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Dynorphin-(1–13), Dopamine and Feeding In Rats

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MORLEY, J. E., A. S. LEVINE, M. GRACE AND J. KNEIP. *Dynorphin-(1–13), dopamine and feeding in rats.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 701–705, 1982.—Intraventricular administration of the dopamine agonist, bromergocryptine, reliably induces feeding over a narrow dose range with a bell-shaped curve. Bromergocryptine (80 μg) induced feeding is inhibited by the dopamine antagonist, haloperidol (0.5 mg/kg) and the opiate antagonist, naloxone (10 and 1 mg/kg). The leucine-enkephalin containing opioid peptide, dynorphin-(1–13) induces feeding which is inhibited by haloperidol (0.5 and 0.1 mg/kg) and by naloxone (1 mg/kg). Of the common satiety factors tested only bombesin (10 μg/kg subcutaneously) inhibited both dynorphin-(1–13) and bromergocryptine induced feeding. Cholecystokinin-octapeptide (10 and 20 μg/kg, subcutaneously), thyrotropin-relasing hormone (10 and 20 μg), ICV) and calcitonin (1 unit, ICV) all failed to inhibit dynorphin-(1–13)-induced feeding. Calcitonin and CCK-8 but not TRH inhibited bromergocryptine-induced feeding. These studies have demonstrated the close interaction between dopaminergic an dopiate systems in the regulation of food intake. The concept of dopamine being primarily responsible for the initiation of chewing behavior and the opiates regulating food ingestion is compatible with the observations reported here.

Bromergocryptine Dynorphin Dopamine Naloxone Opiates TRH Bombesin CCK Calcitonin Appetite

WE have previously proposed an integrated hypothesis to explain the monoaminergic-peptidergic regulation of appetite [26]. 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 was 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 noradrenergic-thyrotropin releasing hormone (TRH) system. It was suggested that gamma amino butyric acid (GABA) stimulated food intake by decreasing the serotonergic inhibitory effect on feeding. in a series of studies, we have pharmacologically examined the interrelationships of a number of the inhibitory peptides and monoamines in a variety of feeding models [19, 30, 31, 35]. In this study we have used a similar pharmacological approach to examine the interrelationship of dopamine and the endogenous opioid peptides and their relationship to the central regulation of appetite.

Although the endogenous opioid peptides have been demonstrated to have a variety of central effects [29], a number of lines of evidence have suggested that they may play a primary role in the regulation of appetite [26,36]. Systemic administration of the opiate antagonist, naloxone, decreases feeding in food deprived animals [4,15] during stress-induced eating [23,27], after muscimol-induced [30], norepinephrine-induced [35] or diazepam-induced [44,45] eating, and in responses on operant schedules for food reinforcement [10]. A number of studies have suggested that the classical opiate, morphine, facilitates food intake [43]. Grandison and Guidotti [12] showed that intra-hypothalamic injection of β-endorphin initiates feeding. The long acting methionine-enkephalin analog, D-ala-met-enkephalin, has also been shown to induce feeding in sated rats [3] and to reverse the satiety effect of a number of putative satiety factors such as cholecystokinin, bombesin and thyrotropin-releasing hormone and its metabolite, histidyl-proline diketopiperazine [28, 31, 34]. Dynorphin-(1–13), a basic opioid peptide, is the most potent opioid peptide isolated to date [11]. Recently we have shown that dynorphin-(1–13) induces spontaneous feeding in rats after intracerebroventricular (ICV) injection and that this initiation of food ingestion is antagonized by concomitant administration of a small dose of naloxone, ICV [37].

A number of studies have implicated dopamine in the regulation of appetite. Dopamine depletion in the nigrostriatal tract duplicates most of the lateral hypothalamic starvation syndrome and dopamine agonists help restore food ingestion [24, 46, 47]. Dopamine antagonists suppress deprivation induced feeding, feeding elicited by injections of 2-deoxy-glucose and stress-induced (tail-pinch) eating [23, 27, 41, 42].

In this study we report that the dopamine agonist, bromergocryptine, induces feeding over an extremely narrow-dose range and that this feeding is antagonized by opiate blockade. In addition, we have examined the effect of dopamine blockade on dynorphin-(1–13) induced eating. These studies allow us to further elaborate on the complex interrelationship of dopamine and the endogenous opioids as a tonic stimulus governing food intake.

TABLE 1
THE EFFECT OF BROMERGOCRYPTINE ON FOOD INGESTION

	n	g/60 min
Vehicle	6	0.2 ± 0.2
Bromergocryptine 40 μg ICV	6	0.2 ± 0.1
60 μg ICV	10	0.5 ± 0.3
80 μg ICV	8	1.9 ± 0.6*
100 μg ICV	8	0.5 ± 0.3
120 μg ICV	6	0.4 ± 0.2
Bromergocryptine 80 μg + Haloperidol (0.5 mg/kg)	8	0 ± 0†
+ Naloxone (10 mg/kg)	4	0.1 ± 0.1†
+ Naloxone (1 mg/kg)	6	0.2 ± 0.1†
+ Naloxone (0.1 mg/kg)	8	0.8 ± 0.2

*$p<0.01$ vs vehicle.
†$p<0.01$ vs bromergocryptine 80 μg ICV.

