppressing peptides remain poorly characterized. We have recently demonstrated that α-MSH opposes the orexigenic effect of MCH after ICV administration in the rat, although responses to these peptides appear to be mediated by distinct receptor pathways (24). GLP-1 has been reported to suppress NPY-induced feeding, an action that is blocked by the coadministration of the GLP-1 antagonist exendin (9). Furthermore, MTII, i.e., Ac-Nle^4-[Asp^5, D-Phe^7, Lys^{10}]α-MSH-(4-10)-NH$_2$, an MSH superagonist, may suppress NPY-induced eating (25). However, the interactions of the appetite-stimulating peptides with the anorectic peptides, using defined feeding paradigms, have not been systematically examined. In the current study, we sought to perform an analysis of potential interactions by studying the functional relationships between orexigenic peptides, specifically MCH or NPY, and anorectic peptides, including α-MSH, neurotensin, and GLP-1.

RESEARCH DESIGN AND METHODS

Animals. Adult male Sprague-Dawley rats weighing 250–300 g were obtained from Taconic (Germantown, NY), bearing a 23-gauge stainless steel cannula aseptically placed into the left lateral ventricle. The animals were housed singly at 20°C, fed standard rat food (Purina rodent chow; St. Louis, MO) and tap water ad libitum, and maintained on a 12-h reverse light cycle with the dark cycle beginning at 9:00 AM. All animals were handled daily for 1 week before initiating studies on food intake in order to minimize handling stress effects on subsequent food intake. Cannula placement was confirmed in all animals by evaluating the response to ICV angiotensin II (50 ng). Only animals drinking more than 5 ml of water in 1 h were used in the feeding studies. Furthermore, cannula placement was verified by histology at the conclusion of the study. Animals were observed daily for signs of illness, such as weight loss, decreased activity, or poor grooming. Such animals were euthanized. The study design was approved by the animal review committee of the Joslin Diabetes Center.

Peptides. The rat/human MCH (19 amino acid [aa]), α-MSH(1-13) amide (13 aa), rat/human NPY (36 aa), neurotensin (13 aa), human GLP-1(7-36) amide (30 aa), and angiotensin II (8 aa) were obtained from Bachem Neurosciences (King of Prussia, PA), dissolved in sterile artificial cerebrospinal fluid (CSF) (NaCl 147 mmol/l, $CaCl_2$ 1.3 mmol/l, $MgCl_2$ 0.9 mmol/l, KCl 4.0 mmol/l), and kept frozen in small aliquots until use. Neuropeptide glycine glutamic acid (NGE) and neuropeptide glutamic acid isoleucine amide (NEI) were synthesized in the protein core facility (Joslin Diabetes Center, Boston, MA) using an automated peptide synthesizer, were similarly dissolved in sterile artificial CSF, and were kept frozen until use.

Feeding studies. Studies involving the use of MCH, NPY, or GLP-1 were performed by using a modification of the method of Stanley and Leibowitz (7,24), according to which a highly palatable food (consisting of 27% sugar, 27% condensed milk, and 36% ground rat food) was provided to all study animals the evening before the study. Food bowls were replaced with a fresh aliquot of the same food 1 h before the ICV injection. This approach induces a reproducible hyperphagia followed by a consistent period of slower sustained food intake. Studies involving the use of either neurotensin or α-MSH alone were performed in animals whose food had been withdrawn the evening before the study, the administration of the same palatable food coinciding with the administration of the peptide animals were exposed to. This led to increased eating by control animals only and provided a wider range in which the anorectic effects of the neuropeptides could be demonstrated. Experimental animals were injected with the respective neuropeptide solution in a volume of 5 µl over 1 min at the beginning of the dark period, returned to their cages, and subsequently left undisturbed. Control animals were included in each experiment and were injected with an equal volume of artificial CSF and were otherwise handled in an identical manner to the experimental animals. Each experimental or control group consisted of five animals, unless mentioned otherwise. Food intake was measured at 2, 4, and 6 h after injection of either neuropeptide or artificial CSF. Injection of neuropeptides was repeated no more frequently than every 72 h, and animals were assigned to groups randomly in each experiment.

