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Hypothalamic Sites Sensitive to Morphine and Naloxone: Effects on Feeding Behavior

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WOODS, J. S. AND S. F. LEIBOWITZ. *Hypothalamic sites sensitive to morphine and naloxone: Effects on feeding behavior.* PHARMACOL BIOCHEM BEHAV **23**(3) 431–438, 1985.—Three experiments investigated the feeding response of brain cannulated rats to hypothalamic injection of norepinephrine (NE), the opiate agonist morphine sulfate (MO) and the opiate antagonist naloxone (NAL). Morphine elicited feeding in a dose-dependent manner when injected into the paraventricular nucleus (PVN) of satiated rats, at doses of 0.78 to 100 nmoles, with a threshold dose of 1.56 nmoles. Naloxone, at doses of 3.13 to 200 nmoles, was injected into the PVN of food-deprived rats and was found to produce a dose-dependent suppression of feeding (threshold dose of 6.25 nmoles). Animals with brain cannulas aimed at the PVN, the perifornical hypothalamus (PFH), the dorsomedial (DMN) and ventromedial (VNM) nuclei were compared for their sensitivity to the feeding stimulatory effects of NE and MO (except in the DMN) and the feeding suppressive effects of NAL. Consistent with earlier reports, the PVN-cannulated animals exhibited a reliable increase in feeding after NE injection; the VMN cannula yielded a small feeding response, whereas the DMN and PFH were insensitive to NE. Morphine, in contrast, strongly stimulated eating after administration into PFH, as well as the PVN, apparently dissociating the NE and MO eating responses. The VMN, however, was generally unresponsive to both MO and NE. With regard to NAL's suppressive effect on feeding, the PVN and PFH, which were sensitive to MO, also exhibited responsiveness to opiate antagonism suggesting the existence in these areas of opiate receptors that modulate feeding. This contrasts with the DMN, where NAL had no effect on feeding and the VMN, where NAL was sensitive in the absence of MO responsiveness. These mapping studies suggest that the opiate receptors affecting eating behavior are most dense within the PVN and PFH but are either less dense or have variable responsiveness within the DMN and VMN.

Morphine Naloxone Norepinephrine Opiate α-Adrenergic Paraventricular nucleus Perifornical hypothalamus Dorsomedial nucleus Ventromedial nucleus Feeding behavior

THE discovery of opioid peptides in the brain has led to a vigorous search for their physiological roles. Several investigators have reported an increase in food intake with injection of opiate agonists or peptides, which has led to the hypothesis that the endogenous opiates are involved in the regulation of feeding behavior [2, 12, 26, 37, 41, 49, 54]. Consistent with this hypothesis, a number of studies have shown that the opiate antagonists produce an attenuation of food consumption in food-deprived rats [14, 15, 16, 18, 31, 41, 49]. This effect is believed to occur specifically at the opiate receptor and appears to be mediated centrally, since the quarternary form of NAL which does not pass the blood-brain barrier has no effect [5].

Although strong evidence has accumulated in support of a brain opiate system for control of food intake, studies involving direct central manipulations with opiate drugs are relatively scarce. Thus, the anatomical location of this hypothesized opiate system remains to be determined. One possible site may be the medial hypothalamus, where a few recent studies have revealed a stimulatory effect of opiates on feeding [12, 26, 37, 54]. One objective of the present study was to analyze more precisely, through a cannula mapping study, the area(s) of the hypothalamus that are sensitive to opiate stimulation. An additional objective was to examine the opiate antagonist NAL in the brain, to determine its effectiveness in altering feeding. To date, most studies which have described a NAL suppression of food intake effect have administered this compound peripherally. Three studies have injected it centrally, namely into the lateral ventricles [15, 18, 62], and conflicting results have been obtained. If opiate receptors do exist in the hypothalamus to potentiate feeding under physiological conditions, then hypothalamic administration of NAL should significantly suppress food intake through opiate receptor antagonism.

In the present study, the opiate agonist MO and the antagonist NAL were tested in the medial hypothalamus, specifically the paraventricular nucleus (PVN), to determine their effects on food intake at a wide range of doses. These compounds, in addition to the α-adrenergic agonist NE which also stimulates feeding, were then tested in four different hypothalamic areas to determine the central site of greatest sensitivity. The results obtained demonstrate that the PVN is sensitive to MO stimulation of feeding at a dose as low as 1.56 nmoles, and NAL suppression of feeding at a low dose of 6.25 nmoles. Furthermore, the PVN, in addition to a more lateral perifornical hypothalamic (PFH) site but in contrast to the ventromedial (VMN) and dorsomedial hypothalamic nuclei (DMN), appear to be the most sensitive sites to local administration of both an opiate agonist and antagonist. These results have been presented in a prelimi-

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nary form at the Proceedings of the 54th Annual Meeting of the Eastern Psychological Association [61].

