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A Probable Role for Norepinephrine in Feeding After Hypothalamic Injection of Morphine[1]

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TEPPERMAN, F. S., M. HIRST AND C. W. GOWDEY. *A probable role for norepinephrine in feeding after hypothalamic injection of morphine*. PHARMAC. BIOCHEM. BEHAV. 15(4) 555–558, 1981.—When morphine is instilled directly into the ventromedial hypothalamus of rats there is a latent period followed by a prolonged bout of feeding. This enhanced activity may be mediated by the release of norepinephrine; for morphine-induced feeding was depressed by the α-adrenergic receptor blocker phentolamine. Several neurotransmitter agonists and antagonists failed to duplicate this action: propranolol, serotonin, methysergide, apomorphine and haloperidol were ineffective in modifying ingestion elicited after morphine. Unlike apomorphine, dopamine augmented morphine's feeding effect. This difference may exist because dopamine acts as a precursor for norepinephrine formation in local ventromedial hypothalamic neurons.

Morphine Ventromedial hypothalamus Norepinephrine Feeding Phentolamine

WE have shown previously that in free-feeding rats small doses of morphine can enhance feeding activity when it is injected into the same site in the ventromedial hypothalamus (VMH) that is associated with norepinephrine (NE)-stimulated feeding [6]. The time-course of the feeding activity produced by these two agents is not identical: following NE injection feeding is rapid in onset and short-lived, whereas following morphine there is a long latency and then prolonged ingestion. Injection of naloxone into the VMH just prior to morphine reduces feeding, but if it is delayed till one hour after the morphine, it has no appreciable effect. These findings led us to postulate that feeding after morphine might be mediated by NE or another neurotransmitter present in the hypothalamus. The effects of noradrenergic, serotonergic and dopaminergic agonists and antagonists on morphine-induced feeding have therefore been investigated.

METHOD

Male Sprague-Dawley rats weighing 250–320 g were housed individually and maintained with ad lib food and water on a 12-hour (08:00 to 20:00) light/dark cycle. A guide cannula extending to the right VMH was implanted stereotaxically in each animal (Pellegrino *et al.* [5], coordinates: +0.4 mm anterior to bregma, 0.5 mm lateral, 8.3 mm depth) while they were anesthetized with Equithesin (3.5 ml/kg). Cannulae were anchored to the skull with stainless steel jeweller's screws and dental acrylic cement. Except during injections, when a 30 gauge cannula was inserted to a depth 0.6 mm below the guide cannula (Fig. 1), a stainless steel obdurator pin was left in the guide cannula. Animals were allowed to recover for at least seven days during which they were handled and sham-injected regularly.

In the morning of a trial day animals were allowed to continue eating and drinking ad lib and were handled. Initial injections were made at approximately 12:30. Injections were always made in 0.5 μl sterile, pyrogen-free saline unless otherwise specified. Following injection(s), food was cleared from the cages and replaced by a pre-weighed quantity of rat chow pellets (Purina). After 1 hour the remaining pellets and the spillage were removed and replaced by fresh pre-weighed food. The food eaten during the first and second hours after the final injections was determined. Trials were never performed on consecutive days.

Since morphine seems to depress activity in rats before it enhances feeding [6], only rats that ate during the second hour following morphine treatment were incorporated into these studies.

Experiment 1

The initial study was performed in 7 rats to determine the dose of phentolamine sufficient to block the effect of a norepinephrine (NE) injection given 5 minutes later. The experimental design was:

[1]Preliminary data from this report were presented at the IVth Meeting, Canadian College of Neuropsychopharmacology, Toronto, Canada, April 23–25, 1981.

	Injection 1	—(5 min later)—	Injection 2
Trial 1	—		saline
Trial 2	saline		NE (30 nmoles)
Trial 3	phentolamine (30,60 nmoles)		NE (30 nmoles)
Trial 4	saline		NE (30 nmoles)

Experiment 2

To determine whether phentolamine would affect feeding due to morphine the following series of injections were given (n=7):

	Injection 1	—(5 min later)—	Injection 2
Trial 1	—		saline
Trial 2	saline		morphine (5.3 nmoles)
Trial 3	phentolamine (60 nmoles)		morphine (5.3 nmoles)
Trial 4	saline		morphine (5.3 nmoles)

