0022-3565/98/2852-0908$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS Vol. 285, No. 2

JPET 285:908–914, 1998 *Printed in U.S.A.*

Intake of High-Fat Food Is Selectively Enhanced by *Mu* Opioid Receptor Stimulation within the Nucleus Accumbens[1]

MIN ZHANG, BLAKE A. GOSNELL and ANN E. KELLEY

Department of Psychology (M.Z.), University of Wisconsin-Madison, and Department of Psychiatry (A.E.K.), University of Wisconsin-Madison Medical School, Madison, Wisconsin; and Neuropsychiatry Research Institute (B.A.G.), Fargo, North Dakota

Accepted for publication January 2, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

The present study was designed to further investigate the nature of feeding induced by opioid stimulation of the nucleus accumbens through an examination of the effects of intra-accumbens (ACB) opioids on macronutrient selection. In 3-hr tests of free-feeding (satiated) rats, intra-ACB administration of the *mu* receptor agonist D-Ala^2,N,Me-Phe^4,Gly-ol^5-enkephalin (DAMGO; 0, 0.025, 0.25 and 2.5 μg bilaterally) markedly enhanced the intake of fat or carbohydrate when the diets were presented individually (although the effect on fat intake was much greater in magnitude). Intra-ACB injections of DAMGO, however, produced potent preferential stimulatory effects on fat ingestion with no effect on carbohydrate ingestion when both fat and carbohydrate diets were present simultaneously. Moreover, this selective stimulation of fat intake was independent of base-line diet preference and could be blocked by systemic injection of naltrexone (5 mg/kg). We also examined the effect of 24-hr food deprivation on the pattern of macronutrient intake in rats with access to both carbohydrate and fat. In contrast to the DAMGO-induced selective enhancement of fat intake, food deprivation significantly increased the intake of both diets to the same extent; however, in this case, only the stimulated fat intake was blocked by systemic naltrexone. Intra-ACB administration of DAMGO in hungry rats produced an effect similar to that observed in free-feeding rats; preference was strongly shifted to fat intake. Similarly, the opioid antagonist naltrexone (20 μg) infused directly into ACB preferentially decreased fat intake in hungry rats. These findings suggest that endogenous opioids within the ventral striatum may participate in the mechanisms governing preferences for highly palatable foods, especially those rich in fat.

Endogenous opioid peptides have been found to be involved in the modulation of feeding behavior in both humans and rats. Since the initial findings that morphine increased food consumption (Flowers *et al.*, 1929; Martin *et al.*, 1963), the role of endogenous opioids in different aspects of feeding behavior, such as caloric intake, taste reactivity, diet preference and the interaction with other neuropeptides, has been investigated extensively. Generally speaking, peripheral or central injections of opioid agonists, including *mu*, *delta* and *kappa* agonists, increase food intake and opioid antagonists decrease food consumption, at least in the short term, under various testing conditions (Holtzman, 1974; Reid, 1985; Levine and Billington, 1989). In addition to modifying total food consumption, opioid peptides influence macronutrient selection. Several studies with dietary self-selection paradigms showed that acute systemic morphine injections preferentially stimulate fat consumption while suppressing carbohydrate intake and having little modulatory effect on protein intake (Marks-Kaufman and Kanarek, 1980; Marks-Kaufman, 1982; Ottaviani and Riley, 1984). A similar pattern has been observed when rats were treated with morphine chronically (Ottaviani and Riley, 1984; Gosnell and Krahn, 1993). In contrast, the opioid antagonist naloxone selectively decreases fat intake when carbohydrate and protein are also available to rats (Marks-Kaufman and Kanarek, 1981, 1990). However, when base-line diet preference of rats was taken into consideration, Gosnell *et al.* (1990) found that morphine primarily increased preferred diet consumption rather than the intake of a specific macronutrient. Evans and Vaccarino (1990) also found that systemic morphine enhances intake of preferred food. These findings seem to support the currently favored hypothesis that opioids play a role in modulating the palatable aspects of food, making food more rewarding (Cooper and Kirkham, 1993).

Most of the studies described above have been conducted with the peripheral or intraventricular administration of morphine. Although a number of studies have demonstrated the involvement of the ACB in opioid-induced hyperphagia, little work has been done to investigate its role in macronutrient selection. In light of evidence implicating the accumbens in the processing of reward-related information, the investigation of how this structure modulates macronutrient preference could be particularly valuable for further under-

Received for publication September 18, 1997.
[1] This work was supported by Grant DA09311 from the National Institute on Drug Abuse.

ABBREVIATIONS: ACB, nucleus accumbens; DAMGO, D-Ala^2,N,Me-Phe^4,Gly-ol^5-enkephalin.

standing of mechanisms underlying opioid-induced hyperphagia. Indeed, it is well documented that the ACB is a crucial brain area responsible for the establishment and maintenance of drug-seeking behavior induced by opiates, psychostimulants and alcohol (Wise and Bozarth, 1987; Koob and Bloom, 1988). Recently, evidence emerging from both clinical surveys and animal studies demonstrated parallels between drug or alcohol addiction and food cravings (Mitchell *et al.*, 1985; Morabia *et al.*, 1989; Krahn and Gosnell, 1991; Bell *et al.*, 1994; Gosnell *et al.*, 1995). Accordingly, it is possible that the ACBs may be involved in both drug reward and the subjective reward derived from food. This is consistent with our previous studies showing that in satiated rats, microinjections of *mu* and *delta* opioid receptor agonists into the ACB stimulate chow feeding and intake of liquid sucrose, effects both of which are blocked by pretreatment with naltrexone (Bakshi and Kelley, 1993a; Kelley *et al.*, 1996; Zhang and Kelley, 1997). Furthermore, the phenomenon of conditioned feeding induced by multiple morphine microinjections into the ACB provides further evidence to suggest the involvement of reward mechanisms (Bakshi and Kelley, 1994; Kelley *et al.*, in press). The current study extended these investigations to examining intra-ACB opioid effects on macronutrient selection. Because our past findings showed that the orexigenic effect of opioids seems to be mediated primarily by *mu* receptors (Bakshi and Kelley, 1993a; Zhang and Kelley, 1997), the current study was focused on the *mu* receptor. In this study, the relationship between base-line diet preference and the feeding stimulatory effect of opioids was investigated. We further compared the pattern of diet selection induced by opioid treatments with that induced by food deprivation. We assumed that intake in food-restricted rats is driven mainly by energy needs, with taste or palatability being relatively less important. In consideration of the notion that opioids facilitate feeding in satiated animals by enhancing the orosensory palatability of food, we predicted a difference in the profile of these two feeding paradigms.

