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Cholecystokinin is necessary for the expression of morphine conditioned place preference

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Abstract

There is evidence that the neuropeptide cholecystokinin (CCK) is important for the rewarding effects of drugs of abuse. However, less is known regarding the role of CCK in drug seeking and craving. The present study investigated whether the CCK_B antagonist L-365, 260 could block morphine-induced drug seeking using the conditioned place preference paradigm and whether the dopaminergic reward pathway contributes to the effect of L-365, 260 on expression of morphine place preference. We found that systemic administration of the CCK_B antagonist L-365, 260 attenuates the expression of morphine-induced drug seeking as assessed using conditioned place preference (CPP) and shows that this effect is mediated by CCK_B receptors in the anterior nucleus accumbens (NAcc). Additionally, we demonstrate that this effect is dependent on D_2 receptor activation in the anterior nucleus accumbens (NAcc). These results indicate that endogenous CCK modulates the incentive-salience of morphine-associated cues and suggest that CCK antagonists may be useful in the treatment of drug craving.

Keywords: Dopamine; Accumbens; Incentive; Reward; Craving; Drug seeking

1. Introduction

Cholecystokinin (CCK) is a neuropeptide that acts in the CNS to modulate appetite, stress, and anxiety (Nemeroff et al., 1978; Willis et al., 1986; Singh et al., 1991; Bhatnagar et al., 2000). Two distinct CCK receptors have been identified: the CCK_A (CCK1) receptor and the CCK_B (CCK2) receptor (Crawley and Corwin, 1994). Under some circumstances CCK acts as an anti-opioid peptide. For example, CCK attenuates the analgesic effects of morphine and other mu opioid receptor agonists (Faris et al., 1983), while CCK antagonists can potentiate the analgesic effects of mu opioid agonists (Watkins et al., 1984; Katsuura and Itoh, 1985). CCK antagonists also impede the acquisition of morphine tolerance (Tang et al., 1984; Dourish et al., 1988) yet have no effect on morphine dependence and withdrawal (Panerai et al., 1987; Baber et al., 1989).

In contrast to its opioid-opposing action in analgesia, there is evidence that CCK contributes to the rewarding effects of opioids, psychostimulants, and ethanol (Higgins et al., 1992; Crespi, 1998). Additionally, a CCK_B antagonist blocks both stress-induced reinstatement of cocaine conditioned place preference (Lu et al., 2002) and drug-primed reinstatement of morphine conditioned place preference (Lu et al., 2001). Importantly, in the absence of drug associated cues and stress, CCK_B antagonists typically have no effect on place preference (Higgins et al., 1992; Lavigne et al., 1992). Taken together, this research suggests that CCK plays a role in drug craving and reinstatement of drug self-administration. Although the contribution of CCK to the acquisition of morphine CPP has been studied (Higgins et al., 1992) its role in the expression of established morphine CPP (i.e. drug seeking) has not.

There is evidence that CCK interactions with dopamine in the NAcc can contribute to goal directed behaviors. CCK is colocalized with dopamine in approximately 80% of neurons in

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the substantia nigra pars compacta (Seroogy et al., 1989) and 40% of neurons in the ventral tegmental area (VTA; Wang et al., 1985). Furthermore, VTA dopaminergic neurons that project to the NAcc contain CCK (Hökfelt et al., 1980). There are also CCK containing projections to the NAcc from the prefrontal cortex (Hökfelt et al., 1988; Brog et al., 1993; You et al., 1998). Finally, systemic morphine administration induces the release of both dopamine and CCK in the NAcc (Hamilton et al., 2000).

The effect of dopamine on NAcc neurons *in vitro* can be either enhanced or suppressed by application of CCK-8 (White and Wang, 1984; Voigt et al., 1986; Wang and Hu, 1986) and microinjection of CCK-8 into the NAcc can both attenuate and potentiate the release of dopamine *in vivo* (Yim and Mogenson, 1991; Derrien et al., 1993; Ladurelle et al., 1993). Taken together, these findings suggest that the modulatory effect of CCK on dopamine transmission is dependent on differential activation of subregions within the NAcc or on variability in the expression of CCK ligands or receptor subtypes.

The conditioned place preference paradigm offers a substantive means of assessing the mechanism by which drug associated cues control appetitive behaviors. In the current study we used CPP to determine the contribution of CCK acting in the NAcc on expression of preference for an environment previously paired with morphine. We examined the effect of both CCK_A and CCK_B selective antagonists on expression of morphine CPP. Since the NAcc is implicated in the expression of morphine CPP (Tzschentke, 1998) we also examined the role of dopamine in CCK actions at this site. Additionally, as previous groups have noted a difference in the actions of CCK in the anterior versus posterior NAcc (Vaccarino and Vaccarino, 1989; Marshall et al., 1991; Ladurelle et al., 1993), we further hypothesized that we would observe differences in the effects of CCK drugs on expression of morphine CPP depending on whether the drug was injected into the anterior or posterior region of the NAcc. Lastly, because a change in affinity at the D_2 binding site has been noted in response to striatal CCK (Murphy and Schuster, 1982; Agnati et al., 1983), we hypothesized that intra-accumbens effects of CCK on expression of morphine CPP would involve dopamine D_2 receptors.