METHOD

General Method

Fifty-seven male albino rats of the Sprague-Dawley strain (400–450 g) were used in this experiment. The animals were individually housed on a 12:12 hour light-dark schedule, (lights on at 6:00 a.m.), with free access to food and water. All animals were stereotaxically implanted with a unilateral 23-gauge cannula under pentobarbital anesthesia. This cannula was aimed at one of four brain sites. These sites and their coordinates were: PVN, −0.2/+0.3/+8.2 with tooth bar 3.1 mm above interaural line; PFH, −2.5/+1.5/−8.9 with tooth bar 3.1 mm above interaural line; DMN −2.5/+0.7/−8.4 with tooth bar in skull fat position; VMN, −2.5/+0.5/−9.5 with tooth bar in skull flat position. In Experiment 1, 10 rats, each with a cannula aimed at the PVN, were tested with MO at doses of 0.78 to 100 nmoles; in Experiment 2, an additional group of 10 rats with PVN cannulas were tested with NAL at doses of 3.13 to 200 nmoles. In Experiment 3, 36 animals were injected with 40 nmoles NE, 25 nmoles MO, and 50 nmoles NAL, into one of 4 intended brain sites. In Experiments 1 and 3, animals were placed on a sweetened milk-mash diet, consisting of 50 g ground pellets, 40 g sugar and 1.3 oz evaporated milk. The animals in Experiment 2 were maintained on Purina lab chow pellets.

During the post-operative recovery week, animals were handled and mock-injected to adapt them to the testing procedures. This process has been found to be particularly helpful in eliminating spontaneous meals which might occur during control vehicle tests, as well as in making the subjects receptive to drug manipulations. Testing was conducted every 2–3 days, with subjects receiving 2 drug injections and 1 vehicle injection per week. Pretest conditions consisted either of satiation with fresh food for 1 hour (Experiments 1 and 3), or food deprivation for 4 hours (Experiments 2 and 3). Drug or vehicle was then centrally administered, and a measured food dish was placed quietly in the corner of the cage. Food consumption was recorded at hourly intervals, except where indicated. For each drug dose or placement site, at least 4 trials were conducted per rat. For the dose-response tests in Experiment 1, the MO injection was begun at a relatively high dose of 25 nmoles and then reduced, by 50%, in a gradually descending order to 0.78 nmoles. The NAL tests for Experiment 2 were started at 100 nmoles and reduced by 50% in a gradually descending order to 3.125 nmoles. At the completion of these dose-response experiments, each rat was once again tested with moderate doses of MO (12.5 nmoles) or NAL (25 nmoles), to confirm stable sensitivity to the drug. For the cannula mapping experiment (Experiment 3), the rats with PVN, PFH or VMH cannulas were first tested under food-satiated conditions, with NE (40 nmoles), MO (25 nmoles) and saline according to a Latin Square sequence. The DMN, in contrast to the other 3 sites, was only tested with NE versus saline. All rats were then introduced to the 4-hour food-deprivation paradigm and tested with NAL (50 nmoles) and saline in counterbalanced order. Upon completion of testing, animals were sacrificed, and histology was performed to evaluate their cannula placements. Serial frozen brain sections (50 μ) were cut and stained with cresyl violet.

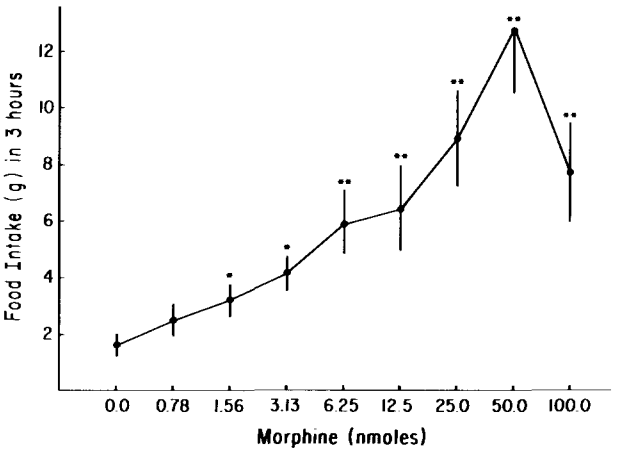

FIG. 1. Dose-response analysis of feeding response elicited by paraventricular nucleus (PVN) injection of morphine (MO) into satiated rats (N = 10). A reliable eating response with MO, compared with saline baseline (0.0 nmoles), was obtained at all doses, except for 0.78 nmoles (*$p<0.05$; **$p<0.01$).

