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Evidence for involvement of neuropeptide Y and melanocortin systems in the hyperphagia of lactation in rats

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Abstract

Hypothalamic neuropeptide Y (NPY) systems are upregulated during lactation in rats. Because NPY is central to the hypothalamic control of energy balance, the present studies tested the hypothesis that NPY contributes to the marked hyperphagia during lactation. A 4-day infusion of [D-tyr (27,36), D-thr (32)] NPY (27–36) (D-NPY$_{27-36}$), a peptide analogue of NPY that antagonizes NPY-induced feeding, into the third ventricle at 1 μg/h transiently inhibited nocturnal feeding in nonlactating female rats. However, this antagonist had no effect on nocturnal feeding, but did transiently reduce food intake during the light hours, when infused into the third ventricle at the same dose in lactating females. An essentially similar pattern of results was obtained with chronic infusion into the third ventricle of the anorexigenic peptide α-melanocyte-stimulating hormone (α-MSH, 1 μg/h), in nonlactating and lactating rats. Both D-NPY$_{27-36}$ and α-MSH transiently reduced nocturnal food intake in lactating rats by approximately 10% when infused at the higher dose of 5 μg/h, and a marked inhibition of approximately 40% of both nocturnal and diurnal feeding was produced by a combined infusion of both at 5 μg/h. These results provide the first pharmacological evidence implicating specific neuromessengers in mediating the hyperphagia of lactation, and suggest that, while an action of NPY may contribute to the increased food intake seen in lactating animals, other systems are also involved. In particular, a reduction in melanocortin signaling during lactation may allow for an increased orexigenic influence of the agouti-related protein (AgRP), which is co-expressed with NPY.

Keywords: Feeding; α-Melanocyte-stimulating hormone; Neuropeptide Y; Rat

1. Introduction

Recent evidence indicates that neuropeptide Y (NPY) exerts several important neuroendocrine actions during lactation in rats. Following the observations that NPY is expressed within a subpopulation of tuberoinfundibular dopamine neurons of the hypothalamic arcuate nucleus during lactation in rats and mice, but is not present in these cells in nonlactating females or males (Ciofi et al., 1991, 1993), previous studies from this laboratory have provided evidence that NPY serves as a co-hypothalamic hormone with dopamine in control of prolactin secretion during lactation (Wang et al., 1996). In addition, the expression of NPY within the nondopamine NPY neuronal population in the arcuate nucleus, and also in cells of the dorsomedial nucleus, increases during lactation (Chen et al., 1999; Li et al., 1999; Malabu et al., 1994; Smith, 1993), and it has been suggested that these changes are important in mediating the altered energy balance characteristic of lactation (Johnstone and Higuchi, 2001; Smith and Grove, 2002).

It is well established that NPY projections from arcuate and dorsomedial nuclei to the hypothalamic paraventricular nucleus (PVN) comprise critical orexigenic pathways (Kalra et al., 1999; Schwartz et al., 2000). For example, central administration of NPY dramatically induces feeding behavior (Clark et al., 1984) and interference with endogenous NPY signaling inhibits deprivation-induced, as well as normal circadian food intake (Akabayashi et al., 1994; Dube et al., 1994; Myers et al., 1995). Thus, it is intriguing that this system is upregulated during lactation, which is characterized by a marked physiological hyperphagia, due to the energy demands of milk production by

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the mammary gland (Vernon, 1989; Williamson, 1980). To date, however, there have been no reports presenting direct evidence that NPY, or any other neurotransmitter or peptide known to influence feeding behavior, participates in the control of food intake during lactation.

Hence, one objective of the present studies was to test the hypothesis that central NPY contributes to the hyperphagia of lactation by examining the effects of an antagonist analogue reported to inhibit NPY-stimulated food intake (Myers et al., 1995). The majority of arcuate NPY neurons involved in feeding behavior also express the agouti-related protein (AgRP) (Chen et al., 1999; Hahn et al., 1998), which also is a potent stimulant of feeding behavior (Rossi et al., 1998; Kalra et al., 1999; Schwartz et al., 2000) and whose expression is also increased in lactation in rats (Chen et al., 1999). Because AgRP antagonizes the anorexigenic action of α-melanocyte-stimulating hormone (α-MSH) (Rossi et al., 1998; Ollmann et al., 1997; Quillan et al., 1998; Yang et al., 1999), and itself may be an inverse agonist, at MC-4 receptors stimulated by α-MSH in the PVN (Nijenhuis et al., 1997; Haskell-Luevano and Monck, 2001), one means of functionally inhibiting AgRP action is through stimulation of MC-4 receptors with α-MSH. Thus, an additional objective was to examine the effects of α-MSH alone and in combination with the NPY antagonist on food intake in lactating female rats.

