Pharmacology Biochemistry & Behavior, Vol. 54, No. 4, pp. 771–777, 1996

0091-3057/96 $15.00 + .00

PII S0091-3057(96)00019-6

Behavioral Effects of Naloxone on Neuropeptide Y-Induced Feeding

JEFFREY M. RUDSKI,¶ MARTHA GRACE,* MICHAEL A. KUSKOWSKI,*
CHARLES J. BILLINGTON*§ AND ALLEN S. LEVINE*†‡§¶[1]

*Veterans Affairs Medical Center, One Veterans Drive,
Research Service (151), Minneapolis, MN 55417
†Departments of Psychiatry, ‡Food Science and Nutrition, §Medicine, ¶Psychology,
Minneapolis, MN 55455

Received 10 May 1995; Revised 3 September 1995; Accepted 22 September 1995

RUDSKI, J. M., M. GRACE, M. A. KUSKOWSKI, C. J. BILLINGTON AND A. S. LEVINE. *Behavioral effects of naloxone on neuropeptide Y-induced feeding.* PHARMACOL BIOCHEM BEHAV **54**(4) 771–777, 1996.—We evaluated the effect of naloxone on neuropeptide Y (NPY)-induced feeding behavior using two methods; operant chambers and observational analysis. In the first study rats were trained on a FR 80 (first pellet) FR 3 (subsequent pellets) reinforcement schedule. Following training, rats were injected with NPY (intraventricular, 5 μg) and various doses of naloxone (subcutaneous, 0, 0.1, 0.3, 1, 3, and 10 mg/kg). NPY significantly increased the number of pellets consumed during the one hour session and naloxone (1, 3, and 10 mg/kg) blocked this effect. NPY failed to alter the time to the first response, but did decrease the time needed to complete the first ratio (FR 80). Naloxone (3 and 10 mg/kg) increased the latency to the first response and blocked NPY's effect on completion of the first ratio. In the second study, we observed rats continually following injection of saline, NPY (5 μg ICV) and/or naloxone (1 mg/kg SC). NPY increased food intake during the 1-h session and naloxone blocked this effect. NPY decreased the latency to eat, but naloxone failed to significantly antagonize this effect. The amount of time spent eating was greater in the NPY group compared to the saline group and naloxone antagonized this effect. Lag sequential analysis indicated that NPY induced a move-eat-move behavioral sequence that disappeared following naloxone administration. These data lend support to the notion that opioids are involved in maintenance of NPY-induced feeding but affect meal initiation in a minor way. Only relatively high doses of naloxone (3 and 10 mg/kg) altered NPY-induced changes in meal initiation.

Naloxone Neuropeptide Y Induced feeding

IT is well known that naloxone decreases nocturnal feeding as well as feeding induced by various neuroregulatory substances and by food deprivation (7,20). The manner in which opioid receptor antagonism decreases feeding is unclear. Kirkham used observation analysis to evaluate the effect of naloxone on eating and other behaviors in rats deprived of food for 6 h (15). Naloxone reduced the latency to approach food and hastened the termination of eating during the test period. In a second study Kirkham used a runway to monitor changes in food motivation and food consumption after naloxone administration (16). Naloxone did not reduce the speed of traversing the runway to acquire food, but decreased the amount of food consumed once the rats were in the goal box. Cooper and Holtzman (6) found that naloxone decreased drinking duration without altering latency to begin drinking. Naloxone has also been shown to shorten the duration of nightime meals and to extend the postmeal intervals without altering meal frequency or eating rate within a meal (17). We found that naloxone failed to affect acquisition time of the first pellet in an FR 80 (first pellet) FR 3 (subsequent pellets) reinforcement schedule, but did decrease the number of pellets consumed (27). These data suggest that naloxone affects maintenance rather than initiation of feeding.

Neuropeptide Y is the most potent orexigenic peptide, enhancing feeding after intracerebroventricular or hypothalamic administration (1). Repeated administration of NPY to rats for 3 weeks increased food intake and resulted in weight gain (34). NPY has been reported to stimulate food reinforced behavior in rats placed on various reinforcement schedules (11). Feeding following NPY administration is characterized by intake initiation within 20 min of injections, increased time spent eating, increased local rate of ingestion, and continuation

[1]To whom requests for reprints should be addressed.

of feeding over an extended time period (8,13,22,23,25,33,35). Thus, NPY appears to be involved in both the initiation and maintenance of feeding. No NPY receptor(s) antagonists have been synthesized that block the effects of NPY on feeding behavior. However, peripheral and central administration of naloxone has been shown to decrease NPY-induced feeding, suggesting that NPY's effect on feeding may be dependent on an opioidergic pathway (21,23,33).

