Peptides that Regulate Food Intake
An orexigenic role for µ-opioid receptors in the lateral parabrachial nucleus

John D. Wilson, Danielle M. Nicklous, Vincent J. Aloyo, and Kenny J. Simansky
Department of Pharmacology and Physiology, Drexel University
College of Medicine, Philadelphia, Pennsylvania 19102

Submitted 4 March 2003; accepted in final form 11 August 2003

Wilson, John D., Danielle M. Nicklous, Vincent J. Aloyo, and Kenny J. Simansky. An orexigenic role for µ-opioid receptors in the lateral parabrachial nucleus. Am J Physiol Regul Integr Comp Physiol 285: R1055–R1065, 2003; 10.1152/ajpregu.00108.2003. The pontine parabrachial nucleus (PBN) has been implicated in regulating ingestion and contains opioids that promote feeding elsewhere in the brain. We tested the actions of the selective µ-opioid receptor (µ-OR) agonist [d-Ala²,N-Me-Phe⁴,Gly⁵-ol]enkephalin (DAMGO) in the PBN on feeding in male rats with free access to food. Infusing DAMGO-induced feeding, with complete blockade by 1.0 µmol/kg h of the µ-OR-selective antagonist [d-Ala²,N-Me-Phe⁴,Gly⁵-ol]enkephalin (DAMGO) in the PBN on feeding in male rats with free access to food. Infusing DAMGO increased food intake. The hyperphagic effect was anatomically specific to infusions within the LPBN, dose and time related, and selective for ingestion of chow compared with (nonnutritive) kaolin. The nonselective opioid antagonist naloxone (0.1–10.0 nmol intra-PBN) antagonized DAMGO-induced feeding, with complete blockade by 1.0 nmol and no effect on baseline. The highly selective µ-opioid agonist D-Phe-Cys-Trp-Arg-Thr-Pen-Thr-NH₂ (CTAP; 1.0 nmol) also prevented this action of DAMGO, but the κ-antagonist nor-binaltorphimide did not. Naloxone and CTAP (10.0 nmol) decreased intake during scheduled feeding. Thus stimulating µ-ORs in the LPBN increases feeding, whereas antagonizing these sites inhibits feeding. Together, our results implicate µ-ORs in the LPBN in the normal regulation of food intake.

Address for reprint requests and other correspondence: K. J. Simansky, Dept. of Pharmacology and Physiology, Drexel Univ. College of Medicine, Mailstop 488, 245 N. 15th St., Philadelphia, PA 19102-1192 (E-mail: simansky@Drexel.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
evidence has demonstrated β-endorphin (18), several forms of dynorphin and enkephalin (17, 62, 74), and endomorphin (particularly EM-1; 58) in the PBN. The extrinsic pathways are partly responsible for the communication of the hypothalamus (including PVN) (62), NTS (74), and spinal cord (83) with this region. Complementary to these observations, the LPBN expresses κ-ORs (55, 89) and especially μ-ORs (1, 5, 14, 55–57).

There is one report of δ-ORs in the PBN (4), but they are very sparse at best (cf. Ref. 55). Taken together, the PBN appears to be an excellent candidate for a region in the brain stem where ORs should modulate feeding.

Several convergent findings have established a role for parabrachial ORs in feeding and possibly more broadly in ingestive reward. In 1991, Carr et al. (13) reported that infusing the relatively nonselective (25) OR antagonist naloxone into the LPBN increased the threshold for electrical stimulation of the lateral hypothalamus to elicit feeding in rats. In contrast, the κ₁-OR-selective antagonist nor-binaltorphimine (norbNI) failed to alter thresholds. Given the poorer affinity of naloxone for δ-ORs than the others and the sparse, at best, presence of δ-ORs in the PBN, these data suggested that activating μ-ORs in the LPBN normally enhances the probability of feeding. Parabrachial infusion of DAMGO into the PBN increased preference of rats to consume saccharin (63), thus supporting this hypothesis. Finally, chronic food restriction downregulated μ-ORs (and upregulated κ-ORs) in the PBN (91). This suggested that physiological status influenced the ingestive roles of opioids within this pontine region. However, the effects of manipulating parabrachial opioid mechanisms on initiation of eating, meal size, and prandial behaviors have not been reported.