2. Materials and methods

2.1. Animals

Sprague–Dawley female rats were obtained from Charles River Laboratories (Wilmington, MA). Cycling females were housed singly, while individual lactating females were housed with their litters on a 12:12-h light/dark cycle (lights on at 06:00 h) and ad libitum food and water. Each litter was culled to an *n* of 8 on postpartum day 2. All animal protocols were approved by the University of Utah Institutional Animal Care and Use Committee.

2.2. Stereotaxic surgery

Animals were anesthetized with sodium methohexital (Brevital) and placed in a Kopf stereotaxic apparatus. In one experiment, a 24-gauge stainless steel guide cannula was implanted unilaterally into the PVN of the hypothalamus and affixed to the skull with dental acrylic and jeweler's screws. In the other studies, a 30-gauge stainless steel infusion cannula was implanted into the third ventricle at the level of the PVN, and was connected via polyethylene (PE20) tubing to an Alzet osmotic minipump (# 2002), which was filled with saline vehicle or test drug as indicated below. Drugs or vehicle were infused into the third ventricle at a rate of 1 μl/h, beginning immediately upon completion of surgery and continuing for the duration of the experiment.

2.3. Experiment 1: effects of an antagonist of NPY-induced feeding

Randomly cycling female rats with cannulas implanted in the PVN 1 week previously received two unilateral microinjections (each at 0.5 μl) into the PVN, 10 min apart, of either (1) saline vehicle, (2) saline followed by NPY (2 μg), (3) [D-tyr (27,36), D-thr (32)] NPY (27–36) (D-NPY_{27-36}, 3 μg), a peptidergic antagonist of NPY-induced feeding (Myers et al., 1995), followed by saline, or (4) D-NPY_{27-36} (3 μg), followed by NPY(2 μg). Injections were made with a 30-gauge injector cannula during the latter half of the light period, and food intake was measured 2 h following drug administration. NPY and D-NPY_{27-36} were obtained from Peninsula Laboratories (Belmont, CA).

2.4. Experiment 2: effects of the NPY antagonist and α-MSH on daily food intake in nonlactating rats

In separate experiments, randomly cycling females were infused into the third ventricle for 4 days with either the NPY feeding antagonist peptide D-NPY_{27-36} (1 μg/μl/h) or α-MSH (1 μg/μl/h; Peninsula Laboratories); in each experiment, saline-infused (1 μl/h) controls were tested concurrently. Preweighed Purina Lab chow pellets were provided at dark onset and light onset, and food intake was measured for both periods. The first measurement of nocturnal feeding thus occurred on the morning following cannula and osmotic minipump implantation.

2.5. Experiment 3: effects of the NPY feeding antagonist peptide and α-MSH on daily food intake in lactating rats

In one study, the same protocol used in Experiment 2 above was employed in lactating rats. Third ventricular cannulas were implanted between days 5 and 7 of lactation, and either saline vehicle, the NPY feeding antagonist peptide D-NPY_{27-36} or α-MSH was infused (each at 1 μg/μl/h) for a 4-day period. Food intake during light and dark periods was measured as above.

In a second experiment, lactating females with third ventricular cannulas were infused for 4 days with either saline vehicle at 1 μl/h, the NPY feeding antagonist at 5 μg/h, α-MSH at 5 μg/h or both the NPY antagonist and α-MSH, each at 5 μg/h. Food intake during the dark and light periods was measured as above.

In each infusion experiment conducted in lactating rats, weights of the dams and litters were recorded each day. At the completion of each study, animals were lightly anesthetized and sacrificed by decapitation. Brains were sectioned in a microtome cryostat and stained to verify correct cannula placement.