Because opioids are thought to be involved in the maintenance of feeding, it is possible that NPY initiates food intake and opioids maintain this feeding. While naloxone decreases NPY-induced feeding, it is not clear whether it does so by affecting initiation or maintenance of the meal, rate of eating, number of meals, or if naloxone induces an alternative behavior that interferes with feeding. Questions regarding NPY's effects on initiation and maintenance of feeding were addressed in the current study by using a reinforcement schedule sensitive to both of these components of feeding; that is, rats were required to emit 80 (FR 80) presses to acquire the first pellet (initiation), and three lever presses (FR 3) to obtain each subsequent pellet (maintenance). Furthermore, the pattern of feeding over a 60-min session was tracked by recording the number of pellets delivered every 2 min. We also continuously observed and recorded feeding and other behaviors exhibited by rats given NPY and naloxone in their home cages.

METHOD

Subjects

Two sets of male Sprague–Dawley rats (Harlan, Madison, WI) were housed in individual wire hanging cages and maintained in a temperature-controlled vivarium (20°C) on a 12 L:12 D cycle (lights on at 0700 h). The first group of rats ($n = 6$) was maintained at 85% of their free-feeding weights (300–325 g) during behavioral training. Following training, free access to food (Teklad lab chow) was reinstated. The second group of rats ($n = 18$) weighed 400–475 g at the start of the experimental procedure. Tap water was available ad lib in home cages.

Apparatus

In the first study, experimental sessions were conducted in six standard operant chambers (Coulbourn Instruments, Inc., Lehigh Valley, PA). In the second study, sessions were conducted in the home cages kept in a quiet room. Operant chambers were enclosed in an isolation cubicle (Model E10-20, Coulbourn Instruments, Inc.) to attenuate outside noise, and equipped with an exhaust fan to supply ventilation. Sessions were controlled and data were recorded by a Zeos 486 computer located in the same room as the chambers.

Surgery

Rats were anesthetized with 60 mg/kg IP Nembutal, and a 20 gauge guide cannula (Plastics One, Roanoke, VA) was implanted in the right lateral ventricle (1.5 mm lateral, 1.0 mm posterior, and 3.5 mm below bregma, incisor bar set 3 mm below the interaural line). Rats were allowed to recover at least 7 days before beginning the experimental procedures. Cannula placement and patency was verified by injecting 0.1 nmol Angiotensin II and recording fluid intake over 15 min. Angiotensin is a potent dipsogenic agent, and if rats consumed more than 6 ml of fluid over 15 min, the cannula was considered to be properly placed and patent. Tests of patency with angiotensin were conducted at the commencement and termination of drug administration. All rats in the present study met this criterion. Experiments were not conducted for at least one day after angiotensin injection.

Drug Preparation and Administration

Neuropeptide Y (Peninsula Laboratories) was diluted in isotonic (0.9%) saline to provide a concentration of 1 μg/μl and stored in aliquots at −20°C. The NPY was thawed prior to injections and administered ICV, injected over 45 s at a constant volume of 5.0 μl, 20 min before sessions. In Experiment 1, naloxone was diluted in isotonic saline and administered subcutaneously (SC) immediately following NPY administration. In Experiment 2, naloxone was injected SC 15 min prior to ICV injection of NPY. Prepared solutions of naloxone were stored at 4°C.

Experimental Design

Experiment 1. In the first experiment, rats were deprived to 85% of their free-feeding weights and trained to press the left lever to obtain food under a FR 80 (first pellet) FR 3 (subsequent pellets) reinforcement schedule. Training took 7 to 10 days. Free access to food in home cages was restored, and rats were run daily under the FR 80–FR 3 reinforcement schedule for 1 week. Rats were injected with NPY (5 μg) 60 min following each of the last two training sessions. A saline-saline control, and NPY (5 μg) administered in conjunction with various doses of SC naloxone (0, 0.1, 0.3, 1.0, 3.0, and 10.0 mg/kg) were administered to each rat over 7 consecutive days in a randomized order. Naloxone injections immediately followed NPY administration and rats were placed in the operant chambers approximately 15 min later.

The effect of NPY alone and NPY-naloxone combinations on latency to initiate responding (i.e., initial latency), time required to complete the initial ratio following the initial response (i.e., FR 80 completion time), and number of pellets consumed over the session were recorded. Rats not responding during the session were assigned an initial response latency and a FR 80 completion time of 60 min. Rats which emitted some initial responses but did not complete the initial ratio were assigned an FR 80 completion time of 60 min. Main effects for initial response latency, subsequent FR 80 completion time, and total number of pellets consumed were analyzed using a repeated measures analysis of variance (RMANOVA).

In order to examine the pattern of responding over the session, the number of pellets consumed after the first reinforcer delivery was accumulated into 2-min bins. If latency to acquire the first pellet precluded access to all bins, they were left empty and not included in the data analysis (e.g., with session length being 60 min, a rat that required 30 min to acquire the first pellet only had data entered in the first 15 2-min bins, with the remaining bins remaining empty for that subject). Due to the extremely large number of post hoc comparisons required in examining 2-min bins, data were grouped into 10-min bins and compared with a RMANOVA (treatment × 10-min bin). Because 3.0 and 10.0 mg/kg naloxone completely eliminated responding in three of six rats, these doses were not included in the statistical analysis. Furthermore, because most of the terminal bins (40–60 min) were left empty due to the time necessary to acquire the initial pellet, sample size was too small to test statistically. Post hoc comparisons were analyzed with Fisher's least significant difference test.

