Paraventricular opioids alter intake of high-fat but not high-sucrose diet depending on diet preference in a binge model of feeding

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Naleid AM, Grace MK, Chimukangara M, Billington CJ, Levine AS. Paraventricular opioids alter intake of high-fat but not high-sucrose diet depending on diet preference in a binge model of feeding. Am J Physiol Regul Integr Comp Physiol 293: R99–R105, 2007. First published April 11, 2007; doi:10.1152/ajpregu.00675.2006.—Previous work from our laboratory indicates that when rats are given a choice between a high-fat and a high-sucrose diet, opioid blockade with naltrexone (NTX) in a reward-related site (central amygdala) inhibits intake of the preferred diet only, whereas NTX injected into a homeostasis-related site, such as the hypothalamic paraventricular nucleus (PVN), inhibits intake of both diets. However, other work suggests that opioids increase intake of fat specifically. The present study further investigates the role of PVN opioids in food choices made by calorically-replete animals. We used a binge model with Chow-maintained rats given 3-h access to a choice of a high-fat or high-sucrose diet 3 days a week. We hypothesized that intra-PVN injection of the μ-opioid agonist, DAMGO (0, 0.025, 0.25, and 2.5 nmol) would enhance, and NTX (0, 10, 30, and 100 nmol) would inhibit intake of both diets to an equal extent. We found that when animals were divided into groups according to sucrose or fat preference, DAMGO increased fat intake in fat-consuming animals, while having no effect on intake of either diet in sucrose-consuming animals. NTX, however, inhibited fat intake in both groups. Intra-PVN NTX did not inhibit intake of sucrose when presented in the absence of a fat choice, but did so when injected peripherally. Furthermore, intra-PVN and systemic NTX inhibited intake of Chow by 24-h food-deprived animals. These results indicate a complex role for PVN opioids in food intake with preference, nutrient type, and energy state affecting the ability of these compounds to change behavior.

Paraventricular nucleus; choice; palatability; reward; naltrexone; DAMGO

It is rare that obese patients can maintain weight loss through a low-calorie diet requiring self-discipline, despite how much they might want to lose weight. It is possible that this is due, in part, to an excessive motivation to eat or overactivity of the reward pathway, which, as in dependent states, would override an individual’s impulse control (31). Mesolimbic opioids play a significant role in the reward response to food, apparently acting in response to the quality of the food or its ability to stimulate motivation to continue eating, rather than the initiation due to hunger (1, 3–5, 17, 18, 20–22). However, the nuances of opioid control of feeding are still not fully revealed.

Some studies have argued that opioids enhance intake of fat, while neuropeptide Y (NPY) triggers intake of carbohydrate, deemed the “one peptide, one nutrient” hypothesis (22). Some of the first findings showed that peripheral injection of morphine increased intake of fat but not carbohydrate or protein when all three were presented together (24). A subsequent study supported this finding by showing that peripheral naloxone, an opioid antagonist, suppressed fat intake over 6 h, while leaving intake of carbohydrate and protein intact (23). Indeed, many newer studies also support a role for opioids in fat appetite (12, 16, 18, 22, 30, 32, 33). These results strongly suggest that opioids mediate fat intake to a greater extent than carbohydrate intake.

Other studies, however, suggest that opioids modulate intake of an animal’s preferred food, regardless of nutrient content (12, 13, 22, 26). One of these studies demonstrated that naloxone inhibited intake of a preferred diet, but not of a nonpreferred diet, when rats were stimulated to eat their choice of fat or carbohydrate by injection of NPY or by 24-h food deprivation (12). Another study showed morphine stimulation of carbohydrate intake in carbohydrate-prefering rats and fat intake in fat-prefering rats (14). Moreover, when given a choice of two types of fat or two types of carbohydrate, opioids appear to enhance intake of the more palatable source (i.e., sucrose vs. cornstarch) (10, 11, 29), indicating that sucrose intake may be more subject to control by opioids than less palatable, isocaloric counterparts. It is possible that providing a more palatable carbohydrate source might alter the finding that opioids selectively enhance intake of fat, as described by some of the earlier studies.