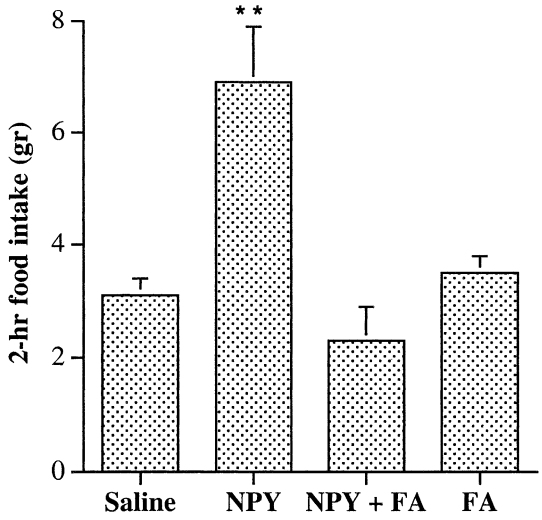

Fig. 1. Effects of administration of the NPY feeding antagonist peptide (FA) [D-tyr (27,36), D-thr (32)] NPY (27–36) (3 μg) on NPY-induced (2 μg) food intake in nonlactating female rats. Peptides were administered unilaterally to the paraventricular nucleus. $n=4–7$ per treatment group. $**P<.01$ vs. all other groups, based on single factor analysis of variance and Fisher's least significant differences test.

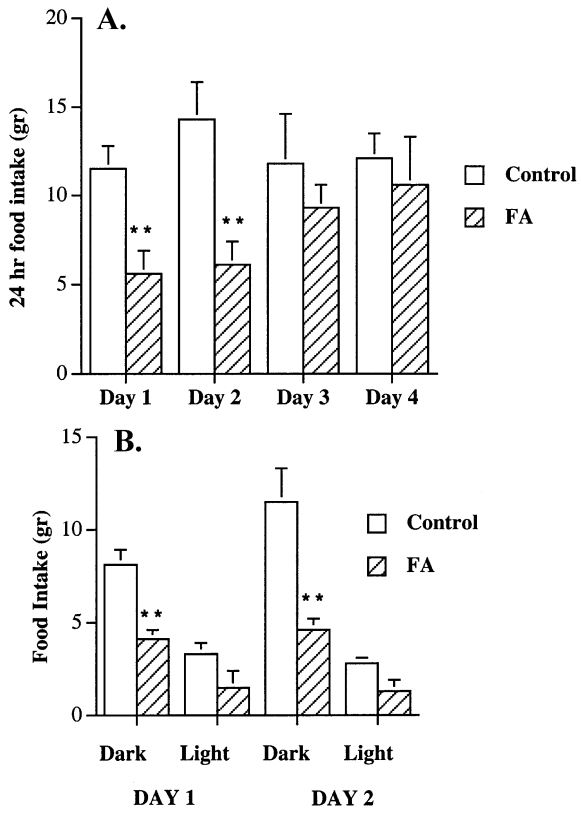

Fig. 2. Effects of third ventricular infusion of the NPY feeding antagonist peptide (FA, 1 μg/h) on food intake in nonlactating female rats. $n=5$ per treatment group. (A) 24-h food intake; $**P<.01$ vs. saline control. (B) Food intake during 12-h dark and 12-h light periods; $**P<.01$ vs. saline control.

2.6. Statistical analysis

Food intake, body weight and litter weight data were analyzed by analysis of variance and individual comparisons were made with Fisher's least significant difference tests, using the Statview program for the Macintosh.

3. Results

The first experiment was intended to confirm that the NPY antagonist analogue D-NPY_{27-36}, which has been reported to inhibit NPY- and deprivation-induced feeding behavior (Myers et al., 1995), was effective in this laboratory. Fig. 1 shows that this agent was without effect when injected alone into the PVN, but completely prevented the significant increase in food intake in response to intra-PVN injection of NPY. Single factor analysis of variance revealed a significant overall treatment effect ($F=10.46$ at 3,16 degrees of freedom, $P<.0005$) and the post-hoc test showed that food intake after NPY alone was significantly higher than the other three groups ($P<.01$).

Fig. 2A shows that when this NPY antagonist was infused into the third ventricle of nonlactating female rats, 24-h food intake was significantly depressed on the first 2 days of treatment, with recovery to normal levels by day 3.