Statistics. Data were analyzed by repeated-measures analysis of variance followed by post hoc analysis of significance by the Bonferroni-Dunn statistic, using the Statview 4.5 software package (Abacus Concepts, Berkeley, CA). Probability (P) values <0.05 were considered statistically significant.

RESULTS

In accordance with our previous data, ICV administration of 5 µg of MCH led to an approximately twofold increase in feeding at all time points (Fig. 1*A*). In contrast, ICV administration of 5 µg of either NGE or NEI, which are encoded in the MCH prepro-mRNA (26) and coexpressed with MCH (27), did not affect food intake (data not shown). We also confirmed the known orexigenic effects of NPY (Fig. 1*B*) as well as the anorectic effects of GLP-1 (Fig. 1*C*). ICV administration of 10 µg of NPY led to an approximately twofold increase in food intake at all time points, and ICV administration of 10 µg of GLP-1 led to an approximately threefold decrease in food intake at all time points. Similarly, we confirmed the previously demonstrated anorectic effects of α-MSH (Fig. 1*D*) and neurotensin (Fig. 1*E*). ICV administration of 10 µg of either α-MSH or neurotensin led to an approximately two- to threefold decrease in food intake at all time points.

We next evaluated the effect of each anorectic neuropeptide (α-MSH, neurotensin, or GLP-1) on either MCH- or NPY-induced hyperphagia. ICV administration of 10 µg of α-MSH had no effect on NPY-induced (10 µg) hyperphagia, as α-MSH + NPY-treated animals ate an amount similar to that of NPY-treated animals (Fig. 2*A*). In contrast, ICV administration of 10 µg of α-MSH abolished the orexigenic effect of 5 µg of MCH, as α-MSH + MCH-treated animals ate approximately three- to fourfold less food than did the MCH-treated animals (Fig. 2*B*).

Furthermore, ICV administration of 10 µg of neurotensin had no effect on NPY-induced (10 µg) hyperphagia, as neurotensin + NPY-treated animals ate an amount similar to that of NPY-treated animals (Fig. 3*A*). In contrast, ICV administration of 10 µg of neurotensin abolished the orexigenic effect of 5 µg of MCH, as neurotensin + MCH-treated animals ate approximately two- to threefold less food than did the MCH-treated animals (Fig. 3*B*).

Interestingly, ICV administration of 10 µg of GLP-1 abolished both NPY-induced (10 µg) and MCH-induced (10 µg) hyperphagia, as GLP-1 + NPY-treated animals ate approximately threefold less food than did NPY-treated animals, and GLP-1 + MCH-treated animals ate approximately twofold less food than did MCH-treated animals (Fig. 4*A* and *B*, respectively).

DISCUSSION

In this study, we aimed at characterizing the interactions between two key orexigenic peptides, MCH and NPY, and three anorectic peptides, α-MSH, neurotensin, and GLP-1. We initially confirmed the previously reported appetite-related effects of these peptides by using our feeding paradigms (7,24). Our current data confirm our previous findings (4), as well as those of others (20), that have suggested a role for MCH as a short-term appetite-stimulating peptide. In contrast, MCH does not appear to lead to a sustained increase in food intake and significant weight gain after long-term ICV administration (20), perhaps as a result of