A further confound in all these studies is the method used to stimulate intake. In some studies, this is done by depriving or restricting intake for a period of time before the experiment, while in others intake is stimulated by drug injection. A recent study by Corwin (6) demonstrated that animals will spontaneously consume significant amounts (up to 6 g, or 55 kcal) of unaltered shortening without having been deprived for 24 h. This finding provides a method for studying a behavior that more strongly resembles human intake of palatable high-fat food. Much of human overeating occurs in the absence of caloric deprivation, so studies using opioid antagonists to inhibit deprivation-induced feeding are not as applicable as this new method. Corwin showed that providing the shortening snack at the end of the light cycle on an unpredictable schedule (3 days a week on varying days was most effective) causes animals to eat large amounts of shortening despite being

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calorically replete. We found that this phenomenon occurs when animals are provided with a nutrient-enriched high-fat or high-sucrose diet (Table 1) and we used this paradigm to test the ability of DAMGO to stimulate or naltrexone (NTX) to inhibit this kind of feeding.

The present investigation attempts to answer some persistent questions about opioid control of feeding: In the paraventricular nucleus (PVN), a site thought to modulate food intake based on energy needs and not necessarily palatability, how do opioids affect animals’ choice of two palatable diets? Do PVN opioids play a role in the spontaneous feeding modeled by Corwin (6) and how does this behavior compare with deprivation-induced feeding? To further address these issues, we hypothesized that intra-PVN DAMGO would stimulate and NTX would inhibit intake of both diets to an equal extent, regardless of initial preference. To test this hypothesis, we prepared animals with bilateral PVN cannulas and divided them into groups according to their apparent preference for a high-fat or high-sucrose diet; then we provided these diets, according to Corwin’s schedule, without deprivation and observed the feeding response to DAMGO or NTX injection. To clarify the results of the first experiment, we also assessed the ability of systemic and intra-PVN NTX to inhibit sucrose intake alone and laboratory chow intake after 24-h food restriction. We introduce a new way to analyze intake of preferred and non-preferred diets to allow comparison of multiple factors simultaneously.

**MATERIALS AND METHODS**

**Animals**

Male Sprague-Dawley rats (Charles River, Wilmington, MA), weighing 270–330 g were individually housed in conventional hanging cages with a 12:12-h light-dark photoperiod (lights on at 0700) in a temperature-controlled room (21–22°C). Animals were handled daily after arrival and after surgery. All protocols and conditions were approved by the Institutional Animal Care and Use Committee at the Minneapolis Veterans Affairs Medical Center.

![Fig. 1. Coronal diagrams of bilateral paraventricular nucleus (PVN) injection locations in animals included in the study. Dots indicate the location of the injector tip.](http://ajpregu.physiology.org/)
Drugs and Injections

For central injections, the injector (33 gauge) extended 1 mm beyond the tip of the cannula. Between surgery and the first injection 7–10 days elapsed. Injections were administered in volume of 0.5 μl over a period of 30 s, with a 30-s wait before removing the injector. DAMGO (Sigma, St. Louis, MO), the μ-opioid agonist (0, 0.025, 0.25, and 2.5 nmol), was injected unilaterally to stimulate feeding. NTX (Sigma), the general opioid antagonist (0, 10, 30, and 100 nmol) was injected bilaterally to inhibit feeding. Both drugs were dissolved in artificial cerebrospinal fluid (aCSF), which was the vehicle used in all central injections. For peripheral injections, NTX (0, 0.03, 0.1 and 0.3 mg/kg body wt) was dissolved in saline and injected subcutaneously (15 min delay to food presentation). Central microinjections were administered with 2 days between injections and in doses in randomized order to minimize any carryover effect of the previous injection.

