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Naloxone, not proglumide or MK-801, alters effects of morphine preexposure on morphine-induced taste aversions

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Abstract

Both cholecystokinin (CCK) antagonists and *N*-methyl-D-aspartate (NMDA) antagonists block or reduce the development of morphine tolerance in several analgesic assays. The present experiments were performed to assess the ability of the CCK antagonist proglumide and the NMDA antagonist MK-801 to affect tolerance to the aversive properties of morphine as indexed by conditioned taste aversion (CTA) learning. Specifically, male Sprague–Dawley rats were exposed to either vehicle or morphine (5 mg/kg) in combination with either proglumide (5 mg/kg; Experiment 1), MK-801 (0.1 mg/kg; Experiment 2) or naloxone (1, 3.2 mg/kg; Experiment 3). Saccharin was then presented and was followed by an injection of either vehicle or morphine (10 mg/kg). Animals preexposed to and conditioned with morphine acquired an attenuated morphine-induced aversion to saccharin. While neither proglumide nor MK-801 had an effect on this attenuation, naloxone blocked the effects of morphine preexposure, suggesting that neither CCK nor NMDA may be involved in the aversive effects of morphine (or their modulation by drug exposure). That the attenuating effects of morphine preexposure on a morphine-induced CTA can be blocked suggests that the weakening of the aversive effects of morphine with chronic use can be prevented, an effect that may have implications for overall drug acceptability.

Keywords: Taste aversion; UCS preexposure; Tolerance; Morphine; Proglumide; MK-801; Naloxone; NMDA; CCK

1. Introduction

Although animals readily acquire an aversion to tastes paired with morphine (see Hutchinson et al., 2000; Mucha and Herz, 1985; Riley et al., 1978; Switzman et al., 1981), these aversions are significantly attenuated if animals have a history of morphine prior to conditioning (Dacanay and Riley, 1982; Domjan and Siegel, 1983; Riley et al., 1984; Simpson and Riley, 2005; Stewart and Eikelboom, 1978), a suggestive effect of tolerance to its aversive effects (see Cappell and LeBlanc, 1977; Riley et al., 1976; though see de Brugada et al., 2004, 2005). Although the effects of drug preexposure are well documented in taste aversion learning in general, and with morphine more specifically (for a review, see Randich and LoLordo, 1979; Riley and Simpson, 2001), it is not known to what extent (if any) these effects can be modulated either behaviorally or pharmacologically. An understanding of what factors can modulate the attenuating effects of drug history on taste aversion learning may be important given that a drug's abuse potential is a function of the balance of its aversive and rewarding effects. Any manipulation that impacts either of these affective properties is likely to affect the vulnerability to the use and abuse of that compound.

Interestingly, tolerance to the analgesic effects of morphine (Dourish et al., 1990; Zhou et al., 1992) has been reported to be blocked by a variety of compounds. For example, both cholecystokinin (CCK) antagonists, such as proglumide, and the non-competitive *N*-methyl-D-aspartate (NMDA) antagonist MK-801 have been shown to block or reduce the development of morphine tolerance in several assessments of analgesia,

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including the hot-plate and tail-flick tests (Bhargava and Matwyshyn, 1993; Dourish et al., 1988, 1990; Hoffmann and Wiesenfeld-Hallin, 1994; Kellstein and Mayer, 1991; Panerai et al., 1987; Singh et al., 1996; Tang et al., 1984; Tortorici et al., 2003; Trujillo and Akil, 1991, 1994; Watkins et al., 1984; Xu et al., 1992). Given the effects of CCK and NMDA antagonists on the development of tolerance to morphine's analgesic effects, it is possible that proglumide and MK-801 could modulate the development of tolerance to the aversive effects of morphine. To explore this possibility, the current studies were performed to investigate the effects of the non-selective CCK antagonist proglumide (Experiment 1), the non-competitive NMDA antagonist MK-801 (Experiment 2) and the non-selective opioid antagonist naloxone (Experiment 3) on the morphine preexposure effect in taste aversion learning. Specifically, animals were exposed to morphine in combination with either proglumide, MK-801 or naloxone prior to taste aversion conditioning during which a novel saccharin solution was paired with morphine on four separate conditioning trials. Saccharin consumption was assessed on each of these days, as well as on a final aversion test.

2. General method

2.1. Subjects

Subjects were 230 experimentally-naïve male Sprague–Dawley rats, approximately 60–100 days in age and approximately 275–500 g in weight at the beginning of the experiments. Animals were housed individually in stainless-steel, wire-mesh cages and maintained on a 12-h light/12-h dark cycle (lights on at 0800 h) and at an ambient temperature of 23 °C for the duration of the experiments. Standard rat chow and water (except where noted) were available ad libitum. Animals were housed for approximately 2 weeks prior to the commencement of each experiment for habituation to their new environment. Procedures recommended by the Institutional Animal Care and Use Committee at American University were followed at all times.