2. Methods

2.1. Subjects

83 male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 275–300 g at the onset of the study were individually housed in a temperature-controlled environment (21 °C) and kept on a 12 h light/dark cycle. Rat chow and water were available *ad libitum*. Animals were tested at the same time during their light cycle each day. All experimental protocols were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH). 22 rats were used for systemic injections following morphine place preference, 15 for systemic injections following food place preference, and the remaining 46 rats were cannulated for NAcc microinjections. Additional animals failed to develop morphine place preference ($n=8$) or food place preference ($n=5$), and were excluded from the study.

2.2. Surgery

Animals were initially anesthetized with a ketamine–xylazine mixture (1 mg/kg) and then maintained on isoflurane (.5 L/min) as needed for the duration of surgery. Animals were placed in a stereotaxic frame and were implanted with bilateral 26-gauge stainless steel chronic guide cannulae (Plastics One, Roanoke, VA) into either the Anterior (AP, 2.2; ML, ±1.1; DV, −5.5) or Posterior (AP, 1.2; ML, ±1.1; DV, −5.5) NAcc. Cannulae were secured to the skull with dental cement. At the end of the surgical procedure, animals were treated with 1 mg/kg i.m. penicillin and topical antibiotics. A stainless steel dummy cannula (Plastics One) was inserted into each guide cannula and remained in place when the guide cannulae were not in use. Animals were allowed a 1 week recovery period prior to behavioral testing.

2.3. Conditioned place preference

Animals were trained in 3 chamber place conditioning boxes (Med Associates) in which 2 chambers (28×21×21 cm) that differed in color (one black, one white), light level, and texture were separated by a neutral gray chamber (12×21×21). During the initial baseline period, animals were placed in the central chamber and were allowed to freely explore all 3 chambers for a period of 30 min. Beam breaks, entries, and time spent in each chamber were automatically recorded using infrared beams. Animals were excluded from the study if baseline data revealed a chamber bias of >250 s or if no CPP was apparent on testing day (<250 s difference between chambers). Animals were administered habituation injections for a minimum of 3 days prior to place preference injections.

2.3.1. Morphine

During each conditioning session, animals were injected with either morphine or saline and were then immediately confined to one of the two larger end-chambers for 60 min. Rats received 2 conditioning sessions per day for 4 days. Conditioning sessions were separated by a minimum period of 5 h. Animals were tested for expression of conditioned place preference 1 day after the final conditioning session. Drug side and treatment order were counterbalanced (unbiased). We have previously shown that this place preference procedure induces a strong and long-lasting place preference to morphine (Kim et al., 2004). As previous studies have demonstrated the persistence of morphine place preference for at least 12 weeks using methodology similar to that reported here, each animal in the microinjection portion of this study was tested multiple times for morphine CPP (Mueller and Stewart, 2000; Mueller et al., 2002). Systemic L-365, 260 was administered 30 min before the onset of CPP testing.

2.3.2. Food

Rats received one conditioning session per day for a period of 25 days. Each conditioning session lasted for 60 min. During each conditioning session, one chamber was paired with 10 g of

dustless precision chocolate pellets (BioServ) and the other chamber with 10 g of dustless precision banana pellets (BioServ). Pellets and chambers were counterbalanced (unbiased). Animals were tested for expression of place preference one day after the final conditioning session. Using this methodology, 15 animals expressed a robust place preference to their preferred flavor. On the following 2 days, animals were injected with either systemic L365, 260 or vehicle, and tested again for food place preference. L365, 260 and vehicle injections were randomized and counter-balanced.