Materials and Methods

Animals. A total of 54 male rats (Harlan-Sprague Dawley, Indianapolis, IN) weighing between 270 and 320 g were used in the present study. Animals were housed two or three to a cage with access to unlimited water and standard normal laboratory chow (Purina Chow) unless they were under specific food deprivation schedule (see details of experimental design). The lights were on at 7:00 A.M. and off at 7:00 P.M.

Surgery. Animals were handled for several days after arrival. For surgery, animals were anesthetized with a mixture of ketamine and xylazine (90 mg/kg ketamine and 9 mg/kg xylazine). Bilateral guide cannulae were secured to the skull with stainless steel screws and light curable dental resin (Dental Supply of New England, Boston, MA). Coordinates for the aimed sites were (in mm, with tooth bar 4 mm below interaural zero): +1.5 from the bregma in the anteroposterior plane, 1.5 from the midline in the lateromedial plane and −5.5 from the skull in the dorsoventral plane. For this study, placements were not aimed specifically at the core or shell and for the most part were localized in an area between these two subregions. After the surgery, wire stylets were placed in the guide cannulae to prevent occlusion.

Drugs and microinjection. DAMGO, the *mu* receptor agonist, and the general opioid receptor antagonist naltrexone were obtained from Research Biochemicals (Natick, MA). Both of these drugs were dissolved in sterile 0.9% saline. The vehicle was always sterile 0.9% saline.

After the stylets were removed, the drugs in a volume of 0.5 μl were infused through 12.5-mm injector cannulae into the ACB by a microdrive pump (Harvard Apparatus, South Natick, MA), connected *via* polyethylene tubing (PE-10). Thus, injector tips extended 2.5 mm beyond the end of the guide cannula, for a final dorsoventral coordinate of −8.0 mm from skull. The rate of injection was 0.32 μl/min with the total duration of infusion being 1 hr 33 min. One additional minute was allowed for diffusion. Injectors then were removed, and the stylets were replaced.

Behavioral testing and experiment design. After 2 to 3 days of recovery, the rats were brought into the testing room and provided daily with the testing diet (high-carbohydrate diet, high-fat diets or both, depending on the experimental design) for 3 hr in a testing cage. As indicated in table 1, both diets contained equal amounts of vitamins, minerals, choline chloride, fiber and protein, when equated on a caloric basis. Diets were obtained from Teklad Diets (Madison, WI). This period lasted for several days until stable food consumption across days was obtained. Except for the daily 3-hr testing period, animals were provided with standard laboratory chow, either *ad libitum* or with food restriction, depending on the experimental design (see below). Before the testing period began, a saline injection and a sham injection were given on separate days to habituate the rats to the injection procedure. Preweighed jars containing diets were attached to the testing cages. At the end of each testing period, the diet jars were removed and weighed, and the corresponding diet intake in calories was calculated and corrected for spillage. Water was available during the entire testing session.

In experiments 1 and 2, non-food-deprived rats were treated with intra-ACB microinjections of DAMGO in doses of 0 (saline), 0.025, 0.25 and 2.5 μg/side in a counterbalanced order. There was at least 1 day spaced between injections. The testing session began immediately after the microinjections. For the first experiment, there were two groups of rats in which the effect of DAMGO on the consumption of high-carbohydrate diet and high-fat diet were evaluated separately ($n = 10$ for carbohydrate group, $n = 14$ for fat group). The diet was weighed every hour over the 3-hr testing period. For experiment 2 ($n = 14$), non-food-deprived rats had simultaneous access to both diets in the testing cage, and the position of diet jars was exchanged daily. After the rats were adapted to the testing diets, base-line intakes over 3 hr were measured for 3 consecutive days; thus, rats were divided into carbohydrate-preferring (CP) and fat-preferring (FP) groups on the basis of the ratio of the average of carbohydrate intake to fat intake. For CP group, the ratio was >1; otherwise, rats were assigned to the FP group. One day after the determination of

TABLE 1
Composition of diets used in experiments

	Diet	
	Carbohydrate/protein	Fat/protein
	g	
Corn starch	917.9	
Dextrin	458.8	
Sucrose	152.9	
Casein[a]	429.2	429.2
DL-Methionine	6.5	6.4
Vegetable shortening		578.0
Safflower oil		101.8
AIN-76A vitamin mix[b]	19.9	19.9
AIN-76 mineral mix[b]	70.0	69.8
Choline chloride	5.0	5.0
Cellulose (Alphacel)	99.8	100.0
Weight (g)	2160.0	1310.1
Total energy (kcal)[c]	7800	7800
Energy density (kcal/g)	3.61	5.95

All components are expressed as weight (g).
[a] Assuming a protein content of 90%.
[b] The vitamin and mineral mixes contain 97% and 12% sucrose, respectively.
[c] Based on energy values of 4, 9 and 4 kcal/g for carbohydrate, fat and protein, respectively.

the base-line preference, rats were tested for the effect of DAMGO on diet preference. Immediately after the microinjections, the rats were put into the testing cages, which contained both of the diets. After the last injection of DAMGO, the rats underwent 1 additional test day in which an intraperitoneal injection of naltrexone (5 mg/kg) was given 15 min before an intra-ACB microinfusion of DAMGO at the dose of 2.5 μg. The intake of high-carbohydrate diet and high-fat diet was measured for 3 hr after DAMGO treatment.