Diet-Choice Experiment

Rats were given continual access to standard rodent chow (Harlan Teklad, Madison, WI) and water at all times. Three days a week, for 3 wk after recovery from surgery, animals were given a jar of fat diet and several pellets of AIN 76 formulated sucrose diet (product no. D1001; Research Diets, New Brunswick, NJ) in their home cages for 3 h a day; water was always available, but access to chow was blocked while the fat and sucrose diets were in the cage. Diet composition is shown in Table 1. Animals’ intake of fat and sucrose was recorded every day after 3 h of exposure until intake had stabilized. Animals were then designated as fat preferring or sucrose preferring by averaging the last 3 days’ ratio of fat intake to sucrose intake, in kilocalories. If the ratio was >1, then the animal was designated a fat consumer and if <1, was labeled a sucrose consumer. After 3 wk, most animals rapidly (within 1 h) consumed up to 10 g of their preferred diet, and a smaller amount of their nonpreferred diet, despite having eaten chow during the dark cycle. Animals were consistent in preference, and this designation yielded roughly eleven fat consumers and six sucrose consumers. To illustrate variability in preference, and this designation yielded roughly eleven fat consumers and six sucrose consumers. To illustrate variability in preference, Table 2 shows each group’s average fat-to-sucrose preference ratio, average favorite-to-total ratio, and average total kilocalories consumed for the last three exposures to diets before testing.

Once preference was established, animals were injected with DAMGO or NTX in a repeated-measures, counterbalanced design to determine the effect of opioid stimulation or antagonism on diet choice. Both diets were weighed at 1, 2, and 3 h after placement within the cage.

Table 2. Profiles of preference for each group

<table>
<thead>
<tr>
<th>Preference Group</th>
<th>No. Rats</th>
<th>Fat-to-Sucrose</th>
<th>Favorite-to-Total</th>
<th>Total Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>11</td>
<td>3.62±3.00</td>
<td>0.74±0.11</td>
<td>56.5±13.88</td>
</tr>
<tr>
<td>Sucrose</td>
<td>6</td>
<td>0.45±0.25</td>
<td>0.71±0.12</td>
<td>57.4±9.98</td>
</tr>
</tbody>
</table>

Preference ratios for experimental groups were determined on the last 3 days of the 10-day diet exposure. All values are calculated from kilocalories (±SD). Because fat-to-sucrose ratios are difficult to interpret, favorite-to-total ratios are also provided. Both groups consumed between 71 and 74% of their calories from their preferred nutrient and ate similar amounts of total calories.

Analysis for diet choice experiment. The experimental design yielded two groups: PVN-cannulated fat consumers and PVN-cannulated sucrose consumers. To determine whether the drugs altered total intake, intake of fat, or intake of sucrose, two parameters were averaged for each of the two groups across doses (total kilocalorie intake and that of sucrose kilocalories subtracted from fat kilocalories, herein called intake difference). With these parameters, total intake can be assessed, as can individual diet intake, as seen in the intake-difference calculation, giving a full representation of the animals’ change in choice of diet or total kilocalorie intake with changing dose. Two graphs are produced from these data: one showing total kilocalorie intake across doses for both groups and another showing the difference of fat kilocalories and sucrose kilocalories across doses, indicating a change in diet choice as dose increases.

Data were analyzed with a least squares means test, to determine whether groups behaved differently, and repeated-measures ANOVA to adjust for within-rat variance. Fisher’s paired least significant difference post hoc test determined whether there was a significant effect of dose within each group. Finally, a simple correlation test was performed between preference ratio and drug effect for all animals together, to determine whether drug effect indeed varies with preference (Table 3).

No-Choice Experiment

For the second set of injections, two sets of animals were cannulated in the PVN as described in Surgery, yielding two groups of eight animals with accurate cannula placement. The first group was trained to consume a palatable treat as done in the diet-choice experiment, but these animals only received a sucrose diet, rather than a choice. Once rats were trained to consume the sucrose within an hour in their home cage, NTX was injected into the PVN in a repeated-measures, counterbalanced order. Finally, under the same conditions, sucrose intake was examined after subcutaneous injection of NTX.

The second group of rats in the no-choice experiment was not trained with sucrose pellets. These animals were food-restricted to 80% of free-feeding intake (roughly 17–18 g of chow) for the 24 h preceding the experiment. They were then injected with NTX into the PVN and provided with chow in their regular hoppers 15 min later. These animals were also injected with NTX subcutaneously in the last portion of the experiment.