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 (Purina rat chow) and water, were used for all experiments. Cannulas were implanted into the lateral ventricles as previously described [28]. The animals were allowed a minimum of 5 days post-operative recovery before experiments were commenced. All animals had free access to food and water until the experiments were performed. The animals were housed in individual cages and all testing was carried out in their home cage. Use of the home cage is important for dynorphin-(1–13) induced eating, as in a preliminary experiment only one of eight animals given 10 μg of dynorphin-(1–13) ICV ate when placed in a novel environment. All testing was carried out between 1400 and 1600 hours. Imemdiately after drug or vehicle administration, animals were returned to their 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/60 minutes). All results are expressed as mean±S.E.M. Results were compared using the two-tailed unpaired Student's *t*-test.

All drugs administered ICV were delivered in a 5 μl bolus. All parenterally administered drugs were given subcutaneously in a 0.2 cc volume. Bromergocryptine (Sandoz Laboratories, East Hanover, NJ) was dissolved in 40% propyleneglycol and dynorphin-(1–13) (Sigma Chemical Co., St. Louis, MO) was dissolved in methanol/0.1 N hydrochloric acid (1:1, v/v). Cholecystokinin octapeptide (CCK-8; Calbiochem-Behring Co., LaJolla, CA) was dissolved in 0.1 mg/ml $NaHCO_3$ and 0.1 mg/ml cysteine. Haloperidol (McNeil Laboratories, Fort Washington, PA), bombesin (Sigma Chemical Co., St. Louis, MO), thyrotropin-releasing hormone (Calbiochem-Behring Corporation, LaJolla, CA) and naloxone (Endo Products, Garden City, NY) were dissolved in saline. Control injections included, where appropriate, the propyleneglycol or methanol carrier and were adjusted to the same pH.

RESULTS

ICV bromergocryptine consistently induced eating at a 80 μg dose (Table 1). The mean latency to initiation of feeding was 19.1±2.6 min. At the 60 and 100 μg doses, bromergocryptine induced feeding in 9 out of 10 animals and 4 out of 8 animals compared to only 1 out of 6 animals receiving the vehicle. Bromergocryptine (80 μg)-initiated eating was inhibited by the dopamine antagonist, haloperidol (0.5 mg/kg, SC) and by the opiate antagonist, naloxone (Table 1). Dynorphin-(1–13) (10μg) produced the expected increase in feeding over that seen in animals receiving the vehicle (Fig. 1). Dynorphine-(1–13) induced feeding was blocked by the opiate antagonist, naloxone (1 mg/kg, SC) and by the dopamine antagonist, haloperidol (Fig. 1). Haloperidol suppresses feeding over a one hour period after 30 hr starvation at 0.5 mg/kg (0.7±0.4 g, n=6, $p<0.01$), at 0.1 mg/kg (1.3±0.2 g, n=14, $p<0.01$) but not at 0.01 mg/kg (2.3±0.2 g, n=16) or

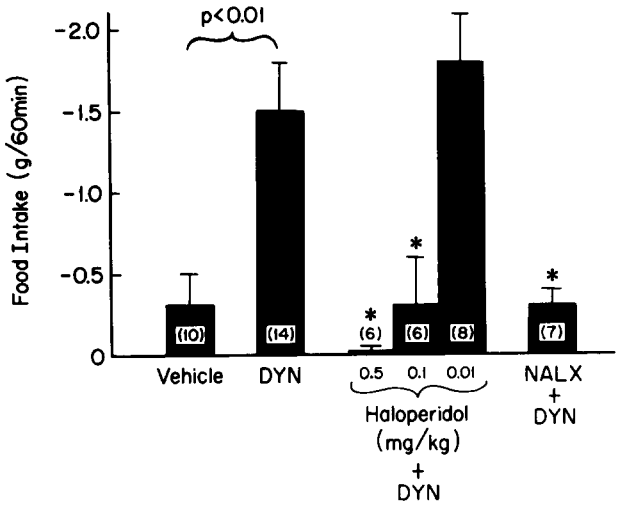

FIG. 1. Effect of the dopamine antagonist, haloperidol, and the opiate antagonist, naloxone, on dynorphin-(1–13)-induced feeding. *$p<0.01$ vs dynorphin-(1–13). DYN=dynorphin-(1–13) 10 μg ICV; NALX=naloxone 1 mg/kg SC.

TABLE 2

EFFECT OF PUTATIVE SATIETY PEPTIDES ON DYNORPHIN-(1–13) INDUCED EATING

	n	Food Intake (g/60 min)	p
Dynorphin (10 μg ICV)	15	1.5 ± 0.2	—
+ Bombesin (10 μg/kg sc)	6	0.1 ± 0.1	0.01
+ CCK-8 (10 μg/kg sc)	6	1.0 ± 0.5	NS
+ CCK-8 (20 μg/kg sc)	6	1.5 ± 0.4	NS
+ TRH (10 μg ICV)	6	0.7 ± 0.3	NS
+ TRH (20 μg ICV)	6	1.4 ± 0.3	NS
+ Calcitonin (1 unit ICV)	14	0.9 ± 0.2	NS

0.001 mg/kg (2.6±0.3 g, n=9) compared to eating in control animals (2.1±0.2 g, n=19).