The food intake scores for drug and control vehicle were analyzed with Repeated Measures ANOVA for Experiments 1 and 2. 1-Way and 2-way ANOVAs were performed on the data from Experiment 3, which involved tests in different brain sites in different groups of rats. To allow for direct comparisons between the drug responses associated with these different brain areas, the raw data were converted to difference scores (drug minus saline). Post-hoc evaluations were carried out using the Newman-Keuls method.

l-Norepinephrine-d-bitartrate (Sigma Chemical), morphine sulfate (Merck and Company), and naloxone (a kind gift from Endo Laboratories) were dissolved in bacteriostatic sodium chloride (0.9%) which also served as control solution. All injections were make in a volume of 0.5 μl, with a Hamilton 701-LT microliter syringe.

RESULTS

Experiment 1

MO sulfate, injected into the PVN of satiated rats, was found to produce a dose-dependent effect on food intake, $F(8,81)=2.8$, $p<0.01$, during 3 hours after injection (Fig. 1). The saline control baseline score was 1.7 g. The administration of 0.78 nmoles of MO appeared to elevate food intake (2.6 g) somewhat in comparison to the control score, but this effect was not statistically significant. The first significant effect was seen at 1.56 nmoles, (3.2 g, $p<0.01$), and this eating response increased to 12.6 g ($p<0.001$) at 50 nmoles of MO and then reduced somewhat to 7.8 g ($p<0.001$) at 100 nmoles. Analysis of the time course of this eating response showed that 80% of the animals initiated their response during the second hour after injection (Fig. 2). Continued, sometimes sporadic, eating was observed during the next hour.

Experiment 2

As shown in Fig. 3, NAL injected into the PVN of mildly food-deprived rats caused a dose-dependent inhibitory effect

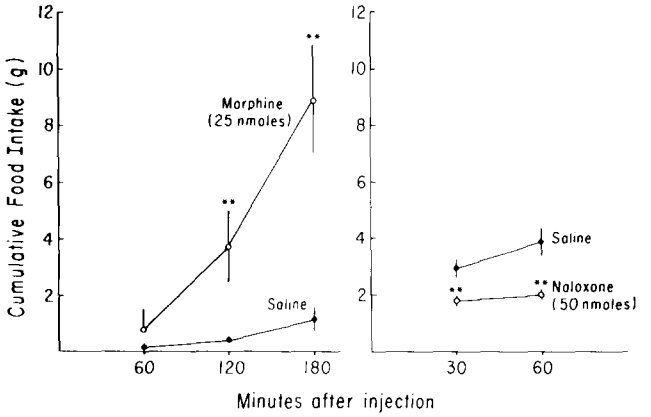

FIG. 2. Time response analysis of feeding behavior changes induced by paraventricular nucleus (PVN) injection of morphine (MO) (25 nmoles) into satiated rats (N=10), and nalaxone (NAL) (50 nmoles) into mildly food-deprived rats (N=10). Statistical comparisons between drug and saline vehicle scores revealed a significant ($p<0.01$) feeding stimulatory effect for MO at 120 and 180 minutes but not at 60 minutes and a feeding suppressive effect for NAL at 30 and 60 minutes ($p<0.01$).

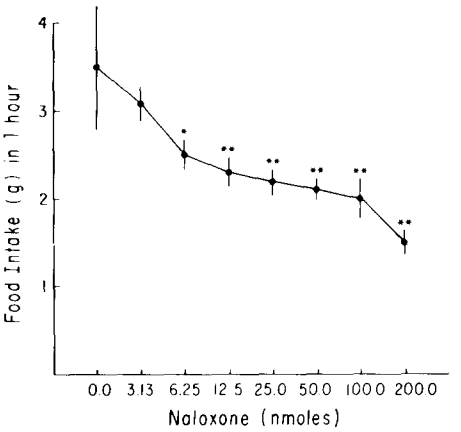

FIG. 3. Dose-response analysis of feeding suppressive effect induced by paraventricular nucleus (PVN) injection of naloxone (NAL) into mildly food-deprived rats (N=10). A statistically reliable effect with NAL, as compared with saline baseline (0.0 nmoles), was obtained at all doses except for 3.13 nmoles (*$p<0.05$; **$p<0.01$).