2.4. Microinjections

All injection sites were based on the atlas of Paxinos and Watson (1998). Bilateral injections were made into either the Anterior (AP, 2.2; ML, ±1.1; DV, −5.5) or Posterior (AP, 1.2; ML, ±1.1; DV, −5.5) NAcc. Each injection was made using a 1 µl syringe (Hamilton, Reno, Nevada) attached to 20 cm of PE 50 tubing connected to a 33-gauge injection cannula (Plastics One). Microinjections were given at a rate of .5 µl/min using a syringe pump (kd Scientific, Holliston, MA). A volume of .5 µl was injected into each side of the NAcc. Injection cannulae extended 2 mm beyond guide cannulae and were left in place for 1 min following microinjections to minimize the backflow of drug solution. 10 min after microinjection was completed, animals were placed into the central chamber of the CPP apparatus and allowed to explore for a period of 30 min. Animals received a maximum of four bilateral NAcc microinjections over seven days. The number of microinjections each animal received was determined by the persistence of place preference. Participation was terminated if an animal's preference score did not return to the pre-injection level within 24 h following a microinjection. Because of this stringent rejection criterion, once animals received a microinjection of L365, 260 into the anterior NAcc, they were excluded from further study (see Results). Microinjection drug order was counterbalanced and began a minimum of one day following the test for expression of morphine CPP (Fig. 1). Animals were tested for expression of morphine CPP 24 h following each microinjection to ensure the return to pre-injection levels. At the conclusion of the experiment, animals were anesthetized with pentobarbital and perfused intracardially through the ascending aorta with .1 M phosphate buffered saline followed by 10% formalin. Brains were sectioned coronally at 50 µm, mounted and stained with neutral red.

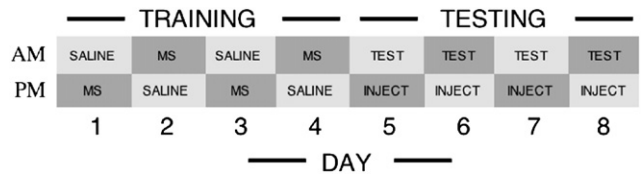

Fig. 1. Timeline of CPP and microinjection procedures. Rats received 1 morphine and 1 saline conditioning session per day for a period of 4 days. Rats were tested for CPP on the mornings of the 5th, 6th, 7th, and 8th days and injected each afternoon with one of the following compounds: L-365, 260, lorglumide, CCK-4, raclopride, or with a combination of L-365, 260 and raclopride or L-365, 260 and CCK-4. Each animal received a maximum of 4 microinjections. All drug orders were counterbalanced.

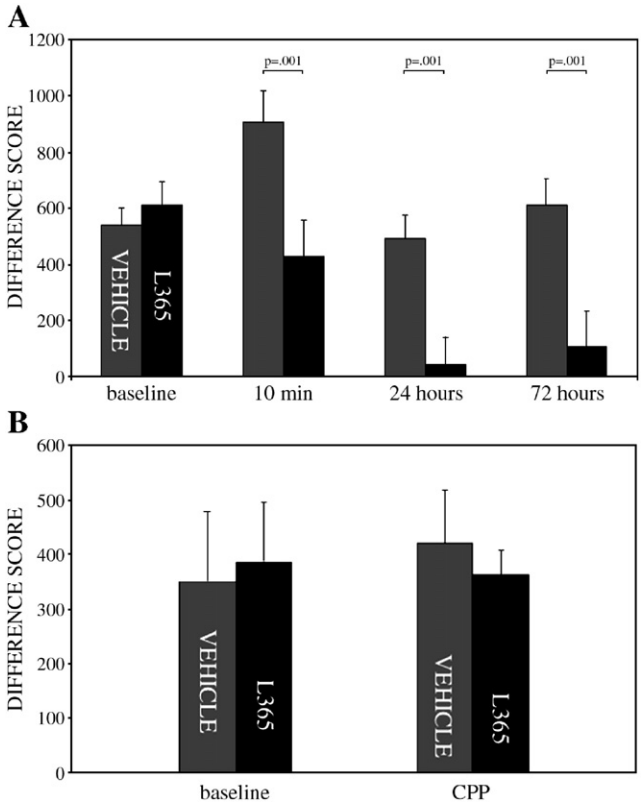

Fig. 2. (A) The effects of the CCK_B antagonist L-365, 260 (1 mg/kg) on the expression of morphine conditioned place preference following systemic administration. L-365, 260 attenuates the expression of morphine CPP when animals ($n=11$) are tested 10 min after injection ($p=.001$), 24 h after injection ($p=.001$) and 72 h after injection ($p=.001$). (B) L-365, 260 has no effect on the expression of food CPP ($n=15$, $p=.84$).

2.5. Drugs

Morphine sulfate powder was provided by the NIDA Drug Supply Program (Research Triangle Park, NC) and was dissolved in physiological saline and injected at a dose of 10 mg/kg s.c.. The CCK_B antagonist L-365, 260 was the gift of ML Laboratories (St. Albans, UK) and was dissolved in 5% DMSO. L-365, 260 was injected i.p. at a dose of 1 mg/kg and was microinjected at a dose of 15 ng/site. CCK-4 (100 ng/site), the CCK-A antagonist lorglumide (500 ng/site), and the D2 antagonist raclopride (4 µg/site) were obtained from Research Biochemicals (Natick, MA) and were dissolved in physiological saline.