In the first part of experiment 3 (n = 16), the effect of mild food deprivation on diet selection was investigated. As in experiment 2, the rats were divided into FP and CP groups based on the same criteria. The rats had unlimited access to normal laboratory chow in the home cages except on the day on which the rats were deprived of food for 24 hr before the testing session. During the testing period, the hungry rats were put into the testing cages containing both high-carbohydrate and high-fat diets. Diet intake over the 3-hr session was measured. Four days later, the same procedure was conducted except that the rats received saline or naltrexone injections (5 mg/kg i.p.) 15 min before the access to carbohydrate and fat. Each rat received both intraperitoneal treatments, which were spaced by ≥4 days.

The effect of stimulating or inhibiting *mu* receptor systems within the ACB, combined with food deprivation, was investigated 7 days after the rats received the last intraperitoneal injection described above. The rats were administered an intra-ACB infusion of DAMGO (2.5 μg) or saline (injections spaced 2 days apart), in combination with the 24-hr food deprivation. One week later, the same group of rats were treated with intra-ACB microinfusion of naltrexone (20 μg) or saline. Diet intake over 3 hr was calculated as noted above.

All experiments used a within-subjects design; therefore, each rat received all dose treatments. All drug doses were administered in a counterbalanced order. The dose ranges used in these studies were chosen on the basis of previous work in the laboratory.

Data analysis. Data were analyzed initially using SuperANOVA software package (Abacus) on a MacIntosh computer. The main dependent variable, food intake (in kcal), was analyzed with a two-factor (dose and time, or dose and diet) or one-factor (dose or treatment) analysis of variance followed by mean value comparison contrasts (Rosenthal and Rosnow, 1991). Planned contrasts compared each drug dose to its vehicle control. To determine the relationship between base-line diet preference and drug effect (experiment 2), difference scores were calculated for each diet and DAMGO dose by subtracting the intake of a particular diet intake after saline injection from the intake of the same diet after each DAMGO treatment. These difference scores were used to compute the correlations (Pearson's r). The same method was used to determine the correlation between the base-line diet preference and the effects of hunger on nutrient intake, except that no difference scores were introduced for Pearson's r (for details, see "Results").

Histology. After the completion of all testing, rats were anesthetized deeply with sodium pentobarbital and perfused transcardially with a 0.9% isotonic saline solution followed by a 10% formalin solution. Brain were removed and stored in a 10% formalin solution for several days to allow for fixation. Before the cutting, the brain were transferred into the 10% sucrose-formalin overnight. For histological preparation, brains were cut into 60-μm sections, mounted and stained with cresyl violet. Sections were examined under the microscope to determine the placement of injector tips. Injections sites were well localized within accumbens approximately on the core/shell border. No animals were eliminated from any of the studies. (Photomicrographs of representative placements are shown in fig. 5.)

Results

Experiment 1: Effect of Intra-ACB *Mu* Opioid Agonist Infusion on Intake of High-Carbohydrate or High-Fat Diets Given Separately

The effect of DAMGO on fat intake. Two-way analysis of variance revealed a significant main effect of dose [$F(3,39) = 39.276$, $P < .01$] and dose × time bin interaction [$F(6,78) = 7.741$, $P < .01$]. Subsequent planned mean contrasts suggested that both the medium dose (0.25 μg) and high dose (2.5 μg) contribute to the significant dose effect ($P < .01$) (fig. 1). Follow-up analysis of simple comparisons between saline and each dose of DAMGO treatment at each level of time bins (first, second and third hour) showed that all of the three doses enhanced fat intake in the first hour ($P < .05$ for 0.025 μg DAMGO; $P < .01$ for 0.25 and 2.5 μg DAMGO). The fat intake enhanced by 2.5-μg dose was still pronounced in the second hour ($P < .01$).

The effect of DAMGO on carbohydrate intake. Infusion of DAMGO elicited an increase in carbohydrate intake (fig. 1). An overall significant dose effect was obtained [$F(3,27) = 5.176$, $P < .01$], for which subsequent contrast analysis demonstrated to result from the dose of 0.25 μg ($P < .01$) and 2.5 μg ($P < .01$). Analysis of the time course of carbohydrate intake indicated a significant interaction between dose and time bin [$F(6,54) = 7.680$, $P < .01$]. In the first hour, both 0.025 ($P < .05$) and 0.25 ($P < .01$) μg enhanced carbohydrate consumption. The effect of 2.5 μg DAMGO peaked in the second hour ($P < .01$).

In summary, when carbohydrate and fat diets were presented separately to rats, stimulation of *mu* receptors within the ACB elicited potent and long-lasting feeding effect on the fat diet, such that the amount of fat eaten in 3 hr increased by as much as 280% after the high DAMGO dose compared with saline control. Compared with the dramatic effect on fat intake, the effect of DAMGO on carbohydrate intake was much smaller.

Experiment 2: Effect of Intra-ACB *Mu* Opioid Agonist Infusion on Diet Preference in Nondeprived Rats

In this test with the two macronutrient-specific diets simultaneously available, free-feeding rats given an intra-ACB infusion of DAMGO displayed a strong preference for fat compared with the carbohydrate diet (fig. 2). Significant effects of dose condition [$F(3,39) = 22.029$, $P < .01$], diet [$F(1,13) = 9.851$, $P < .01$] and dose × diet interaction [$F(3,39) = 14.141$, $P < .01$)] were found. As shown in figure 2 (top), the 0.25- and 2.5-μg doses of DAMGO significantly increased total caloric intake (the sum of fat intake and carbohydrate intake) ($P < .01$) and fat intake ($P < .01$), whereas carbohydrate consumption remained unchanged. Furthermore, the enhancement induced by 2.5 μg DAMGO was completely blocked by naltrexone treatment ($P < .01$).