2.2. Drugs and solutions

Morphine sulfate, naloxone hydrochloride (both generously provided by the National Institute on Drug Abuse) and MK-801 were dissolved in distilled water. Proglumide (obtained from Panos Therapeutics) was suspended in a solution of 1.2% DMSO in 7.0 pH buffer solution. All drugs were administered by intraperitoneal (i.p.) injection and prepared in the following concentrations: morphine (5 mg/ml), MK-801 (10 mg/ml), naloxone (1 mg/ml), proglumide (5 mg/ml). Saccharin (0.1% sodium saccharin, Sigma Chemical Company) was prepared as a 1 g/l solution in tap water.

2.3. Procedure

Phase 1: Water habituation. Following 23-h water deprivation, animals were given 20-min access to water. This procedure was repeated each day until all subjects drank within 2 s of water presentation.

Phase 2: Preexposure. At the outset of this phase, animals were assigned to a drug preexposure group based on the average amount of water consumed over the last 3 days of water habituation. On Day 1 of this phase, animals received their regular 20-min access to water. Five hours later, all subjects were injected with one of the aforementioned compounds (proglumide, MK-801, naloxone or vehicle), and subjects then received injections of either morphine or vehicle. These drug combinations were administered every other day for a total of five preexposure days. Animals received 20-min access to water on the recovery day between each preexposure session, as well as on the day following the last drug preexposure. No injections were administered following water access on these days.

Phase 3: Conditioning. On the second day after the last drug preexposure session in Phase 2, all animals received 20-min access to a novel saccharin solution. Immediately following access to saccharin, each preexposure group was divided in half such that saccharin consumption was comparable between groups. Each animal then received an i.p. injection of either morphine (10 mg/kg) or the distilled water vehicle. On the following three water-recovery days, all animals received 20-min access to water. No injections followed water access on those days. This alternating procedure of conditioning followed by three water-recovery days was repeated for four complete cycles.

Phase 4: Final aversion test. On the day after the final water-recovery session of Phase 3, animals received 20-min access to saccharin in a final test of the aversion to saccharin. No injections followed saccharin access on this test day.

2.4. Group names

Group names are such that the first set of letters refers to the first (proglumide (P), MK-801 (Mk), naloxone (n, 1 mg/kg; N, 3.2 mg/kg), vehicle (V)) and second (morphine (M), vehicle (V)) drugs administered during preexposure. One group of animals received an injection of proglumide followed immediately by morphine (PiM). The last letter refers to animals administered morphine (M) or vehicle (V) during conditioning.

2.5. Statistical analysis

For each experiment, repeated measures analyses of variance (ANOVAs) with one between-group factor (Group) and one within-group factor (Preexposure Day) were performed on mean water consumption during preexposure. A Group × Trial repeated measures ANOVA was performed to assess differences in saccharin consumption among the control groups (i.e., all animals receiving vehicle during conditioning) within each experiment. There were no significant differences among any of these groups. Consequently, the control groups within each experiment were combined for all further analyses. For each experiment, a repeated measures ANOVA with one between-group factor (Group) and one within-group factor (Trial) was performed to compare saccharin consumption during conditioning. Post-hoc analyses were conducted using

Tukey HSD pairwise comparisons. Significance was based on $p<0.05$.

3. Experiment 1: proglumide

3.1. Procedure

During the drug preexposure phase, animals received i.p. injections of either proglumide (5 mg/kg) or its vehicle (see above) followed 15 min later by an i.p. injection of either morphine (5 mg/kg) or its vehicle. One group of animals received an injection of proglumide followed immediately by morphine (PiM) in order to consider a time–response effect between administration of proglumide and morphine. As noted above, during the conditioning phase, animals received an i.p. injection of either morphine (10 mg/kg) or its vehicle.

3.2. Results

3.2.1. Preexposure

A 5×5 (Group×Preexposure Day) repeated-measures ANOVA revealed a significant effect of Group ($F(4,67)=3.881$, $p=0.007$) and Preexposure Day ($F(4,268)=9.075$, $p<0.001$). Subjects in each preexposure group consumed approximately 15 ml of water on the first drug preexposure day and continued to have high intake on subsequent preexposure days. There was no significant Group×Preexposure Day interaction ($F(16,268)=1.283$, NS).

3.2.2. Conditioning

Fig. 1 illustrates the mean (±S.E.M.) consumption of saccharin over repeated conditioning trials (Panel A) and on the Test Day (Panels A and B). A 6×5 (Group×Trial) repeated measures ANOVA revealed significant main effects for Group ($F(5,77)=39.974$, $p<0.0001$) and Trial ($F(4,308)=13.207$, $p<0.0001$) as well as a significant Group×Trial interaction ($F(20,308)=18.533$, $p<0.0001$).

