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ORIGINAL INVESTIGATION

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Intake of saccharin, salt, and ethanol solutions is increased by infusion of a mu opioid agonist into the nucleus accumbens

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Abstract *Rationale:* Endogenous opioids have been implicated in the hedonic evaluation of food and palatability. Opioids may also be involved in alcohol intake, as there is a positive correlation between alcohol drinking and preference for sweets and fats. Our previous studies have shown that mu opioid stimulation of the nucleus accumbens preferentially augments intake of palatable food containing sucrose and fat. *Objective:* The first goal of the present study was to further explore the nature of the involvement of mu opioids within the nucleus accumbens in ingestive behavior by investigating the importance of orosensory reward in opioid-mediated feeding, using non-caloric tasty substances (saccharin and salt). Second, we investigated whether mu opioid receptors within the nucleus accumbens also regulate alcohol consumption. *Methods:* The mu agonist, D-Ala2, NMe-Phe4, Glyol5-enkephalin (DAMGO; 0, 0.025 and 0.25 µg/0.5 µl per side), was microinfused into the nucleus accumbens, and intake of 0.6% saline, 0.15% sodium saccharin, water, and 6% ethanol was measured. *Results:* Microinfusion of DAMGO into the nucleus accumbens increased the drinking of salt and saccharin solutions in non-deprived rats. However, water intake was not increased by this treatment in water-deprived rats. Mu opioid stimulation of the nucleus accumbens also augmented ethanol intake in rats not deprived of fluid, while leaving water intake unchanged when water was concurrently available. *Conclusion:* These results provide evidence to suggest that the mu opioid system within the ventral striatum regulates ingestive behavior via a mechanism related to the hedonic assessment of taste. In addition, the nucleus accumbens may be a key brain area where ethanol interacts with endogenous opioid systems, and thus may be a common neural substrate for both food palatability and alcohol drinking.

Keywords Mu opioid receptor · Enkephalin · Taste · Palatability · Ingestive behavior · Alcohol · Nucleus accumbens · Ventral striatum

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Introduction

Although feeding behavior has been widely studied in the context of energy homeostasis, attention has also been drawn to the hedonic aspects of food intake, where food is ingested solely for its pleasurable effects, and reduction of energy needs is not the main purpose of food consumption (Cabanac 1979). Indeed, a primary motivating force governing eating, at least in humans, is the positive feelings associated with intake of desirable foods. A likely neural substrate for this state is the endogenous opioid system, which has been implicated in the positive affective state generated by eating palatable food containing high fat and sugar (Cooper and Kirkham 1993; Berridge 1996).

The involvement of brain opioid peptides in food palatability and taste pleasantness has been supported by several lines of behavioral, anatomical and physiological studies. In animal studies, one of the most frequently reported phenomenon is that the potency of opioid agonists and antagonists on food consumption is highly associated with the degree of palatability of food (Cooper and Turkish 1989; Kelley et al. 1996; Weldon et al. 1996; Zhang and Kelley 1997; Giraudo et al. 1999). Moreover, eating of a sweet or high fat diet has been found to induce alterations in the sensitivity, synaptic levels and gene expression of opioid systems (Dum et al. 1983; Welch et al. 1996; Rudski et al. 1997; Carr et al. 1999; Shabir and Kirkham 1999). Evidence emerging from clinical studies further supports the existence of a functional link between brain opioids and perceived pleasantness of palatable food. For example, opioid antagonists, such as naloxone and naltrexone, decreased hedonic rat-

ings of salty and sweet solutions, and of solid food containing a high content of sugar, fat and protein, in both normal control subjects and binge eaters (Yeomans and Wright 1991; Drewnowski et al. 1992; Yeomans and Gray 1996).

Food intake is altered by microinfusion of opioid agonists and antagonists into brain areas traditionally associated with feeding, such as the paraventricular nucleus, lateral and dorsomedial hypothalamus, nucleus of solitary tract, and parabrachial nucleus (Leibowitz and Hor 1982; Gosnell et al. 1986; Stanley et al. 1988; Koch et al. 1995), as well as reward-related areas such as nucleus accumbens, amygdala, and ventral tegmental area (Gosnell 1988; Stanley et al. 1988; Bakshi and Kelley 1993a, 1993b; Badiani et al. 1995; Kelley et al. 1996; Zhang and Kelley 1997; Zhang et al. 1998). Of these areas, the nucleus accumbens is particular interesting, given its important role in linking motivational state to adaptive motor behaviors, as well as the evidence that stimulation of opioid receptors in this structure preferentially facilitates intake of fat and sucrose (Zhang et. al 1998).