Data for this no-choice experiment were analyzed using a one-way repeated-measures ANOVA.

RESULTS

Diet-Choice Experiment

DAMGO total intake. At the highest dose (2.5 nmol), DAMGO increased total kilocalorie intake by 33% in fat consumers. There was a significant difference from aCSF treatment in the fat-preferring group at the two highest doses (0.25 nmol DAMGO, P = 0.05; 2.5 nmol DAMGO, P = 0.0001; both compared with aCSF treatment) (Fig. 2A). There was a main effect of dose [F(3,84) = 7.43, P = 0.0002] and interaction of preference and dose [F(3,84) = 4.80, P = 0.004]. Sucrose consumers showed no change in total kilocalorie intake.

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DAMGO intake difference. At the highest dose, DAMGO increased the intake difference (i.e., increased fat intake) by threefold in fat consumers. There was a significant difference from aCSF treatment in the fat-preferring group at the two highest doses (Fig. 2B) [0.25 nmol DAMGO, P = 0.003; 2.5 nmol DAMGO, P < 0.0001; both compared with aCSF treatment]. We found a main effect of preference [F(1,28) = 47.5, P < 0.0001] and dose [F(3,84) = 14.8, P < 0.0001]. There was a nonsignificant interaction of preference and dose [F(3,84) = 2.27, P = 0.09]. There was no significant change in intake difference in the sucrose-preferring group.

NTX total intake. NTX decreased total intake in fat consumers by 25%, and this was significantly different from aCSF treatment at the highest dose (Fig. 3A) [100 nmol NTX, P = 0.0085, compared with aCSF treatment], but there was no effect of NTX on total intake by sucrose consumers. However, there was a nearly significant main effect of dose [F(3,99) = 2.51, P = 0.06].

NTX intake difference. The highest dose of NTX decreased intake of fat relative to sucrose by 37% in fat consumers [100 nmol NTX, P = 0.0085, compared with aCSF treatment] and threefold in sucrose consumers, in fact reversing preference in the latter group [10 nmol NTX, P = 0.041; 30 nmol NTX, P = 0.01; 100 nmol NTX, P = 0.01; all compared with aCSF treatment] (Fig. 3B). There was a significant main effect of preference [F(1,33) = 17.66, P = 0.0002] and nearly significant effect of dose [F(3,99) = 2.55, P = 0.06].

No-Choice Experiments

Group 1. Intra-PVN-injected NTX failed to inhibit spontaneous daytime sucrose intake when sucrose was presented alone to ad libitum chow-fed rats [F(8,32) = 0.427, P = 0.79] (Fig. 4A). However, upon subcutaneous NTX injection, there was a near-significant effect of dose [F(8,24) = 2.89, P = 0.056] (Fig. 4B).

Group 2. Intra-PVN-injected NTX did suppress food-restriction-induced chow intake, with a significant main effect of dose [F(8,32) = 3.42, P = 0.02] with the three highest doses causing significant decreases in intake compared with aCSF controls (Fig. 5A). Subcutaneous NTX also suppressed restriction-induced chow intake with an effect of dose [F(8,24) = 5.12, P = 0.001] and the two highest doses significantly decreased intake (Fig. 5B).

DISCUSSION

We hypothesized that PVN opioids modulate intake of calories in general, rather than intake of a preferred food. We approached this question by dividing animals into groups according to diet preference, injecting DAMGO or NTX into the PVN, and observing changes in total calorie intake and intake of fat relative to sucrose. The findings were surprising. Injection of DAMGO stimulated fat intake primarily in fat-preferring animals. Injection of NTX, however, inhibited fat intake when injected into the PVN in both sucrose and fat consumers, but in this case, the total number of calories taken in was only decreased significantly in fat consumers. Sucrose consumers presumably compensated for the decreased calorie intake from fat by increasing their intake of sucrose.