Experiment 2. In this experiment we evaluated the effect of NPY with or without naloxone on rat behavior by direct

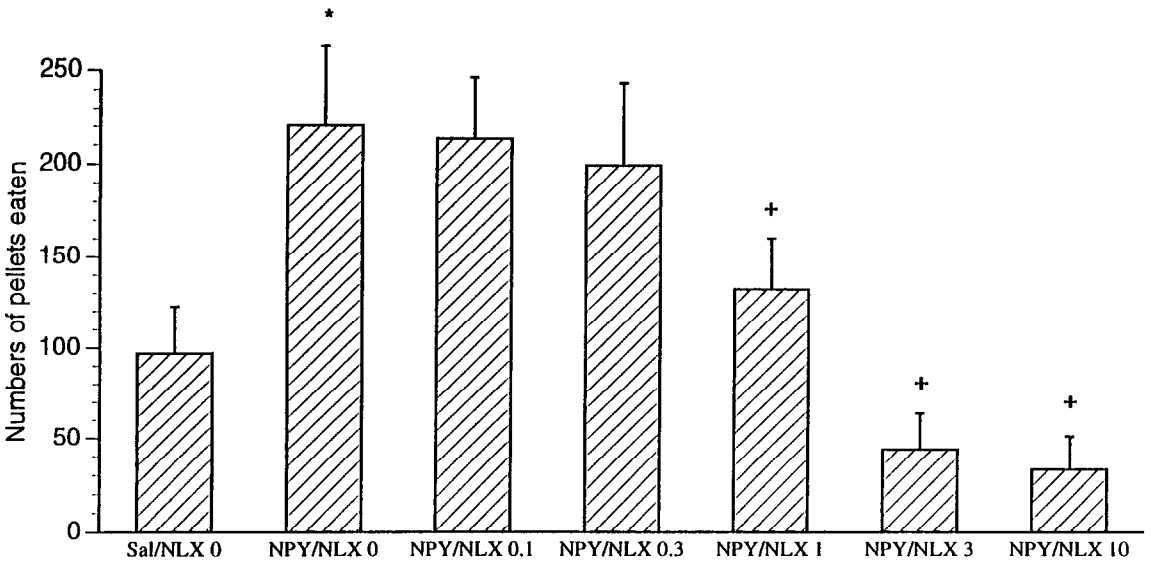

FIG. 1. Effect of naloxone on number of pellets (45 mg) ingested following intraventricular injection of NPY (5 μg) to rats responding on an FR 80 FR 3 reinforcement schedule. $*p < 0.05$ compared to saline/NLX 0 control group; $+p < 0.05$ compared to NPY/NLX 0 group.

observation. Rats were divided into three groups of six each and injected with either saline (ICV, 5 μl) , NPY (ICV, 5 μg/5 μl) or NPY (ICV, 5 μg/5 μl) + naloxone (1 mg/kg SC). Rats were pretreated with naloxone 15 min prior to injection of NPY or saline. After NPY or saline administration rats were returned to their home cages (hanging wire cages), which contained preweighed food pellets (Teklad lab chow) and a water bottle. A trained observer, unaware of the order of treatment examined each rat's behavior continually for a 60-min time period. Behaviors were recorded using a bar code scanner (Time Wand Optical Bar Code: Videx Inc., Corvalis, OR), which the observer scanned across various bar codes printed on a sheet of paper. The data collected with a battery operated Time Wand was downloaded to a Macintosh computer. The time wand recorded the onset of a given behavior and time spent on each activity was calculated by subtracting the start time of one behavior from the start time of the next behavior. Food intake was measured at the end of each 60-min time period. No more than four rats were studied each day between 0900–1400 h. Behaviors that were recorded included eating (food chewed and swallowed), drinking (licking of sipper tube and apparent swallowing), grooming (licking of paws, stomach, hindquarters; rubbing paws over face; scratching), moving body (moving around cage), moving head (moving head, but not body), resting (not moving, eyes open or shut), rearing (standing on rear legs), sniffing (moving nares without moving about cage), and teeth chatter (movement of jaw in an oscillating manner). Data were recorded as the amount of time spent on each activity and were converted to percent time spent on each activity. These data are expressed as the means ± SEM and were analyzed by RMANOVA. Also, latency to the first meal (at least 16 s of eating behavior) was recorded. The average number of meals, intermeal interval, length of eating episodes and rate of intake (g/min) were calculated from the observed results. Means were compared using Fisher's protected least significant difference test.

Behavioral patterns were analyzed using lag sequential analysis as previously described (22,30). Lag sequential analysis is a nonparametric strategy for testing the independence of behavioral states in sequential data that allows the identification of significantly likely (or unlikely) behavior sequences following a criterion behavior. It involves a comparison of the probability of occurrence of a given behavior with the probability of that behavior's occurrence conditional on its following (or preceding) some criterion behavior. A behavioral sequence is said to be established if the conditional probability is significantly greater than the unconditional probability. This analysis requires a large number of observations; therefore, we collapsed observational data across all animals within a given treatment condition.