Experiment 3

Since phentolamine in sufficient dosage to block the effects of NE given 5 minutes later did not block morphine feeding, it was decided to allow a one-hour interval between injection of morphine and the test drugs. The reason for this was that our earlier experiments [6] had indicated a latent period of about an hour before morphine feeding began. Therefore, the following design was used to test various agonist and antagonist drugs:

	Injection 1	—(1 hr later)—	Injection 2
Trial 1	saline		—
Trial 2	drug		—
Trial 3	morphine (5.3 nmoles)		saline
Trial 4	morphine (5.3 nmoles)		drug
Trial 5	morphine (5.3 nmoles)		saline

Seven drugs were tested in as many groups of rats: phentolamine (60 nmoles, n=6), propranolol (60 nmoles, n=5), serotonin(5-HT) (30 nmoles, n=5), methysergide (28.3 nmoles, n=5), dopamine (30 nmoles, n=7), haloperidol (8 nmoles given in 1 μl, n=6) and apomorphine (25 nmoles given in 1 μl, n=7). The haloperidol was dissolved in a small amount of acetic acid and made up to volume with saline and bicarbonate. The pH of all solutions was approximately 5.5.

Cannula placements were verified histologically. Results were analyzed for significance by the Randomized Block Analysis of Variance. Where significance was present ($p \leq 0.05$), results were further assessed by the Studentized range test [2].

Norepinephrine HCl and dopamine HCl were purchased from Sigma and serotonin (5-hydroxytryptamine) from BDH Chemicals. The authors would like to thank the following companies for their kind donations: May & Baker for morphine sulfate; CIBA Pharmaceuticals for phentolamine HCl; Ayerst Laboratories for propranolol HCl; Sandoz for methysergide bimaleate; Mr. R. Graham, Health and Welfare, Canada, for apomorphine HCl; and McNeil Laboratories for haloperidol base.

RESULTS

Norepinephrine-induced feeding is significantly depressed by 60 nmoles of the α-adrenergic receptor antagonist phentolamine, $F(2,12)=8.49$, $p \leq 0.05$ (Fig. 2a). A smaller

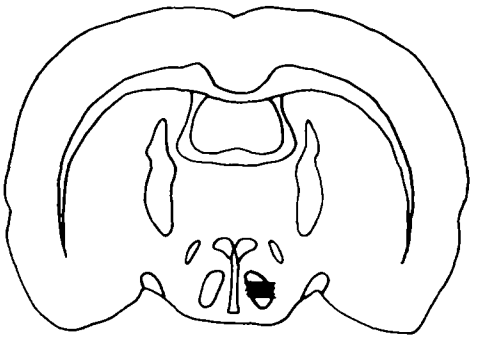

FIG. 1. Frontal diagram of the rat brain. Hatched lines indicate the area in the ventromedial hypothalamus (VMH) where injections were made.