The observation that DAMGO only stimulated feeding in animals that prefer fat is remarkable, but not without precedent. The finding suggests that there is a difference in the opioid system of fat-preferring animals compared with sucrose-

Table 3. Correlation between intakes with highest drug doses and preference ratio

<table>
<thead>
<tr>
<th>Drug Treatment and Intake</th>
<th>Fat-to-Sucrose Preference Ratio</th>
<th>R Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAMGO total (2.5 nmol)</td>
<td>0.251</td>
<td>0.337</td>
<td></td>
</tr>
<tr>
<td>DAMGO difference (2.5 nmol)</td>
<td>0.552</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>NTX total (100 nmol)</td>
<td>−0.356</td>
<td>0.163</td>
<td></td>
</tr>
<tr>
<td>NTX difference (100 nmol)</td>
<td>0.340</td>
<td>0.185</td>
<td></td>
</tr>
</tbody>
</table>

Correlation between intake at highest DAMGO or naltrexone (NTX) dose and fat-to-sucrose preference ratio for all animals grouped together (n = 17 rats).
preferring animals, since they responded differently to an injected opioid. Studies showing that rats that prefer ethanol are more likely to prefer fat (19) or morphine (25) may support this claim. Since ethanol and fat activate the mesolimbic reward pathway, a common mechanism for fat preference and ethanol preference could be the opioid system in these sites, which have connections with the PVN via the lateral hypothalamus (2). A recent study by Olszewski et al. (27) showed that nociceptin/orphanin FQ, a ligand for the orphan receptor ORL1, which behaves similarly to opioids, also altered intake in fat consumers but had no effect on sucrose consumers, which lends strength to our conclusion that a difference in preference may indicate a different susceptibility to the effects of injected opioids or similar compounds. The lack of effect of DAMGO on sucrose intake in either group supports the findings of previous studies that opioids do not alter sucrose intake even in sucrose-prefering animals.

Intra-PVN NTX decreased total calorie intake at the highest dose in fat consumers but decreased the amount of fat eaten relative to sucrose in both fat and sucrose consumers. Why would DAMGO be ineffective in sucrose consumers, while NTX is able to curb their intake of fat? Since DAMGO is a μ-selective opioid agonist, and NTX is a nonselective general opioid antagonist, we may be seeing a difference in the ratio of μ to other opioid receptors between fat consumers and sucrose consumers. NTX blockade of non-μ-receptors in sucrose consumers may be responsible for inhibition of their fat intake. DAMGO’s effectiveness in fat consumers may reflect an increased population of μ-receptors compared with sucrose consumers.

According to the present findings, activation of opioid receptors in the PVN is necessary for fat intake in this paradigm, in both fat and sucrose consumers, apparently contradicting the finding of Glass et al. (8) that NTX in the PVN inhibits intake of both preferred and nonpreferred diets. However, if the present data is analyzed differently, grouping fat consumers’ fat intake together with sucrose consumers’ sucrose intake (thus creating a preferred diet category), the result is the same as found by Glass et al. That is, NTX in the PVN inhibited fat intake in both fat consumers and sucrose consumers, but had no effect on sucrose intake in either group. When the animals are grouped together, the fat consumers’ fat intake creates the result found by Glass et al. that NTX in the PVN inhibits intake
present analysis allows for direct comparison, rather than inference. Furthermore, the least squares mean test allows for statistical analysis of the interaction of factors when there is variance within animals (due to the repeated-measures dosing) and between preference groups. Standard ANOVA is not capable of accurately comparing groups when this design is employed. This type of analysis allows for more complex design of experiments with multiple factors, such as preference, cannulation site, and drug treatment and perhaps others.