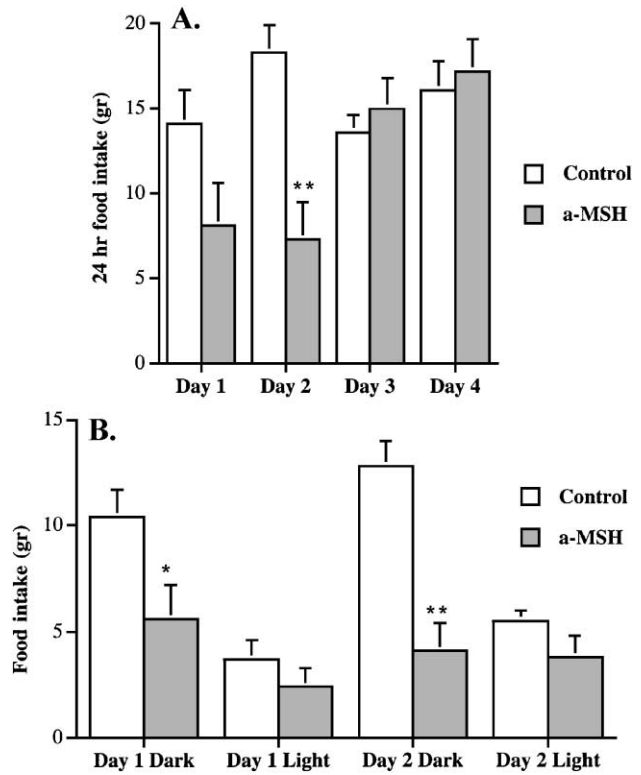

Fig. 3. Effects of third ventricular infusion of α-MSH (1 μg/h) on food intake in nonlactating female rats. $n=5$ per treatment group. A. 24-h food intake; $**P<.01$ vs. saline control. (B) Food intake during 12-h dark and 12-h light periods; $*P<.05$, $**P<.01$ vs. saline control.

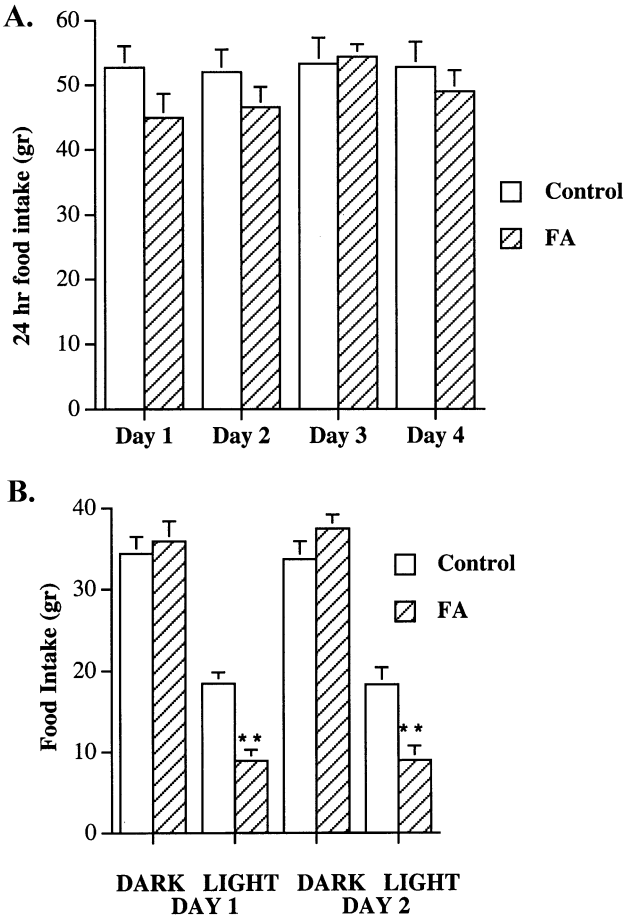

Fig. 4. Effects of third ventricular infusion of the NPY feeding antagonist peptide (FA, 1 μg/h) on food intake in lactating female rats. $n=5$ per treatment group. (A) 24-h food intake. (B) Food intake during 12-h dark and 12-h light periods; $**P<.01$ vs. saline control.