on food intake, $F(7,73)=10.3$, $p<0.001$. A significant suppression of feeding (28%, $p<0.01$) was observed at a dose as low as 6.25 nmoles. This inhibition increased to 55% ($p<0.01$) with dose increases up to 200 nmoles. The doses between 12.5 and 100 nmoles appeared equally effective in producing a suppression of food consumption. Analysis of the time course of this feeding suppression (Fig. 2) showed its latency to be approximately 10–20 minutes, and its duration at least 60 minutes.

Experiment 3

Histological analysis of the brain-cannula placements revealed that 24 of the 36 rats studies in this experiment had "on-target" cannulas aimed at one of the 4 intended brain sites. Twelve rats had "off-target" cannulas aimed at miscellaneous sites, either in the thalamus or the caudal hypothalamus and were therefore eliminated from the study. The precise cannula placements and food intake data for the animals with PVN (N=10), PFH (N=5), DMN (N=4) and VMN (N=5) cannulas, are presented in Fig. 4. Representative histological examples of these cannula placements are shown in Fig. 5. The PVN cannulas, illustrated in Fig. 4 at the level of 5340 μ according to König and Klippel [22], generally fell within the borders of the nucleus (Fig. 5a) or along its dorsal or ventral surface. The PFH cannulas (Figs. 4 and 5b) were located somewhat caudal to the PVN (at A4620 μ) and fell within 0.3 mm of the fornix, just lateral to the DMN and dorsolateral to the VMN. The VMN cannulas (Figs. 4 and 5c) were located in the anteriomedial portion of the nucleus (A4850 μ), just caudal and ventral to the PVN. Since these VMN cannulas were implanted using skull-flat coordinates, the tract made by the cannula implant did not penetrate the PVN but instead could be seen just caudal to the PVN, penetrating the rostral portion of the DMN. The cannulas aimed directly at the DMN (Figs. 4 and 5d) fell within the middle to caudal portion of this nucleus, at A4380 μ. The tips of these cannulas were positioned approximately 1.0 mm from the PVN cannulas.

Analysis of the food intake data for NE (40 nmoles), MO (25 nmoles) and NAL (50 nmoles) injected into the different brain areas (Fig. 4), revealed significant overall drug effect for each compound, $F(5,138)=6.7$, $p<0.001$. This effect of these compounds on feeding behavior occurred in the absence of any detectable changes in non-consummatory behaviors. Direct comparisons between the saline and drug scores for PVN-injected animals yielded statistical significance for the strong eating responses to NE ($p<0.01$) and MO ($p<0.001$), and for the suppression of feeding with NAL (−32%, $p<0.001$). These effects of the opiate drugs in the PVN are similar in magnitude to those observed in Experiments 1 and 2, in which multiple doses were examined. In the PFH, MO also significantly stimulated feeding ($p<0.001$), and NAL inhibited feeding by 37% ($p<0.001$). In contrast, NE was totally ineffective. The VMN responded strongly to NAL, which reduced feeding by 49% ($p<0.001$). This nucleus, however, failed to respond significantly to MO and yielded only a small eating response to NE ($p<0.05$). No significant drug effects were observed with either NE or NAL injected into the DMN.

To permit direct comparisons between the drug responses observed in the different brain areas, the raw data were converted to drug minus saline difference scores, which were then analyzed using an analysis of variance. Significant values for drug, $F(3,72)=50.3$, $p<0.001$, brain site, $F(3,72)=11.7$, $p<0.001$, and drug × brain site interaction, $F(3,72)=5.9$, $p<0.001$, were obtained. Direct comparisons between the drug scores of the different brain sites showed that: (1) The NE-elicited eating response was significantly greater ($p<0.05$) in the PVN (8.2 g) than in the VMN (2.8 g). The PFH and DMN appeared totally insensitive to NE. (2) MO was the most effective in eliciting eating when injected into the PVN and the PFH. Both brain areas responded similarly to MO, yielding a large increase in eating of over 10 g, $F(3,23)=10.7$, $p<0.001$. This response contrasts with a small, insignificant eating response with MO in the VMN (6.3 g compared with a saline score of 4.3 g). (3) NAL's suppressive effect on feeding was significantly observed with

injection into the PVN, PFH and VMN, but not into the DMN. The VMN exhibited the largest feeding suppression (−49%), which was somewhat greater than that observed in the PVN (−32%) and PFH (−37%).

