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Peptidergic Regulation of Norepinephrine Induced Feeding

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MORLEY, J. E., A. S. LEVINE, S. S. MURRAY AND J. KNEIP. *Peptidergic regulation of norepinephrine induced feeding.* PHARMAC, BIOCHEM. BEHAV. 16(2) 225–228, 1982.—The inhibitory effect of a variety of substances on feeding induced by norepinephrine (20 μg ICV) was studied. Subcutaneous administration of the opiate antagonist, naloxone, inhibited norepinephrine-induced eating at 10 and 5 mg/kg, but not a 1 mg/kg. Intraventricular administration of the GABA antagonist, bicuculline, produced a dose related decrease in food ingestion. The putative satiety hormones, bombesin (10 μg/kg; subcutaneously) and cholecystokinin octapeptide (10 μg/kg; subcutaneously) also reduced norepinephrine induced eating, as did ICV administration of calcitonin (2 units). Neither thyrotropin-releasing hormone (1 μg ICV) nor its metabolits, histidyl-proline diketopiperazine (1 μg ICV) altered norepinephrine-induced feeding. The studies reported here suggest a neuromodulatory role of peptides in the central regulation of norepinephrine-induced feeding.

Norepinephrine Bombesin Opiates Cholecystokinin Naloxone TRH GABA
Histidyl-proline diketopiperazine Bicuculline Appetite

WE HAVE previously proposed an integrated hypothesis to explain the monoaminergic-peptidergic regulation of appetite [12]. We suggested that the hypothalamus acts as a neuroendocrine transducer with the control of food intake involving a delicate balance between a number of neuropeptides and monoamines. It was suggested that food intake is initiated by a tonic signal produced by a dopamine-enkephalinergic mechanism in the area of the lateral hypothalamus and that this signal is governed by inhibitory inputs from the medial hypothalamic area including a serotonergic-cholecystokinin (CCK) and a nor-adrenergic-thyrotropin releasing hormone (TRH) system.

Historically, norepinephrine (NE) has been implicated as an important hypothalamic factor in the activation of feeding [8,21] although it has been demonstrated that its function after injection into some areas of the lateral hypothalamus can produce the opposite effect [8]. In this report we have used norepinephrine induced feeding as a pharmacological model to examine the interrelationships of the monoaminergic and peptidergic substances involved in the hypothalamic regulation of appetite.

METHOD

Male Sprague-Dawley rats (200–250 g) kept under standard lighting conditions (12 hr/day artificial light—6 am to 6 pm) and given free access to a standard rat diet and water, were used for all experiments. Cannulas were implanted into the lateral ventricle as previously described [14]. The animals were allowed a minimum of 5 days postoperative recovery before experiments were commenced. Norepinephrine (Sigma Chemical Co., St. Louis MO) was freshly dissolved in slightly acidified saline and administered in a 5 μl volume ICV. All other drugs and peptides were dissolved in saline and administered in a 5 μl volume ICV or in a 0.5 cc volume subcutaneously. All feeding antagonists were administered immediately before norepinephrine was given. Substances were obtained from the following sources: histidyl-proline diketopiperazine (Dr. Chandan Prasad, Louisiana State University, School of Medicine, New Orleans); naloxone (Endo Products, Garden City, New York); phentolamine (Ciba Pharmaceutical, Summit New Jersey); cholecystokinin-octapeptide (Peninsula Labs, Inc., San Carlos, CA); bombesin (Sigma Chemical Co., St. Louis MO); TRH (Calbiochem-Behring Corporation, LaJolla, CA); calcitonin (Armour Pharmaceuticals, Phoenix, AZ) and bicuculline methiodide (Vega Biochemicals, Tucson, AZ).

All animals had free access to food and water until the experiments were performed. All tests were performed between 1300 to 1500 hours. Immediately after drug administration animals were put in a new cage together with 2 pellets of pre-weighed Purina rat chow (7–10 g). In all studies, food intake is expressed as grams eaten (to the nearest 0.1 g/30 minutes.