The present study, therefore, analyzed the effects of stimulating and antagonizing μ-ORs in the LPBN on food intake and feeding. We employed the selective μ-agonist DAMGO to elicit feeding and the antagonists naloxone (nonselective) and d-Phe-Cys-Trp-Arg-Thr-Pen-Thr-NH₂ (CTAP; μ-selective) (2, 44) to inhibit feeding. The results demonstrate a robust hyperphagic action of DAMGO that is anatomically and behaviorally selective. Conversely, blocking μ-receptors in the LPBN inhibits feeding. Some of these data have been presented in abstract form (81).

MATERIALS AND METHODS

Animals and Surgery

Male Sprague-Dawley rats weighing 350–450 g were housed in wire-mesh hanging cages (43 cm long × 22 cm wide × 18 cm high). Rats were obtained from Taconic Farms (Germantown, NY) unless indicated otherwise (Harlan Industries; Indianapolis, IN). The animals were housed in a temperature-controlled room (23 ± 1°C) with a 12:12-h light-dark cycle (lights on at 0630). Standard pelleted rat chow (Purina, St. Louis, MO) and tap water were available as indicated below. Rats were anesthetized with Equithesin (3.5 ml/kg ip) and placed in a Kopf stereotaxic apparatus (Kopf Instruments, Tujunga, CA). A single 26-gauge stainless steel cannula (Plastics One, Roanoke, VA) was implanted, aimed to end 1 mm above the lateral parabrachial nucleus (LPBN).

The stereotaxic coordinates were determined from the rat atlas of Paxinos and Watson (69) and were 9.5 mm posterior to bregma, 1.8 mm lateral to the midline suture, and −4.8 mm ventral, with the skull level between lambda and bregma. The animals were allowed 10 days to recover from the surgery before testing.

Infusions were made in a total volume of 0.5 μl of 0.9% sterile saline (vehicle) using a Harvard infusion pump (Harvard Apparatus, Cambridge, MA) with a remote 10-μl Hamilton microsyringe (Hamilton, Reno, NV) attached to a 33-gauge injector with PE-20 polyethylene tubing. The drugs and vehicle solutions were made just before the infusion. The injectors extended 1 mm beyond the end of the guide cannula. The 90-s infusions began between 1000 and 1100; the injector was left in place for 30 s after delivery of drug or vehicle to minimize backflow.

Testing Procedure

Food (~70 g; daily ad libitum intake was 28–30 g) and fresh tap water were provided ad libitum for all experiments except in the studies that determined the effects of OR blockade on scheduled feeding (see below). The food was removed from the cage before the infusion. After the infusion, the preweighed food was placed into the cage. Food remaining in the cage and spillage under the cage were weighed 30, 120, and 240 min after infusion. In the experiment assessing the behavioral profile after infusing DAMGO ([d-Ala², N-Me-Phe⁴, Gly⁵-ol]enkephalin; mol wt = 514; Peninsula Laboratories, Belmont, CA), food and spillage were measured only at the end of the 120-min test. The rats received infusions of vehicle on successive days until baselines stabilized (3–4 days). Once the testing cycle started, at least one baseline test was completed before each drug test, with drug treatments every 4th day. All procedures for these studies were performed in compliance with the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society (3) and were approved by the Institutional Animal Care and Use Committee (IACUC) of MCP Hahnemann University (now, Drexel University).

Experimental Design

Actions of DAMGO on food intake: time course and dose effect. The actions of DAMGO on food intake were tested in two groups of eight rats. The first group received 2 nmol of peptide. In the second group, we administered DAMGO into the PBN in the order 2.0, 0.5, 1.0, and 4.0 nmol to determine a dose-effect curve. After this sequence, attempts to test 8.0 nmol in four rats produced catalepsy, and no additional tests were performed with this higher dose.