Analysis of variance on 24-h food intake data on the first 2 days of treatment revealed a significant main effect of drug treatment ($F=33.49$ at 1,16 degrees of freedom, $P<.0001$), with no main effect of days or drug by days interaction. Fig. 2B shows the effect of D-NPY$_{27-36}$, infusion with respect to the diurnal variation in food intake on the first 2 days of treatment. This antagonist significantly reduced food intake during the dark time periods on these 2 days ($P<.01$); food intake was also reduced by D-NPY$_{27-36}$, somewhat during the light hours, but the reduction did not achieve statistical significance. Analysis of variance of food intake data each day showed significant main effects of time ($P<.001$), drug treatment ($P<.001$) and time by treatment interaction ($P<.05$).

As seen in Fig. 3, infusion of α-MSH into the third ventricle of nonlactating female rats produced inhibitory effects on food intake very similar to that produced by the NPY feeding antagonist peptide. An overall analysis of variance on 24-h food intake for the first 2 days of treatment showed a significant main effect of drug treatment ($F=20.52$ at 1,16 degrees of freedom, $P<.0003$), with no significant main effect of day or day by treatment interaction. Individual comparisons indicated that the inhibitory effect of α-MSH on 24-h food intake just missed statistical significance on day 1, but was significant on day 2 ($P<.01$; Fig. 3A). Twenty-four-hour food intake was unaffected by α-MSH on the third and fourth days of infusion. Also similar to the effect of D-NPY$_{27-36}$, the inhibitory effect of α-MSH was evident during the dark periods on the first 2 days of treatment ($P<.05$ on day 1, $P<.01$ on day 2), while food intake during the light periods was unaffected (Fig. 3B).

As presented in Fig. 4, lactating female rats consumed in excess of 50 g of food over the 24-h period, compared to the 10–15 g normally eaten by nonlactating rats. When infused into the third ventricle at the same 1 μg/h dose that inhibited feeding in nonlactators, D-NPY$_{27-36}$ was much less effective in inhibiting food intake in lactating animals compared to nonlactators. Thus, no inhibitory effect of this antagonist peptide was observed over the 24-h period, and when food intake was measured over the dark and light periods, a significant reduction was observed only during the light period of days 1 and 2 of treatment, with normal food intake thereafter. Analysis of variance on food intake during the light periods of treatment days 1 and 2 showed a significant main effect of treatment ($F=49.05$ at 1,16 degrees of

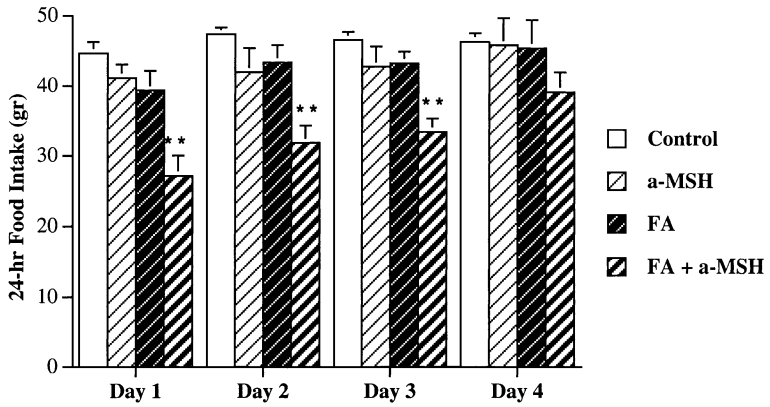

Fig. 5. Effects of third ventricular infusion of α-MSH (5 μg/h), the NPY feeding antagonist peptide (FA, 5 μg/h), or both (each at 5 μg/h) on 24-h food intake in lactating female rats. $n=6–8$ per treatment group. $**P<.01$ vs. saline control.

freedom, $P<.0001$), and the individual comparisons showed that the antagonist peptide significantly reduced food intake compared to saline controls on days 1 and 2 ($P<.01$).

In an additional study (not shown), a 4-day infusion of α-MSH at the 1-μg/h dose that was effective in nonlactators was without effect on food intake in lactators. In addition, neither D-NPY$_{27-36}$ nor α-MSH significantly affected body weight gain or litter weight gain over the 4-day treatment period.