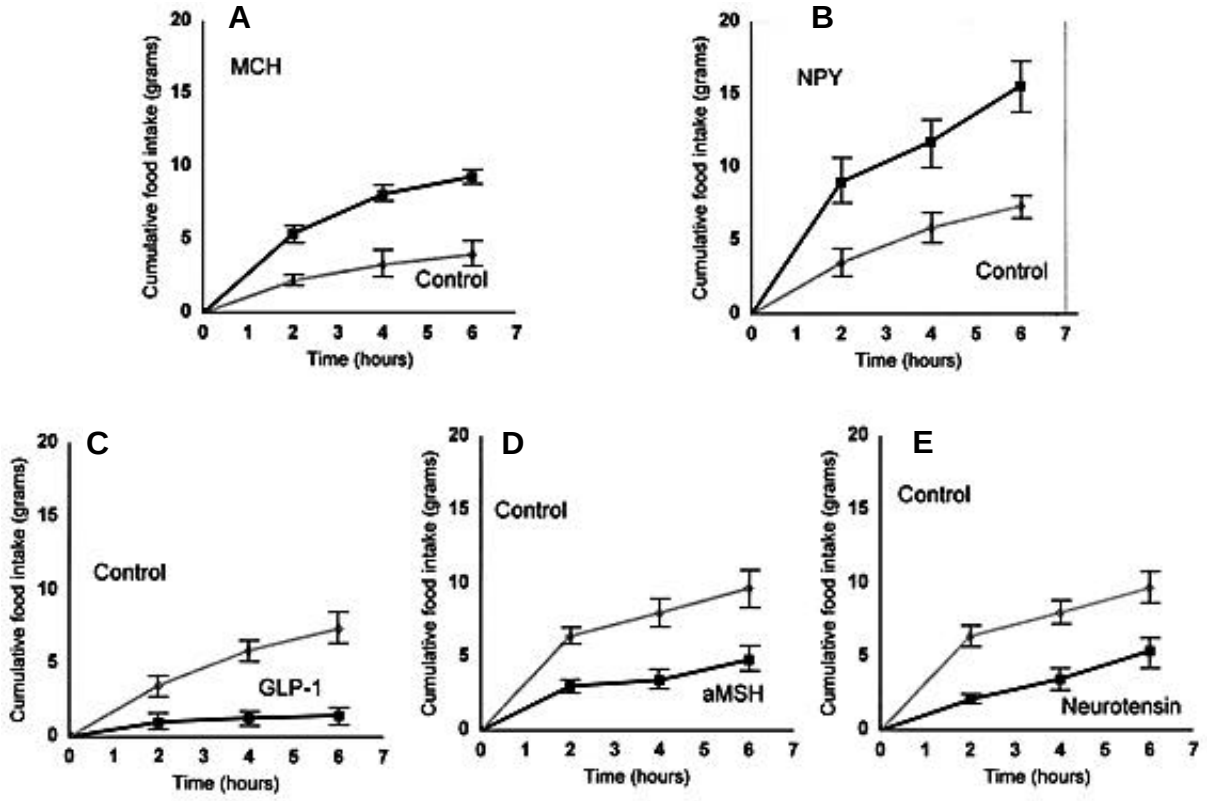


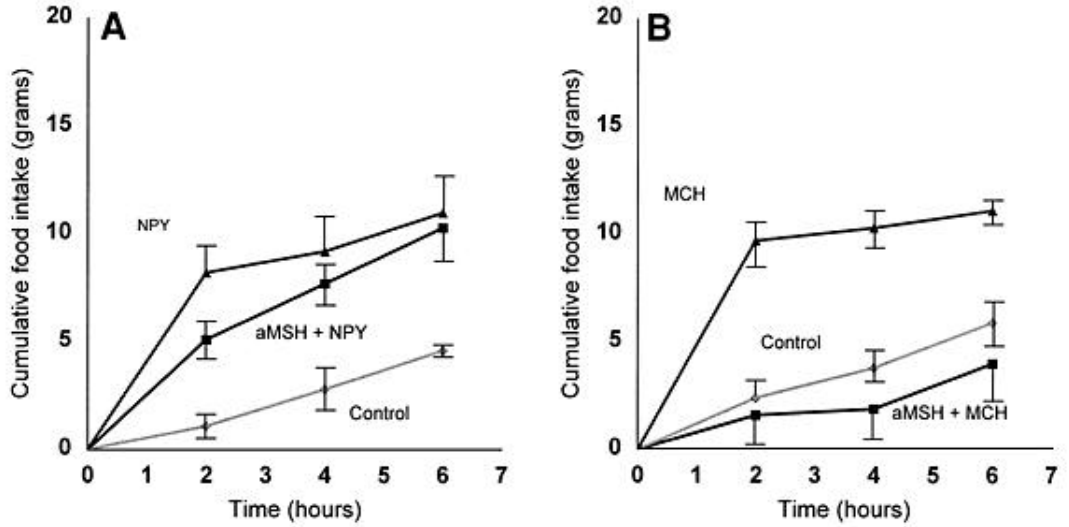
FIG. 1. *A*: **Orexigenic effect of ICV MCH in the rat (MCH-treated vs. control animals, $P = 0.0001$). MCH-treated animals (5 µg); control animals (5 µl artificial CSF) ($n = 5$ each group). At 2 h, MCH-treated animals consumed 5.4 ± 1.1 g of food (mean ± SE) compared with 2.2 ± 0.5 g for the control animals. Similarly, at 4 h, MCH-treated animals consumed 8.1 ± 0.6 g of food compared with 3.3 ± 1.0 g for the control group. At 6 h, MCH-treated animals consumed 9.3 ± 0.6 g of food compared with 4.0 ± 1.3 g for the control animals.** *B*: **Orexigenic effect of ICV NPY in the rat (NPY-treated vs. control animals, $P < 0.0001$). NPY-treated animals (10 µg); control animals (5 µl artificial CSF) ($n = 5$ each group). At 2 h, NPY-treated animals consumed 9.0 ± 2.1 g of food compared with 3.5 ± 0.8 g for the control animals. Similarly, at 4 h, NPY-treated animals consumed 11.8 ± 2.0 g of food compared with 5.9 ± 0.9 g for the control animals. At 6 h, NPY-treated animals consumed 15.6 ± 2.4 g of food compared with 7.4 ± 0.9 g for the control animals.** *C*: **Anorectic effect of ICV GLP-1 in the rat (GLP-1–treated vs. control animals, $P = 0.003$). GLP-1–treated animals (10 µg); control animals (5 µl artificial CSF) ($n = 5$ each group). At 2 h, GLP-1–treated animals consumed 1.0 ± 0.6 g of food compared with 3.5 ± 0.8 g for the control animals. Similarly, at 4 h, GLP-1–treated animals consumed 1.3 ± 0.6 g of food compared with 5.9 ± 0.9 g for the control animals. At 6 h, GLP-1–treated animals consumed 1.5 ± 0.6 g of food compared with 7.4 ± 0.9 g for the control animals.