Post-hoc Tukey HSD analyses revealed that there were no significant differences among the groups on the first trial (all p's>0.05), with all groups drinking approximately 11.8 ml. Significant differences among groups emerged over the remaining conditioning trials. Specifically, Groups VV-M and PV-M consumed significantly less saccharin than Controls on Trials 2, 3 and 4 as well as on the Test Day (all p's<0.05), indicating a significant morphine-induced taste aversion. Consumption for Group VM-M was also significantly less than that of Controls on Trials 2, 3, 4 and on the Test Day (all p's<0.05), but it was significantly greater than that of nonpreexposed animals in Group VV-M on Trials 2, 3 and 4 as well as on the Test Day (all p's<0.05), indicating that preexposure to morphine significantly attenuated the aversion to morphine.

Groups PiM-M and PM-M also consumed significantly less saccharin than Controls on Trials 2 (Group PiM-M only), 3 and 4 as well as on the Test Day (all p's<0.05), but consumed significantly more saccharin than Group PV-M on Trials 2 (Group PM-M only), 3 and 4 as well as on the Test Day (all p's<0.05). These findings indicate that preexposure to morphine attenuated the aversion to morphine, even in subjects receiving proglumide either immediately or 15 min prior to morphine drug preexposure.

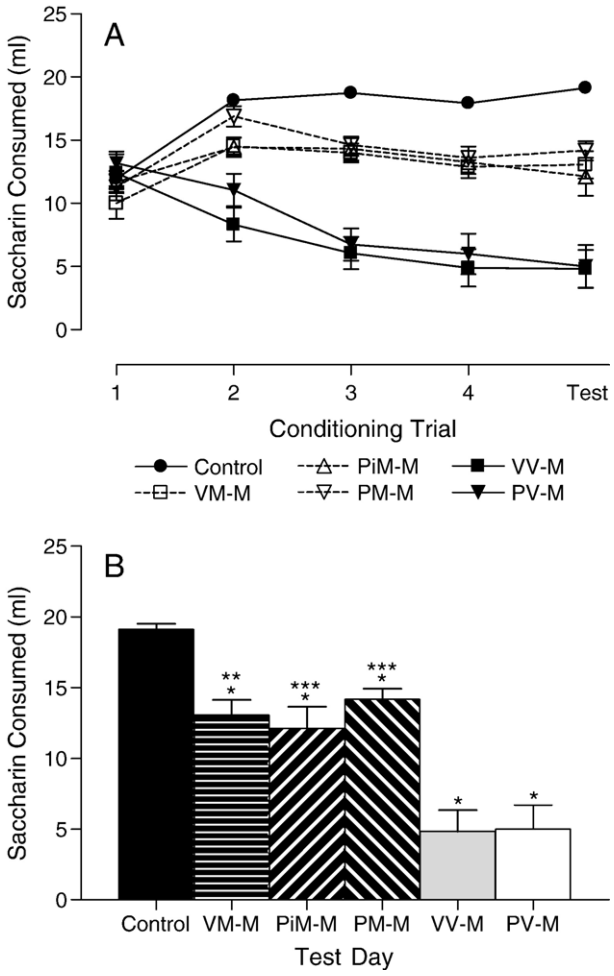

Fig. 1. The mean (±S.E.M.) consumption of saccharin over repeated conditioning trials (Panel A) and on the Test Day (Panels A and B). Each group is designated by three letters. The first two letters refer to the injections given during preexposure, i.e., proglumide (P; 5 mg/kg i.p.) or vehicle (V), followed by either morphine (M; 5 mg/kg i.p.) or vehicle (V). PiM refers to the group that was injected with proglumide immediately followed by morphine. The last letter refers to the injection given during conditioning, i.e., morphine (M; 10 mg/kg i.p.). As there were no differences between any of the groups administered vehicle (V) during conditioning, the control groups were combined (Control) for all analyses. Panel B: * Significant difference from Control Group, ** significant difference from Group VV-M, *** significant difference from Group PV-M, $p<0.05$.

There were no significant differences in saccharin consumption between Groups PV-M and VV-M, subjects not preexposed to morphine but conditioned with morphine, on any trial (all p's>0.05), indicating that preexposure to proglumide alone did not affect the development of a morphine-induced taste aversion. Further, there were no differences in saccharin consumption on any trial among Groups VM-M, PM-M and PiM-M, subjects preexposed to morphine or a combination of proglumide and morphine and conditioned with morphine (all p's>0.05), again indicating that proglumide did not alter the effect of morphine preexposure on a morphine-induced CTA.

4. Experiment 2: MK-801

4.1. Procedure

During preexposure, animals received i.p. injections of either MK-801 (0.1 mg/kg) or its vehicle followed 30 min later by an i.p. injection of either morphine (5 mg/kg) or its vehicle. During the conditioning phase, animals received an i.p. injection of either morphine (10 mg/kg) or its vehicle.