For the experiment presented in Figs. 5 and 6, the dosages of D-NPY$_{27-36}$ and α-MSH were increased to 5 μg/h for the 4-day infusion period in lactating animals. Fig. 5 shows that neither D-NPY$_{27-36}$ nor α-MSH alone significantly affected 24-h food intake at this higher dose level, but food intake was significantly reduced by the combined infusion of D-NPY$_{27-36}$ and α-MSH. An overall analysis of variance showed a significant drug treatment effect ($F=26.1$ at 3,88 degrees of freedom, $P<.0001$), but no effect of days or days by treatment interaction. Individual comparisons showed that the combined D-NPY$_{27-36}$ and α-MSH infusion significantly decreased food intake compared to the saline controls or each peptide alone on days 1–3 of treatment ($P<.01$), with no effect on day 4.

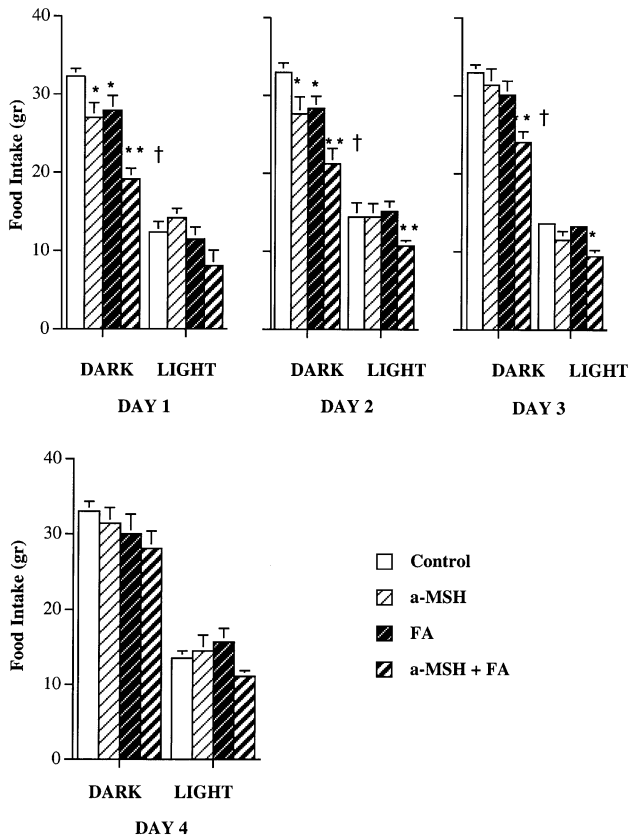

Fig. 6. Effects of third ventricular infusion of α-MSH (5 μg/h), the NPY feeding antagonist peptide (FA, 5 μg/h), or both (each at 5 μg/h) on food intake during the 12-h dark and 12-h light periods in lactating female rats. $n=6–8$ per treatment group. $^{*}P<.05$, $^{**}P<.01$ vs. saline control. $^{\dagger}P<.01$ vs. FA alone or α-MSH alone.

Table 1
Effects of third ventricular infusion of α-MSH (5 μg/h) on the NPY feeding antagonist peptide (FA, 5 μg/h) or both (each at 5 μg/h) on body weight gain (g) of the lactating females and litters

Group	Body weight gain in lactators	Litter weight gain
Control	12.7 ± 4.5	41.3 ± 1.8
α-MSH	13.2 ± 3.3	33.0 ± 3.8
NPY FA	11.8 ± 3.0	38.0 ± 3.6
α-MSH + FA	11.9 ± 3.0	26.8 ± 4.0**

** $P<.01$ vs. saline control; single factor analysis of variance and Fisher's least significant difference test.

When the diurnal rhythm in food intake was analyzed, additional effects of the peptides became apparent. As shown in Fig. 6, D-NPY$_{27-36}$ and α-MSH each significantly reduced food intake during the dark period for the first 2 days of the 4-day infusion period ($P<.05$), while neither peptide affected food intake during the light period. However, marked decreases in feeding behavior were observed in response to the combined infusion of D-NPY$_{27-36}$ and α-MSH during the dark periods on days 1–3 ($P<.01$), with recovery to normal on day 4; the reductions in food consumption in animals infused with the two agents together appeared to be additive and were significantly greater than the decreases seen with either infused alone ($P<.01$). In addition, the combined treatment also significantly decreased food intake during the light hours on days 2 and 3 ($P<.01$ and $P<.05$, respectively).