Behavioral profile after infusion of DAMGO. To characterize the behavioral effects of DAMGO (2 nmol), we used forced time-sampling observations (e.g., Ref. 47) every 30 s for 120 min after infusion of peptide or vehicle, in a new group of six rats. The behaviors monitored were feeding, drinking, locomotion, rearing, sniffing, standing, and resting. Locomotion, sniffing, and rearing were combined into a single measure called “total activity.” The total number of observations for each rat, per behavioral measure, was summed, and the mean and SE were calculated for each 15-min time interval. The drug and vehicle infusions were prepared by another investigator, and a counterbalanced order was used to ensure that the observer was unaware of the treatments of the individual rats.
Behavioral specificity of ingestion. An additional group of rats (Harlan, IN) was infused with vehicle, followed on the next day with DAMGO (2.0 nmol), and presented simultaneously with standard chow and a nonnutritive substance (kaolin; Refs. 54, 86) to test the specificity of the hyperphagic effect to real food. Kaolin (aluminum silicate, Sigma-Aldrich, St. Louis, MO) was hydrated and formed into chow-sized pellets that were dried before presentation. The resulting pellet was slightly lighter in color than chow, but similar in size and weight. Intakes of the chow and kaolin pellets, corrected for spillage, were determined at 30-, 120-, and 240-min intervals.

Effects of delaying food presentation. On the day after completing the experiment with kaolin, the same rats were adapted to waiting 30 min after the parabrachial infusion of vehicle before chow (only) was placed in the cage. Once baselines stabilized (~4 days), we tested the effects of 2 nmol of DAMGO on food intake 30, 120, and 240 min after food was provided. Thus the rats had access to food during the interval from 30 min to 4.5 h after infusion. We then tested the rats on the standard schedule used in the other studies in which food was provided. Thus the rats had access to food during the interval from 30 min to 4.5 h after infusion. Under these latter conditions, the rats had access to food from 0 to 4 h after administration of drug or vehicle.

Pharmacological mechanisms for opioid-related feeding in the PBN. Antagonism of DAMGO-induced hyperphagia. Rats from the previous experiment were infused with the following combinations of treatments: vehicle + vehicle; vehicle + DAMGO (2 nmol); the nonselective opioid antagonist naloxone hydrochloride (10 nmol; mol wt = 364; Sigma, St. Louis, MO) + vehicle; and naloxone + DAMGO. The interinfusion interval was 15 min, and food was placed in the cage immediately after the second infusion. In another group of six animals, naloxone (0.01, 0.1, and 1.0 nmol) or vehicle was infused to determine the potency of this antagonist to inhibit the hyperphagic effect of DAMGO (2.0 nmol). Next, these rats were infused with the κ-selective antagonist nor-BNI dihydrochloride (mol wt 735; Sigma) before DAMGO. The dose of nor-BNI (1.0 nmol) was equimolar to the dose of naloxone that prevented the hyperphagic action of DAMGO in the dose-response study. Finally, in a new group of five rats, we tested whether the μ-or-selective antagonist CTAP (1.0 nmol; mol wt 1,104; d-Phe-Cys-Trp-Arg-Thr-Pen-Thr-NH₂; Tocris Cookson, Ellisville, MO) would block the effect of DAMGO to increase food intake. The rats were tested under each of the following conditions: vehicle + vehicle; vehicle + DAMGO; CTAP + vehicle; and CTAP + DAMGO.

Inhibition by naloxone and CTAP of feeding elicited by limited access to chow. Another group of rats (n = 6) was restricted to 30 g of food per day (~100% of normal daily intake) for 10 days. They were provided with fresh food at the same time each day (1000). Under these conditions, the rats consumed a reliable amount of food during the baseline tests (~12 g/240 min). After this adaptation period, we evaluated whether infusion of naloxone alone (10 nmol) would reduce food intake under these conditions. A second experiment was conducted with 10 additional rats in which the μ-selective antagonist CTAP (10 nmol) was used instead of naloxone.

Histological Analysis

After the completion of each experiment, the rats were perfused with 0.9% saline followed by 10% neutral buffered formalin (Fischer Scientific; King of Prussia, PA). The brains were removed and placed in the buffered formalin until staining with cresyl violet for histological examination with reference to the atlas of Paxinos and Watson (69). Place-
motor nucleus of the trigeminal nerve (corresponding to 8.7 mm posterior to bregma in the atlas of Paxinos and Watson (69)) to caudally at the level 9.3 mm posterior to bregma. Cannulas in the nonresponders were placed lateral, dorsal, medial, and ventral to the PBN at different coronal levels.