Of the putative satiety factors tested only bombesin suppressed dynorphin-(1–13) induced eating with cholecystokinin-octapeptide, thyrotropin-releasing factor (TRH) and calcitonin being ineffective (Table 2). Bombesin, calcitonin and CCK-8 all suppressed bromergocryptine induced feeding while TRH was ineffective (Table 3).

DISCUSSION

The dopamine agonist, bromergocryptine, induced spontaneous eating over an extremely narrow range with a bell shaped dose response curve. The bromergocryptine induced eating was inhibited by the dopamine antagonist, haloperidol. It is well recognized that destruction of the dopaminergic fibers in the nigro-striatal tract inhibits feeding [5, 25, 46].

Previous studies involving the administration of dopamine agonists have been reported to either increase or decrease feeding depending on the agonist involved and the site of administration (see [16] for a review). The major effect of dopamine agonist administration appears to be increased locomotor activity and an oral stereotypy of which gnawing is one of the main manifestations [6,14]. Further evidence suggesting a major role of dopaminergic mechanisms in the regulation of gnawing behavior comes from the studies on stress-induced (mild tail pinch) eating which has been demonstrated to be secondary to activation of the endogenous dopaminergic sytems [1,2]. Recent evidence has suggested that the primary tail pinch behavior may be chewing with eating representing an epiphenomenon ([17, 18, 22, 38] and unpublished observations). The steep dose-response seen with bromergocryptine may be due to small concentrations of dopamine being capable of stimulating feeding *per se* whereas slightly higher concentrations may cause oral stereotypy to become the predominant behavior, producing disruption of normal feeding patterns. This could explain the conflicting results previously reported for the effect of dopamine agonists on feeding behavior [16].

There is a large body of biochemical and pharmacological evidence suggesting a close interrelationship of dopamine and the endogenous opiates in the central nervous system [29]. The inhibition of bromergocryptine induced feeding by naloxone is consistent with our previously published model of central appetite regulation [26]. We had suggested that dopamine initiates feeding by activating the endogenous opiates and activating gnawing behavior by an alternative mechanism [20].

Dynorphin-(1–13) is newly isolated potent endogenous opioid peptide [11]. Besides its effects on feeding behavior, it produces analgesia [13], catalepsy [13], excessive grooming [37,49], alterations in centrally stimulated gastric acid secretion [32] and mild hyperthermia (unpublished observations). We have previously shown that dynorphin-(1–13) induced eating is blocked by simultaneous central administration of a small dose of naloxone (5 μg) suggesting that its effects on feeding are mediated through opiate receptors [37]. In this study we have suggested that dynorphin-(1–13) induced eating is also blocked by the dopamine antagonist, haloperidol. This finding could still be consistent with our previously proposed model of appetite regulation as the dopamine antagonist could be predominantely blocking chewing behavior and if the animal cannot chew, it cannot eat. In studies reported here we have shown that in the same strain of animals the doses of haloperidol that suppress dynorphin induced eating, also suppress starvation-induced feeding. We have previously shown that naloxone blocks stress-induced eating while not suppressing stress-induced chewing [27,31]. Further studies with other dopamine antagonists will be necessary to confirm the specificity of this effect.

Cholecystokinin [7,39], bombesin [8,33], TRH [28,48] and calcitonin [9,21] are considered putative satiety hormones. We administered these substances at pharmacological doses previoulsy demonstrated to suppress eating at pharmacological doses previously demonstrated to suppress eating in a

TABLE 3

EFFECT OF PUTATIVE SATIETY PEPTIDES ON BROMERGOCRYPTINE INDUCED EATING

	n	Food Intake (g/60 min)	p
Bromergocryptine (80 μg ICV)	15	1.1 ± 0.2	—
+ Bombesin (10 μg/kg sc)	6	0.2 ± 0.1	0.025
+ CCK-8 (10 μg/kg sc)	6	0.5 ± 0.2	0.05
+ TRH (10 μg ICV)	10	0.9 ± 0.2	NS
+ TRH (20 μg ICV)	6	1.4 ± 0.2	NS
+ Calcitonin (1 unit ICV)	8	0.1 ± 0.1	0.01

variety of feeding models. Only bombesin suppressed both bromergocryptine and dynorphin induced eating. CCK-8, TRH and calcitonin were ineffective at suppressing dynorphin-(1–13) induced eating whereas only TRH was ineffective at suppressing bromergocryptine induced eating. These data suggest that CCK and calcitonin produce satiety by inhibiting the dopaminergic feeding system whereas bombesin has a direct effect on the opioid feeding drive. Further studies will be necessary to unravel the complex interactions of the satiety factors on the dopamine-opioid system responsible for the initiation of food intake. In conclusion, these studies have demonstrated the close interaction between the dopaminergic and opiate systems in the regulation of food intake. The concept of dopamine being primarily responsbile for the initiation of chewing behavior and the opiates regulating food ingestion is compatible with the observations reported here.

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