4.2. Results

4.2.1. Preexposure

A 4×5 (Group×Preexposure Day) repeated-measures ANOVA revealed a significant effect of Preexposure Day ($F(4,236)=3.537$, $p=0.008$). Animals consumed approximately 15 ml on the first drug preexposure day and continued to have high intake throughout preexposure. There was no significant effect of Group ($F(3,59)=1.029$, NS), nor was there a significant Group×Preexposure Day interaction ($F(12,236)=1.053$, NS).

4.2.2. Conditioning

Fig. 2 illustrates the mean (±S.E.M.) consumption of saccharin over repeated conditioning trials (Panel A) and on the Test Day (Panels A and B). A 5×5 (Group×Trial) repeated measures ANOVA revealed significant main effects for Group ($F(4,59)=45.849$, $p<0.0001$) and Trial ($F(4,236)=4.231$, $p=0.003$) and a significant Group×Trial interaction ($F(16,236)=28.084$, $p<0.0001$).

There were no significant differences among groups on the first conditioning trial (all p's>0.05), as indicated by post hoc Tukey HSD analyses, with all groups drinking approximately 12 ml. Significant differences emerged among groups over conditioning. Groups VV-M and MkV-M consumed significantly less saccharin than Controls on Trials 2, 3 and 4 as well as on the Test Day (all p's<0.05), indicating a significant morphine-induced taste aversion. Consumption for Group VM-M was also significantly less than that of Controls on Trials 2, 3, 4 and on the Test Day (all p's<0.05), but it was significantly greater than that of nonpreexposed animals in Group VV-M on Trials 2, 3 and 4 as well as on the Test Day (all p's<0.05), indicating that preexposure to morphine significantly attenuated the aversion to morphine.

Group MkM-M also consumed significantly less saccharin than that of Controls on Trials 2, 3 and 4 as well as on the Test Day (all p's<0.05), but consumption for Group MkM-M was significantly greater than that of nonpreexposed animals in Group MkV-M on Trials 2, 3 and 4 as well as on the Test Day (all p's<0.05), indicating that preexposure to morphine attenuated the aversion to morphine, even in subjects receiving MK-801 along with morphine during drug preexposure.

Groups MkV-M and VV-M, subjects not preexposed to morphine but conditioned with morphine, showed no significant differences in saccharin consumption over conditioning (all p's>0.05), indicating that preexposure to MK-801 alone did not affect the morphine-induced taste aversion. Further, there were no differences in saccharin consumption on any trial between Groups VM-M and MkM-M, subjects preexposed to morphine or a combination of MK-801 and morphine and conditioned with morphine (all p's>0.05), indicating again that MK-801 did not alter the effects of morphine preexposure on a morphine-induced CTA.