Behavioral analysis of DAMGO-induced feeding. The results of the time-sampling analysis revealed that DAMGO produced a main effect of drug treatment \[F(1,5) = 43.8, P < 0.01\] and time \[F(7, 35) = 3.4, P < 0.01\] (Fig. 4). DAMGO increased total nonfeeding activity above baseline during the first 75 min. Feeding began later and increased significantly during the period from 30 to 75 min. Resting emerged as feeding and other activity waned. Vehicle-infused animals rested for the majority of the time and more than after DAMGO, from 30 to 105 min. There was no significant difference in resting between the two groups during the last 15-min interval of the 120-min session.

Based on the time-sampling analysis, kaolin was presented simultaneously with food to clarify the behavioral specificity of the hyperphagic effect. DAMGO increased food intake above baseline during the 240-min test \([\text{DAMGO}, 3.1 \pm 0.4 \text{ g vs. vehicle, } 0.4 \pm 0.1 \text{ g, } P < 0.01, F(1,5) = 3.03, P < 0.01]\). In contrast, rats did not eat kaolin (Fig. 5).

As just described, DAMGO-induced feeding began after a latency of ~30 min when rats had access to food immediately after infusion of this peptide. It was possible that contact with food, or with cues related to food, for this amount of time was necessary to enable the orexigenic action of DAMGO. If so, then delaying food presentation should further retard feeding. Figure 6 shows, however, that the animals increased consumption of food immediately (i.e., within the initial 30-min measurement interval) when the chow was placed in the cage 30 min after infusion. As in the earlier experiments, the same rats did not eat during the first 30-min period when chow was provided immediately after infusion, \(F(1,5) = 8.21, P < 0.05\). Total intake for the 4-h period after food presentation did not differ between conditions (no delay, 4.8 ± 0.5 g; delayed, 4.7 ± 0.7 g).

Opioidergic Mechanisms for Feeding Elicited by DAMGO and Food Deprivation

Naloxone and CTAP block the hyperphagic action of DAMGO. Naloxone (10 nmol) administration by itself did not affect intake in nondeprived rats. This antagonist, however, blocked the hyperphagic effect of DAMGO during the 240-min test, \(F(3,15) = 22.9, P < 0.01\) (Fig. 7). Dose-response analysis showed intrapBN infusion of naloxone reduced the hyperphagic effect, with 1.0 nmol of the antagonist blocking the hyperphagia completely (Fig. 8). In contrast, the \(\kappa\)-OR-selective antagonist nor-BNI (1.0 nmol) did not alter the hyperphagic effect of DAMGO. Finally, the \(\mu\)-OR-
selective antagonist CTAP did prevent DAMGO-elicited feeding (Fig. 9).

Naloxone and CTAP reduce deprivation-induced feeding. The ad libitum schedule for feeding used in the previous studies produced expected low baselines for food intake. To assess the action of opioid blockade on feeding, we adapted the rats to a schedule in which they received a ration of 30 g (100% of daily free intake) at the same time each day. This scheduled, rationed feeding produced much higher, reliable baselines (Table 1). On this schedule, infusion of naloxone (10 nmol) decreased food intake, $F(1,5) = 21.0, P < 0.01$. The cumulative intake was smaller at each interval, $F(2,10) = 5.9, P < 0.01$, with a significant treatment by time interaction during the 240-min test. Naloxone did not inhibit consumption of chow in the first measurement period but did thereafter. CTAP did reduce intake significantly during the initial period and this effect persisted for the 4-h test [$F(1,9) = 15.11, P < 0.01$]. Separate ANOVAs of the amounts ingested within each interval, rather than cumulatively, revealed that naloxone reduced intake only during the second interval and CTAP only during the first, with no compensatory overeating in the latter periods.