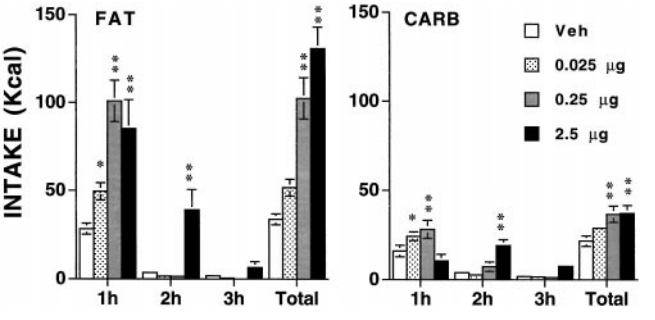

Fig. 1. The effect of intra-ACB injection of vehicle and difference doses of DAMGO on fat and carbohydrate intake when these two diets were presented to rats separately for 3 hr. Bars represent mean ± S.E.M. **$P < .01$, *$P < .05$, compared with vehicle control. Carb, carbohydrate; kcal, kilocalories.

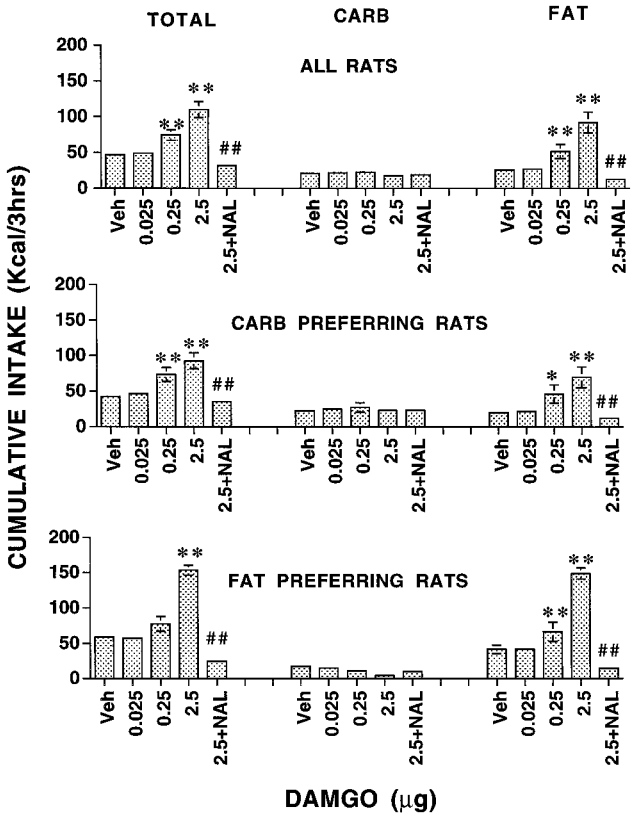

Fig. 2. Three-hour cumulative intake of diets after intra-ACB injections of vehicle, DAMGO and cotreatment of 2.5 μg of DAMGO with intraperitoneal injection of naltrexone (5 mg/kg), when the diets were presented to rats concurrently. Top, effect of the treatments on diet selection when all of the rats were considered as one group. Middle and bottom, intake when rats were divided into carbohydrate-preferring and fat-preferring group, respectively. See text for method of grouping. Bars represent mean ± S.E.M. $^{**}P < .01$, $^{*}P < .05$, compared with vehicle control; ##$P < .01$, compared with 2.5 μg DAMGO.

The correlation between the intake of each diet and base-line diet intake was shown in table 2, which suggested increased fat intake elicited by the 2.5-μg dose was positively correlated with base-line fat intake ($P < .01$) and negatively correlated with base-line carbohydrate intake ($P < .05$). To investigate the relationship between drug effect and base-line preference in detail, the data were reanalyzed after dividing the rats into CP and FP groups based on the criteria mentioned earlier. As demonstrated in figure 2 (middle and bottom), the 0.25- and 2.5-μg doses significantly facilitated fat feeding in both CP and FP groups. In contrast, no effect was observed on carbohydrate intake in both groups. Moreover, the effect on fat intake induced by the 2.5-μg dose was blocked by naltrexone pretreatment ($P < .01$). These results indicated that DAMGO exerted a pronounced preferential influence on fat intake, regardless of preexisting diet preference.

Experiment 3: Effect of Food Deprivation Alone and Food Deprivation in Combination with Intra-ACB *Mu* Agonist or Antagonist on Diet Selection

Food-deprivation alone. The mean value of intake across 3 consecutive days before the first test day was calculated and served as base-line intake in the satiated condition. The cumulative 3-hr diet intake was analyzed with two-way analysis of variance: feeding condition (satiation *vs.* hunger) × diet (fat *vs.* carbohydrate). In contrast to the results in experiment 2, only the main effect of feeding condition was found significant [$F(1,15) = 82.819$, $P < .01$], with no interactions or effects of diet content. This profile indicates that 24-hr food deprivation produced a significant increase in caloric intake compared with the satiated feeding condition but, more importantly, that the magnitude of this increase was similar across both diet conditions as shown in figure 3 (top). Similarly to experiment 2, however, only enhanced fat intake was blocked by intraperitoneal injection of naltrexone ($P < .01$).

In contrast to the selective effect of DAMGO on fat preference demonstrated in experiment 2, food deprivation produced no preferential alterations in macronutrient consumption in relation to base-line preference. Food deprivation-induced diet consumption was significantly correlated with corresponding base-line diet preference (table 3). As shown in figure 3 (middle and bottom), food deprivation increased consumption of both diets to the same degree in both FP ($P < .01$ for both diets) and CP groups ($P < .01$ for carbohydrate; $P < .05$ for fat).