RESULTS

Experiment 1

NPY administration increased food intake (Fig. 1), an effect suppressed by naloxone doses of 1.0 mg/kg and greater, $F(6, 41) = 8.58, p < 0.0001$. NPY administration was characterized by continued eating throughout the session ($F = 6.87$, $p < 0.0001$) (Fig. 2). Naloxone doses (0.1, 0.3 mg/kg) that did not suppress NPY's effect on intake produced an intake pattern throughout sessions similar to NPY, whereas 1.0 mg/kg naloxone produced intake patterns statistically similar to the control condition. Three of the six rats injected with 3.0 mg/kg and 10.0 mg/kg naloxone did not complete the initial ratio. Rats responding following high naloxone doses (3 and 10 mg/kg) showed little feeding late in the session (data not shown).

NPY administration did not alter time to first response (initial response latency) (Table 1). Coadministration of naloxone at doses of 3.0 mg/kg and higher increased latency to respond ($F = 3.91$, $p < 0.005$), although this may have been artifactual, resulting from the default value of 60 min being assigned to rats not responding. Once responding commenced, NPY administration decreased FR 80 completion time (Table 1). Naloxone (3 mg/kg or higher) also suppressed NPY's effect

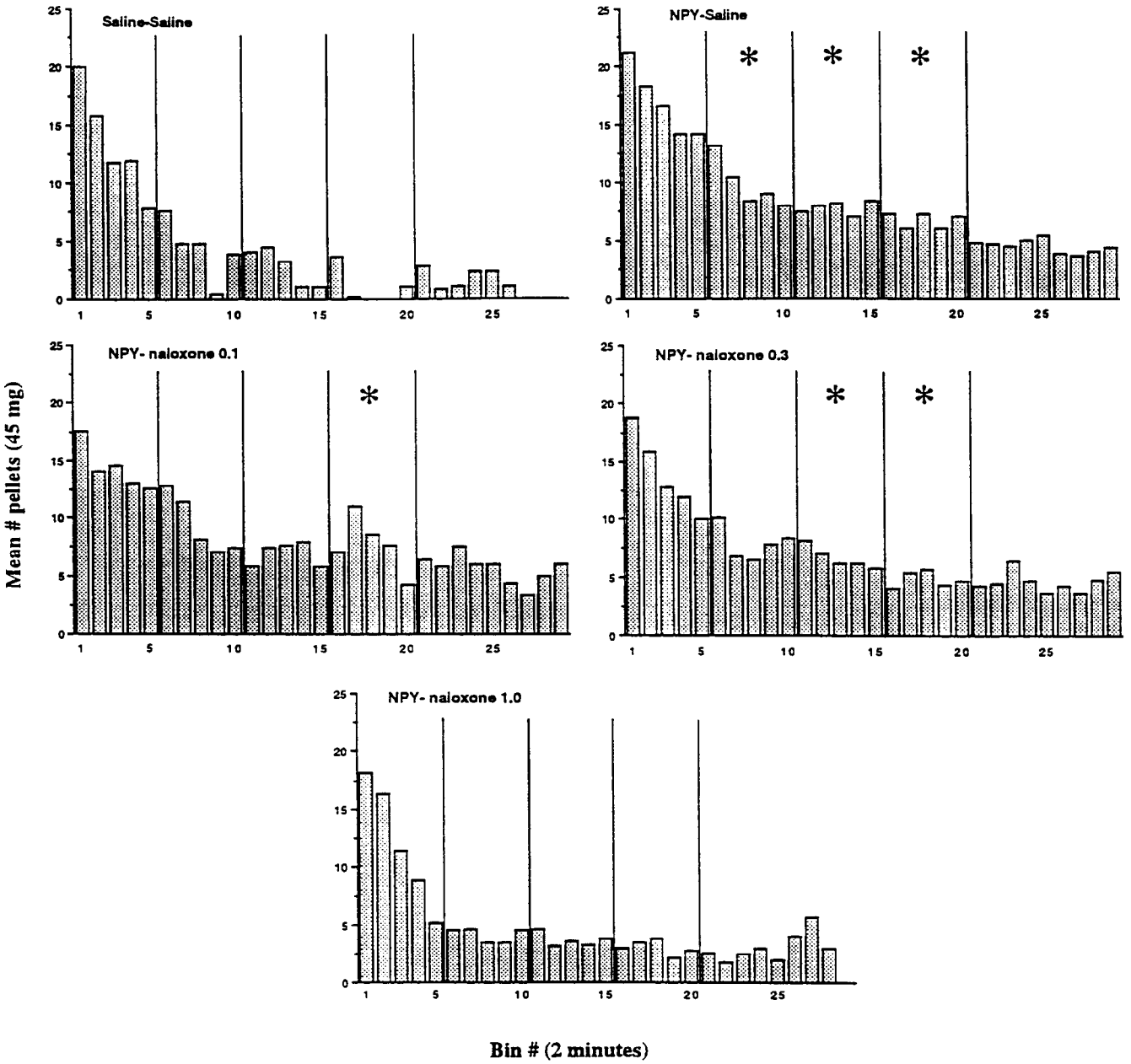

FIG. 2. Effect of naloxone on number of pellets (45 mg) ingested every 2 min following intraventricular injection of NPY (5 μg) to rats responding on an FR 80 FR 3 reinforcement schedule. $*p < 0.05$ compared to saline/NLX 0 control group.