** *D*: **Anorectic effect of ICV α-MSH in the rat (α-MSH–treated animals vs. control animals, $P = 0.0002$). α-MSH–treated animals (10 µg); control animals (5 µl artificial CSF) ($n = 5$ each group). At 2 h, α-MSH–treated animals consumed 3.0 ± 0.6 g of food compared with 6.4 ± 0.6 g for the control animals. Similarly, at 4 h, α-MSH–treated animals consumed 3.4 ± 0.8 g of food compared with 8.0 ± 1.2 g for the control animals. At 6 h, α-MSH–treated animals consumed 4.8 ± 1.4 g of food compared with 9.7 ± 1.7 g for the control animals.** *E*: **Anorectic effect of ICV neurotensin in the rat (neurotensin-treated vs. control animals, $P = 0.0002$). Neurotensin-treated animals (10 µg); control animals (5 µl artificial CSF) ($n = 5$ each group). At 2 h, neurotensin-treated animals consumed 2.1 ± 0.4 g of food compared with 6.4 ± 0.6 g for the control animals. Similarly, at 4 h, neurotensin-treated animals consumed 3.5 ± 1.0 g of food compared with 8.0 ± 1.2 g for the control animals. At 6 h, neurotensin-treated animals consumed 5.4 ± 1.4 g of food compared with 9.7 ± 1.7 g for the control animals.**

compensatory changes in other neurotransmitter or neuropeptide systems. Our data apparently contradict the findings of another study (28), which suggested that MCH inhibits food intake in rodents. However, the amounts of MCH administered in the latter study were significantly lower, and therefore, a biphasic effect of MCH on appetite and food consumption cannot be excluded. Finally, our findings suggest that acute administration of either NEI or NGE, which are encoded in the MCH prepro-mRNA (26) and cosecreted with MCH (27), does not affect food intake.