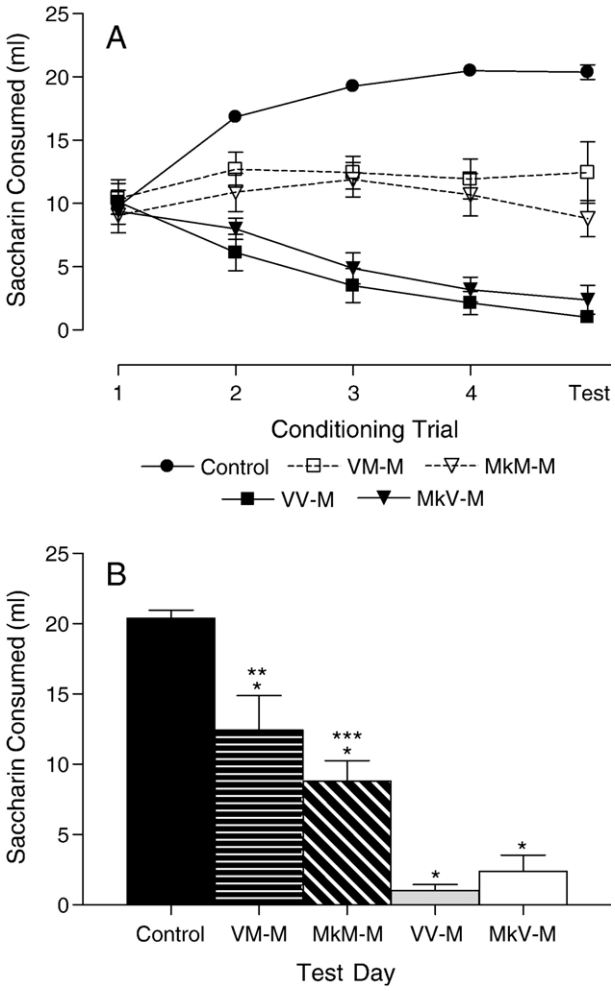

Fig. 2. The mean (±S.E.M.) consumption of saccharin over repeated conditioning trials (Panel A) and on the Test Day (Panels A and B). Each group is designated by three letters. The first two letters refer to the injections given during preexposure, i.e., MK-801 (Mk; 0.1 mg/kg i.p.) or vehicle (V), followed by either morphine (M; 5 mg/kg i.p.) or vehicle (V). The last letter refers to the injection given during conditioning, i.e., morphine (M; 10 mg/kg i.p.). As there were no differences between any of the groups administered vehicle (V) during conditioning, the control groups were combined (Control) for all analyses. Panel B: * Significant difference from Control Group, ** significant difference from Group VV-M, *** significant difference from Group MkV-M, $p<0.05$.

5. Experiment 3: naloxone

5.1. Procedure

During preexposure, animals received i.p. injections of either naloxone (1 or 3.2 mg/kg) or its vehicle followed immediately

by an i.p. injection of either morphine (5 mg/kg) or its vehicle. Over conditioning trials, animals received an i.p. injection of either morphine (10 mg/kg) or its vehicle.

5.2. Results

5.2.1. Preexposure

A 5×5 (Group×Preexposure Day) repeated-measures ANOVA revealed a significant effect of Preexposure Day ($F(4,304)=9.098$, $p<0.001$). Animals consumed approximately 16 ml on the first drug preexposure day and continued to have high intake on subsequent preexposure days. There was no significant effect of Group ($F(4,76)=1.215$, NS) nor was there a significant Group×Preexposure Day interaction ($F(16,304)=0.907$, NS).

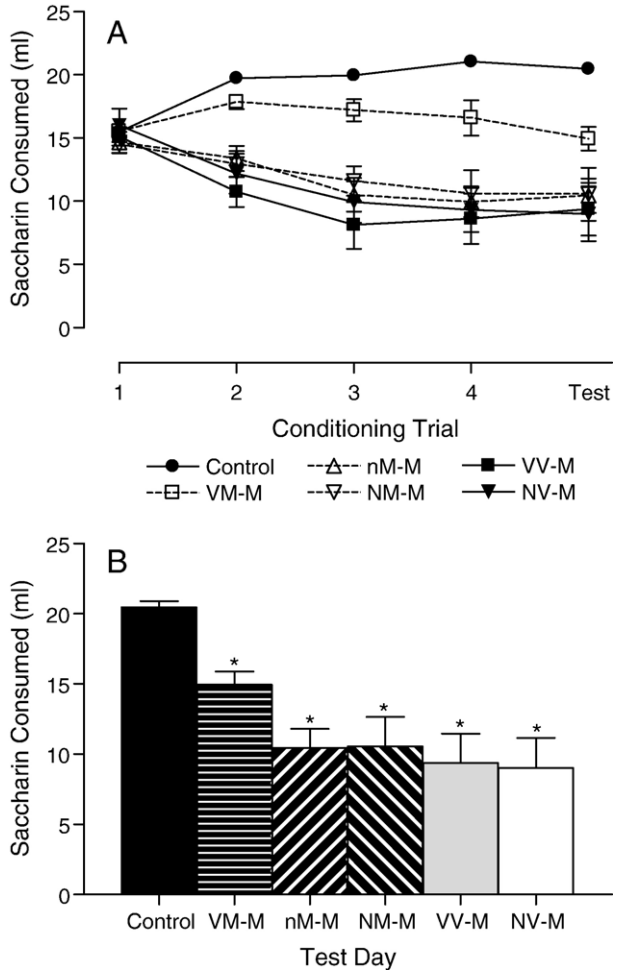

Fig. 3. The mean (±S.E.M.) consumption of saccharin over repeated conditioning trials (Panel A) and on the Test Day (Panels A and B). Each group is designated by three letters. The first two letters refer to the injections given during preexposure, i.e., naloxone (n, 1 mg/kg i.p.; N; 3.2 mg/kg i.p.) or vehicle (V), followed by either morphine (M; 5 mg/kg i.p.) or vehicle (V). The last letter refers to the injection given during conditioning, i.e., morphine (M; 10 mg/kg i.p.). As there were no differences between any of the groups administered vehicle (V) during conditioning, the control groups were combined (Control) for all analyses. Panel B: * Significant difference from Control Group, $p<0.05$.

5.2.2. Conditioning

Fig. 3 illustrates the mean (±S.E.M.) consumption of saccharin over repeated conditioning trials (Panel A) and on the Test Day (Panels A and B). A 6×5 (Group×Trial) repeated measures ANOVA revealed significant main effects for Group ($F(5,77)=25.611$, $p<0.0001$) and Trial ($F(4,308)=16.831$, $p<0.0001$) and a significant Group×Trial interaction ($F(20,308)=14.293$, $p<0.0001$).

