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OPPOSING EFFECTS OF INTRA-NUCLEUS ACCUMBENS MU AND KAPPA OPIOID AGONISTS ON SENSORY SPECIFIC SATIETY

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Abstract—Mu opioid (MOP) agonists acting in the nucleus accumbens (NAcc) robustly enhance consumption of palatable foods. In addition, the effect on consumption of palatable foods produced by MOP agonists acting in the NAcc depends on both recent flavor exposure and the availability of a choice between different-flavored foods. In contrast, kappa opioid (KOP) agonists have variable effects on feeding and KOP agonists have MOP opposing behavioral actions when microinjected at several brain sites. We previously demonstrated that NAcc MOP agonists reverse the devaluation (satiety) effect of pre-feeding for a given flavor; in fact, NAcc MOP agonists selectively increase consumption of a recently sampled food. In contrast, in the present study, we found that the selective KOP agonist U50488 injected into the NAcc of rats reduced consumption of a recently sampled flavor while increasing consumption of the flavor that was not pre-fed. Intra-NAcc U50488 did not affect overall consumption or flavor preference in the absence of pre-feeding. The present data, in conjunction with our previous findings, highlight the robust and opposing role of NAcc MOP and KOP opioid receptors in palatability-based food choice and consumption and raise the possibility that an endogenous KOP agonist acting in the NAcc contributes to the phenomenon of sensory specific satiety.

Key words: opioids, nucleus accumbens, feeding, palatability, choice.

Flavor information guides decisions about food consumption that are critical for survival. In the setting of choice, the palatability of a food item (i.e. the reward value of a food, as signaled by orosensory cues (Rolls, 2001)) is essential to decision-making (e.g. what and how much of an item will be consumed), but the neural mechanisms underlying food preference are poorly understood. The opioid system is critical for the rewarding action of palatable foods. Mu opioid (MOP) receptor agonists induce robust feeding in the rat (Martin et al., 1963) by increasing the consumption of palatable food (Berridge, 1996). Accordingly, in humans, non-selective opioid antagonists reduce the positive hedonic effect of food but leave taste recognition thresholds unaffected (Yeomans and Gray, 2002).

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Abbreviations: DAMGO, D-Ala^2,N,Me-Phe^4,Gly-ol^5-enkephalin; Dyn, dynorphin; KOP, kappa opioid; MOP, mu opioid; NAcc, nucleus accumbens; SSS, sensory specific satiety.

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The nucleus accumbens (NAcc) is a critical site for opioid actions on palatability. It contains high densities of both MOP and kappa opioid (KOP) receptors as well as enkephalinergic and dynorphinergic fibers (Mansour et al., 1988; Van Bockstaele et al., 1995). Microinjection of D-Ala^2,N,Me-Phe^4,Gly-ol^5-enkephalin (DAMGO, a MOP receptor selective agonist) into the NAcc preferentially increases consumption of palatable items (Zhang et al., 1998; Zhang and Kelley, 2002) and preferred flavors (Woolley et al., 2006) as well as positive affective orofacial reactions to sweet tastes (Pecina and Berridge, 2005). Additionally, opioid antagonists in the NAcc selectively reduce consumption of palatable foods, indicating that endogenous opioid release modulates feeding (Segall and Margules, 1989). We have previously shown that MOP agonists within the NAcc can condition taste preferences (Woolley et al., 2007) but the effects of KOP agonists at this site are unclear. KOP antagonists in the NAcc can alter consumption under certain conditions but the range of effects differs from those of MOP antagonists (e.g. both MOP and KOP antagonists reduce deprivation and glucoprivic-induced feeding but only MOP antagonists reduce consumption of sucrose in non-deprived rats) (Bodnar et al., 1995). On the other hand, KOP agonist microinjection into the NAcc does not alter consumption of chow or a palatable sucrose solution (Majeed et al., 1986; Bakshi and Kelley, 1993; Zhang and Kelley, 1997). Furthermore, KOP agonists often have behavioral actions that are distinct from and often oppose MOP agonist actions when injected into the same brain region ((Mucha and Herz, 1985) and see (Pan, 1998) for review). To address this issue, we used a sensory specific satiety (SSS) paradigm to compare the actions of intra-NAcc MOP and KOP agonists on short term flavor conditioning and the reward value of specific tastes.

EXPERIMENTAL PROCEDURES

Animals

A total of 41 male rats (Long Evans, Charles River Laboratories, Wilmington, MA, USA) weighing between 270 and 450 g were used in the present studies. All procedures were approved by the UCSF Animal Care and Use Committee and conformed to international guidelines on the ethical use of animals. Every attempt was made to minimize the number of animals used and their suffering. Animals were individually housed in conventional hanging cages in a temperature- and humidity-controlled room on a 12-h light/dark cycle. Animals had *ad libitum* access to water at all times and *ad libitum* access to chow at all times except during testing.