Diet selection induced by intra-ACB DAMGO or naltrexone in hungry rats. As for the effects of DAMGO on diet choice after food deprivation, two-way analysis of variance showed significant main effects for dose [$F(1,15) = 13.536$, $P < .01$), and diet [$F(1,15) = 9.367$ $P < .01$]. There also was a significant dose × diet interaction [$F(1,15) = 7.552$, $P < .05$]. Further analysis of simple comparisons showed that the selective stimulatory effect of DAMGO on fat consumption ($P < .01$) was responsible for the interaction (fig. 4).

An intra-ACB infusion of naltrexone suppressed food intake, shown in figure 4. Analysis of variance revealed a significant main effect of drug treatment [$F(1,15) = 54.219$, $P < .01$] and a significant drug × diet [$F(1,15) = 13.593$, $P < .01$] interaction. Follow-up simple comparison between drug and saline treatment at each level of diet demonstrated that naltrexone treatment dramatically inhibited fat consumption ($P < .01$) while producing less effect on carbohydrate intake ($P < .05$).

TABLE 2
Correlation coefficients (Pearson's *r*) for the relationship between base-line diet intake and the effect of DAMGO on diet intake

	Effect of DAMGO on the intake of each diet (difference from control intake)					
	Fat			Carbohydrate		
Base-line intake (μg)	0.025	0.25	2.5	0.025	0.25	2.5
Fat	0.137	0.231	0.723*r*[a]	−0.246	−0.502	−0.467
Carbohydrate	−0.096	−0.426	−0.550[b]	0.368	0.406	0.419

[a] $P < .01$ and [b] $P < .05$ indicate significant correlation.

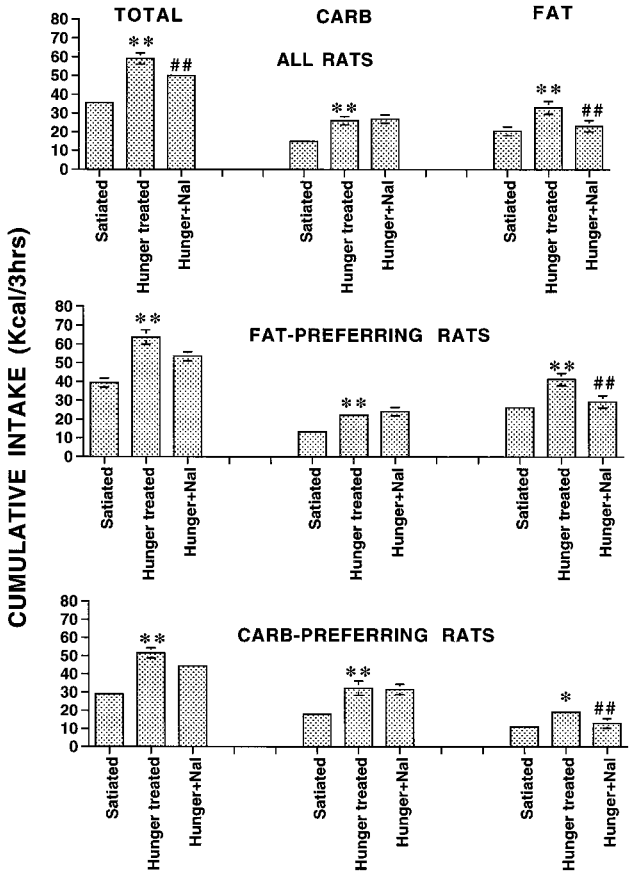

Fig. 3. The effect of 24-hr food deprivation or the cotreatment of food deprivation with the injection of naltrexone (5 mg/kg i.p.) on diet selection. Top, Intake when rats were treated as one group. Middle, Intake in fat-preferring group. Bottom, Intake in carbohydrate-preferring group. Bars represent mean ± S.E.M. $^{**}P < .01$, $^{*}P < .05$, compared with the satiated rats; $^{\#\#}P < .01$, compared with rats treated with 24-hr food deprivation. Nal, naltrexone.

TABLE 3

Pearson's *r* for the relationship between base-line diet intake and the effect of food deprivation on diet intake

	Effect of food deprivation on diet intake	
Base-line intake	Fat	Carbohydrate
Fat	0.806[a]	−0.496[b]
Carbohydrate	−0.555[a]	0.546[b]

[a] $P < .01$ and [b] $P < .05$ indicate significant correlation.

Discussion

The main purpose of this study was to investigate the role of ACB *mu* opioid receptors in macronutrient preference and to obtain further information concerning the mechanisms underlying opioid-induced feeding. Our data demonstrate that within this brain area, stimulation of *mu* receptors exerts a powerful preferential facilitory effect on fat consumption. Furthermore, this effect is independent of preexisting diet preference and is blocked by cotreatment with naltrexone. Finally, the difference in the profiles between DAMGO- and food deprivation-induced diet selection suggests that endogenous opioids, at least within the ACB, may play a key role in the factors that govern food intake other than energy deficits, particularly palatability.

There is some debate with regard to the nature of opioid-induced feeding in terms of whether such feeding is nutrient specific or rather specific to individual base-line preferences. A selective increase in fat intake was reported by several studies with rats undergoing acute (Marks-Kaufman and Kanarek, 1980; Marks-Kaufman, 1982; Romsos *et al.*, 1987) or chronic treatment with morphine under different feeding conditions (Ottaviani and Riley, 1984; Marks-Kaufman and Kanarek, 1990; Gosnell and Krahn, 1993). Consistent with the present results, naltrexone or naloxone selectively decreased fat intake in a self-selection paradigm (Marks-Kaufman and Kanarek, 1980; Marks-Kaufman, 1982). On the other hand, some researchers have argued that opioid-induced feeding is preference specific rather than macronutrient specific, finding that the effects of morphine on nutrient intake were modified when base-line preference was taken into consideration. Three studies provide evidence to support the preference-related hypothesis. The most direct evidence is provided by the study of Gosnell *et al.* (1990), who found that systemic injection of morphine preferentially increased fat intake in fat-preferrers and carbohydrate intake in carbohydrate-preferrers. They concluded that morphine caused a selective increase in the intake of preferred foods. Several differences may account for the discrepancies between that study and the present data. First, in the study of Gosnell *et al.*, morphine was administered systemically, therefore stimulating many regions and subtypes of opiate receptors. Second, rats were allowed 24-hr access to the different diets compared with only 3-hr access in the present experiments. Consequently, the restricted base-line tests may be more strongly influenced by taste preference or palatability. Furthermore, it is noteworthy that in the study of Gosnell *et al.*, although no significant effect was found on carbohydrate intake in fat-preferring rats, morphine still produced a significant stimulatory effect on fat intake, even in the carbohydrate-preferring rats. This finding suggests that morphine tends to produce a relatively greater effect on fat intake, although the size of the effect may be dependent on base-line preference. An additional study reported that systemic administration of morphine facilitated intake of carbohydrate in carbohydrate-deprived rats and protein in protein-deprived rats (Evans and Vaccarino, 1990). Furthermore, Glass *et al.* demonstrated that the anorexic effect of naloxone on