DISCUSSION

Our results support the concept that brain opiate systems are involved in feeding behavior. This study has shown that hypothalamic injection of MO and NAL are effective in altering feeding, and that these effects are site specific within the brain. Most studies conducted to date have used the peripheral route of drug injection to examine opiate effects on feeding. In these studies, it has been demonstrated that peripherally injected MO, at doses of 1–30 mg/kg, has a potent stimulatory effect on food intake in satiated rats [2, 16, 50, 51, 62]. Other studies have reported that at the higher doses within this range (>5 mg/kg), MO may under certain conditions suppress food intake, possibly due to the increasing sedative effects of this drug at the higher doses [8, 41, 51]. This sedative effect is most pronounced during the first hour after injection, such that a feeding suppression may frequently occur at this time, followed by vigorous feeding during the next few hours [16, 24, 33, 34]. In contrast to this MO-induced feeding response, the opiate antagonist NAL, peripherally injected at doses of 1–30 mg/kg, causes a dose-dependent suppression of feeding in food-deprived rats [5, 14, 16, 50].

In contrast to the numerous studies conducted with peripheral injection of opiate agonists and antagonists, there are relatively few investigations which have employed the central route of drug administration, for the purpose of identifying the central brain site(s) and mechanism(s) involved in mediating opiate control of food intake. A few investigations using intraventricular injection of opiate agonists have consistently revealed a potentiation of feeding with these compounds [36,43]; however, the brain site mediating these responses has yet to be determined. McLean and Hoebel [37] have recently reported a dose-dependent feeding response with PVN injection of MO, for doses between 1.5 and 15.0 nmoles. With saline baseline taken into account, the food intake scores obtained by McLean and Hoebel were very similar to those reported in Fig. 1 of the present investigation. A tendency towards a slightly smaller feeding response in their study is very likely due to their use of a shorter test interval (135 min), which would fail to include the final stages of the MO elicited eating response (see Fig. 2). Although doses greater than 15.0 nmoles were not tested by McLean and Hoebel, we have established in the present study that the most robust feeding responses (between 5 and 15 g of food intake) occur at the somewhat higher doses of 25 and 50 nmoles, with a sharp decline at 100 nmoles due to increasing sedative effects. With regard to the threshold dose for PVN MO-elicited feeding, it would appear to be in the range of 0.15 nmoles (McLean and Hoebel's lowest effective dose) and 1.5 nmoles (the lowest effective dose in Fig. 1). These doses fall between 3 and 4 orders of magnitude lower than the MO doses effective with peripheral injection [16,51]. This dose differential supports the proposal that centrally, as well as peripherally, administered MO are acting within the brain and possibly at similar hypothalamic sites. It is noteworthy that the latency for the elicited eating response obtained with peripheral MO injection (60–90 minutes) [16,24] is roughly comparable to that observed with PVN injection (Fig. 2). Furthermore, we have found no evidence

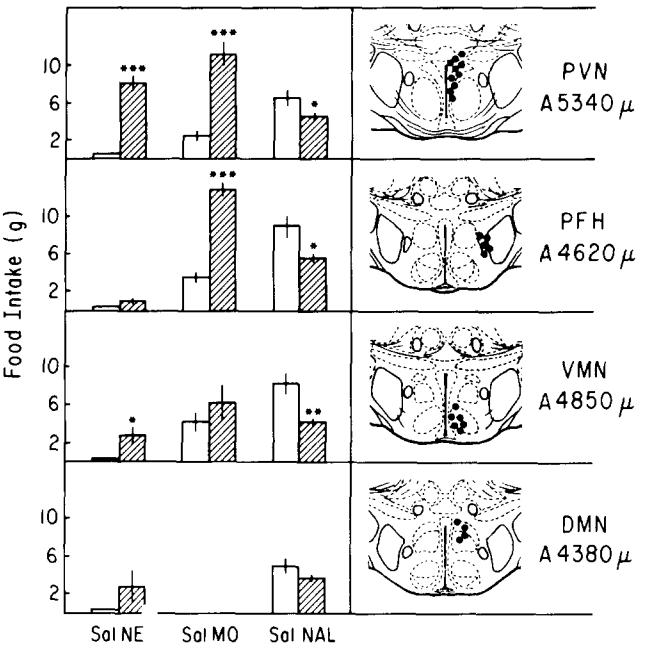

FIG. 4. Cannula mapping study of feeding effects induced by hypothalamic injection of norepinephrine (NE, 40 nmoles), morphine (MO, 25 nmoles) in satiated rats and naloxone (NAL, 50 nmoles) in mildly food-deprived rats. Four hypothalamic areas, namely the paraventricular nucleus (PVN), lateral perifornical hypothalamus (PFH), ventromedial nucleus (VMN), and dorsomedial nucleus (DMN), were examined. Results of histological analyses are illustrated to the right, on coronal drawings of the rat brain (taken from Konig and Klippel, [21]). Statistical comparisons between drug and saline scores yielded significance at $*p<0.05$; $**p<0.01$; and $***p<0.001$.