2.6. Statistical analysis

Difference scores were calculated by subtracting the time spent in the saline paired chamber from the time spent in the morphine paired chamber during a test session. A positive score is therefore indicative of place preference while a negative score indicates place aversion. Values are given as arithmetic means± SEM. Statistical significance was set at $p<.05$. For systemic

injections, a two-way ANOVA followed by Fisher's LSD was used to compare across drug treatment and time. As multiple tests were performed to assay the expression of morphine place preference in animals used for NAcc microinjections, paired comparisons between groups were conducted using a student's *t*-test (two-tailed) to analyze each drug condition. Between groups comparisons were conducted using unpaired two-tailed *t*-tests. Statistical measures were chosen based on consultation with the UCSF Division of Biostatistics. All statistical tests were conducted using commercially available software (Microsoft Excel and SPSS v. 11).

3. Results

When administered systemically following the acquisition of conditioned place preference, a two-way ANOVA revealed significant main effects of Treatment ($F=12.58$, $p=.002$) and Day ($F=8.491$, $p<.0001$) as well as a significant Treatment × Day interaction ($F=3.93$, $p=.012$). Specifically, the CCK_B antagonist L-365, 260 (1 mg/kg) attenuated the expression of morphine CPP (Fig. 2). Interestingly, this effect was not only significant when animals were tested immediately after antagonist injection ($p=.001$, compared to vehicle) but also when animals were tested 24 h ($p=.001$ and $p<.0001$, compared to vehicle and baseline, respectively) and 72 h ($p=.001$ and $p<.0001$, compared to vehicle and baseline, respectively) after antagonist injection. This demonstrates that CCK antagonists produce a prolonged change in the expression of morphine conditioned place preference and suggests that CCK contributes to drug seeking induced by morphine associated cues.

In contrast to morphine CPP, systemic injection of the CCK_B antagonist L-365, 260 did not affect the expression of food place preference ($t=-.25$, $p=.84$). Vehicle injections were also without effect ($t=.55$, $p=.60$). These data suggest that the L-365, 260-induced attenuation of expression of morphine place preference is specific to morphine associated cues and that L-365, 260 does not produce a generalized impairment of memory or approach behaviors elicited by reward associated cues.

NAcc microinjection sites are illustrated in Fig. 3. In order to determine the contribution of CNS reward circuitry to this effect, L-365, 260 (15 ng) was microinjected into either the anterior or posterior NAcc (Fig. 4A). L-365, 260 microinjected into the anterior NAcc attenuated the expression of morphine