The current study was designed to explore further the nature of opioid-mediated enhancement of food intake. Although it is likely that the nucleus accumbens may provide a neural substrate for coding the reward value of food, questions remain as to the mechanism underlying this role. For example, it is not clear whether the increased intake induced by opioid stimulation of the accumbens is attributed to orosensory alteration or a generally enhanced motivational state to eat or drink, independent of a gustatory component. In order to address this question, in the first two experiments, we evaluated the effects of microinfusion of opioids into the nucleus accumbens on drinking of two palatable solutions, saccharin and salt solutions, which have no caloric content, in rats that were not food- or water-deprived. In the third experiment, the role of intra-accumbens infusion of an opioid receptor agonist on water intake was assessed in water-deprived rats. Because it has been shown that the orexigenic effect of opioids appears to be mediated primarily by mu receptors (Bakshi and Kelley 1993a; Zhang and Kelley 1997; Zhang et al. 1998), the current study was focused on mu receptors within the nucleus accumbens.

In a fourth experiment, the involvement of mu opioid receptors within the accumbens in the intake of another solution with a distinct taste, alcohol, was assessed. There is an intriguing relationship between alcohol intake and the endogenous opioid system arising from a number of observations in the literature (for review, see Reid 1990). Similarly to the effects on drinking sweet solutions, opioid agonists increase ethanol consumption, while opioid antagonists decrease ethanol intake, and alcohol drinking induces alterations in the activity of endogenous opioid systems as does eating of sweets (Froehlich and Li 1994; Froehlich 1996; Herz 1997). Furthermore, taste and diet preferences have been found to be positively correlated with alcohol drinking in both animal and human studies. For example, saccharin-preferring rats tended to consume more alcohol (Gosnell and Krahn 1992; Bell et al. 1994). Rats preferring fat to carbohydrate also self-administered more alcohol compared to rats preferring carbohydrate to fat (Krahn and Gosnell 1991). Studies with humans also demonstrate that individuals with a positive history of alcoholism are more likely to prefer stronger sucrose solution (Kampov-Polevoy et al. 1997, 1998). These data suggest the possibility that enjoyment of sweet solutions may share similar neural substrates with alcohol consumption. Thus, we investigated whether the nucleus accumbens modulates alcohol drinking via an opioid-related mechanism.

Materials and methods

Animals

A total of 38 male rats (Harlan-Sprague Dawley, Madison, Wisc., USA) weighing between 270 and 320 g were used in the present study. Animals were housed two or three to a cage with access to standard normal laboratory chow (Purina chow) and unlimited water unless they were under a specific water-deprivation schedule (see experimental design). The lights were on at 7:00 a.m. and off at 7:00 p.m. All procedures pertaining to the use of animals were carried out in strict accordance with institutional and NIH guidelines.

Surgery

Animals were handled for several days after arrival. For surgery, animals were anesthetized with a mixture of ketamine and xylazine (ketamine, 90 mg/kg; xylazine, 9 mg/kg), and treated with atropine sulfate (0.54 mg/ml). Bilateral guide stainless steel cannula (23 gauge, 10 mm) were stereotaxically implanted into the nucleus accumbens. Guide cannula were secured to the skull with stainless steel screws and light curable dental resin (Dental Supply of New England, Boston, Mass., USA). Coordinates for the aimed site were (in mm, with tooth bar 5 mm above interaural zero): +3.0 from bregma in the anteroposterior (A-P) plane, and ±1.5 from midline in the lateromedial (L-M) plane, and –5.7 from skull in the dorsoventral (D-V) plane. After the surgery, wire stylets were placed in the guide cannula to prevent occlusion. The present study did not specifically aim at the core or shell subregions of accumbens, although histology indicated that the injection sites were generally in the core, or on the core-shell border, just medial to the decussation of the anterior commissure (see Fig. 1).