of decreasing FR 80 completion time ($p < 0.05$), although again, this might have been artifactual.

Experiment 2

Food intake was significantly increased ($p < 0.05$) by central administration of 5 μg of NPY compared to the vehicle control and naloxone administration (1 mg/kg) blocked this increase in food intake (Table 2). NPY significantly decreased the latency to eat compared to the control group and naloxone partially antagonized this effect ($p < 0.05$ compared to saline group but not compared to NPY/sal group). There was a suggestion that drug administration altered the number of meals (meal $\geq$ 16 s spent eating) (Table 2). Based on the Fisher's least significant difference test ($p < 0.05$), NPY increased the number of meals compared to the saline control but naloxone did not reverse this effect. There was no effect of drug administration on intermeal interval, length of each eating episode or on the rate of food intake (data not shown). Also, there was no effect of naloxone on the amount of time to the last eating episode either during the first 30 min of the study (NPY: 20 ± 3 s; NPY/naloxone: 17 ± 4 s) or during the entire 60-min session (NPY: 36 ± 9 s; NPY/naloxone: 46 ± 7 s).

The results of the lag sequential analysis demonstrated a difference in behavioral pattern between groups. There was a significantly increased probability ($p < 0.01$) that moving

TABLE 1

EFFECT OF NALOXONE ON TIME TO FIRST RESPONSE AND TIME TO COMPLETE THE FIRST RATIO (FR 80) FOLLOWING INTRAVENTRICULAR INJECTION OF NPY(5 μg).

Drug Treatment	Time to 1st Response	Time to Complete FR 80
Sal/NLX 0	96 ± 42 s	1125 ± 632 s
NPY/NLX 0	91 ± 27 s	592 ± 521 s
NPY/NLX 0.1	46 ± 8 s	372 ± 177 s
NPY/NLX 0.3	139 ± 65 s	254 ± 170 s
NPY/NLX 1	166 ± 97 s	495 ± 185 s
NPY/NLX 3	1459 ± 613 s*	2049 ± 638 s*
NPY/NLX 10	902 ± 261 s*	2276 ± 595 s*

*$p < 0.05$ compared to NPY/NLX 0 group.

followed or preceded eating in the NPY group. The unconditional probability that a rat would move was 0.39, whereas the conditional probability that a rat would move after eating was 0.53 and 0.48 before eating. In contrast, neither the saline nor NPY + naloxone groups demonstrated an increase in probability of moving after eating. In the control group no one behavior was more likely to occur before or after eating, whereas NPY induced a move-eat-move sequence. This NPY-induced shift in behavioral sequence was antagonized by naloxone administration.

The amount of time spent eating was greater in the NPY group compared to the saline group and naloxone blocked this effect (Fig. 3). The time spent on other behaviors was unaltered by drug administration (Fig. 3).

DISCUSSION

NPY administration increased food intake contingent upon operant responding. This effect was suppressed by administration of naloxone doses of 1.0 mg/kg or higher. Both initiation and maintenance of feeding were stimulated by NPY, and relatively high doses of naloxone (3 and 10 mg/kg) affected both of these components.

NPY-induced increases in free feeding are characterized by decreased latency to begin eating, more time spent eating, increasing meal size, and duration (8,19,22,23,32,33), as well as with an increased rate of feeding (4,13,25). The current study produced a similar profile. Examination of intake in 10-min bins indicated that NPY increased feeding relative to controls as sessions progressed, indicating that NPY prolonged time spent eating.

NPY's effects on food reinforced behavior appear to be different from that induced by orexigenic doses of opiates. Whereas NPY successfully increases food intake contingent upon operant responding in satiated animals, opiate administration typically has no effect (28), with the only positive response obtained with butorphanol (26) or buprenorphine (29). Butorphanol and buprenorphine increased food intake contingent upon a reinforcement schedule identical to the one used in the current study. Neither of these opiates decreased the time necessary to complete the first ratio (FR 80); however, buprenorphine did decrease the latency to the first lever press. Also, both butorphanol and buprenorphine increased food intake across the experimental session. These data support the idea that the opiates/opioids have their major effects on maintenance rather than initiation of feeding (15,16). In contrast to opioids, NPY also appears to be involved in meal initiation. NPY levels in the PVN increase immediately preceding meals and fall in response to that meal (14). Furthermore, endogenous hypothalamic levels and release of NPY correspond to an animal's circadian rhythm of feeding; increasing at the beginning of the dark cycle, the time of day when most ingestion occurs (12,24).