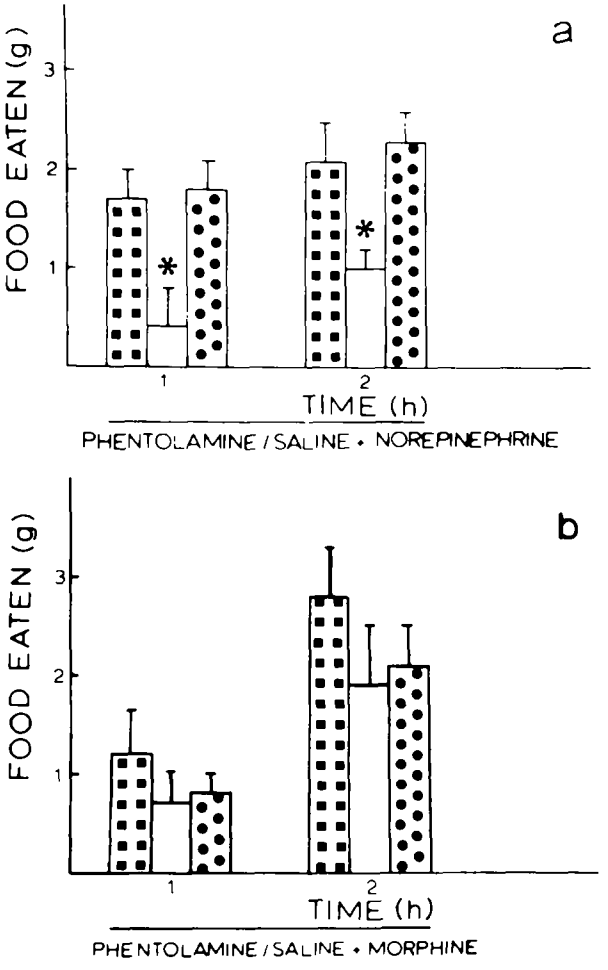

FIG. 2. (a) Cumulative food intake at 1 and 2 hr following injections of: saline followed after 5 min by NE (bars with closed squares); phentolamine followed after 5 min by NE (open bars); and a later trial with saline followed after 5 min by NE (bars with closed circles) (n=7). (b) Cumulative food intake at 1 and 2 hr following injections of: saline followed after 5 min by morphine (bars with closed squares); phentolamine followed after 5 min by morphine (open bars); and a later trial with saline followed after 5 min by morphine (bars with closed circles) (n=7). In (a) and (b), vertical lines represent S.E.M. Significant differences ($p \leq 0.05$) between open bars and filled bars are indicated as *.

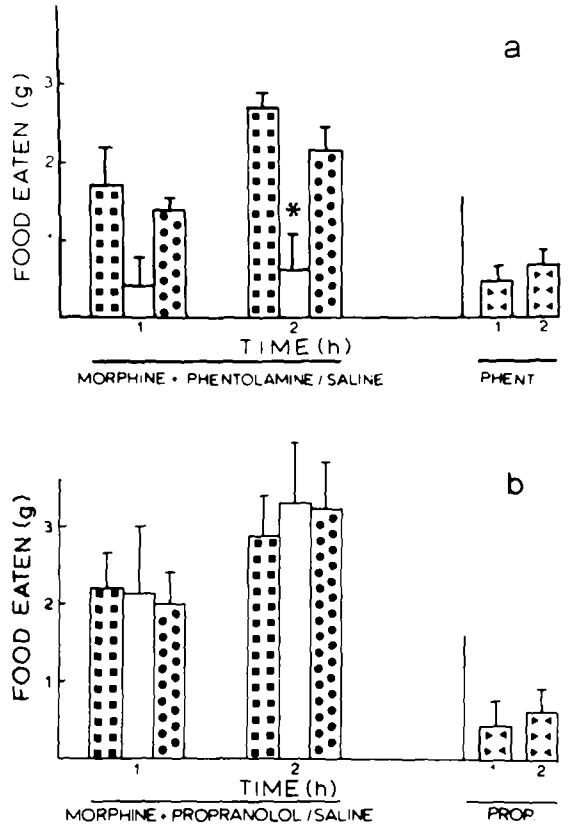

FIG. 3. Cumulative food intake at 1 and 2 hr following injections of: morphine followed after 1 hr by saline (bars with closed squares); morphine followed after 1 hr by drug treatment (open bars); a later trial with morphine followed after 1 hr by saline (bars with closed circles); and drug treatment alone (bars with closed triangles). In 3a the trial drug was phentolamine (n=6); in 3b the trial drug was propranolol (n=5). Vertical lines represent S.E.M. Significant differences ($p \leq 0.05$) between morphine + drug treatment and morphine + saline treatments are indicated as *