For each experiment a new batch of animals was used. Concurrent controls demonstrating the effect of norepinephrine on eating were used for each experiment. No rat was used more than three times, with a minimum of 48 hours between each time the animal was tested. Controls and each of the treatments used in a particular experiment were run concurrently with a cross-over design so that approximately equal numbers of animals received norepinephrine alone or

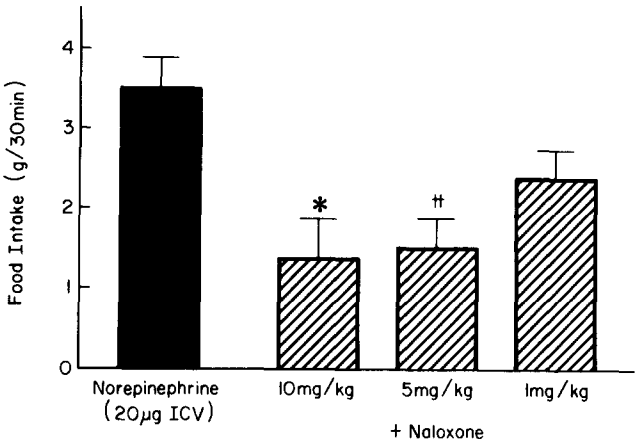

FIG. 1. Effect of the opiate antagonist, naloxone, on norepinephrine induced eating (*$p<0.01$, ††$p<0.005$). Naloxone was administered subcutaneously. n=7–10 for each treatment and controls.

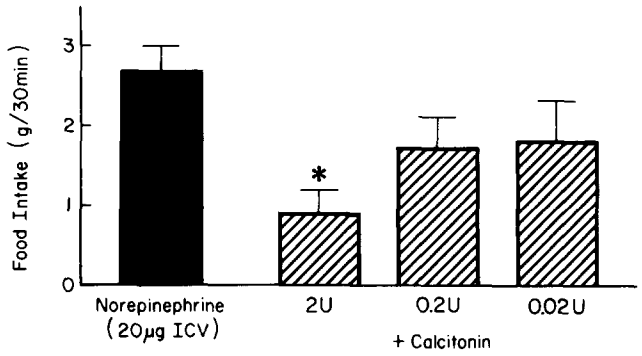

FIG. 2. Effect of the putative satiety hormone, calcitonin, on norepinephrine-induced eating (*$p<0.01$). Calcitonin was administered intracerebroventricularly. n=15 for controls and 8 for each calcitonin treatment.

TABLE 1

EFFECT OF INTRAVENTRICULAR ADMINISTRATION OF THE GABA-ANTAGONIST BICUCULLINE-METHIODIDE ON NOREPINEPHRINE (20 μg ICV)-INDUCED FEEDING

	n	g/30 minutes	p
Norepinephrine (20 μg ICV) + saline	8	2.7 ± 0.4	—
NE + bicuculline methiodide (100 μg)	10	2.4 ± 0.4	NS
NE + bicuculline methiodide (250 μg)	6	0.7 ± 0.3	0.01
NE + bicuculline methiodide (500 μg)	10	0.4 ± 0.2	0.001

TABLE 2

EFFECT OF SUBCUTANEOUS ADMINISTRATION OF BOMBESIN AND CHOLECYSTOKININ-OCTAPEPTIDE (CCK-3) ON NOREPINEPHRINE-INDUCED FEEDING

	n	g/30 minutes	p
Norepinephinre (20 μg ICV) + saline	10	3.6 ± 0.4	—
NE + bombesin (10 μg/kg SC)	10	2.1 ± 0.4	0.05
NE + CCK-8 (10 μg/kg SC)	10	2.3 ± 0.3	0.05

in combination with another treatment on each experimental day. A total of 75 animals were used for these studies. All results are expressed as mean±S.E.M. Results were compared using the two-tailed unpaired Student's *t*-test.

RESULTS

Control animals receiving acidified saline demonstrated no eating over the 30 minute test period. We feel that this failure to eat during the control period is due to the inhibitory effect of a novel environment on feeding in the sated animal. Parenterally administered naloxone reduced norepinephrine induced eating at 10 and 5 mg/kg but not at 1 mg/kg (Fig. 1). The α-antagonist, phentolamine (100 μg ICV) produced the expected reduction in norepinephrine-induced (20 μg) eating. The rats ate 3.7±0.5 g/30 minutes after norepinephrine alone (n=6) compared to 0.7±0.2 g/30 minutes after norepinephrine and phentolamine (n=8; $p<0.01$). Intraventricular administration of the GABA antagonist, bicuculline methiodide, produced a dose related decrease in food ingestion (Table 1).