Post-hoc Tukey HSD analyses revealed that there were no significant differences among groups on the first trial (all p's>0.05), with all groups drinking approximately 16.3 ml. Significant differences emerged among groups over conditioning. Specifically, Groups VV-M and NV-M consumed significantly less saccharin than Controls on Trials 2, 3 and 4 as well as on the Test Day (all p's<0.05), indicating the development of a morphine-induced taste aversion. Consumption for Group VM-M was also significantly less than that of Controls on Trial 4 and on the Test Day (all p's<0.05), but it was significantly greater than that of nonpreexposed animals in Group VV-M on Trials 3 and 4 (all p's<0.05), indicating that preexposure to morphine significantly attenuated the aversion to morphine.

Groups nM-M and NM-M did not differ from Group NV-M on any trial nor on the Test Day, illustrating that while preexposure to morphine attenuated the aversion to morphine, naloxone, at both 1 and 3.2 mg/kg, blocked this attenuation.

There were no significant differences in saccharin consumption between Groups NV-M and VV-M, subjects not preexposed to morphine but conditioned with morphine, on any trial (all p's>0.05), indicating that naloxone administered alone during preexposure did not affect the development of a morphine-induced aversion. However, there were significant differences in saccharin consumption between Groups nM-M and NM-M and Group VM-M on Trials 2, 3 and 4 (p's<0.05), again indicating that naloxone blocked the effect of morphine preexposure on the morphine-induced CTA.

6. Discussion

Given that CCK antagonists, such as proglumide, and the non-competitive NMDA antagonist MK-801 have been shown to block and/or reverse the development of tolerance to morphine's analgesic effects, the current research examined the effects of these compounds on changes in the aversive effects of morphine following repeated morphine exposure (Riley et al., 1976). As described, although animals preexposed to and conditioned with morphine displayed an attenuated morphine aversion relative to nonpreexposed subjects, neither proglumide nor MK-801 had an effect on this attenuation, indicating that proglumide and MK-801 at these doses did not block the development of tolerance to the aversive effects of morphine. The inability of these compounds to block the effects of morphine preexposure is not necessarily a function of the design in which these assessments were made. As described, the opiate antagonist naloxone (1 and 3.2 mg/kg) did block the effects of morphine preexposure. That is, animals given naloxone concurrent with morphine during preexposure acquired aversions comparable to those of nonpreexposed

subjects. These doses of naloxone are in the range of effective doses within CTA learning, conditioned place preference, and the conditioned taste aversion baseline of drug discrimination learning (both as a discriminative stimulus and as an agent to block the stimulus effects of morphine) (Mucha and Walker, 1987; Sherman et al., 1980; Smurthwaite et al., 1992; Stevenson et al., 1992, 2000; Van der Kooy and Phillips, 1977).

The fact that neither proglumide nor MK-801 was able to block the development of tolerance to the aversive effects of morphine is interesting in the context that both of these compounds have been reported to block the development of tolerance to the analgesic effects of morphine. It may be the case that while both CCK and NMDA are involved in the analgesic effects of morphine, they play no role in the aversive properties of morphine and/or the chronic effects of morphine within this response system. Studies have been performed to investigate the effects of both MK-801 and CCK antagonists on the rewarding properties of morphine. For example, MK-801 has been shown to enhance morphine self-administration and intracranial self-stimulation (Carlezon and Wise, 1993; Glick and Maisonneuve, 1998; although see Semenova et al., 1999). Further, MK-801 blocks morphine-induced place preferences (Del Pozo et al., 1996; Kim et al., 1996; Suzuki et al., 2000; Tzschentke and Schmidt, 1995; Yonghui et al., 2006; but see Ribeiro Do Couto et al., 2004). In relation to CCK, the CCK_B receptor antagonist L-365,260, but not the CCK_A antagonist devazepide (MK-329), blocked the development of a morphine-induced place preference when administered before morphine during conditioning (Lu et al., 2000). However, Higgins et al. (1991, 1992a) reported opposite findings, in that devazepide, but not L-365,260, attenuated a morphine-induced place preference.