Peripheral and central naloxone administration decrease NPY-induced feeding (2,21,23,33). Previous studies examining NPY's operant effects have not examined whether naloxone suppresses NPY's orexigenic effects. The current study indicates that this is, in fact, the case. Naloxone suppressed NPY's effects on operant responding in both decreasing the time required to complete the initial ratio (initiation, 3.0 and 10.0 mg/kg doses) and total number of pellets consumed (maintenance, 1.0, 3.0, and 10.0 mg/kg doses). We previously found that in deprivation induced feeding, naloxone affected the overall time to acquire the first pellet (FR 80) and maintenance of feeding (FR3); however, the effect on initiation was very mild (27). Furthermore, the time to acquire the first pellet was unaffected by naloxone, at doses as high as 10 mg/kg in food-restricted rats. The pattern of intake in the current study suggests that naloxone (1.0 mg/kg and higher) decreases NPY-induced feeding by blunting NPY's potency throughout the feeding session, even at the 1 mg/kg dose (Fig. 2).

In the home cage study NPY also increased feeding, an effect blocked by coadministration of naloxone (1.0 mg/kg). The finding that NPY induced a move-eat-move sequence substantiates previous findings indicating that NPY increases activity compared to food deprived rats (22). Administration of naloxone eliminated this behavioral pattern. In the current study naloxone failed to block the NPY-induced decrease in latency to eat. However, the latency to eat was still longer than the saline control group. NPY appeared to increase the number of meals, but naloxone did not alter this effect. No other measures of feeding behavior such as intermeal interval, rate of food intake, or length of meals were affected by naloxone. Naloxone did not alter any other behavior than time spent eating. Thus, the behavioral analysis data are consistent with findings in the operant environment; that is, that relatively low naloxone doses (1 mg/kg) fail to alter initiation of feeding, but do decrease total food intake apparently by decreasing intake throughout the experimental session. In contrast, Kirkham reported that naloxone (5 mg/kg) decreased the latency to the end of the final eating bout, thus shortening the eating

TABLE 2

EFFECT OF NAXOLONE (1 mg/kg) ON NPY (5 μg)-INDUCED FEEDING PARAMETERS

	Vehicle	NPY	NPY+NLX	ANOVA
Food intake (g)	1.1 ± 0.5	4.1 ± 0.8*	1.5 ± 0.4†	$F = 16.4$, $p = 0.013$
Latency to 1st meal (min)	34.9 ± 9.3	2.7 ± 0.7*	8.4 ± 3.4*	$F = 10.4$, $p = 0.004$
# meals	2.7 ± 1.3	15.3 ± 5.1*	7.5 ± 5.7	$F = 3.28$, $p = 0.081$

*$p < 0.05$ compared to vehicle group, †$p < 0.05$ compared to NPY group.

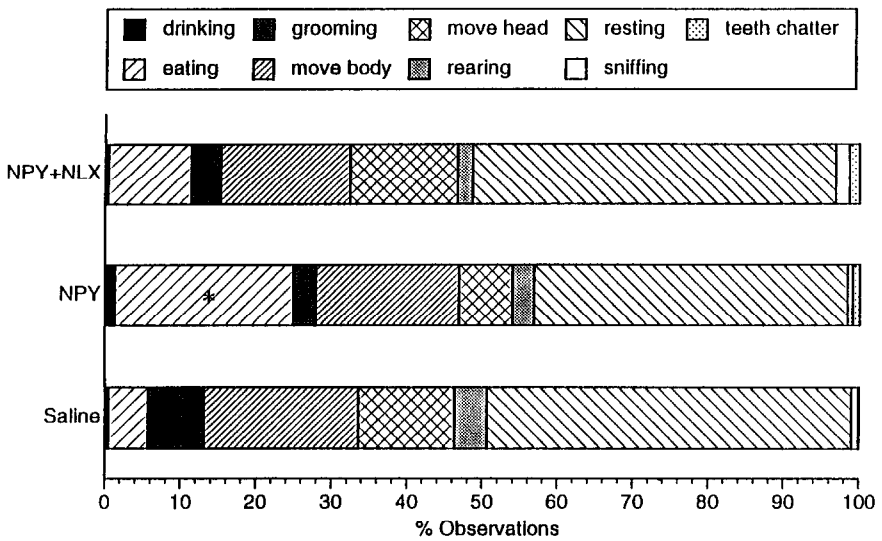

FIG. 3. Effect of NPY (5 μg) and/or naloxone (1 mg/kg) on portion of time spent on various behaviors. $*p < 0.05$ compared to saline control group.

session (15). While lower doses decreased feeding, such doses had no effect on other parameters of feeding behavior including eating rate, duration of eating, latency to onset of eating, or latency to the end of the final eating bout.