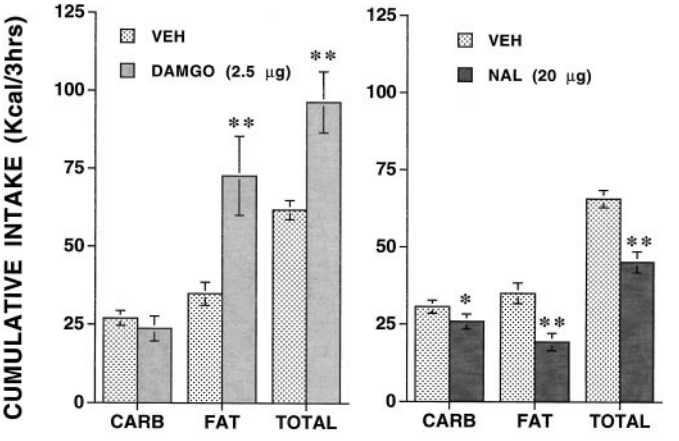

Fig. 4. The effect of intra-ACB infusion of DAMGO (2.5 μg) or naltrexone (20 μg) on diet selection between fat and carbohydrate in rats with 24-hr food deprivation. Bars represent mean ± S.E.M. $^{**}P < .01$, $^{*}P < .05$, compared with vehicle control.

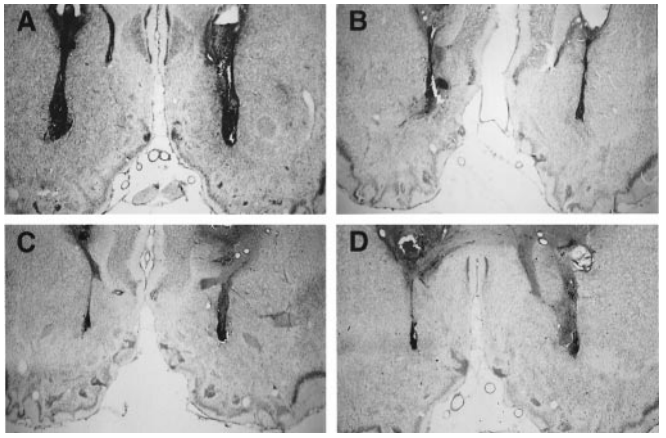

Fig. 5. Photomicrographs of cannula placements within ACB of representative animals from the different experiments. A, Experiment 1, fat group. B, Experiment 1, carbohydrate group. C, Experiment 2, choice test, satiated rats. D, Experiment 3.

neuropeptide Y-induced feeding of carbohydrates and fats was dependent on diet preference. In these cases, however, the base-line preference of the given nutrient was determined by either nutrient deficit or neuropeptide Y-induced stimulation, both of which introduce a variety of factors that could mask the pure effects of opioids on nutrient selection.

The data from the present experiments help to clarify this debate. They suggest, at least for the ACB, that *mu* receptor stimulation preferentially enhances intake of foods that have inherently high palatability, such as fat. This effect is mostly clearly observed when animals have a choice of macronutrients or foods. When given a choice between carbohydrate and fat, ACB opioid stimulation enhanced only fat intake. Similarly, when rats are presented with both sweetened water and unsweetened water, only intake of the sweetened solution is enhanced (Zhang and Kelley, 1997), and when given a choice between chow and sugar, only sugar intake is enhanced (Evans and Vaccarino, 1990). However, because all foods that rats eat naturally have some inherent palatability, such as rat chow or carbohydrate, DAMGO also is able to stimulate intake when such foods are present alone. Furthermore, intra-ACB administration of DAMGO not only increases sucrose drinking (Zhang and Kelley, 1997) but also enhances the intake of salt solution (unpublished observation). It has also been reported that morphine suppresses aversive reactions to a bitter solution such as pure quinine (Doyle *et al.*, 1993). Taken together, these experiments suggest that ACB opioids are involved in modulating the palatable response to food across a broad range of macronutrients and tastes. This idea may appear somewhat at odds with our present finding that the opioid effect was relatively fat specific. However, it is highly likely that the extent of enhanced palatability is correlated with the content of the fat and sugar because in general, highly preferred foods contain combinations of fat and sugar (or fat and salt) (Drewnowski *et al.*, 1992b). In support of this notion, there is evidence of a correlation between taste palatability and activation of endogenous opioid systems. For instance, consumption of highly palatable food rich in fat and sugar causes an increase in the release of β-endorphin, (Brennan *et al.*, 1994; Welch *et al.*, 1996). Additionally, an interesting interaction has been found between drug self-administration and food or taste preference. For example, fat-preferring rats consume more ethanol than carbohydrate-preferring rats (Forsander, 1988; Krahn and Gosnell, 1991). In an earlier study, it was found that the rats with high preference for fat ultimately became morphine drinkers, whereas those with low preference for fat did not consume the drug (Marks-Kaufman and Lipeles, 1982). Rats that have a high preference for sweet taste show a greater tendency to self-administer morphine (Gosnell *et al.*, 1995). These lines of evidence suggest the existence of a close relationship between the opioid reward system and neural circuits processing taste reactivity and palatability. More specifically, we hypothesize that opioid systems within the ACB are the major region in which this processing occurs; stimulation of opioid receptors in this region results not only in hyperphagia but also in reinforcing effects as demonstrated in the place preference paradigm or intracerebral self-administration (Van der Kooy *et al.*, 1982; Goeders *et al.*, 1984; Mucha and Iversen, 1986; Zhang and Kelley, 1997).