DISCUSSION

These data strongly implicate μ-ORs in the lateral parabrachial region of the pons in the excitatory modulation of feeding in rats. Infusion of the opioid peptide agonist DAMGO into this site robustly increased food intake in nondeprived rats. DAMGO has very high affinity for μ-ORs (0.14 nM) and displays at least 1,000-fold selectivity in binding to these vs. κ- and δ-opioid recognition sites (25). In autoradiographic studies, DAMGO was a full agonist at activating G proteins in the PBN. This cellular action was blocked by the nonselective opioid antagonist naloxone but not by the potent κ-OR-selective antagonist nor-BNI or by the δ-selective antagonist ICI-174,864 (78).

These observations in vitro mimicked our results in which naloxone but not nor-BNI blocked the orexigenic action of DAMGO. A dose of 1.0 nmol of naloxone reduced intake to baseline levels. An equimolar dose of the highly selective μ-OR antagonist CTAP also blocked completely the action of DAMGO, but the same dose of the selective κ-OR antagonist nor-BNI did not. In one representative estimate (60), naloxone had binding affinities of 3.9, 95, and 16 nM for the μ-, δ-, and
κ-ORs, respectively. In comparison, the highly κ-selective antagonist nor-BNI displayed affinities of 97, 244, and 0.8 nM. Nor-BNI selectively blocks the κ₁-OR subtype, although it retains significant antagonist activity vs. κ₂-OR subtypes; naloxone is less selective and less potent (31). Thus, in our experiment, 1.0 nmol of nor-BNI should have occupied the κ-ORs as much or more than the equimolar dose of naloxone. These data argue that κ-ORs are not involved in the orexigenic effect of DAMGO in the PBN. CTAP is 10-fold more potent than naloxone at blocking κ-ORs and has >1,000-fold higher affinity for κ- than δ-ORs with poor affinity also for δ-ORs (2, 31, 44). Rich concentrations of μ-ORs exist in the external lateral and external medial subnuclei with dendritic labeling extending further dorso-laterally into the LPBN (14, 56). Our results would appear to establish that activating the δ-OR subtype in the lateral parabrachial region elicits feeding. Interestingly, nor-BNI has inhibited feeding elicited by DAMGO in the lateral ventricles (52), shell of the NAC (72), and ventral tegmental area (45). Blocking δ-ORs antagonized DAMGO-induced feeding in the ac—
cumbens shell (72). The ability of non-\(\mu\)-antagonists to block the hyperphagic action of DAMGO has implied a complex interrelationship among the ORs in feeding. Our data would appear to eliminate \(\kappa\)-ORs (at least \(\kappa_1\) and \(\kappa_2\)) in this role in the PBN. It remains possible that DAMGO can recruit parabrachial \(\delta\)-ORs indirectly through a serial, polysynaptic mechanism. Agonists at \(\delta\)-ORs exert electrophysiological actions at parabrachial synapses (15), but the PBN has at most a very sparse population of this subtype (4).

DAMGO increased food intake during the measurement interval from 30 to 120 min after infusion but not earlier. These results corresponded to the 30-min latency for the onset of feeding that was determined by behavioral observations. DAMGO certainly did not in-
capacitate the rats initially because motor activity other than feeding was elevated at the 15-min timepoint. The frequency of nonfeeding motor behaviors at 30 min (when feeding did emerge) was similar to that in the first interval. Thus it was unlikely that competition from hyperactivity explained the delayed ingestion. We entertained the possibility that DAMGO elicited a stage of foraging that required contact with food to enable feeding behavior. However, rats ate beginning 30 min after DAMGO whether or not food was present immediately after infusion. Similar observations have been reported for increased ingestion of chow or sucrose solution despite elevated motor activity after infusion of DAMGO into striatum and NAC (9, 95). Delayed feeding occurs also after administration of DAMGO into the NTS (42).