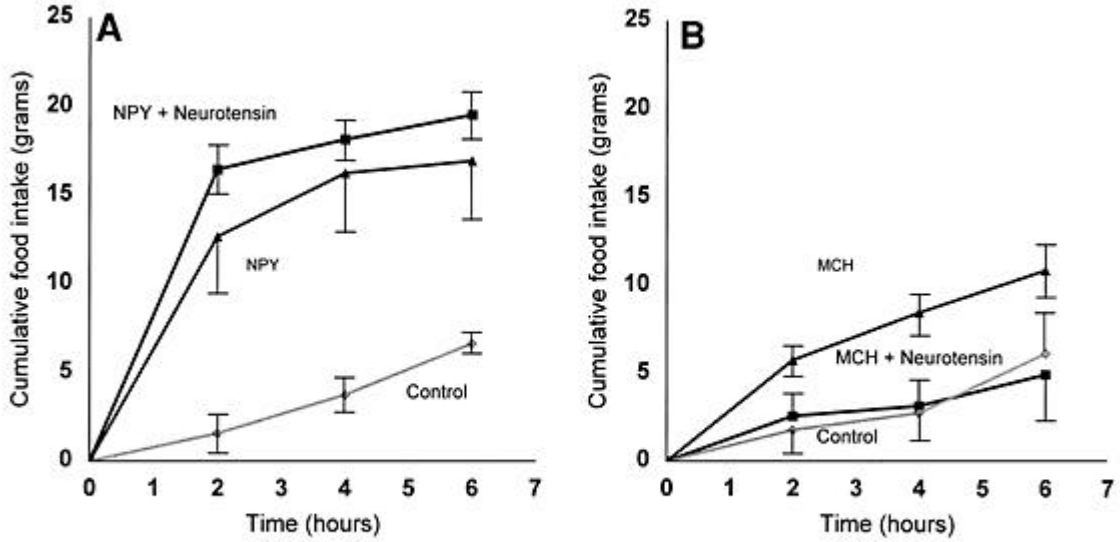
Considerable evidence also suggests that NPY plays a pivotal role in the regulation of food intake. ICV or intrahypothalamic administration of NPY leads to hyperphagia acutely (7) and to obesity after chronic administration (14). Furthermore, ICV or direct paraventricular nuclear administration of antibody to NPY leads to a reduction in feeding (29,30), suggesting an important role for endogenous NPY in the regulation of food intake. However, the role of NPY in modulating food intake has not been thoroughly elucidated, as NPY knockout mice have no feeding phenotype (5), and the obese brown adipose tissue–deficient (uncoupling protein–diphtheria toxin A) mice have decreased hypothalamic expression of NPY mRNA (31).

The potential interactions of various orexigenic and anorectic peptides are also poorly understood. In part, this is due to the fact that few studies have systematically examined the effects of ICV coinjection of different neuropeptides under similar conditions.

After confirming the previously reported appetite-stimulating effects of NPY (7,14) and MCH (4,20), as well as the anorectic effects of α-MSH (8), neurotensin (21), and GLP-1 (9,32), we evaluated the functional interactions between