Drugs and microinjection

D-Ala2, NMe-Phe4, Glyol5-enkephalin (DAMGO), the selective mu receptor agonist, was obtained from Research Biochemicals (Natick, Mass., USA). The drug was dissolved in sterile 0.9% saline. The vehicle control was always sterile 0.9% saline. After the stylets were removed, the drug or vehicle in a volume of 0.5 µl was infused through 12.5 mm 33 gauge injector cannula into the nucleus accumbens. Infusions were delivered with a microdrive pump (Harvard Apparatus, South Natick, Mass., USA), connected via polyethylene tubing (PE-10), while animals were gently handheld. Thus, injector tips extended 2.5 mm beyond the end of the guide cannula, for a final D-V coordinate of –8.2 mm. The rate of injection was 0.32 µl/min with the total duration of infusion being 93 s. One additional minute was allowed for diffusion. Injectors then were removed, and the stylets were replaced. In all experiments, vehicle and two doses of DAMGO (see below) were administered to all rats, on separate test days, in a randomized order.

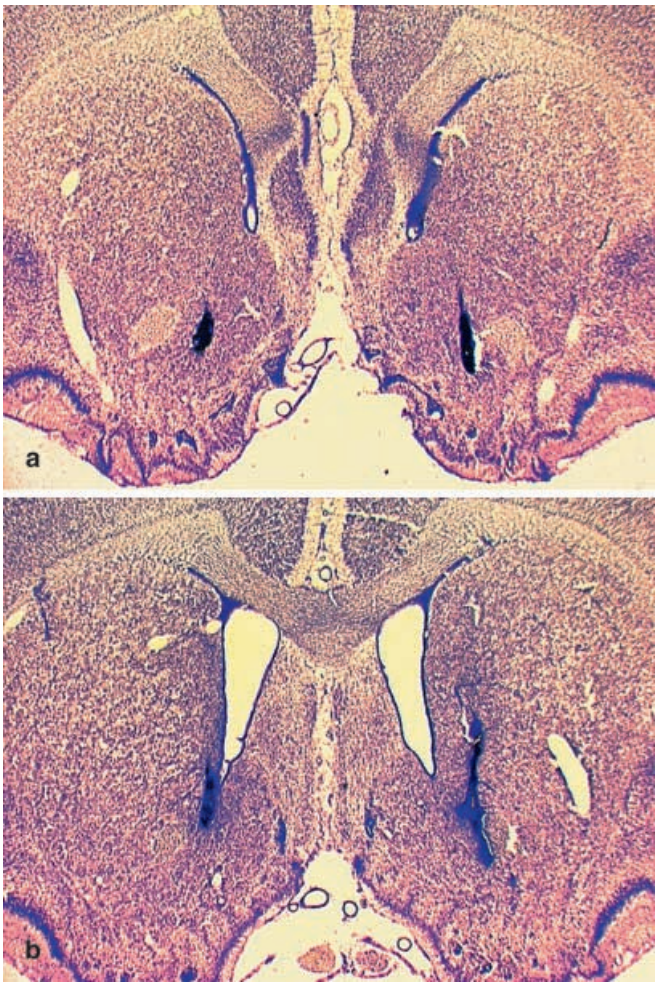

Fig. 1a, b Examples of cannulae track placements from two representative animals, placed within the nucleus accumbens (nissl-stained coronal sections at the level of the striatum). In the present study, no specific attempt was made to differentiate between the core and shell subregion of the nucleus accumbens. **a** Section from alcohol group; **b** section from saccharin group

Behavioral testing and experimental design

All behavioral testing took place in an animal testing room distinct from the animal colony, in individual hanging wire cages. Three to 4 days following surgery, the rats in Experiments 1, 2 and 3 were brought into the testing room and provided with the test solution, 0.6% (w/v) salt solution for Experiment 1 (n=10), 0.15% (w/v) sodium saccharin for Experiment 2 (n=8), or water for Experiment 3 (n=8), for 3 h in the test cages. This habituation period lasted for several days until stable solution drinking was obtained. Preweighed bottles containing the test solutions were attached to the testing cages. The bottles were removed and weighed at the end of habituation session, and corresponding amount of solution drinking was calculated. The rats had unlimited access to water and chow in home cages, except for those in Experiment 3, which were deprived of water from 11:00 p.m. to 9:00 a.m. prior to the habituation session.

The rats in Experiment 4 (n=10) were trained to drink 6% ethanol (v/v) with a sucrose-fading approach (Samson 1986). In brief, the rats had free access to both water and the fluid, which contained 6% ethanol and 3% sucrose at night in home cages. Gradually, the content of sucrose in ethanol was reduced to 1%, then reduced to zero. Chow was always available. After stable drinking of 6% ethanol at night was obtained, the rats were provided with 6% ethanol and water simultaneously for 3 h every day during the daytime in the testing cages. Except for the 3-h habituation period each day, the rats had access to chow and water ad libitum in home cages. The rats underwent surgery after the intake of ethanol across days was stable. The testing period began after the rats recovered from surgery and baseline of ethanol drinking was restored.