Table 1 presents mean body weight gains for the lactating rats and their litters over the course of this experiment. No differences in body weight gain of the lactating females were noted in any of the treatment groups during the 4-day infusion. Neither α-MSH nor D-NPY$_{27-36}$ alone affected weight gain of the litters; however, the combined infusion did significantly reduce litter weight gain by approximately 40%.

4. Discussion

The present studies provide the first direct evidence using a pharmacological approach for involvement of specific neuromessenger systems in mediating the marked hyperphagia of lactation. Specifically, the present findings suggest that hypothalamic NPY contributes to the increased food intake during lactation. However, it is also likely that an action of NPY alone cannot account for the dramatic increase in feeding, and that NPY systems may interact with melanocortin signaling pathways, involving α-MSH, and by inference, AgRP.

Although its interaction with specific NPY receptor subtypes remains undefined, a previous report (Myers et al., 1995) indicated that the NPY analogue D-NPY$_{27-36}$ inhibits NPY- and deprivation-induced feeding in rats. The present studies confirmed the inhibition of NPY-induced food intake and demonstrated further that this peptide inhibits physio-

logical food intake over the circadian cycle in both lactating and nonlactating rats. However, there were differences in the efficacy of this NPY antagonist in the two groups of animals. When infused into the third ventricle of nonlactating animals at 1 μg/h, D-NPY$_{27-36}$ substantially inhibited 24-h food intake, with the effect largely due to suppression of nocturnal feeding. In contrast, this same regimen had relatively little effect on feeding in lactating rats; nocturnal feeding was unaffected, while diurnal feeding was inhibited by approximately 50%. It is interesting to note that, in both types of animals, the inhibitory effects of the NPY antagonist occurred on the first 2 days of infusion, with recovery to normal levels of feeding thereafter.

The reason for the differential effect on nocturnal vs. diurnal feeding in the two groups of animals is not clear at present, although it may relate to the fact that in these studies, lactating animals consumed approximately as much food during the light hours as nonlactating animals did in the entire 24-h period. Supporting this view, D-NPY$_{27-36}$ was somewhat more effective in inhibiting food intake in lactating rats at the higher infusion dose of 5 μg/h, and the pattern of effect, i.e., primarily inhibition of nocturnal feeding, resembled that seen in nonlactating animals. However, the inhibitory effect of D-NPY$_{27-36}$ infusion at 5 μg/h on nocturnal feeding in lactating animals was only on the order of an approximately 10% reduction, which was much less in magnitude than produced by the lower infusion dose in nonlactators. It is possible that the lack of effect of D-NPY$_{27-36}$ during light hours feeding in nonlactating animals is due to the low baseline level of intake, but this would not account for the lack of effect on light hours feeding in lactating animals, which is substantial. It is conceivable that the influence of NPY over food might be different in dark vs. light hours, perhaps in interaction with other neural systems.

It is also highly likely that other neurochemical systems participate in stimulating the marked hyperphagia of lactation in an interaction with the NPY system. The present studies show for the first time that α-MSH, which is well known to inhibit feeding in various animal models (Kalra et al., 1999; Schwartz et al., 2000), also decreases food intake in both cycling and lactating rats. It is interesting to note the similar pattern of effects, including the differences in efficacy between lactators vs. nonlactators, produced by α-MSH and the NPY antagonist in the present studies. Thus, as with the NPY antagonist D-NPY$_{27-36}$, infusion of α-MSH at 1 μg/h to nonlactating females inhibited feeding during the dark hours for the first 2 days of infusion, followed by recovery on days 3 and 4, and the efficacy of this dose in lactators was also much reduced compared to nonlactators. Further, the 5-μg/h infusion of α-MSH alone in lactating females resulted in essentially the same degree of inhibition of food intake as did the infusion of the NPY antagonist.