IG. 2. *A*: Lack of an effect of ICV α-MSH on the orexigenic effect of ICV NPY in the rat (NPY vs. α-MSH + NPY-treated animals, *P* = 0.18; NPY-eated animals vs. control animals, *P* < 0.0001; and α-MSH + NPY-treated animals vs. control animals, *P* = 0.0005). α-MSH + NPY-treated animals 10 μg α-MSH and 10 μg NPY); NPY-treated animals (10 μg); control animals (5 μl artificial CSF) (*n* = 5 each group). At 2 h, α-MSH + NPY-treated nimals consumed 5.1 ± 1.2 g of food (mean ± SE), and NPY-treated animals consumed 8.2 ± 1.1 g of food compared with 1.1 ± 0.2 g for the con-ol animals. At 4 h, α-MSH + NPY-treated animals consumed 7.7 ± 1.6 g of food and NPY-treated animals consumed 9.2 ± 1.7 g of food compared ith 2.8 ± 1.1 g for the control animals. At 6 h, α-MSH + NPY-treated animals consumed 10.3 ± 1.6 g of food, and NPY-treated animals ate 11.0 ± .2 g of food compared with 4.6 ± 1.4 g for the control animals. *B*: Abolition of the orexigenic effect of ICV MCH by coadministration of α-MSH the rat (MCH + α-MSH–treated animals vs. control animals, *P* = 0.63; and either MCH vs. α-MSH + MCH-treated animals or MCH-treated ani-als vs. control animals, *P* < 0.0001). α-MSH + MCH-treated animals (10 μg α-MSH and 5 μg MCH) (*n* = 6); MCH-treated animals (5 μg) (*n* = 9); ontrol animals (5 μl artificial CSF) (*n* = 11). At 2 h, α-MSH + MCH-treated animals consumed 1.6 ± 1.2 g of food (mean ± SE), and MCH-treated nimals consumed 9.7 ± 1.3 g of food compared with 2.4 ± 0.8 g for the control animals. At 4 h, α-MSH + MCH-treated animals consumed 1.9 ± 1.4 of food, and MCH-treated animals consumed 10.3 ±1.0 g of food compared with 3.8 ± 1.1 g for the control animals. At 6 h, α-MSH + MCH-treated nimals consumed 4.0 ± 1.6 g of food, and MCH-treated animals ate 11.1 ± 0.9 g of food compared with 5.9 ± 1.4 g for the control animals. Fig. 2*B* as adapted from Ludwig et al. (24) and included for the purpose of comparison.



IG. 3. *A*: Lack of an effect of ICV neurotensin on the orexigenic effect of ICV NPY in the rat (NPY vs. neurotensin + NPY-treated animals, *P* = .25; NPY or neurotensin + NPY-treated animals vs. control animals, *P* < 0.0001). Neurotensin + NPY-treated animals (10 μg neurotensin and 10 g NPY); NPY-treated animals (10 μg); control animals (5 μl artificial CSF) (*n* = 5 each group). At 2 h, neurotensin + NPY-treated animals con-umed 16.5 ± 1.4 g of food (mean ± SE), and NPY-treated animals ate 12.7 ± 3.2 g of food compared with 1.6 ± 1.3 g for the control animals. Sim-arly, at 4 h, neurotensin + NPY-treated animals consumed 18.2 ± 1.1 g of food, and NPY-treated animals ate 16.3 ± 3.4 g of food compared with .8 ± 0.8 g for the control animals. At 6 h, neurotensin + NPY-treated animals consumed 19.6 ± 1.0 g of food, and NPY-treated animals consumed 7.0 ± 3.2 g of food compared with 6.7 ± 0.6 g for the control animals. *B*: Abolition of the orexigenic effect of ICV MCH by coadministration of eurotensin in the rat (MCH + neurotensin-treated animals vs. control animals, *P* = 0.99; MCH vs. neurotensin + MCH-treated animals or MCH-eated animals vs. control animals, *P* < 0.0001). Neurotensin + MCH-treated animals (10 μg neurotensin and 5 μg MCH); MCH-treated animals 5 μg); control animals (5 μl artificial CSF) (*n* = 5 each group). At 2 h, neurotensin + MCH-treated animals consumed 2.6 ± 0.5 g of food, and MCH-eated animals consumed 5.8 ± 1.2 g of food compared with 1.8 ± 0.7 g for the control animals. Similarly, at 4 h, neurotensin + MCH-treated ani-als consumed 3.2 ± 0.9 g of food, and MCH-treated animals consumed 8.5 ± 1.4 g of food compared with 2.8 ± 1.1 g for the control animals. At 6 , neurotensin + MCH-treated animals consumed 5.0 ± 1.5 g of food, and MCH-treated animals consumed 10.9 ± 1.9 g of food compared with 6.2 2.4 g for the control animals.