Suppression of NPY-induced feeding by naloxone might reflect a general depression of food-motivation following its administration, or a more specific opioid involvement in the antagonism of NPY's effects. Naloxone administration decreases feeding induced by a variety of measures including food deprivation, tail-pinch, availability of palatable food, benzodiazepines, clonidine, neuropeptide Y, 2-deoxyglucose, or electrical brain stimulation, across many species (7,20). Thus, food intake in the current study might have reflected an interaction of increased intake due to NPY and decrease due to naloxone, with independent physiological mechanisms. However, a case can also be made for a specific NPY-opioid interaction. Naloxone is more potent in suppressing NPY induced feeding than that induced by norepinephrine or muscimol (21). In addition to suppressing NPY's effects on food intake, naloxone also alters NPY's effects on energy expenditure (18) and memory (5). Naloxone administration has been reported to increase in vitro release of NPY in a dose-dependent manner (31) and to increase plasma levels of NPY (3). Furthermore, there appears to be anatomical overlap between the two systems with opioidergic and NPYergic synapses occurring in close spatial proximity (9,10).

In summary, as in studies examining its effects on free-feeding, NPY-induced feeding in operant chambers is characterized by effects on both initiation and maintenance of feeding. Naloxone has similar effects on NPY-induced changes in operant-contingent food intake and NPY-induced changes in free feeding. These effects of naloxone on NPY-induced feeding appear to resemble those observed in free feeding and restriction or deprivation-induced feeding; that is, a limited effect on initiation and a more potent effect on meal maintenance. The anorectic effect of naloxone seems to simply represent a blunting of the amount of food ingested throughout the experimental session.

ACKNOWLEDGEMENTS

This work was supported by the Department of Veterans Affairs, by National Institute of Drug Abuse Grant DA-03999, and by the National Institutes of Diabetes and Digestive and Kidney Diseases Grant DK-42698.

REFERENCES

1. Billington, C. J.; Levine, A. S. Hypothalamic neuropeptide Y regulation of feeding and energy metabolism. Curr. Opin. Neurobiol. 2:847–851; 1992.
2. Borisova, E. V.; Sabo, G.; Zhuravlev, B. V.; Telegdi, G. Effects of neuropeptide Y on behavior and noradrenaline level in the brain. Biull. Eksp. Biol. Med. 108:132–135; 1989.
3. Chang, C. L.; Tsai, Y. C.; Lin, S. S.; Cheng, J. T. Effect of premedication on the changes of neuropeptide Y (NPY) in anesthesia. Ma Tsui Hsueh Tsa Chi Anaesthesiol. Sinica 30:147–152; 1992.
4. Clark, J. T.; Kalra, P. S.; Crowley, W. R.; Kalra, S. P. Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. Endocrinology 115:427–429; 1984.
5. Cleary, J.; Semotuk, M.; Levine, A. S. Effects of neuropeptide Y on short-term memory. Brain Res. 653:210–214; 1994.
6. Cooper, S. J.; Holtzman, S. G. Patterns of drinking in the rat following the administration of opiate antagonists. Pharmacol. Biochem. Behav. 19:505–511; 1983.
7. Cooper, S. J.; Jackson, A.; Kirkham, T. C.; Turkish, S. Endorphins, opiates and food intake. In: Rodgers, R. J.; Cooper, S. J., eds. Endorphins, opiates and behavioural processes. London: John Wiley & Sons, Ltd.; 1988:143–186.
8. Corp, E. S.; Melville, L. D.; Greenberg, D.; Gibbs, J.; Smith, G. P. Effect of fourth ventricular neuropeptide Y and peptide YY on ingestive and other behaviors. Am. J. Physiol. 259:R317–R323; 1990.
9. Csiffary, A.; Gores, T. J.; Palkovits, M. Neuropeptide Y innervation of ACTH-immunoreactive neurons in the arcuate nucleus of rats: A correlated light and electron microscope double immunolabelling study. Brain Res. 506:215–222; 1990.
10. Horvath, T. L.; Naftolin, F.; Kalra, S. P.; Leranth, C. Neuropep-