Before the experimental testing period began, a sham injection and a vehicle injection were given on separate days to adapt the rats to the injection procedure. No data were collected on these days. During testing days, DAMGO in the dose of 0, 0.025 or 0.25 µg/0.5 µl per side was microinfused into the nucleus accumbens and solution consumption was measured every 30 min. In Experiment 1, there was a 30-min delay between the microinfusion and the onset of the test, and the test session lasted for 150 min. In the other experiments, tests lasted for 180 min and began right after the microinfusion. Since Experiment 1 in the current study was a continuation of our previous study in which the effect of DAMGO on sucrose drinking was investigated with a 30-min interval between the microinfusion and the onset of feeding (Zhang and Kelley 1997), we originally planned to use the same experimental design in the present study. However, after the first microinfusions in Experiment 1, we observed a strong inhibitory effect on saline intake within the first 30-min testing period in the rats treated with 0.25 µg DAMGO. In Experiments 2, 3 and 4, it was therefore decided to extend the testing duration to 180 min in order to unmask the stimulatory effect of the higher DAMGO dose, and to begin testing immediately following infusions.

All experiments utilized a within-subjects design. Therefore, each rat received all dose treatments. All drug doses were administered in counterbalanced order. The dose range used in current study was based on previous studies conducted in the same laboratory.

Data analysis

All data were analyzed using SuperANOVA software package (Abacus) on a MacIntosh computer. The main dependent variable, solution drinking, was analyzed with a two-factor (dose×time, for Experimentals 1, 2 and 3) and three-factor (dose×solution×time, for Experimental 4) analysis of variance followed by planned contrast of means. The criteria for statistical significance were set at P<0.05.

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. Brains were moved and stored in a 10% formalin solution for several days to allow for fixation. Before the cutting, the brains 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. Photomicrographs of representative sections are shown in Fig. 1.

Results

Experiment 1: effect of nucleus accumbens mu opioid stimulation on the intake of salt solution in non-deprived rats

Administration of DAMGO into the nucleus accumbens elicited an overall increase in the consumption of 0.6% saline following the higher dose (0.25 µg) treatment, as shown in Fig. 2. Two-factor analysis of variance revealed a significant dose effect [F(2,22)=10.933, P<0.01], as well as dose×time interaction [F(8,88)=8.565, P<0.01].

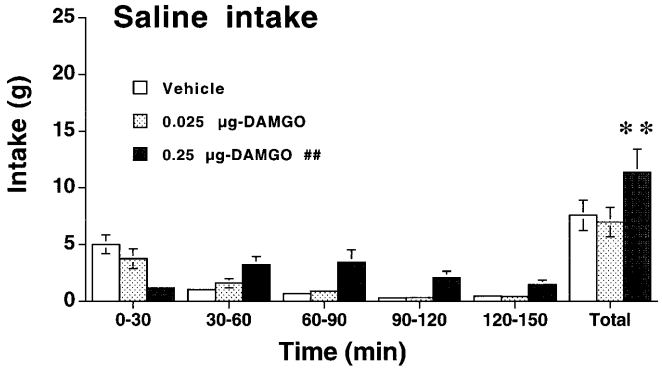

Fig. 2 Effect of microinfusion of mu opioid receptor agonist, DAMGO, into the nucleus accumbens on the intake of 0.6% salt solution in non-deprived rats. The figure shows the time course of solution drinking and total intake induced by DAMGO treatment. *Bars* represent means±SEM. ***P*<0.01, significant dose effect compared to saline treatment. ##*P*<0.01, significant dose×time partial interaction compared to saline treatment

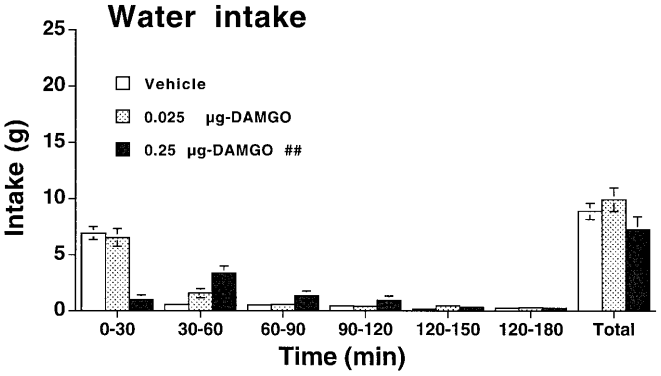

Fig. 4 Effect of microinfusion of mu opioid receptor agonist, DAMGO, into the nucleus accumbens on the intake of water in mildly water-deprived rats. The figure shows the time course of solution drinking and total intake induced by DAMGO treatment. *Bars* represent means±SEM. ##*P*<0.01, significant dose×time partial interaction compared to saline treatment