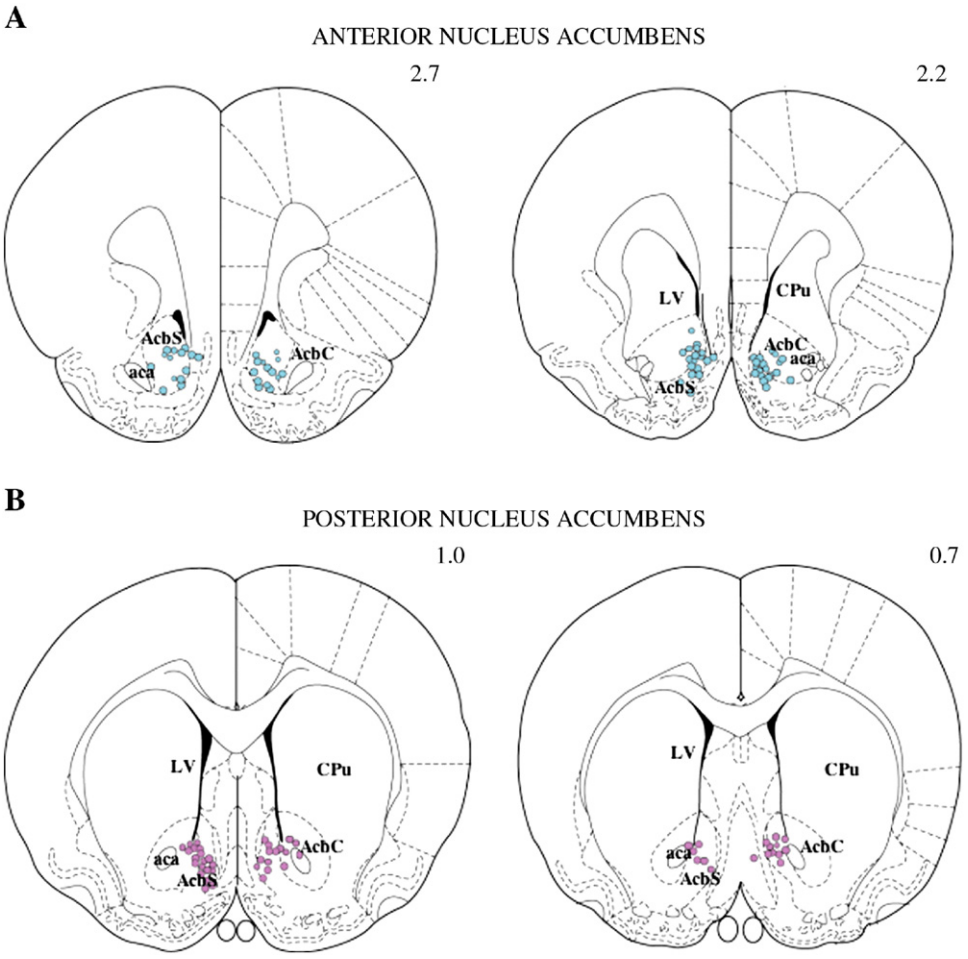

Fig. 3. Anterior (A) and posterior (B) NAcc injection sites ($n=46$). Coordinates in the upper right refer to distance from bregma. CPu, caudate putamen; aca, anterior commisure; AcbS, accumbens shell; AcbC, accumbens core; LV, lateral ventricle. The diameter of each circle indicates the extent of gliosis at each injection site. Injection sites were marked for each animal in every section on which they were visible.

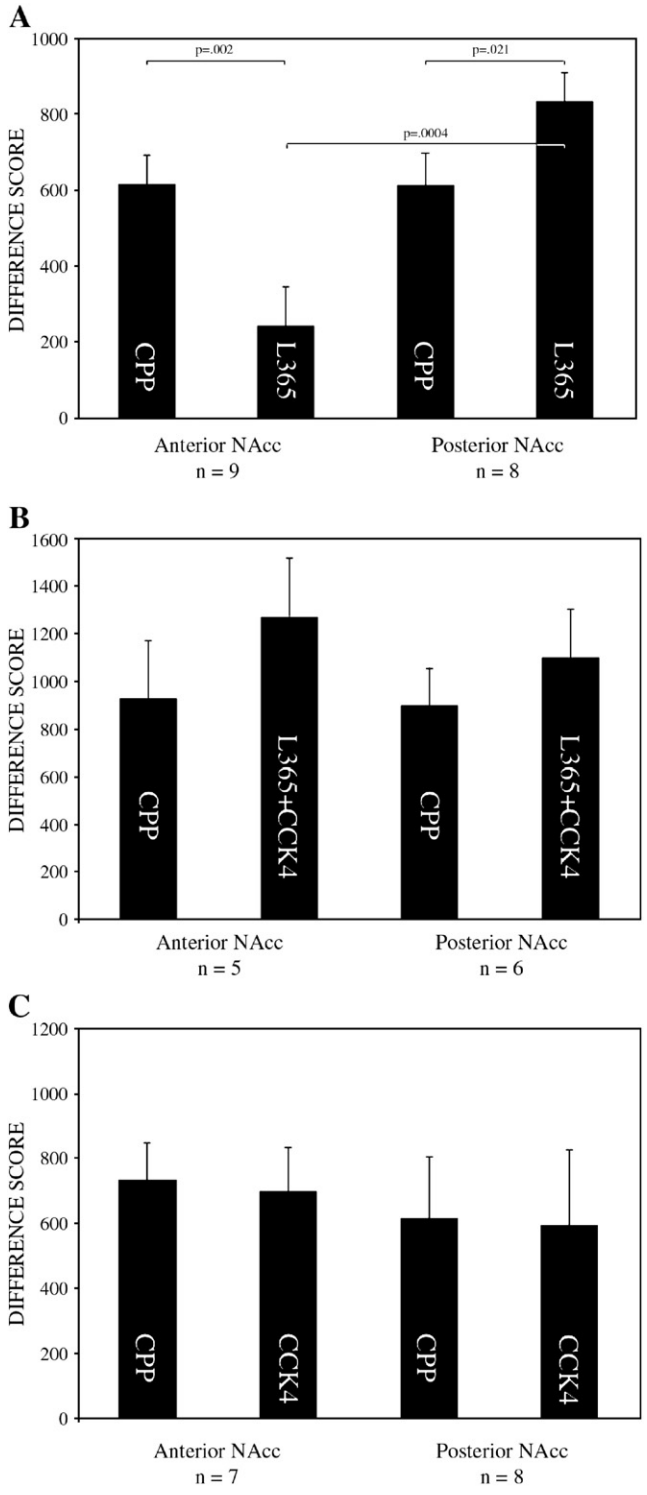

Fig. 4. (A) L-365, 260 (15 ng) microinjected into the anterior NAcc attenuates the expression of morphine CPP ($n=9$, $p=.002$) and L-365, 260 microinjected into the posterior NAcc potentiates the expression of morphine CPP ($n=8$, $p=.021$). Both effects are blocked by co-injection of 100 ng CCK-4 ($n=11$, B), which has no effect on the expression of morphine CPP when injected independently ($n=15$, C).