It is of interest to note the difference in the profiles of DAMGO-induced and food deprivation-induced diet intakes. In nondeprived rats with diets presented concurrently, DAMGO augmented only fat consumption without an effect on carbohydrate intake. However, 24-hr food deprivation enhanced feeding of both diets to the same degree. When base-line intake of the two diets was taken into consideration, the effect of DAMGO on fat intake was positively correlated with base-line fat intake, whereas no correlation was found between DAMGO-induced carbohydrate intake and base-line carbohydrate preference. In contrast to the DAMGO-induced selective change in diet preference, food deprivation produced no effect on diet preference; indeed, the degree of intake of each diet after deprivation corresponded to the initial base-line preference in the nondeprived state. Support for this idea is provided by the finding that naltrexone infusion was able to decrease both fat and carbohydrate intake when infused into the ACB in hungry rats. Granted, the effect of intra-ACB naltrexone was less potent on carbohydrate than on fat, and in the initial part of that experiment, systemic naltrexone did not affect deprivation-induced carbohydrate intake. This discrepancy may be accounted for by the higher concentration of antagonist in the intracerebral injection. Because naltrexone is not entirely selective, it may also be possible that blockade of *delta* receptors accounts for the decrease in carbohydrate intake. A number of studies, including our own, have shown that infusion of *delta* agonists into ACB or ventral tegmental area enhances food intake (Majeed *et al.*, 1986; Bakshi and Kelley, 1993a; Noel and Wise, 1995; Zhang and Kelley, 1997).

These data also raise the question concerning the relationship between hunger and palatability. Although many foods have inherent high palatability and are consumed for their pleasurable effects, rather than to relieve an energy deficit, hunger clearly increases palatability of all foods. Thus, it is important to note that opioids likely play a role in palatability both in the nondeprived and food-deprived states. This hypothesis is consistent with clinical studies in control and obese patients, which show that opioid antagonists decrease the intake of foods, especially those rich in sugar and fat, even though hunger and satiety ratings are not affected (Yeomans *et al.*, 1990; Drewnowski *et al.*, 1992a; Yeomans and Gray, 1996). In these reports, the finding that naloxone preferentially decreases intake of sweet and/or high-fat food

by reducing the "pleasantness" rating of these food items lends support to the hypothesis that opioids play an important role in palatability.

Foods containing fat and sugar have high caloric density, and thus the preferential effect of opioids on consumption of these foods might have an adaptive significance from the standpoint of evolution. Throughout evolution, famine or food scarcity, rather than obesity, was likely to be a problem. To survive in these conditions, organisms would have to develop strategies to prepare for impending food shortages due to seasonal or climactic variations or, at times, starvation. Because calorie-dense food (especially fats, which are beneficial for long-term energy storage) has a highly rewarding taste, this would ensure that animals obtain and store energy whenever such food might be available. This notion is consistent with our findings that in hungry rats, DAMGO and naltrexone preferentially increased and suppressed the intake of fat, respectively. In modern Western societies, however, in which the food supply is abundant and overweight rampant, overactivation of the endogenous opioid peptides may contribute partly to human obesity, binge eating and uncontrollable craving for sweet and high-fat foods. The present data suggest that in particular, activation of *mu* opioid receptors within the ventral striatum may participate in the selection and consumption of highly palatable foods.

References

Bakshi VP and Kelley AE (1993a) Feeding induced by opioid stimulation of the ventral striatum: role of opiate receptor subtypes. *J Pharmacol Exp Ther* **265:** 1253–1260.

Bakshi VP and Kelley AE (1993b) Striatal regulation of morphine-induced hyperphagia: an anatomical mapping study. *Psychopharmacology* **111:**207–214.

Baskshi VP and Kelley AE (1994) Sensitization and conditioning of feeding following multiple morphine microinjections into the nucleus accumbens. *Brain Res* **648:** 342–346.

Bell SM, Gosnell BA, Krahn DD and Meisch RA (1994) Ethanol reinforcement and its relationship to saccharin preference in Wistar rats. *Alcohol* **11:**141–145.

Brennan G, Bachus SE and Jhanwar-Uniyal M (1994) Involvement of gene expression for hypothalamic dynorphin in dietary preference. *Soc Neurosci Abstr* **20:**818.

Cooper SJ and Kirkham TC (1993) Opioid mechanisms in the control of food consumption and taste preferences. *In* Handbook of Experimental Pharmacology: 104/II, Opioids II, ed. by A. Herz, pp. 239–262, Springer-Verlag, Berlin.

Doyle TG, Berridge KC and Gosnell BA (1993) Morphine enhances hedonic taste palatability in rats. *Pharmacol Biochem Behav* **46:**745–749.

Drewnowski A, Krahn DD, Demitrack MA, Nairn K and Gosnell BA (1992a) Taste responses and preferences for sweet high-fat foods: Evidence for opioid involvement. *Physiol Behav* **51:**371–379.

Drewnowski A, Kurth C, Holden-Wiltse J and Saari J (1992b) Food preferences in human obesity: carbohydrate versus fats. *Appetite* **18:**207–221.

Evans KR and Vaccarino FJ (1990) Amphetamine- and morphine-induced feeding: evidence for involvement of reward mechanisms. *Neurosci Biobehav Rev* **14:**9–22.