The anatomic distribution of the sites that are sensitive to the orexigenic action of DAMGO argues against an explanation based on the need for diffusion to a remote, sensitive locus (9). It might be necessary for DAMGO to recruit neurons in a serial fashion in a network. The concept of such a distributed network has been proposed (24; see also Ref. 90). That same re-

Table 1. Naloxone and CTAP reduce cumulative food intake on a rationed feeding schedule

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>30</th>
<th>120</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>6</td>
<td>5.4±0.7</td>
<td>9.6±1.6</td>
<td>11.6±1.5</td>
</tr>
<tr>
<td>Naloxone (10 nmol)</td>
<td>6</td>
<td>4.4±0.5</td>
<td>5.8±0.9*</td>
<td>8.6±1.3*</td>
</tr>
<tr>
<td>CTAP (10 nmol)</td>
<td>10</td>
<td>5.1±0.8</td>
<td>6.8±1.0</td>
<td>9.8±1.4</td>
</tr>
<tr>
<td>CTAP (10 nmol)</td>
<td>10</td>
<td>2.8±0.6*</td>
<td>5.2±0.8*</td>
<td>7.7±1.2*</td>
</tr>
</tbody>
</table>

Values are g of chow consumed (means ± SE) by separate groups of rats after parabrachial infusion of indicated treatments. **P < 0.01; Student-Newman-Keuls test after ANOVA.
search group, however, demonstrated bidirectional communication mediated by μ-opioidergic mechanisms (20, 21; see Introduction). Thus, although a network of coordinated regions probably does exist, it does not appear to provide the basis for retarded feeding. It is possible that behaviorally prepotent testing conditions, such as more palatable food, might accelerate feeding after parabrachial infusion of DAMGO. Examination of data from other regions of the brain, such as the NAC, however, does not support that prediction yet (e.g., compare Refs. 9, 95). Finally, the kinetics of changes in second messengers and other cellular mechanisms linked to these receptors may influence response latency (e.g., see discussions in Refs. 9, 39). This possibility has not been addressed.

The PBN receives afferents from the NTS (29, 74) and the area postrema (46). Gastrointestinal toxicants, especially lithium chloride, increase c-fos translation in the LPBN (92). Thus this region is part of the circuitry that supports alimentary responses to aversive states. Rats eat kaolin in response to gastrointestinal distress (86), and some agents, including the potent orexigen neuropeptide Y (54), have been shown to provoke consumption of this compound when offered simultaneously with chow. Nonetheless, infusion of DAMGO stimulated the intake of regular chow but not pellets of kaolin. This observation argues that μ-receptor activation selectively recruited true feeding. Rats drank at the spout relatively few times after DAMGO in the present study. In a recent experiment (66), rats did consume more water after parabrachial administration of DAMGO during a 2-h test with food present, but not when food was absent. Thus any increase in water intake after parabrachial DAMGO is prandial and secondary to the hyperphagic action of this μ-agonist. Analogously, infusion of this peptide into the NAC has been reported to selectively increase sucrose but not water intake (95).

Bilateral excitotoxic lesions of the LPBN, but not the medial PBN, blocked conditioned taste aversion and place aversion, but not place preference, in which systemically administered morphine served as the unconditional stimulus (10). In comparison, lesions of the tegmental pedunculopontine nuclei blocked place preference. This argues that the LPBN mediates neurotransmission that relays aversive stimuli generated by opiate stimulation in the gut. It does not necessarily argue that ORs within the LPBN are essential for that function, although certainly that is a reasonable, testable hypothesis. The results suggest also that appetitively rewarding actions of opiates do not involve the LPBN.

These data would appear to conflict with our results demonstrating a positive appetitive response to local activation of μ-ORs in the LPBN. The specific methods and experimental questions, of course, are quite different. Indeed, it is interesting to note that infusion of naloxone, but not nor-BNI, into the LPBN increased the threshold for electrical stimulation of the lateral hypothalamus to elicit feeding (13). This would appear to converge with our findings and support a positive role for μ-receptors in the LPBN in motivated behavior. Evidence that the external lateral PBN subserves some aspects of the acquisition of flavor preferences agrees with this view (93).

Our data showed that naloxone and CTAP were much more effective (probably also more potent) in blocking feeding elicited by DAMGO than scheduled feeding of chow. This comparison suggests that μ-ORs within the PBN may play only a partial role in feeding when standard food is the target. OR antagonism in other sites within the brain (e.g., 11, 41, 43, 49) has inhibited feeding. Evidence suggests that the testing conditions greatly influence the degree to which such pharmacological manipulations decrease food intake. For example, foods that might be expected to enhance the activation of endogenous opioid pathways (e.g., highly palatable diet) may also enhance the potency or efficacy of receptor blockade (16, 23, 53). It remains to be determined whether the potency and efficacy of opioid antagonists in the PBN to reduce consumption increases with a more palatable test diet.