CPP ($t=4.47$, $p=.002$) while L-365, 260 microinjected into the posterior NAcc *potentiated* the expression of morphine CPP ($t=-2.95$, $p=.021$). The effects of L-365, 260 in the anterior

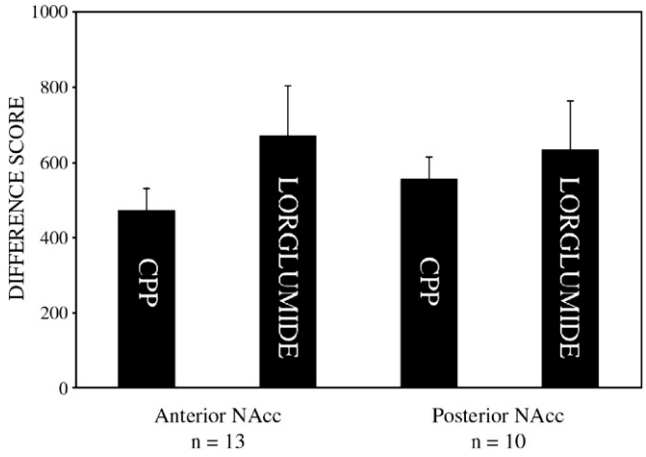

Fig. 5. The specific CCK_A antagonist lorglumide (15 ng) has no effect on expression of morphine CPP when microinjected into either the anterior ($n=13$) or posterior ($n=10$) NAcc.

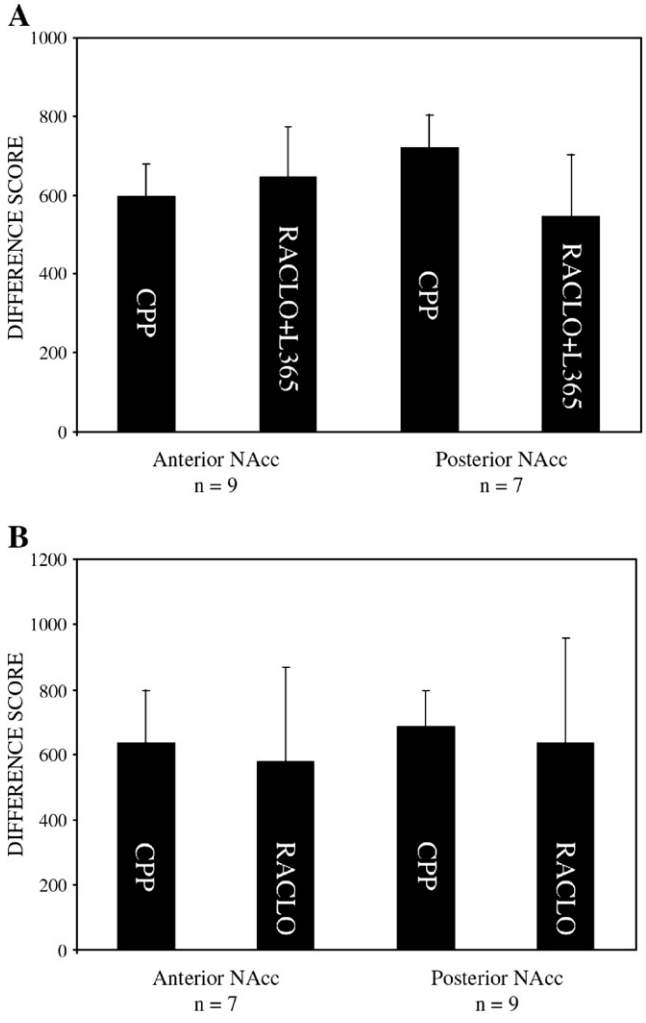

Fig. 6. Raclopride (4 μg) blocks the effect of L-365, 260 in both the anterior ($n=9$) and posterior ($n=7$) NAcc (A) but has no effect on morphine CPP when microinjected independently into either the anterior ($n=7$) or posterior ($n=9$) NAcc (B).

and posterior NAcc were significantly different from each other ($t=4.52$, $p=.0004$). Both effects on expression of morphine CPP were blocked by the co-injection of 100 ng of CCK-4 (anterior: $t=-1.64$, $p=.18$ and posterior: $t=-1.12$, $p=.31$, Fig. 4B), which when injected by itself had no effect on the expression of morphine CPP (anterior: $t=.49$, $p=.64$ and posterior: $t=.11$, $p=.91$, Fig. 4C).