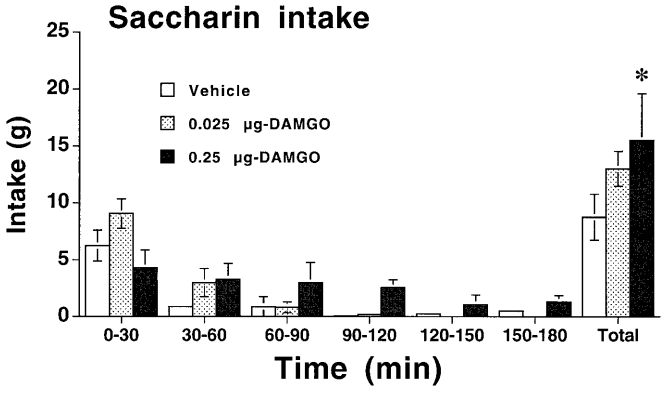

Fig. 3 Effect of microinfusion of mu opioid receptor agonist, DAMGO, into the nucleus accumbens on the intake of 0.15% sodium saccharin solution in non-deprived rats. The figure shows the time course of solution drinking and total intake following microinfusion. *Bars* represent means±SEM. **P*<0.05, significant dose effect compared to saline treatment

Subsequent planned contrasts of dose effect indicated that the 0.25 µg dose significantly increased the intake of salt solution ($P<0.01$), compared to vehicle treatment. Analysis of partial interactions revealed that a dose×time interactions for 0.25 µg DAMGO compared to vehicle [$F(4,88)=15.738$, $P<0.01$] contributed to the overall significant dose×time interaction. The time course of drinking revealed a long-lasting and biphasic effect of DAMGO, especially at the dose of 0.25 µg, with a strong inhibitory effect on salt solution drinking at the early stage of testing and a facilitatory effect appearing later.

Experiment 2: effect of nucleus accumbens mu opioid stimulation on the consumption of saccharin solution in non-deprived rats

Figure 3 shows the effect of DAMGO on total drinking and time course of saccharin solution drinking after microinfusion into the nucleus accumbens. Overall two-factor analysis variance did not result in a significant main effect of dose treatment. However, planned means comparisons still revealed a significant effect on saccharin drinking following DAMGO treatment at the dose of 0.25 µg ($P<0.05$). Although an overall dose×time interaction was found [$F(10,70)=2.580$, $P<0.05$], due to an interaction between the two DAMGO dose treatments, no significant partial interactions were found for either of the two dose treatments versus vehicle treatment.

Experiment 3: effect of nucleus accumbens mu opioid stimulation on water intake in water-deprived rats

Overall, two factor analysis (dose×time) of variance revealed a significant main effect of dose [$F(2,14)=3.741$, $P<0.05$] and a significant dose×time interaction [$F(10,70)=20.461$, $P<0.01$]. As shown in Fig. 4, subsequent analysis of main comparisons of dose effects revealed that neither 0.025 µg nor 0.25 µg DAMGO treatment augmented water intake, compared to vehicle treatment (the main effect of dose was due to a difference between the means of the two DAMGO dose treatments). Follow-up analysis of partial interactions demonstrated a dose×time interaction between 0.25 µg DAMGO group and vehicle-treated group [$F(5,70)=33.558$, $P<0.05$]. It can be observed from Fig. 4 that the highest dose of DAMGO strongly suppressed drinking initially, when it was normally elevated in the vehicle-treated rats. The response then recovered somewhat in the DAMGO-treated rats, in the second and third time periods.