Both bombesin (10 μg/kg SC) and cholecystokinin octapeptide (10 μg/kg SC) significantly reduced norepinephrine induced eating. Intraventricular administration of calcitonin decreased norepinephrine induced eating (Fig. 2). TRH (1 μg ICV; 2.2±0.2 g/30 minutes, n=15) and histidyl-proline diketopiperazine (1 μg ICV; 1.9±0.3 g/30 minutes, (n=12) failed to suppress norepinephrine induced eating when compared to concurrently tested controls (NE 20 μg ICV; 2.1±0.3, n=14).

DISCUSSION

This study demonstrates that a number of substances are capable of reducing the feeding induced by norepinephrine. A large number of studies have demonstrated that the opiate antagonist, naloxone, is capable of inhibiting spontaneous starvation-induced, stress-induced and muscimol-induced feeding [1, 6, 7, 12, 13, 15]. Other studies have shown that intraventricular administration of the endogenous opiates induced feeding [18]. The ability of naloxone to reduce norepinephrine induced feeding provides further confirmatory evidence of the central role that the opiates play in the initiation of the feeding drive [9, 12, 13].

The finding that the GABA antagonist, bicuculline, suppressed norepinephrine-induced eating confirms the observation of Grandison and Guidotti [3] who found a similar

result after intrahypothalamic injection of these two substances. Both they and our group [18] have previously demonstrated that the α-antagonist, phentolamine, fails to block eating induced by the GABA-agonist, muscimol. These observations suggest that the site of GABA action to induce feeding is after that of the α-adrenergic system.

Both cholecystokinin [4,20] and bombesin [5,16] are considered putative satiety hormones. The dose we used for both hormones (10 μg/kg) is a dose that consistently inhibits starvation-induced feeding. Our data confirms the observations of McCaleb and Myers [10] that cholecystokinin acts on the hypothalamic noradrenergic system involved in feeding and extends it by showing that bombesin has a similar action. We have previously shown that neither bombesin (5 μg/kg) nor cholecystokinin (5 μg/kg) alter muscimol induced feeding [18] and thus conclude that these two putative satiety hormones act by modulating the norepinephrine stimulatory effect on GABA. However, as the doses of CCK and bombesin that inhibited norepinephrine induced eating also inhibit deprivation induced eating, there is still some question as to whether these peptides actually do interact directly with norepinephrine.

Both thyrotropin-releasing hormone (TRH) [14, 24, 25] and its metabolite, histidyl-proline diketopiperazine [17] have been shown to suppress spontaneous, starvation-induced and stress-induced eating in a dose range of 10^{-6} to 10^{-8} mole. TRH is well recognized as interacting with noradrenergic systems [11]. It was thus somewhat surprising that neither TRH (1 μg ICV) nor histidyl-proline diketopiperazine (1 μg ICV) had any effect on norepinephrine-induced feeding. It is possible that TRH produces opposite effects on feeding in the venteromedial hypothalamus and the lateral hypothalamus. A similar situation has been postulated for norepinephrine [8] and GABA [22]. In that case a nonspecific effect may cause the predominance of one site of action over the other after intraventricular injection. Microinjection of TRH and norepinephrine into hypothalamic areas should be undertaken to assess this possibility.

Calcitonin has been demonstrated to be a potent inhibitor of food ingestion after parenteral and intraventricular injection [2, 19, 23]. The studies undertaken here show that calcitonin inhibits norepinephrine induced eating but at much higher concentrations (2 units ICV) than are necessary to suppress 24 hour spontaneous (0.2 units ICV) or stress-induced eating (0.002 units ICV). The reason for the insensitivity of calcitonin as a suppressor of norepinephrine induced feeding compared to its effect in other feeding models is not apparent. Calcitonin (2 units ICV) also inhibits muscimol-induced eating suggesting that calcitonin may play a role as a major central satiety substance.

In conclusion, the studies reported here suggest a neuromodulatory role of peptides in the central regulation of norepinephrine induced feeding. We have previously argued that the hypothalamic control of feeding represents a complex interaction of monoamines and neuropeptides [12]. The studies reported here together with those examining the effects of satiety factors on muscimol-induced feeding [18] represent an attempt to pharmacologically probe this complex interaction. We recognize that there are a large number of stimuli that will interfere with eating behavior in a nonspecific manner and may have no meaningful relationship to central control processes for food intake. However, the peptides examined in this study represent those which are widely recognized as playing an important role in the regulation of feeding and whose effects are considered most probably to be specific (see [12] for a review). Future studies, including a complete parametric study of the different eating models, testing the substances at a range of doses to compare their relative effectiveness in inhibiting the different eating responses, will be necessary before the possible physiological significance of the studies reported here can be fully determined.

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