Flowers SH, Dunham ES and Barbour HG (1929) Addiction edema and withdrawal edema in morphinized rats. *Proc Soc Biol Med* **26:**572–574.

Forsander OA (1988) The interaction between voluntary alcohol consumption and dietary choice. *Alcohol* **23:**143–149.

Goeders NE, Lane JD and Smith JE (1984) Self-administration of methionine enkephalin into the nucleus accumbens. *Pharmacol Biochem Behav* **20:**451–455.

Gosnell BA and Krahn DD (1993) The effects of continuous morphine infusion on diet selection and body weight. *Physiol Behav* **54:**853–859.

Gosnell BA, Krahn DD and Majchrzak MJ (1990) The effects of morphine on diet selection are dependent upon baseline diet preferences. *Pharmacol Biochem Behav* **37:**207–212.

Gosnell BA, Lane KE, Bell SM and Krahn DD (1995) Intravenous morphine self-administration by rats with low versus high saccharin preferences. *Psychopharmacology* **117:**248–252.

Holtzman SG (1974) Behavioral effects of separate and combined administration of naloxone and *d*-amphetamine. *J Pharmacol Exp Ther* **189:**51–60.

Kelley AE, Bakshi VP and Holahan MR (1998) Conditioned feeding induced by repeated opioid stimulation of the nucleus accumbens: A pharmacological investigation. *Psychopharmacology*, in press.

Kelley AE, Bless EP and Swanson CJ (1996) Investigation of the effects of opiate antagonists infused into the nucleus accumbens on feeding and sucrose drinking in rats. *J Pharmacol Exp Ther* **278:**1499–1507.

Koob GF and Bloom FE (1988) Cellular and molecular mechanisms of drug dependence. *Science* **242:**715–723.

Krahn DD and Gosnell BA (1991) Fat-preferring rats consume more alcohol than carbohydrate-preferring rats. *Alcohol* **8:**313–316.

Levine AS and Billington CJ (1989) Opioids. Are they regulators of feeding? *Ann NY Acad Sci* **575:**209–219.

Majeed NH, Przewlocka B, Wedzony K and Przewlocki R (1986) Stimulation of food intake following opioid microinjection into the nucleus accumbens septi in rats. *Peptides* **7:**711–716.

Marks-Kaufman R (1982) Increased fat consumption induced by morphine administration in rats. *Pharmacol Biochem Behav* **16:**949–955.

Marks-Kaufman R and Kanarek RB (1980) Morphine selectively influences macronutrient intake in the rat. *Pharmacol Biochem Behav* **12:**427–430.

Marks-Kaufman R and Kanarek RB (1981) Modifications of nutrient selection induced by naloxone in rats. *Psychopharmacology* **74:**321–324.

Marks-Kaufman R and Kanarek RB (1990) Diet selection following a chronic morphine and naloxone regimen. *Pharmacol Biochem Behav* **35:**665–669.

Marks-Kaufman R and Lipeles BJ (1982) Patterns of nutrient selection in rats orally self-administering morphine. *Nutr Behav* **1:**33–46.

Martin WR, Wilker A, Eades CG and Pescor FT (1963) Tolerance to and physical dependence on morphine in rats. *Psychopharmacologia (Berl)* **4:**247–260.

Mitchell JE, Hatsulami D, Eckert ED, Pyle RL (1985) Characteristics of 275 patients with bulimia. *Am J Psychiatr* **142:**482–488.

Morabia A, Fabre J, Chee E, Ieger S, Orsat E, Robert A (1989) Diet and opiate addiction: Quantitative assessment of the diet of non-institutionalized opiate addicts. *Br J Addict* **84:**173–180.

Mucha RF and Iversen SD (1986) Increased food intake after opioid microinjections into nucleus accumbens and ventral tegmental area of rat. *Brain Res* **397:**214–224.

Noel MB and Wise RA (1995) Ventral tegmental injections of a selective mu and delta opioid enhance feeding in food-deprivated rats. *Brain Res* **673:**304–312.

Ottaviani R and Riley AL (1984) Effect of chronic morphine administration on the self-selection of macronutrients in the rat. *Nutr Behav* **2:**27–36.

Reid LD (1985) Endogenous opioid peptides and regulation of drinking and feeding. *Am J Clin Nutr* **42:**1099–1132.

Romsos D, Gosnell BA, Morley JE and Levine AS (1987) Effects of kappa opiate agonists, cholecystokinin and bombesin on intake of diets varying in carbohydrate to fat ratio in rats. *Nutr Behav* **117:**976–985.

Rosenthal R and Rosnow RL (1991) Essentials of Behavioral Research: Methods and Data Analysis. McGraw-Hill, New York.

Van der Kooy D, Mucha RF, O'Shaughnessy M and Bucenieks P (1982) Reinforcing effects of brain microinjections of morphine revealed by conditioned place preference. *Brain Res* **243:**1077–1117.

Welch CC, Kim EM, Grace MK, Billington CJ and Levine AS (1996) Palatability-induced hyperphagia increases hypothalamic dynorphin peptide and mRNA levels. *Brain Res* **721:**126–131.

Wise RA and Bozarth MA (1987) A psychomotor stimulant theory of addiction. *Psychol Rev* **94:**469–492.

Yeomans MR, Wright P, Macleod HA and Critchley JA (1990) Effects of nalmefene on feeding in humans: Dissociation of hunger and palatability. *Psychopharmacology* **100:**426–432.

Yeomans M and Gray RW (1996) Selective effects of naltrexone on food pleasantness and intake. *Physiol Behav* **60:**439–446.

Zhang M and Kelley A-E (1997) Opiate agonists microinjected into the nucleus accumbens enhance sucrose drinking in rats. *Psychopharmacology* **132:**350–360.

Send reprint requests to: Min Zhang, Department of Psychiatry, University of Wisconsin-Madison Medical School, 6001 Research Park Blvd., Madison, WI 53719.