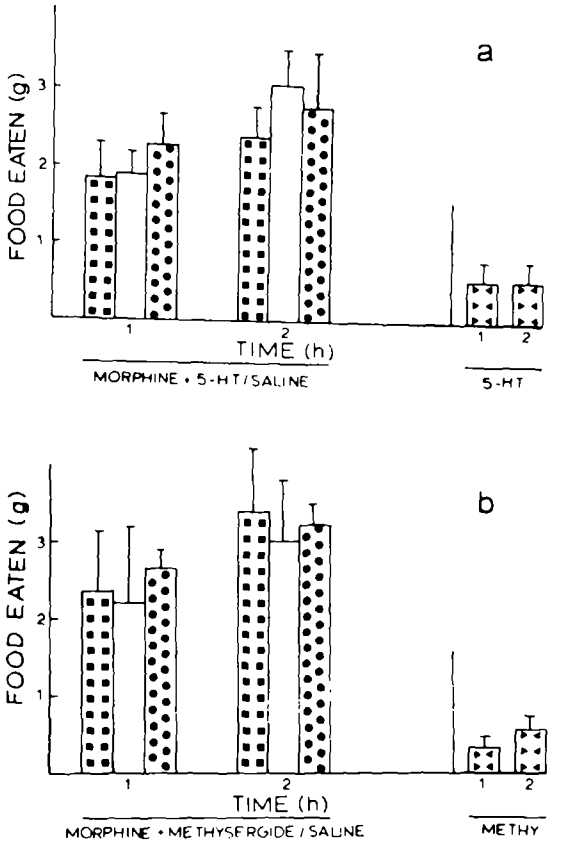

FIG. 4. Cumulative food intake at 1 and 2 hr following injections of: morphine followed after 1 hr by saline (bars with closed squares); morphine followed after 1 hr by drug treatment (open bars); a later trial with morphine followed after 1 hr by saline (bars with closed circles); and drug treatment alone (bars with closed triangles). In 4a the trial drug was 5-HT (serotonin) (n=5); in 4b the trial drug was methysergide (n=5). Vertical lines represent S.E.M. Significant differences ($p \leq 0.05$) between morphine + drug treatment and morphine + saline treatments are indicated as *.

dose (30 nmoles) of phentolamine did not have a significant effect. The same dose of phentolamine (60 nmoles) will block morphine-induced feeding when it is injected one hour after, but not just before the morphine (Figs. 2b, 3a). The later injection of phentolamine tended to suppress feeding during the first hour ($p<0.10$) and significantly reduced the 2-hour food intake, $F(2,8)=7.60$, $p<0.05$. On the other hand, the β-adrenergic receptor antagonist, propranolol, did not block feeding when given 1 hour after morphine (Fig. 3b).

Most other monoamine agonists and antagonists had little effect on feeding activity. Neither serotonin (5-HT) nor its antagonist methysergide significantly affected morphine-induced feeding (Fig. 4). Again, neither the dopamine-receptor agonist apomorphine nor its antagonist haloperidol had any significant effect on feeding due to morphine (Fig. 5). However, dopamine itself showed some tendency to increase ($p<0.10$) feeding during the hour following injection and significantly elevated the 2-hour food intake, $F(2,12)=7.57$, $p<0.05$ (Fig. 6).

None of the drug treatments caused any apparent irreversible changes because the feeding induced by the morphine trial following the trial of the test drug was never statistically different from that found with the morphine trial preceding the test drug trial.

The food intake in the 2 hours following injection of the test drugs by themselves (Figs. 3–6) was small. The mean ingestion following saline control injections never exceeded 0.8 g over the 2-hour measurement period (range 0–0.8 g).

DISCUSSION

In a previous paper [6] we have shown that morphine instilled into the ventromedial hypothalamus (VMH) of rats will cause feeding that is delayed in onset and very prolonged. One reason for the delay might be that the effect of morphine is indirect in that it induces a change in levels of an intermediary substance, which in turn stimulates feeding. Evidence from the present paper suggests that this intermediary substance may well be norepinephrine. Doses of norepinephrine and morphine were chosen to produce roughly similar levels of feeding activity. The results show that the same dose of phentolamine could significantly sup-