Experiment 4: effect of nucleus accumbens mu opioid stimulation on ethanol and water intake in non-deprived rats

As shown in Fig. 5, administration of DAMGO into the nucleus accumbens increased ethanol intake, while no el-

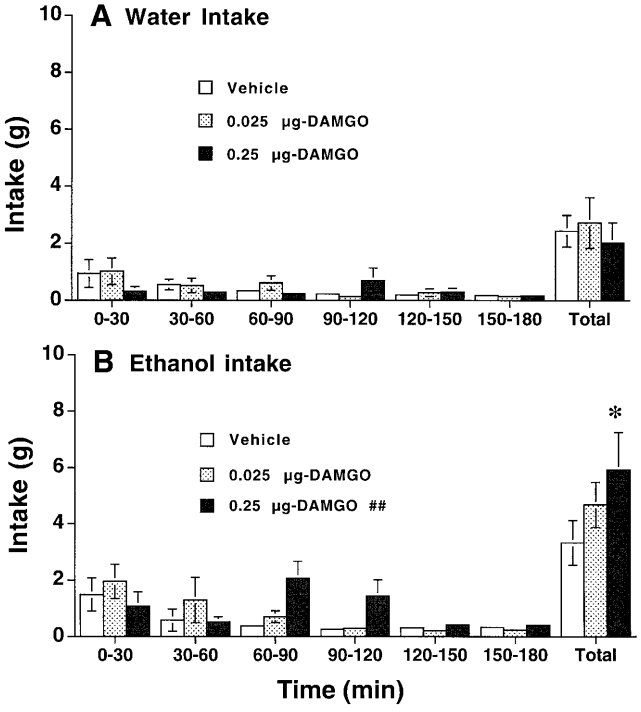

Fig. 5A, B Effect of microinfusion of mu opioid receptor agonist, DAMGO, into the nucleus accumbens on the intake of the concurrently presented 6% ethanol and water in non-deprived rats. **A** and **B** show the total intake and time course of water and ethanol drinking, respectively, following DAMGO treatment. *Bars* represent means±SEM. **P*<0.05, significant dose effect compared to saline treatment. ##*P*<0.01, significant dose×time partial interaction compared to saline treatment

evation in water intake was observed. Three factor analysis (dose×time×solution) of variance revealed a significant main effect of solution [$F(1,9)=6.489$, $P<0.05$], time [$F(5,45)=8.062$, $P<0.01$] and significant treatment×time [$F(10,90)=2.598$, $P<0.01$] interaction. Planned simple comparisons revealed a significant enhancement of ethanol drinking induced by the treatment of 0.25 µg DAMGO ($P<0.05$), compared to vehicle treatment. This stimulatory effect on ethanol drinking was most evident in the third and fourth time periods. This profile was reflected statistically by a significant partial time×dose interaction between 0.25 µg treated and vehicle-treated groups for ethanol intake [$F(5,90)=2.148$, $P<0.01$]. In contrast, no significant effect on water intake was found between saline-treated group and any DAMGO-treated group.

Discussion

The above findings demonstrate that the stimulation of mu opioid receptors within the nucleus accumbens facilitates the consumption of saccharin and salt solution, as well as ethanol, in non-deprived rats. However, water intake was not affected in water-deprived rats. In general, the data, considered together with our previous work with sucrose and fat intake (Kelley et al. 1996; Zhang and Kelley 1997, 2000; Zhang et al. 1998), suggest that opioid peptide-containing neurons within accumbens are part of a system governing positive affective valence associated with food or fluid intake.

It is interesting to note the characteristics of the time course of saline and alcohol drinking behavior following microinfusion of DAMGO into the nucleus accumbens. As demonstrated in the results, the temporal profile of saline and alcohol drinking following administration of the lowest dose DAMGO was similar to that of vehicle treatment, in which drinking peaked in the first thirty minutes and gradually terminated. However, the time course of feeding following 0.25 µg DAMGO treatment was characterized a biphasic inhibitory-stimulatory effect on saline, water and alcohol intake. This biphasic profile has been frequently found in our previous behavioral studies with high doses of mu opioid receptor agonists (Bakshi and Kelley 1993a; Zhang and Kelley 1997). It is possible that this profile resulted from the changes in locomotor activity induced by the mu opioid receptor agonist morphine in high doses, which was reported many years ago (Babbini and Davis 1972). We also observed that locomotor activity was strongly inhibited early and activated later following microinfusion of 0.25 µg DAMGO into the accumbens, while no locomotor inhibition was observed after low dose treatment (results not shown). In addition, the general biphasic effect on locomotor activity also may explain why a similar temporal profile was found for water drinking, although total water intake was not influenced following DAMGO treatment. Infusion of 0.25 µg DAMGO into the nucleus accumbens also tended to inhibit saccharin drinking in the first 30 min, although the biphasic inhibitory-stimulatory effects on saccharin drinking were not as evident as that observed in the drinking of other solutions such as saline and alcohol. What is not clear from these and previous data is that while the overt measure of food intake indicates a biphasic affect, it is not clear whether the palatability affect per se follows a monophasic or biphasic time course. This is difficult to assess since the initial motor inhibition interferes with the expression of feeding behavior.