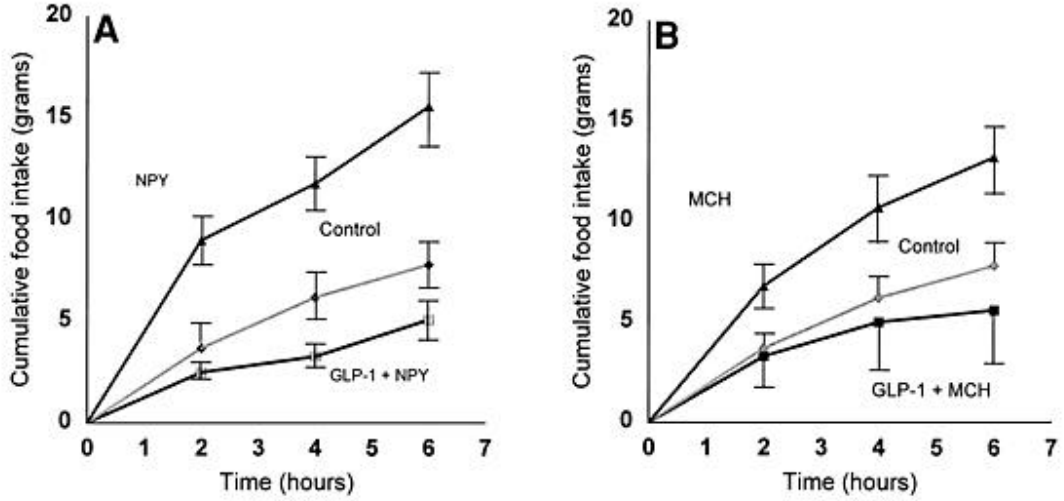


FIG. 4. *A*: **Abolition of the orexigenic effect of ICV NPY by the coadministration of GLP-1 in the rat (GLP-1 + NPY-treated animals vs. control animals, $P = 0.16$; GLP-1 + NPY vs. NPY-treated animals, $P < 0.0001$). GLP-1 + NPY-treated animals (10 µg GLP-1 and 10 µg NPY); NPY-treated animals (10 µg); control animals (5 µl artificial CSF) ($n = 5$ each group). At 2 h, GLP-1 + NPY-treated animals consumed 2.5 ± 0.2 g of food (mean ± SE), and NPY-treated animals consumed 9.0 ± 2.4 g of food compared with 3.7 ± 1.1 g for the control animals. Similarly, at 4 h, GLP-1 + NPY-treated animals consumed 3.3 ± 0.4 g of food, and NPY-treated animals ate 11.8 ± 2.2 g of food compared with 6.2 ± 1.1 g for the control animals. At 6 h, GLP-1 + NPY-treated animals consumed 5.1 ± 0.9 g of food, and NPY-treated animals consumed 15.6 ± 2.7 g of food compared with 7.8 ± 1.1 g for the control animals.** *B*: **Abolition of the orexigenic effect of ICV MCH by the coadministration of GLP-1 in the rat (GLP-1 + MCH-treated animals vs. control animals, $P = 0.24$; GLP-1 + MCH vs. MCH-treated animals, $P = 0.0001$). GLP-1 + MCH-treated animals (10 µg GLP-1 and 5 µg MCH); MCH-treated animals (5 µg); control animals (5 µl artificial CSF) ($n = 5$ each group). At 2 h, GLP-1 + MCH-treated animals consumed 3.3 ± 1.2 g of food, and MCH-treated animals consumed 6.8 ± 1.1 g of food compared with 3.7 ± 0.7 g for the control animals. Similarly, at 4 h, GLP-1 + MCH-treated animals consumed 5.0 ± 2.2 g of food, and MCH-treated animals consumed 10.7 ± 1.4 g of food compared with 6.2 ± 1.1 g for the control animals. At 6 h, GLP-1 + MCH-treated animals consumed 5.6 ± 2.5 g of food, and MCH-treated animals consumed 13.2 ± 1.5 g of food compared with 7.8 ± 1.1 g for the control animals.**