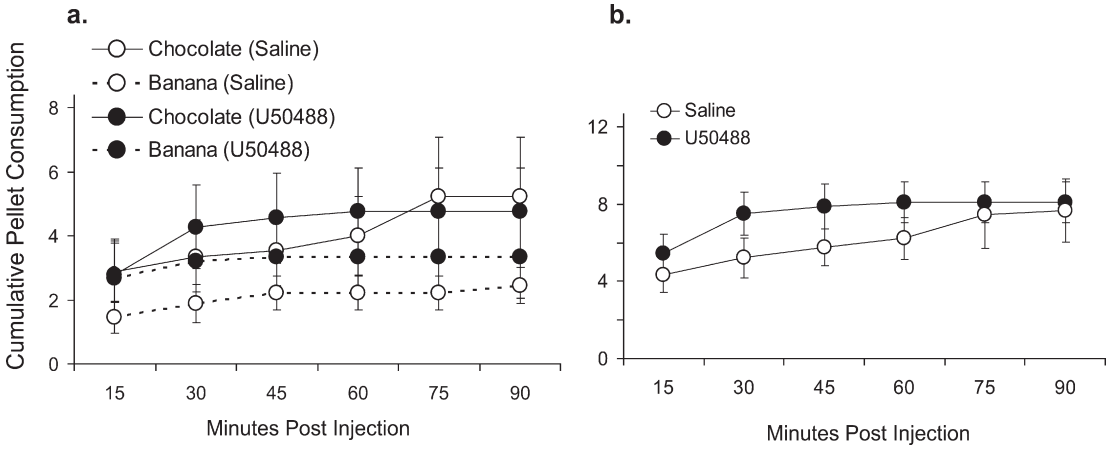

Fig. 1. Effect of intra-NAcc KOP receptor agonist on consumption in a choice paradigm. (a) The number of flavored pellets consumed following saline or U50488 microinjection is shown for each 15 min postinjection. Since both flavors were available after microinjection, closed circles represent data from one test session while open circles represent data from a separate test session. (b) Total cumulative consumption (chocolate plus banana) following microinjection of saline and U50488 is shown.

Surgery

Animals were anesthetized with isoflurane, their heads placed in a stereotaxic device and then, following a small craniotomy, bilateral guide cannulae were stereotactically placed and then secured to the skull with stainless steel screws and dental cement. Coordinates for the target sites were 1.5 mm anterior, 1.1 mm lateral and 5.5 mm ventral from Bregma. For this study, the cannulae were not directed specifically at the core or the shell regions of the NAcc. For control microinjections, coordinates for the target sites were 1.5 mm anterior, 1.1 mm lateral and 3.5 mm ventral from Bregma. Animals were allowed 4 days recovery postsurgery.

Drugs and injections

For microinjections, U50488, the selective KOP receptor agonist was obtained from Sigma Pharmaceuticals (Monticello, IA, USA). U50488 was dissolved in 0.9% sterile saline (3.25 μg per side which is equivalent to 8 nmol/μl, the highest concentration used in a previous study (Bakshi and Kelley, 1993)). First, the stylet was removed from the guide cannulae and the injector cannulae were inserted. The injector cannulae protruded 2 mm past the end of the guide cannula for a final distance of 7.5 mm ventral to Bregma. U50488, in a volume of 0.5 μl of saline, was infused through 12.5 mm injector cannulae connected to a microdrive pump by polyethylene tubing. The rate of infusion was 0.25 μl/min. The injector cannulae remained in place an additional minute after the infusion to allow for diffusion. Injectors were then removed and the stylets were replaced. For s.c. injections, U50488 was diluted in 0.9% sterile saline at a concentration of 2 mg/kg and injected s.c. with a 1 ml syringe. This concentration was chosen because it has been shown to increase consumption of palatable food in non-deprived rats (Cooper et al., 1985b).

Behavioral testing and experimental design

After recovery from surgery (4 days), animals were extensively handled. In order to overcome taste neophobia, rats were brought into the testing room on four separate days and given 1 h simultaneous access to both flavors of pellets (chocolate and banana). After this initial exposure, all rats avidly consumed the pellets when available. The two types of flavored 1 g pellets were made from the same meal substrate and were thus matched for all macro- and micro- nutrients (Bio-Serv, Frenchtown, NJ, USA). Pellets were always delivered in test tube dispensers. Rats were required to grab the pellets with their teeth and forcibly remove them from a hole in the bottom of the tube. This amount of effort encouraged the rats to take only what they would eat and greatly facilitated consumption quantification. Every 15 min postinjection, the number of pellets remaining in the dispenser was counted and a visual inspection of the cage for dropped pellets was made. Rats were removed from their home cages for the duration of the microinjection and then immediately returned. Testing sessions were separated by at least 48 h. The SSS paradigm consisted of a pre-feeding phase that consisted of *ad libitum* access to pellets of one flavor for 1 h, an injection phase, and a post-injection test phase where rats were given simultaneous access to pellets of both flavors for 1.5 h. Before the pre-feeding phase, rats were in an *ad libitum* feeding, non-deprived state.

To determine whether intra-NAcc U50488 affects consumption in the absence of pre-feeding when rats are allowed to choose between flavors, rats (n=9) were microinjected with U50488 or saline into the NAcc and given 1.5 h simultaneous *ad libitum* access to both chocolate- and banana-flavored pellets. All rats underwent both conditions and injection orders were randomized.

To determine whether intra-NAcc U50488 differentially alters consumption of a flavor that has just been consumed, a SSS paradigm was used. Rats (n=16) were given 1 h *ad libitum* pre-feeding access to either banana or chocolate pellets. At the end of this hour, rats were microinjected with either U50488 or saline. Rats were then given 1.5 h simultaneous *ad libitum* access to both chocolate and banana flavored pellets. All rats underwent all four conditions and injection and flavor orders were randomized. As a site control, U50488 was injected 2 mm dorsal to the NAcc injection target (n=8). To further explore the role of NAcc KOP receptors, we repeated the same SSS paradigm but instead of injecting U50488 into the NAcc, we delivered it s.c. (n=18).

Data analysis

All data are expressed as mean±S.E.M. (standard error of the mean). Data were analyzed using repeated measures ANOVA with pharmacologic manipulation, pre-feeding and flavor as repeated measures. Post hoc comparisons were made using the Bonferroni correction.

Histology

After the completion of all testing, rats were anesthetized deeply with sodium pentobarbital and transcardially perfused with a 0.9% isotonic saline solution followed by 10% formalin solution. Brains were removed and stored in 10% formalin for several days fol-