Upon discovering that intra-PVN NTX had no effect on sucrose intake, even in animals that preferred sucrose, we wondered whether this treatment would affect sucrose intake when presented alone without a fat choice. We found that none of the intra-PVN NTX doses were able to decrease spontaneous sucrose intake. However, subcutaneous injection of NTX did suppress sucrose intake, indicating not only that the drug was effective, but also that opioid control of this type of intake may not be occurring in the PVN. Since we have seen decreases in other types of feeding with injection of NTX in the PVN, we reexamined the ability of intra-PVN NTX to inhibit 24-h-food-restriction-induced chow intake. Indeed, as seen previously (8), both intra-PVN and subcutaneous NTX inhibited restriction-induced feeding. These two findings that intra-PVN NTX does not inhibit sucrose intake but does inhibit restriction-induced chow intake may suggest that PVN opioids control intake based on caloric need, rather than hedonics. However, in diet-choice experiment, we have shown that intra-PVN NTX inhibits spontaneous fat intake, which we had considered to be a hedonic-mediated type of feeding. Perhaps this is not an appropriate interpretation. Perhaps dietary fat intake is controlled by opioids in the PVN because it is more calorie-dense than any other nutrient, and therefore is more relevant to an energy-control site like the PVN.

Previous work in our lab (30) has demonstrated that peripherally injected naloxone, an opioid antagonist like NTX, inhibits intake of palatable carbohydrates in nonfood restricted animals, but has no effect on intake of a cornstarch diet. Conversely, when animals were food restricted, naloxone inhibited intake of all diets, including the less-palatable diets. The conclusion of this study was that opioids, in general, modulate the portion of food intake that is done simply for pleasure (the final bites of a meal after being restricted, the excess calories taken in without restriction). Our findings that PVN opioids appear to control only surplus fat intake or restriction-induced chow intake but not sucrose intake, indicate that while overall opioid receptor blockade can inhibit reward-based sucrose intake when that blockade is localized to the PVN opioids are ineffective. This may denote a difference in opioid function depending on site of action. Further studies will address the same questions in different brain sites.

A somewhat novel method employed in this study is the presentation of palatable diets to sated animals on a restricted-access basis. Based on Corwin’s design (6), this model of palatable food ingestion approaches the concept of a binge in humans. Another model addresses the binge-like intake of highly palatable diet after caloric restriction and stress, as in the human experience of dieting (15). Both models appear to be much more relevant to human behavior than the rapid ingestion following a 24-h deprivation. This method has been used for years to determine the role of neuropeptides, like opioids, in feeding, by injection of an antagonist just before

![Graph A](image1.png)

Graph A: Intra-PVN (A) and subcutaneous (B) NTX significantly inhibited 24-h restriction-induced intake of standard chow. Rats receiving highest doses (black bars) were significantly different from saline controls (white bars) P < 0.05; n = 8 rats; repeated-measures design.

![Graph B](image2.png)

Graph B: Subcutaneous Naltrexone Dose (mg/kg) vs. 1-Hour Chow Intake (g).
reinstatement of food. With these new models of ingestion of large amounts of palatable food, we can now determine the role of neuropeptides in modern human eating behavior.

This study demonstrated that opioid receptor activation affects rats differently depending on their initial preference for fat or sucrose diets. Fat consumers responded to injection of DAMGO into the PVN by eating more fat, but not changing their sucrose intake. Sucrose consumers did not respond to DAMGO injection at all. Injection of NTX into the PVN inhibited intake of fat in both fat and sucrose consumers. We conclude that animals that prefer fat respond differently to an exogenously administered opioid and therefore may have a uniquely organized opioid receptor system when compared with sucrose consumers. Furthermore, we conclude that endogenous opioid signaling in the PVN is necessary for the excessive intake of fat in both fat and sucrose consumers. Even when sucrose is presented alone, PVN opioids do not appear to be necessary for spontaneous sucrose intake. However, they are required for restriction-induced Chow intake, suggesting that the PVN controls intake during times of caloric need rather than reward. The role of opioids in the PVN, therefore, appears to be control of fat intake and intake of Chow in times of restriction, but not sucrose intake. The finding that peripheral injection of NTX inhibited sucrose suggests that opioids in some other brain site may control intake of sucrose when it is presented alone.

Further studies should address this new model of intake, the nature of the neurological differences between fat consumers and sucrose consumers, and perhaps, with the new method of analysis, include the variable of connectivity between sites regarding this behavior, using two-site injection studies. It is important that we continue to investigate the role of reward in overeating and that we use models applicable to modern human experience.

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