To further investigate the receptor subtype selectivity of the L-365, 260 effects within the NAcc, the CCK_A-specific antagonist lorglumide (15 ng) was microinjected into either the anterior ($n=13$) or posterior ($n=10$) NAcc (Fig. 5). Lorglumide had no significant effect on expression of morphine CPP in either the anterior or posterior NAcc ($t=-1.60$, $p=.13$ and $t=-.52$, $p=.61$, respectively, Fig. 5). This finding is consistent with previous in situ hybridization studies suggesting the absence of the CCK_A receptor in the NAcc, and indicates that the effect of CCK on expression of morphine CPP in the NAcc is mediated primarily through the CCK_B receptor.

In order to ascertain the contribution of the dopamine system to the effects of CCK on expression of morphine CPP, the D_2 specific antagonist raclopride was microinjected into the anterior and posterior NAcc. Raclopride (4 μg) blocked the effects of L-365, 260 in both regions (anterior: $t=-.34$, $p=.74$ and posterior: $t=1.21$, $p=.27$, Fig. 6A) but had no effect on morphine CPP when microinjected independently into either the anterior or posterior NAcc ($t=.30$, $p=.77$ and $t=.20$, $p=.85$, respectively, Fig. 6B) suggesting that the effect of CCK on expression of morphine CPP requires the action of dopamine at the D_2 receptor.

4. Discussion

We have shown that systemic injection of the CCK_B antagonist L-365, 260 induces a long-lasting D_2 dopamine receptor dependent attenuation of the expression of morphine conditioned place preference and that this effect can be replicated by L-365, 260 microinjection in the anterior, but not the posterior, NAcc. Previous work demonstrated that activation of D_2 presynaptic autoreceptors decreases nucleus accumbens dopamine release (Dugast et al., 1997; Phillips et al., 2002). Additionally, CCK acts at CCK_B receptors in the nucleus accumbens to decrease D_2 receptor binding (Murphy and Schuster, 1982; Agnati et al., 1983; Li et al., 1995). Consequently, reduction of this D_2 autoreceptor-mediated inhibition by a CCK_B agonist would be expected to increase dopamine release (Ladurelle et al., 1993; Ferraro et al., 1996). The current study suggests that a similar mechanism may regulate the expression of morphine conditioned place preference (CPP) in the anterior NAcc. By increasing binding affinity of D_2 receptors, a CCK_B antagonist should decrease the amount of dopamine released from presynaptic terminals. We propose that this decrease in dopamine release translates into a decrease in the predicted reward value associated with morphine conditioned sensory cues, resulting in attenuation in the expression of morphine CPP.

Microdialysis studies suggest that under basal conditions there is tonic release of CCK in the NAcc (Maidment et al., 1991; You et al., 1994), which might explain the lack of a CCK agonist effect on behavior in the absence of a CCK receptor antagonist. If CCK receptors are already saturated, microinjection of additional CCK should be unable to alter expression of CPP. Under the present conditions, the CCK antagonist seems to be acting as a brake on expression of morphine CPP suggesting an action on dopamine dependent incentive cues (Yun et al., 2004). We propose that by decreasing dopamine release, a CCK_B antagonist decreases the value of incentive cues in a drug-paired environment.

The anterior NAcc receives a projection from the prefrontal cortex that includes CCK containing glutamatergic neurons (Meyer et al., 1982; Morino et al., 1994). In contrast, the medial-posterior NAcc receives CCK projections that originate from the VTA and co-contain dopamine (Hökfelt et al., 1980). Interestingly, the VTA dopaminergic projection to the anterior NAcc does not appear to contain CCK (Hökfelt et al., 1980; Seroogy et al., 1989; Ladurelle et al., 1993). Furthermore, the CCK containing projection from the VTA terminates in the NAcc shell while the CCK containing dopamine projection from the substantia nigra pars compacta terminates in the NAcc core (Lanca et al., 1998). Together with the present results, these findings are consistent with the idea that in the anterior NAcc shell CCK released from the terminals of prefrontal cortex neurons acts presynaptically at CCK_B receptors on the terminals of dopaminergic fibers that originate in the VTA and enhances dopamine release.

Our results suggest that the decreased expression of morphine CPP following L-365, 260 administration may be due to a change in the reinforcing or reward predictive properties of the drug associated context and that this change involves a mechanism within the anterior nucleus accumbens, which receives CCK projections from brain regions relevant to craving and relapse. These results are in keeping with previous research suggesting a rostrocaudal gradient of motivated behaviors within the NAcc, in which the anterior accumbens encodes the positive motivational function of reward and the posterior NAcc encodes negative motivational valence (Reynolds and Berridge, 2001). Of particular interest is the long-lasting nature of the effects reported here following the administration of L-365, 260. One possibility is that by attenuating the amount of anterior NAcc dopamine released in the drug associated context, there is a long-lasting or permanent change in the salience of drug-associated stimuli similar to that noted during extinction.