for tolerance to the feeding effects of centrally injected MO at any dose tested, similar to the findings obtained with peripheral MO injection [16,24].

Using a different site of MO injection, namely the VMN, Tepperman *et al.* have also revealed a dose-dependent feeding response with this opiate agonist [54,56]. A primary purpose of the present investigation was to compare the sensitivity of different hypothalamic areas to MO, in an effort to establish: (1) whether any particular site might be considered most crucial in mediating the opiate response; and (2) whether the sensitivity of one site might be attributed to the spread of MO to some alternative site. The results of the mapping study (Fig. 4) demonstrate that MO's stimulatory effect on feeding is to some extent site specific with the PVN and PFH responding equally to MO by yielding a large (9–10 g) increase in eating over saline baseline. The VMN, in contrast, was relatively insensitive to MO, showing only a slight increase of 2 g. To reconcile these findings with those of Tepperman *et al.* [54,55], we may suggest that the apparent sensitivity of the VMN to MO in their experiments was actually due to drug spread up the cannula shaft to a more

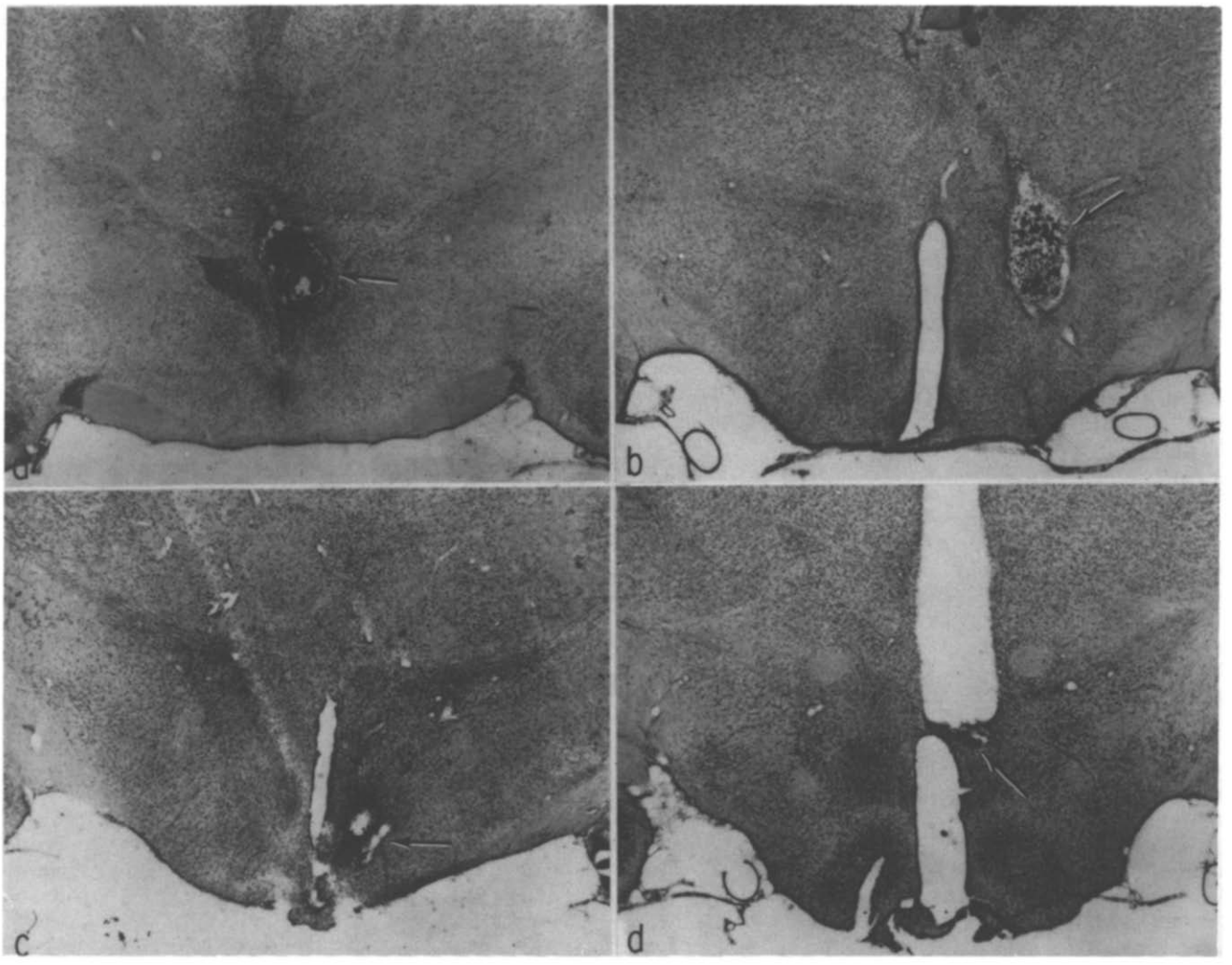

FIG. 5. Microphotographs of frontal sections of the rat brain showing representative injection sites (indicated by arrows) in the area of the paraventricular nucleus (a), perifornical hypothalamus (b), ventromedial nucleus (c), and dorsomedial nucleus (d). See Fig. 4 for diagrammatic representation of these and their respective behavioral sensitivity to norepinephrine, morphine and naloxone.

sensitive area dorsal to the VMN, possibly the PVN. Based on the stereotaxic coordinates described by Tepperman *et al.* (with bregma dorsal to lambda), one would predict that their implanted cannula had passed through the PVN on its ventral course to the VMN. In the present study, specific skull-flat coordinates were used to avoid this contact, and subsequent histological analyses of the cannula tract confirmed that no damage had occurred to the PVN and that the implant fell approximately 0.5 mm caudal to this nucleus (Figs. 4 and 5c). This dissociation of the PVN and VMN injection sites is further substantiated by our additional result, consistent with earlier findings [29], showing that the VMN implant was relatively insensitive to NE injection, whereas the PVN implant responded strongly to NE. It is clear from Fig. 4 that the PVN was not the only brain site sensitive to MO. A strong response was exhibited by a more lateral area, the PFH, which lies approximately 1 mm caudolateral to the PVN, within 0.5 mm of the insensitive VMN injection site.