approximately equimolar amounts of these appetite-stimulating peptides and an excess (~3-fold nmol/l for α-MSH or neurotensin and ~1.5-fold nmol/l for GLP-1) of these appetite-suppressing neuropeptides. First, our data demonstrate that α-MSH does not inhibit the orexigenic effect of NPY after ICV coadministration. This is in contrast to the effect of the melanocortin agonist MTII, which has been shown to inhibit NPY-induced hyperphagia and has been considered as a melanocortin "superagonist" (25). Furthermore, we have previously demonstrated that α-MSH inhibits the orexigenic effect of MCH after ICV coadministration (24). Second, we similarly demonstrated that neurotensin inhibits the orexigenic effect of MCH, but not that of NPY, after ICV coadministration. These data suggest that the functional interactions between exogenous melanocortins and neurotensin, on one hand, and orexigenic peptides, on the other, are specific. Finally, our data suggest that GLP-1 inhibits the orexigenic effect of both MCH and NPY. The latter effect has been previously reported (9) but is particularly significant, since NPY is the most potent orexigenic peptide known. Additionally, our data indicate that to some degree both NPY and MCH may counteract the anorectic effect of GLP-1.

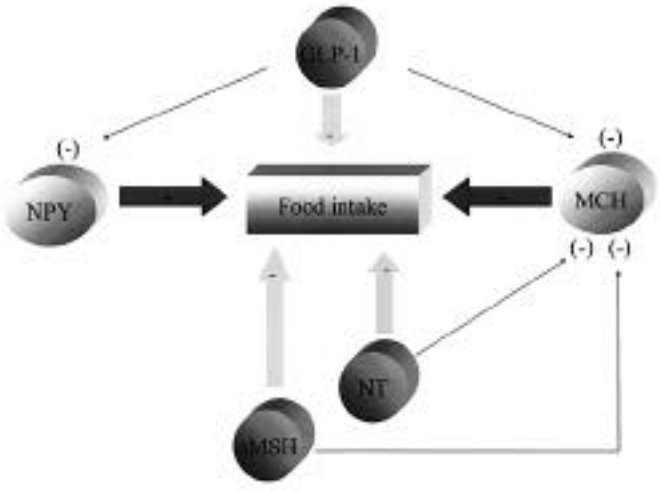


FIG. 5. Hypothetical model depicting the functional interactions between NPY, MCH, α-MSH, neurotensin (NT), and GLP-1 secreting central nervous system pathways. Grey arrows denote inhibitory influences on appetite and food intake; black arrows denote positive influences.

The underlying mechanisms for these interactions remain unclear at present. However, we speculate that a similar functional organization of the corresponding peptidergic neurons may exist in vivo (Fig. 5). It is possible that the effects of melanocortins and neurotensin may occur downstream of those of MCH in the appetite-regulating pathways, and those of NPY are further downstream. Finally, GLP-1 would act at the farthest point of the pathway in this model. Detailed immunohistologic analysis would be required to establish the architecture of these pathways and the validity of such a model. Alternatively, our findings may be explained by presuming modulation of the actions of one neuropeptide by another at a receptor or postreceptor level, more specifically, MCH receptors or postreceptor signaling events by either neurotensin, GLP-1, or α-MSH. A similar modulation of the effect of NPY has been reported as a result of ICV leptin coadministration (33), and this may result from an effect of leptin on NPY binding to its receptors that are critical for feed-

ıg and, possibly, on NPY signal transduction mechanisms 34,35). However, in the case of MCH, testing these hypothe-es would be particularly difficult at present, since both the MCH receptor and signaling mechanisms mediating its effects remain poorly understood. Finally, it should be noted hat we have not examined the effects of other neuropeptides hat have been implicated in the regulation of food intake, uch as galanin and CRH (36), in the context of our study, even hough it is likely that important interactions exist between ther neuropeptides and the peptides we studied.

In conclusion, we have investigated the functional inter-ctions between intracerebroventricularly administered rexigenic and anorectic peptides. We confirmed the previ-usly known appetite-stimulating effects of NPY and MCH and ave demonstrated that the orexigenic effect of MCH is ıhibited by the ICV coadministration of either neurotensin, ;LP-1, or (as we have previously shown) α-MSH, whereas that f NPY is inhibited only by GLP-1 and not by α-MSH or neu-otensin, although the underlying mechanisms responsible for ıese interactions remain poorly understood. Thus, further ıvestigation will be necessary to elucidate them and fur-ıer our understanding of both the regulation of appetite and nergy balance as well as the pathophysiology of obesity.

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Author Queries (please see Q in margin and underlined text)

Q1: AU: Unit of measure correct for 1.5-fold mol/l?