Previous studies show that opioid stimulation of the nucleus accumbens enhances the intake of normal laboratory chow, sucrose and diets containing high carbohydrate or fat in non-deprived rats (Evans and Vaccarino 1990; Bakshi and Kelley 1993a, 1993b; Bodnar et al. 1995; Kelley et al. 1996; Zhang and Kelley 1997; Zhang et al. 1998). Since intake of foods containing sugar and fat is preferentially influenced by opioid manipulation of the nucleus accumbens, it is tempting to speculate that orosensory reward is the primary driving factor in this phenomenon. However, these foods have a high caloric content, which may also be an important factor in stimulating increased food intake. The current study addressed this issue by examining the opioid influence on intake of palatable solutions with no caloric value. Our findings resulting from this study are consistent with several lines of evidence supporting the notion that palatability, rather

than energy needs, is modulated by opioids. For example, the anorectic effect of naloxone was found to be more potent on feeding driven by palatability than by energy needs (Welch et al. 1996), and in that study the activity of endogenous opioids was found to be more likely affected by consumption of sweet/high fat food than by energy deficit. Additional evidence from clinical studies shows that hunger and satiety ratings are not affected by opioid antagonists, while "pleasantness rating" of food is decreased (Yeomans and Wright 1991; Drewnowski et al. 1992; Yeomans and Gray 1996). The present data are also concordant with studies reporting that systemic or central administration of opioid agents enhances consumption of sweet, salt and HCL solutions in non-deprived rats (Levine et al. 1982; Calcagnetti and Reid 1983; Siviy and Reid 1983; Gosnell and Majchrzak 1990) and in rats with gastric cannulae, a method which minimizes postingestive factors (Kirkham and Cooper 1988a, 1988b). Finally, motivation for water appears unchanged following mu opioid stimulation of the nucleus accumbens, since water intake was not affected by opioids, overall, in thirsty rats presumably motivated to drink. This observation provides evidence for specificity of the opioid modulatory effect on ingestive behavior. However, it should also be noted that the effect of accumbens opioid stimulation on intake of non-caloric substances is smaller than that observed for caloric substances; the percent increase reported here is considerably lower than that seen for sucrose or fat (Zhang and Kelley 1997; Zhang et al. 1998). Thus, while opioids may affect intake of all foods to some degree, intake of calorically dense foods (particular fat) may be preferentially increased.

In several theoretical articles, Robinson and Berridge have postulated that the neural circuits involved in the processing of incentive stimuli are dissociable into a "wanting" system for incentive motivation and "liking" system for affective responses, the latter being frequently equated to palatability, orosensory reward and taste pleasure in ingestive behavior paradigms (Robinson and Berridge 1993; Berridge 1996). It is postulated that endogenous opioid systems are involved in the "liking" processes, based on the finding that morphine enhances positive taste reactivity to sweet solution (Pecina and Berridge 1995; Berridge 1996; Rideout and Parker 1996). Pecina and Berridge (2000) also reported recently that morphine infusion into the accumbens enhanced taste palatability in the taste reactivity test. The current study provides further evidence to support the idea that taste palatability, rather than incentive motivation per se, is modulated by brain opioids. However, it is important to note that while opioids may play a central role in the affective response to food, in energy-depleted motivational states, incentive and affective processes are closely integrated. Indeed, food deprivation strongly enhances palatability, and satiation diminishes positive affective responses to food (Cabanac and Lafrance 1990; Berridge 1991; Laeng et al 1993; Warwick and Synowski 1999)

The pleasantness of taste is positively correlated with the concentration of sweet solutions in a certain range. Studies using preference-aversion curves with different concentrations of saccharin demonstrated that morphine shifts the curve to the left, resembling the effect of increasing saccharin concentration (Calcagnetti and Reid 1983). In contrast, naloxone's effect appeared to mimic the attenuation of intake rate produced by sucrose dilution (Leventhal et al. 1995). It is conceivable, then, that the observed responses to intra-accumbens administration of DAMGO could result from increased taste sensitivity. For example, 0.15% saccharin maybe perceived as the concentration of 0.2%; taste palatability is, therefore, enhanced. This appears unlikely, however, as naltrexone and naloxone have been reported to have no impact on the rating of sweetness and saltiness, as well as taste detection thresholds, while perceived palatability was decreased in these studies of human beings (Yeomans and Gray 1996). Furthermore, the failure of naloxone to alter the discrimination of sucrose concentration gradient was also reported in rats trained to discriminate different concentration of sucrose from water (O'Hare et al. 1997). Therefore, the subjective rating of food palatability and its influence by opioid systems appears to be independent of the psychophysical perception of taste. Rather, positive tastes may activate central opioids, which then results in the generation of a positive affective response.