tide-Y innervation of β-endorphin containing cells in the rat mediobasal hypothalamus. Endocrinology 131:2461–2467; 1992.
11. Jewett, D. C.; Levine, A. S.; Cleary, J.; Schall, D. W.; Thompson, T. The effect of intracerebroventricular (ICV) NPY on food reinforced behavior. Soc. Neurosci. Abstr. 15:894, 1989.
12. Jhanwar-Uniyal, M.; Beck, B.; Burlet, C.; Leibowitz, S. F. Diurnal rhythm of neuropeptide Y-like immunoreactivity in the suprachiasmatic arcuate and paraventricular nuclei and other hypothalamic sites. Brain Res. 536:331–334; 1990.
13. Kalra, S. P.; Dube, M. G.; Kalra, P. S. Continuous intraventricular infusion of neuropeptide Y evokes episodic food intake in satiated female rats: Effects of adrenalectomy and cholecystokinin. Peptides 9:723–728; 1988.
14. Kalra, S. P.; Dube, M. G.; Sahu, A.; Phelps, C. P.; Kalra, P. S. Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. Proc. Natl. Acad. Sci. USA 88:10931–10935; 1991.
15. Kirkham, T. C.; Blundell, J. E. Dual action of naloxone on feeding revealed by behavioural analysis: Separate effects on initiation and termination of eating. Appetite 5:45–52; 1984.
16. Kirkham, T. C.; Blundell, J. E. Effect of naloxone and naltrexone on the development of satiation measured in the runway: Comparisons with *d*-amphetamine and *d*-fenfluramine. Pharmacol. Biochem. Behav. 25:123–128; 1986.
17. Kirkham, T. C.; Blundell, J. E. Effects of naloxone and naltrexone on meal patterns of freely feeding rats. Pharmacol. Biochem. Behav. 26:515–520; 1987.
18. Kotz, C. M.; Grace, M. K.; Briggs, J.; Levine, A. S.; Billington, C. J. Effects of opioid antagonists naloxone and naltrexone on neuropeptide Y-induced feeding and brown fat thermogenesis in the rat: Neural site of action. J. Clin. Invest. 96:163–170; 1995.
19. Leibowitz, S. F.; Alexander, J. T. Analysis of neuropeptide Y-induced feeding: Dissociation of Y_1 and Y_2 receptor effects on natural meal patterns. Peptides 12:1251–1260; 1991.
20. Levine, A. S.; Billington, C. J. Opioids. Are they regulators of feeding? Ann. N.Y. Acad. Sci. 575:209–219; 1989.
21. Levine, A. S.; Grace, M.; Billington, C. J. The effect of centrally administered naloxone on deprivation and drug-induced feeding. Pharmacol. Biochem. Behav. 36:409–412; 1990.
22. Levine, A. S.; Kuskowski, M. A.; Grace, M.; Billington, C. J. Food deprivation-induced vs. drug-induced feeding: A behavioral evaluation. Am. J. Physiol. 260:R546–R552; 1991.
23. Levine, A. S.; Morley, J. E. Neuropeptide Y: A potent inducer of consummatory behavior in rats. Peptides 5:1025–1029; 1984.
24. McKibbin, P. E.; Rogers, P.; Williams, G. Increased neuropeptide Y concentrations in the lateral hypothalamic area of the rat after the onset of darkness: Possible relevance to the circadian periodicity of feeding behavior. Life Sci. 48:2527–2533; 1991.
25. Paez, X.; Nyce, J. W.; Myers, R. D. Differential feeding responses evoked in the rat by NPY and NPY_{1-27} injected intracerebroventricularly. Pharmacol. Biochem. Behav. 38:379–384; 1991.
26. Rudski, J. M.; Billington, C. J.; Levine, A. S. Butorphanol increases food-reinforced operant responding in satiated rats. Pharmacol. Biochem. Behav. 49:843–847; 1994.
27. Rudski, J. M.; Billington, C. J.; Levine, A. S. Naloxone's effect on operant responding depend upon level of deprivation. Pharmacol. Biochem. Behav. 49:377–383; 1994.
28. Rudski, J. M.; Schaal, D. W.; Thompson, T.; Cleary, J.; Billington, C. J.; Levine, A. S. Methadone and feeding: Sources of differences between home cage and operant chamber assessment procedures. 49(1):143–146; 1994.
29. Rudski, J. M.; Thomas, D.; Billington, C. J.; Levine, A. S. Buprenorphine increases intake of freely available and operant-contingent food in satiated rats. Pharmacol. Biochem. Behav. 50:271–276; 1995.
30. Sackett, G. P. Measurement in observational behavior. In: Sackett, G. P. ed. Observing behavior: Data collection and analysis methods. Baltimore, MD: University Park Press; 1978:25–43.
31. Sahu, A.; Crowley, W. R.; Kalra, S. P. An opioid-neuropeptide-Y transmission line to luteinizing hormone (LH)-releasing hormone neurons: A role in the induction of LH surge. Endocrinology 126:876–883; 1990.
32. Sahu, A.; Dube, M. G.; Kalra, S. P.; Kalra, P. S. Bilateral neural transections at the level of mesencephalon increase food intake and reduce latency to onset of feeding in response to neuropeptide Y. Peptides 9:1268–1273; 1988.
33. Schick, R. R.; Schusdziarra, V.; Nussbaumer, C.; Classen, M. Neuropeptide Y and food intake in fasted rats: Effect of naloxone and site of action. Brain Res. 552:232–239; 1991.
34. Stanley, B. G.; Anderson, K. C.; Grayson, M. H.; Leibowitz, S. F. Repeated hypothalamic stimulation with neuropeptide Y increases daily carbohydrate and fat intake and body weight gain in female rats. Physiol. Behav. 46:173–177; 1989.
35. Stanley, B. G.; Leibowitz, S. F. Neuropeptide Y injected in the paraventricular hypothalamus: A powerful stimulant of feeding behavior. Proc. Natl. Acad. Sci. USA 82:3940–3943; 1985.