The significance of these mapping results, relative to endogenous opiate processes controlling ingestive behavior, remains to be established. Anatomical studies have indicated that the four hypothalamic areas examined here are moderately to densely innervated by various opiate systems [1, 10, 19, 20, 23, 44, 45, 52, 60]. Terminals containing β-endorphin, enkephalins and dynorphins have been identified in these areas. Furthermore, autoradiographic studies have revealed the existence of μ, δ, and κ opiate receptors throughout these areas [10, 11, 44, 45]. These investigations, therefore, provide clear anatomical support for the proposed existence of specific hypothalamic opiate systems involved in feeding control. Although our MO mapping studies focus our attention on the medial PVN and lateral PFH areas as being most crucial to this behavior, the additional involvement of the VMN needs to be considered.

Our mapping results obtained with centrally injected NAL further address this question of site specificity. Previous investigations with intraventricular NAL administration have demonstrated a significant feeding suppression (40–50%) with this opiate antagonist, at doses of 100–400 μg (300–1200 nmoles). These doses, however, are only slightly lower than the threshold dose for the feeding suppressive

effect of peripherally injected NAL [14, 15, 32, 50]. In one study, intraventricular injection of 15 μg (40 nmoles) of NAL was shown to be effective [18], whereas doses of 0.5 to 50 μg (140 nmoles) were found to be ineffective in another study [15]. With injection into the PVN, Experiment 2 has demonstrated that centrally administered NAL can indeed be effective as a feeding suppressant and requires considerably lower doses (6.25 nmoles or 2.2 μg) than peripherally injected NAL. This provides the first evidence that specific hypothalamic opiate receptors blocked by NAL are essential to normal feeding. Unfortunately, the pattern of distribution of these receptors within the hypothalamus remains to be clearly established. Our mapping results, revealing total insensitivity of the DMN to NAL (Fig. 4), leads us to question whether this nucleus is involved in opiate control of feeding. This interpretation, however, is inconsistent with the findings of Bellinger *et al.* [3], that DMN lesions attenuate the feeding suppressive effects of peripheral NAL injection.