Further experiments will be required to determine the mechanism underlying the recovery of normal food intake during the continual exposure to either D-NPY$_{27-36}$ or α-MSH. In the case of α-MSH, this could relate to agonist-induced desensitization of the MC-4 receptor, while the converse effect of receptor upregulation could occur in response to continued receptor blockade with D-NPY$_{27-36}$. Additional potential mechanisms could also include altered hypothalamic NPY gene expression and release in response to the inhibited feeding, in view of the observations that food deprivation increases NPY synthesis and release in hypothalamus (Dube et al., 1992; Sahu et al., 1992; White and Kershaw, 1990). It may also be the case that, with chronic infusion, there is an induction of peptidase activity, resulting in increased clearance of the peptides from the critical brain areas.

Clearly, the most substantial inhibition of food intake in lactating rats was observed during the combined infusion of D-NPY$_{27-36}$ and α-MSH, which reduced both nocturnal and diurnal feeding for three of the 4 treatment days. The largest decrease of approximately 40% occurred on the first full day of infusion, and the effect gradually waned over the next 2 days of infusion, even though it remained statistically significant. This finding strongly suggests that hyperphagia during lactation is mediated, at least in part, by both an NPY stimulatory tone, as well as removal of the inhibitory melanocortin signaling. The combined infusion also significantly impaired litter weight gain over the 4-day experiment, suggesting that the reduced food intake may have translated into reduced milk production and/or that secretion of the hormones of lactation, prolactin and oxytocin may have been impaired. However, the combined infusion did not affect maternal weight gain over this period; this argues against factors such as nonspecific illness or malaise produced by the peptide infusions.

Moreover, the present findings could be interpreted to support involvement of AgRP signaling through the melanocortin receptor in contributing to the hyperphagia of lactation. AgRP has been considered a functional antagonist of α-MSH action because it binds to the same MC-4 receptor as α-MSH and prevents its inhibitory action (Rossi et al., 1998; Ollmann et al., 1997; Quillan et al., 1998; Yang et al., 1999). More recently, evidence has been presented that AgRP may actually be an inverse agonist at this receptor (Nijenhuis et al., 1997; Haskell-Luevano and Monck, 2001). Hence, it may be the case that during lactation, a reduction in anorexigenic tone exerted by α-melanotropin may allow for the strong orexigenic action of AgRP, which in turn adds with the stimulatory effect of NPY, exerted through its own receptors. Thus, the limited efficacy of antagonizing NPY alone in lactating rats seen in the present studies could be explained by an unrestrained orexigenic action of AgRP; the greater effectiveness of the combined NPY antagonist/α-MSH treatment in reducing feeding would then reflect inhibition of the AgRP influence.

Studies on expression of the NPY, AgRP, and proopiomelanocortin genes during lactation provide support

for this hypothesis. Thus, the levels of NPY and AgRP mRNAs are increased, while proopiomelanocortin mRNA is decreased, in arcuate nucleus during lactation in rats (Chen et al., 1999; Li et al., 1999; Malabu et al., 1994; Smith, 1993). It is interesting to note that proopiomelanocortin mRNA expression in arcuate neurons is also decreased in another animal model of hyperphagia produced by neurotoxin lesioning of the medial basal hypothalamus (Dube et al., 2000), suggesting that removal of this strong inhibitory influence may be a prerequisite for hyperphagia. If these changes in mRNA expression reflect increased transmission by NPY and AgRP and decreased signaling by α-MSH, then the pharmacological results of the present studies are consistent with the hypothesis that increased NPY and AgRP orexigenic signals, concomitant with a reduction in anorexigenic actions of α-MSH, are critical for the profound hyperphagia of lactation. Further investigations will also be needed to identify and characterize the actions and interactions of other orexigenic and anorexigenic neuromessengers during lactation.

The present studies, when considered together with previous work from this laboratory (Wang et al., 1996), suggest that increased synthesis and release of NPY in hypothalamic systems may integrate critical neuroendocrine and behavioral adaptations during lactation. Thus, the novel expression of NPY in a subgroup of tuberoinfundibular dopamine neurons of the arcuate nucleus may provide an additional inhibitory signal to the lactotrophe to help shape the episodic release of prolactin in response to the suckling stimulus. In addition, physiological signals associated with lactation, e.g., reduced circulating leptin (Pickavance et al., 1998; Johnstone and Higuchi, 2001), appear to evoke changes in expression of critical orexigenic and anorexigenic neupeptides in hypothalamus that in turn regulate appetitive behaviors to meet the enhanced energy needs associated with milk synthesis.

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