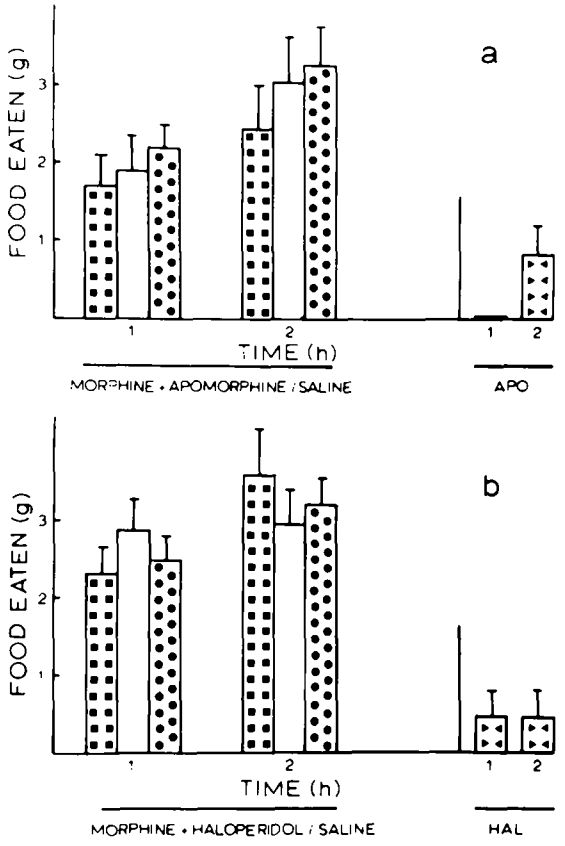

FIG. 5. Cumulative food intake at 1 and 2 hr following injections of: morphine followed after 1 hr by saline (bars with closed squares); morphine followed after 1 hr by drug treatment (open bars); a later trial with morphine followed after 1 hr by saline (bars with closed circles); and drug treatment alone (bars with closed triangles). In 5a the trial drug was apomorphine (n=7); in 5b the trial drug was haloperidol (n=6). Vertical lines represent S.E.M. Significant differences ($p \leq 0.05$) between morphine + drug treatment and morphine + saline treatments are indicated as *.

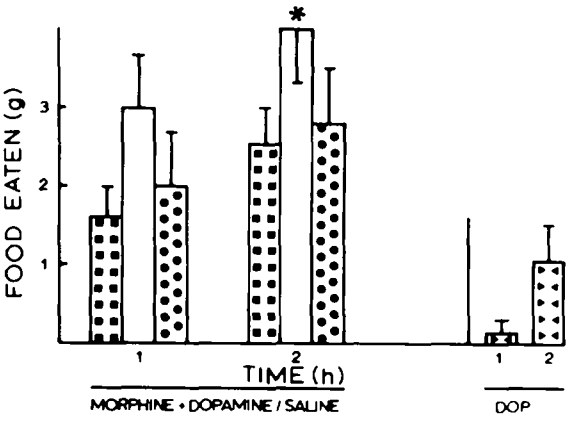

FIG. 6. Cumulative food intake at 1 and 2 hr following injections of: morphine followed after 1 hr by saline (bars with closed squares); morphine followed after 1 hr by dopamine (open bars); a later trial with morphine followed after 1 hr by saline (bars with closed circles); and dopamine alone (bars with closed triangles) (n=7). Vertical lines represent S.E.M. Significant differences ($p \leq 0.05$) between morphine + dopamine and morphine + saline treatments are indicated as *.

press both NE-induced and morphine-induced feeding. (Three of five morphine-treated rats did not eat following the phentolamine treatment.) That this effect is due to a general behavioural depression caused by phentolamine is unlikely because following injection of this drug alone, animals did eat a small quantity of food, and more importantly the same dose of phentolamine given just prior to morphine had no effect on feeding. Perhaps phentolamine is effective after, but not before, morphine because the latent period to feeding is generally more than 30 minutes, by which time the local phentolamine concentration may be insufficient to block liberated NE. These results also reconfirm the finding [4] that α- and not β-adrenergic receptors are involved in stimulation of feeding in the VMH, for propranolol was ineffective against morphine-induced feeding.

Although serotonin (5-HT) appears to have a role in some feeding behaviour [1,3], neither it nor its antagonist methysergide appear to be involved in the feeding evoked by morphine given at this site.

Dopamine augments food intake when given in conjunction with morphine. However, apomorphine and haloperidol have no effect. The dopamine might therefore simply be acting as additional substrate for conversion to NE in local noradrenergic neurons. Confirmation of the proposed role of NE in morphine-induced feeding requires measurement of alterations in NE levels and turnover at the site of the morphine injection.

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REFERENCES

1. Goldman, H. W., D. Lahr and E. Friedman. Antagonistic effects of alpha and beta-adrenergically coded hypothalamic neurons in consummatory behaviour in the rat. *Nature* **231**: 453–455, 1971.
2. Goldstein, A. *Biostatistics: An Introductory Text*. New York: Macmillan, 1964, p. 71.
3. Hoebel, B. S. Pharmacologic control of feeding. *A. Rev. Pharmac. Toxicol.* **17**: 605–621, 1977.
4. Leibowitz, S. F. Reciprocal hunger-regulating circuits involving alpha- and beta-adrenergic receptors located, respectively, in the ventromedial and lateral hypothalamus. *Proc. natn. Acad. Sci. U.S.A.* **67**: 1063–1070, 1970.
5. Pellegrino, L. J., A. S. Pellegrino and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain*. New York: Plenum Press, 1979.
6. Tepperman, F. S., M. Hirst and C. W. Gowdey. Hypothalamic injection of morphine: feeding and temperature responses. *Life Sci.* **28**: 2459–2467, 1981.