The ability of both MK-801 and CCK antagonists to affect morphine dependence and withdrawal has also been assessed. For example, MK-801 has been shown to block the development of morphine dependence (as measured by the ability of naloxone to condition a place aversion via precipitated withdrawal) (Zhu and Barr, 2001) or the expression of naloxone-precipitated withdrawal in morphine-dependent animals (assessed by naloxone-induced place aversion or withdrawal) (Higgins et al., 1992b; Kawasaki et al., 2005; Maldonado et al., 2003; Trujillo and Akil, 1991; Watanabe et al., 2002). Regarding CCK antagonists, Valverde and Roques (1998) reported that chronic administration of the CCK_B antagonists L-365,260 and PD-134,308 decreased and blocked, respectively, the development of dependence in opiate-exposed subjects, assessed by naloxone-induced place aversion. The CCK_B agonist BC 264 and the CCK_A selective antagonist devazepide had no effect on morphine dependence. Lu et al. (2000) reported that pretreatment with the CCK_B-selective antagonist L-365,260 (but not the CCK_A-selective antagonist devazepide) suppressed the signs of naloxone-induced withdrawal in morphine-dependent rats, again suggesting a role of CCK acting through the CCK_B receptor subtype in these effects. Although MK-801 and the CCK_B antagonists were effective in these preparations, these studies used naloxone-precipitated withdrawal to assess morphine tolerance and dependence. It is not clear to what extent the motivational properties of morphine (either positive or negative) were assessed in such designs. Consequently, it is not clear if the present results can be compared to these earlier assessments in terms of the role of NMDA or CCK in the aversive effects of morphine.

Although the present results do not support the position that either CCK or NMDA mediate (or modulate) tolerance to the aversive effects of morphine, there are several caveats to this conclusion. First, it can only be concluded that these compounds are without effects under the specific parameters tested. It is certainly possible that had different doses of proglumide and MK-801 or different CCK or NMDA antagonists been used, direct evidence of the involvement of these systems in the effects of morphine preexposure may have been observed. It is important to note, however, that the doses of proglumide and MK-801 used in the present studies, as well as lower doses, are effective in other preparations in which morphine has been examined, i.e., these compounds are effective in altering the effects of morphine (for example, see Ben-Horin et al., 1984; Bhargava and Matwyshyn, 1993; Del Pozo et al., 1996; Glick and Maisonneuve, 1998; Higgins et al., 1992b; Kim et al., 1996; Suzuki et al., 2000; Trujillo and Akil, 1994; Tzschentke and Schmidt, 1995; Watkins et al., 1984, 1985; Yonghui et al., 2006). Specifically, in other reports, proglumide at doses lower than that used in Experiment 1 do modulate morphine's effects, including morphine-induced analgesia and hypokinesia (Ben-Horin et al., 1984; Watkins et al., 1985). There is only one study assessing the ability of proglumide to block morphine's chronic effects, where 15 mg/kg proglumide partially blocked the development of tolerance to morphine's analgesic effects (Tang et al., 1984). Lower doses of proglumide are also effective in blocking CCK's effects, as 2 mg/kg subcutaneous blocks the effect of CCK-induced suppression of locomotion scores (Katsuura et al., 1984). Further, 5 mg/kg proglumide, the dose used in Experiment 1, blocks CCK-induced reduction of passive avoidance latency (Deupree and Hsiao, 1987).

In order to confirm that this dose of proglumide would be effective under the conditions in Experiment 1 (e.g., strain of rats, gender, route of administration) utilizing another behavioral assessment, an additional study was performed to investigate the ability of 5 mg/kg proglumide to block or attenuate CCK-induced suppression of feeding. Specifically, following 23 h of food deprivation, animals were injected with either saline, 5 mg/kg proglumide, 3 μg/kg CCK or a combination of proglumide followed by CCK. Animals were then allowed access to food for 60 min, and the amount of food consumed at the end of the 60-min period was measured. Following saline injection, mean consumption was approximately 8.1 g. Administration of 3 μg/kg CCK significantly decreased feeding relative to this saline baseline, with animals consuming approximately 3.7 g. Proglumide alone did not significantly alter feeding compared to baseline, with subjects eating approximately 7.2 g. However, proglumide administered before 3 μg/kg CCK did block its suppression of feeding. Specifically, following the combination of proglumide and CCK, consumption was approximately 6.2 g, significantly more

food than that consumed by animals administered CCK alone (data not shown). Thus, although ineffective in Experiment 1, 5 mg/kg proglumide has been shown to be effective in blocking CCK's effects within other behavioral paradigms and is certainly within the range of effective doses.

Further, the dose of 0.1 mg/kg MK-801 used in Experiment 2 is effective in numerous preparations, including reversing (Del Pozo et al., 1996; Yonghui et al., 2006) and blocking (Kim et al., 1996; Suzuki et al., 2000; Tzschentke and Schmidt, 1995; Yonghui et al., 2006) the development of a morphine place preference, in decreasing morphine self-administration (Glick and Maisonneuve, 1998) and in reducing heroin reinforcement (Xi and Stein, 2002). This same dose of MK-801 is also effective in blocking the development of morphine dependence (Zhu and Barr, 2001) and the expression of naloxone-induced withdrawal in morphine-dependent animals (Higgins et al., 1992b; Trujillo and Akil, 1991). Further, 0.1 mg/kg MK-801 is effective in blocking the development of tolerance to the analgesic effects of morphine (Bhargava and Matwyshyn, 1993; Trujillo and Akil, 1991, 1994). As such, had MK-801, and thus NMDA, been involved in the development of tolerance to the aversive effects of morphine, the dose used in the current study would likely have been effective.