Much evidence suggests that the reinforcing properties of alcohol are due, in part, to activation of the endogenous opioid system. Naltrexone has been showed to be an effective pharmacological adjunct in the treatment of alcoholism, and has been reported to be effective in reducing alcohol drinking, alcohol craving, euphoriant effects of alcohol, and frequency of relapse in animal and human studies (Froehlich et al. 1990; O'Malley et al. 1992; Volpicelli et al. 1992, 1995; O'Brien et al. 1996; Williams et al. 1998). Our finding that mu opioid stimulation of the nucleus accumbens increased alcohol drinking suggests that nucleus accumbens may be one of brain areas where alcohol interacts with the endogenous opioid system. The role of opioidergic involvement of the nucleus accumbens in alcohol drinking has been mostly demonstrated in rats selectively bred for high or low alcohol drinking. For example, alcohol-preferring rats showed low basal level of proenkephalin but more potent increase of proenkephalin in the nucleus accumbens in response to the challenge of alcohol, compared to alcohol-avoiding rats (Nylander et al. 1994; Li et al. 1998). The correlation between high responsiveness of intra-accumbens opioid systems and high alcohol drinking is also demonstrated at opioid receptor level. Higher binding of mu opioid receptors has been shown in alcohol-preferring rats than in alcohol-non-preferring rats (McBride et al. 1998). Consistent with this finding, Myers and Robinson reported that mu receptor antisense injected into the nucleus accumbens suppressed high alcohol drinking in genetic drinking HEP (high-ethanol preferring) rats (Myers and Robinson 1999). There-fore, the opioid activation in the nucleus accumbens is correlated with alcohol drinking and may

thus play a critical role in the genetic basis of aberrant drinking of alcohol.

What is left uncertain in our study is whether opioids mediate increased alcohol intake through enhancement of the positive aspects of the taste of the alcohol, in a manner similar to that proposed above for food, or whether opioids rather affect the central euphoriant effects of ethanol. Clearly, rats ingest the alcohol but it is unlikely that it is perceived as a pleasant taste to begin with. Since 6% ethanol is reliably found to have both sweet and bitter components (Di Lorenzo et al. 1986; Lawrence and Kiefer 1987), it is possible that opioid stimulation reduces the aversive properties and enhances the positive ones. Support for this notion is provided by recent work showing that naltrexone reduces ingestive responses and increases aversive responses in a taste reactivity test for ethanol (Hill and Kiefer 1997). Further, it has been found that morphine can effectively reduce the aversiveness of quinine solutions (Clarke and Parker 1995). On the other hand, the palatability explanation fails to account for a study reporting that naltrexone reduced IV self-administration of ethanol (Williams et al. 1998). Perhaps central opioids influence both taste and other central effects of alcohol, a notion that may explain why naloxone can be such an effective treatment for alcoholism (O'Brien et al. 1996). A further important consideration is that in the sucrose fading procedure, the ethanol taste may become a positive conditional stimulus (CS^+) after repeated pairings with sucrose; if this is the case, opioids could act to enhance the hedonic properties of the CS^+. Finally it is also important to note that in addition to opioids, other neurotransmitter systems within the nucleus accumbens, particularly GABAergic (Heibreder and De Witte 1993; Hodge et al 1995; Hodge and Alken 1996; June et al 1998); and dopaminergic (Levy et al 1991; Samson and Hodge 1993; Samson et al. 1999; Kaczmarek and Keifer 2000) systems may also play a critical role in the overall control of alcohol intake.

In conclusion, we postulate that enkephalinergic fibers and mu opioid receptors within the nucleus accumbens play a role in ingestive behavior via a mechanism involving the hedonic assessment of taste, regardless of whether the food has caloric content. In addition, our results further suggest that the nucleus accumbens is one of the brain areas where ethanol interacts with endogenous opioid systems, and thus demonstrate a neural substrate common to both palatable eating and alcohol drinking.

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