A difference in the effects of CCK compounds in the anterior versus posterior NAcc has been noted previously (Vaccarino and Vaccarino, 1989; Marshall et al., 1991; Ladurelle et al., 1993). One possible explanation for this dichotomy is a difference in CCK receptor localization between the anterior and posterior NAcc. Previous studies have established that the CCK_B receptor is localized both pre and postsynaptically (Ferraro et al., 1996), yet no information currently exists on the localization of CCK_B receptors within subregions of the NAcc. Our results could be explained by a presynaptic CCK_B receptor effect on dopaminergic neurons in the anterior NAcc and a postsynaptic CCK_B receptor effect on GABAergic neurons in the medial-posterior NAcc. Indeed, Tanganelli et al. (2001) have suggested a similar model to explain the modulatory

effects of CCK on pre and postsynaptic D_2 receptors in the NAcc. However, it is impossible to determine the precise mechanism for CCK-dopamine receptor interactions without conducting additional research into the exact location of CCK_B receptors in the NAcc.

In contrast with the present results, several groups have noted effects on locomotion, exploration, and learning of conditioned reward following the infusion of either CCK-8 or CCK_A selective antagonists into the posterior NAcc (Crawley, 1992; Derrien et al., 1993; Ladurelle et al., 1993; Josselyn et al., 1996a). Additionally, CCK antagonizes the synaptic effects of dopamine in posterior NAcc *in vivo* (Yim and Mogenson, 1991). These results are also difficult to reconcile with the cytochemical evidence that CCK_A receptors are absent in the posterior NAcc (Honda et al., 1993; Zajac et al., 1996; Lodge and Lawrence, 2001). One possible explanation is that microinjected CCK is able to diffuse into the anterior accumbens where it then acts on CCK_B receptors. Other explanations include the possibility that there are additional novel CCK receptors, or that autoradiography is not the most effective method for visualizing the distribution of CCK_A receptors within the NAcc and that immunohistochemical data might be more sensitive (Mercer and Beart, 2004).

Microinjection of CCK-8 into the accumbens does not affect feeding behavior in rats (Blevins et al., 2000). Additionally injection of a CCK_B antagonist into the NAcc has no effect on bar pressing in response to stimuli associated with food reward (Josselyn et al., 1996b). Taken together with the present data, these results suggest that the effects of L-365, 260 are specific to drug seeking, and may not generalize to natural rewards.

CCK agonists affect learning and memory. Several studies have shown that acute administration of CCK_B agonists can improve memory retention and recall (Gerhardt et al., 1994; Sebret et al., 1999) while other studies have found that CCK_B agonists actually attenuate memory or that memory effects are highly agent or time dependent (Derrien et al., 1994; Shlik et al., 1998; Lena et al., 1999). L-365, 260 has no effect on short-term memory in humans (Grasing et al., 1996) but enhances olfactory memory in rats (Lemaire et al., 1994). Importantly, there is no evidence to suggest that L-365, 260 could be impairing memory recall in the CPP test or that the attenuation in expression of morphine CPP reported here is the result of an amnesic effect of the compound. However, given the relationship between CCK_B and D_2 receptors in the anterior NAcc in spatial recognition (Lena et al., 2001) and the well-established attenuation of fear and anxiety induced by CCK_B antagonism (Adamec et al., 1997; Tsutsumi et al., 1999) it is possible that L-365, 260 works by attenuating the CCK-mediated stress induced by drug associated cues such that they become less salient in the CPP task, which relies, at least in part, on spatial memory.

L-365, 260 cannot be differentiated from saline in animal discrimination studies (Jackson et al., 1994) and has been safely administered to humans with no significant side effects (Murphy et al., 1993; Sramek et al., 1994; Bradwejn et al., 1994; Kramer et al., 1995; Lines et al., 1995; van Megen et al., 1996; Bertoni et al., 2002; McCleane, 2003), thus making it a good compound for human drug abuse treatment. The present results indicate that further research to determine if CCK_B antagonists are viable treatments for drug craving in humans would be valuable.

In conclusion, we have found that the CCK_B antagonist L-365, 260 significantly attenuates the expression of morphine conditioned place preference, but not food place preference, through a mechanism involving D_2 receptors in the anterior nucleus accumbens and that this attenuation can be long-lasting. This result indicates that an active modulatory effect of endogenous CCK contributes to the incentive motivational effect of morphine-associated cues. Consequently, CCK_B receptor antagonists may be useful therapeutics in the treatment of drug abuse and cue induced craving.

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