A second caveat concerns the issue of tolerance itself. The present study was set up under the premise that exposure to morphine prior to conditioned taste aversion training resulted in tolerance to morphine which attenuated its ability to condition a taste aversion. Although this has been presented as a basis for the effects of morphine preexposure (see Cappell and LeBlanc, 1977; Hunt et al., 1985; Riley et al., 1976; Riley and Simpson, 2001), there are other explanations for the preexposure effect in taste aversion learning that do not assume pharmacological tolerance. Under one such interpretation, it is argued that environmental stimuli present at the time of the drug administration become associated with the drug and its effects and then block the ability of saccharin to be associated with the drug during subsequent taste aversion conditioning (see Randich and LoLordo, 1979; Riley and Simpson, 2001). Such blocking is well established in traditional classical conditioning and has been shown to mediate the effects of drug preexposure in taste aversion conditioning with specific drugs, e.g., LiCl (see Batson and Best, 1979; Dacanay and Riley, 1982; de Brugada et al., 2004, 2005). Although clearly a possibility, it should be noted that evidence for blocking with morphine has not been reported when such tests of blocking have been made, i.e., when taste aversion conditioning is attempted in the presence or absence of stimuli associated with morphine during preexposure, the same degree of attenuation is reported (see Dacanay and Riley, 1982; Domjan and Siegel, 1983; Riley et al., 1984; Stewart and Eikelboom, 1978). Further, tolerance to repeated exposure to morphine within the taste aversion preparation is also observed (Farber et al., 1976; Jacquet, 1973; Siegel et al., 1995). For example, Siegel et al. (1995) noted a waning of morphine's ability to condition taste aversions with repeated trials, an effect possibly due to the adaptation or tolerance to morphine's aversive effects. It remains a possibility, however, that the effects of preexposure to morphine may be mediated by a different process than tolerance which itself is not affected by antagonism of CCK or NMDA.

A final caveat of the present results concerns the assumption that the preexposure effect in taste aversion learning with morphine reflects changes in morphine's aversive effects, i.e., the aversive effects of morphine are weakened with drug preexposure. This assumption rests on the traditional position that conditioned taste aversion learning is an index of the aversive properties of the conditioning drug. Although the avoidance of tastes associated with drugs are often described in terms of aversions (see Garcia and Ervin, 1968), more recently it has been argued that the avoidance may be mediated by other processes, e.g., novelty, reward. One position that has received considerable attention is that of Grigson and her colleagues (see Grigson, 1997) who have argued that the avoidance of a taste paired with one of a number of drugs of abuse, including morphine, is a function of the rewarding properties of morphine (and not its aversive effects). In this scenario, animals given a novel taste prior to an injection of morphine come to anticipate morphine when subsequently given saccharin. Given that the relative rewarding value of saccharin pales in comparison to morphine, animals display anticipatory contrast and avoid the saccharin solution. Although there are some concerns with this position and its account of taste avoidance in animals that do not readily self-administer recreational drugs (see Lancellotti et al., 2001; Pescatore et al., 2005), it nonetheless argues that what has traditionally been defined as a taste aversion may not necessarily reflect any aversive effect of the drug.

As noted above, the affective properties of morphine (and other abused drugs) include its aversive and rewarding effects, the balance of which may influence patterns of drug taking such that increased acceptability may lead to increased drug usage. Interestingly, with chronic opioid administration, it has been reported that the rewarding effects of the drug increase (i.e., sensitization; see Lett, 1989; Shippenberg et al., 1996; Simpson and Riley, 2005) and its aversive effects decrease (i.e., tolerance; see Cannon et al., 1975; Hunt et al., 1985; Riley et al., 1976; Stewart and Eikelboom, 1978; Simpson and Riley, 2005). Consequently, abuse potential increases with repeated drug experience (Gaiardi et al., 1991). Any pharmacological manipulation that is able to decrease the overall acceptability of morphine would be beneficial as a treatment for opioid abuse. More specifically, if MK-801 or proglumide had blocked the development of tolerance to morphine's aversive effects, then these compounds may have been useful pharmacotherapeutics for chronic pain victims who can develop dependence to opioids during medical treatment (Bannwarth, 1999; Kouyanou et al., 1997; Longo et al., 2000). The present results suggest that although both CCK antagonists and the NMDA antagonist MK-801 are able to block and/or reverse the development of tolerance to the analgesic effects of morphine, they do not appear to play a similar role in the development of tolerance to the aversive effects of morphine, as neither of these compounds was able to block the attenuation of a morphine-induced CTA when administered with repeated preexposures to morphine.

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