With regard to the three other hypothalamic areas tested, the VMN seems to be particularly sensitive to NAL, although it was relatively insensitive to MO. In contrast, the PVN and PFH were responsive to both the opiate agonist and antagonist. The pattern of opposing effects observed with these drugs in the PVN and PFH provides a strong foundation for the proposal that opiate receptors involved in feeding stimulation do exist in these two hypothalamic areas. Central mapping studies of naloxone's suppressive effect on water intake have found sites in the lateral hypothalamus and zona incerta to be sensitive to opiate blockade [8,53]. (Medial hypothalamic sites were not tested.) Although this hypodipsic effect of naloxone may be related to its hypophagic effect we have observed with lateral PFH injection, preliminary results obtained with PVN naloxone injection (Woods and Leibowitz, unpublished data) revealed a potent suppression of food intake in the absence of any effect on water intake. The effectiveness of NAL as a feeding suppressant in the VMN, an area relatively insensitive to MO, suggests either that centrally injected NAL has a wider area of spread, affecting distant sites not reached by MO, or that VMN-injected NAL is acting within this nucleus on receptors different from MO. The first suggestion, that NAL may be spreading to a brain area (e.g., the PVN or PFH) outside the VMN, appears more likely, in light of the fact that NAL is highly lipid soluble [40], more so than MO [13], and would thus be expected to have higher degree of tissue permeability and anatomical spread. Further, it has been demonstrated that the anorexic effect of peripherally injected NAL remains unaffected by VMN lesions [21].

Concerning the nature of the hypothalamic receptors affected by MO and NAL, the available evidence indicates that these compounds act on a common set of receptors, possibly of the μ subtype. In a variety of pharmacological systems, MO has been characterized as a potent μ-receptor agonist with particular sensitivity to NAL antagonism [6, 7, 30, 35, 46, 57]. More specifically, studies focused on ingestive behavior have shown the eating response elicited by centrally or peripherally injected MO to be effectively blocked by low doses of NAL [12, 39, 49, 56]. Further support for a common receptor mediating these drugs' actions is provided by the evidence that MO and NAL have their opposing effects on total food intake through a specific alteration in fat consumption [33,34]. Although the receptor mediating these effects appears to be μ in nature [48,54], the possible additional involvement of the κ receptor needs to be considered [41].

While the PFH area has received little attention in anatomical and physiological studies of opiate receptors, studies of the PVN have shown this nucleus to contain a relatively dense population of opiate receptors of the μ subtype [10,11]. Electrophysiological experiments have found PVN cells to respond to MO and NAL, with the opiate agonist depressing neuronal activity and NAL antagonizing this effect [47]. In light of this evidence and the finding that PVN lesions cause an increase of food intake and weight gain [28], one might suggest that the opiate system in the PVN enhances feeding by inhibiting the discharge of local "satiety" neurons. A similar hypothesis has been proposed for an α-noradrenergic system which has been localized to the PVN and has also been shown to play a role in feeding stimulation [27,29].

Recent pharmacological studies have suggested that noradrenergic and opiate control of feeding may be linked at the level of the hypothalamus, possibly in a synergistic but dependent fashion [26, 42, 55, 56]. This hypothesis is consistent with the findings that MO increases the turnover of NE in the PVN [17] and that the eating response elicited by peripheral PVN MO injection, like that induced by PVN NE, is attenuated by adrenalectomy [4,25], or that the anorexic effect of peripheral NAL, similar to NE-elicited feeding [27], is attenuated by peripheral atropine injection [18]. However, the implications of these findings, that MO elicits feeding in part through the release of PVN NE, has yet to be directly tested. Our present finding, that MO may act at a brain site (i.e., the PFH) which is insensitive to NE, provides an anatomical dissociation of the opiate and α-noradrenergic systems. It has also been established that the eating response induced by PVN injection of enkephalin [37], or IVT injection of dynorphin [43], in contrast to that induced by peripheral MO [4], remains intact in adrenalectomized animals. Furthermore, eating elicited by PVN NE injection is associated with a specific preference for carbohydrate [58], while eating induced by peripheral MO injection is associated with an increased preference for fat and decreased preference for carbohydrate [33]. Further work will need to examine the possibility that the opiate and α-noradrenergic systems may interact under certain conditions and in certain brain areas, but may become dissociated under other conditions. These results are in agreement with a recent finding that lateral hypothalamus and VMH injections of naloxone or naltrexone attenuate food intake in deprived rats [58].

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