Direct injection of morphine into the PBN (more medially than the LPBe) produced discriminative stimulus effects but not flavor preference (34, 35). These data would appear to eliminate the PBN as a site where opioids produce appetitive reward. DAMGO differs pharmacologically in several ways from morphine. DAMGO has higher affinity than morphine for μ-ORs. DAMGO is 10-fold more selective than morphine for binding to μ- compared with κ- or δ-ORs (25). DAMGO is a full agonist at μ-ORs, and morphine is a partial agonist (78). Finally, morphine is glucuronidated in the liver and brains of several species, including rats, to morphine-3-glucuronide and morphine-6-glucuronide (65). The latter metabolite potently stimulates eating after administration into the lateral ventricles (50, 51).

Importantly, intraventricular morphine-6β-glucuronide stimulates a different μ-receptor than DAMGO and morphine itself to produce hyperphagia (50). Thus potential differences between behavioral actions of DAMGO and morphine (given systemically, as above; Ref. 10; or into the LPBN) might consider the role of this active metabolite and its cellular transduction mechanism. More likely, morphine appears to exert both positive, μ-OR-related, and negative, κ-OR-related, properties when administered to the PBN (63, 64). It is not surprising, then, that parabrachial morphine might provide distinctive behavioral cues without producing flavor preference. A role for parabrachial μ-ORs in appetitive conditioning and ingestive reward remains a viable question.

The PBN is a complex region that has been segregated into 13 subnuclei based on differences in cytoarchitectonic, connectivity, and functional characteristics (19, 75). The LPBN includes subnuclei that process, sort, and project viscerosensory and gustatory information (see Introduction). The LPBN also serves as a target where second-order afferents from these feeding-related systems converge and interact physiologically (7, 8, 30, 38). ORs, especially the dense population of the μ-subtype, can serve multiple roles in this area.
For example, the lateral parabrachial region modulates feeding elicited by antinutielibol of fatty acid utilization (12, 28, 33, 87). Intraventricular injections of opioid antagonists or antiserum to OR mRNA reduced lipoprivic feeding (84), whereas infusion of DAMGO into the NAC enhanced consumption of fat (90, 94). Parabrachial μ-receptors may serve a similar function to modulate consumption of fat. The LPBN has been assigned many other roles pertinent for feeding, however, and more precise anatomic and behavioral studies must be conducted to appreciate the full significance of our current findings.

In conclusion, the results of the present study demonstrate that stimulating μ-ORs in the LPBN increases, whereas blocking those sites decreases, food intake in rats. These pharmacological data, therefore, provide direct support for the parabrachial region as a locus within the brain where opioids subserve the distributed neural network modulating normal feeding behavior. Other studies have implicated serotonin (48, 80), GABA (32, 82), neuropeptide FF (66, 79), and perhaps CCK (85). The functions mediated by each of these neuromediators and by their interactions in the regulatory physiology of the PBN are inviting targets for investigation.

These data, except for the CTAP experiments, were submitted by J. D. Wilson in partial fulfillment of the requirements for the Master of Science degree in Neuroscience at MCP Hahnemann University (now, Drexel University).

DISCLOSURES

Research grants from the National Institute of Mental Health (MH-41987) and the National Institute of Diabetes and Digestive and Kidney Diseases (DK-58669) to K. J. Simansky supported this work.

REFERENCES


27. Herbert H, Moga MM, and Saper CB. Connections of the parabrachial nucleus with the nucleus of the solitary tract and...


68. Ragnauth A, Moroz M, and Bodnar RK. Multiple opioid receptors mediate feeding elicited by \( \mu \) and \( \delta \) opioid receptor
82. Söderpalm AHV and Berridge KC. The hedonic impact and intake of food are increased by midazolam microinjection in the parabrachial nucleus. Brain Res 877: 288–297, 2000.