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Lobeline produces conditioned taste avoidance in rats

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Abstract

Previous results indicate that pretreatment with lobeline attenuates methamphetamine (METH) self-administration in rats, but not by acting as a substitute reinforcer. Given these findings, it has been suggested that lobeline may serve as a useful pharmacotherapy for psychostimulant abuse. However, because lobeline produces emesis and nausea in humans, the present study examined whether lobeline has direct effects on taste avoidance behavior in rats within the same dose range shown previously to decrease METH self-administration. Two experiments utilized a Pavlovian conditioning procedure to determine if lobeline produces conditioned taste avoidance (CTA) in rats. In Experiments 1 and 2, rats consumed either novel milk or salt solutions, respectively, and within 10 min, were injected with lobeline (0.3–3.0 mg/kg) or METH (0.3–3.0 mg/kg). A single-bottle test conducted 48 h after flavor–drug pairings indicated that the dose of lobeline that reduced METH self-administration in a previous study (i.e., 3.0 mg/kg) also produced reliable CTA for milk and salt solution. These findings suggest a need to develop lobeline analogs that reduce METH self-administration, but do not produce CTA following the consumption of a novel solution.

Keywords: Lobeline; Methamphetamine; Conditioned taste avoidance; Rat

1. Introduction

Lobeline is a lipophilic, alkaloidal constituent of lobelia inflata (Dwoskin and Crooks, 2002). Previous studies demonstrate that lobeline decreases methamphetamine (METH)-induced hyperactivity, decreases the discriminative stimulus effect of METH, and attenuates METH self-administration in rats (Miller et al., 2001, 2003; Harrod et al., 2001). The lobeline-induced decrease in responding for METH in the self-administration studies was not the result of nonspecific suppressant effects. Importantly, acute lobeline pretreatment also decreased responding for sucrose reinforcement; however, responding for sucrose returned to baseline levels following repeated lobeline pretreatment, indicating development of tolerance. In contrast, rats repeatedly pretreated with lobeline prior to METH self-administration did not become tolerant to the attenuating effect of lobeline. Furthermore, lobeline pretreatment did not potentiate self-administration of lower doses of METH, nor was the effect of lobeline surmounted by increasing METH doses, consistent with a noncompetitive mechanism (Harrod et al., 2001).

Recent results demonstrate that lobeline did not decrease METH self-administration by substituting for the reinforcing effects of METH (Harrod et al., 2003). For example, lobeline (0.015–0.15 mg/kg/infusion) was not self-administered by rats trained previously to lever press for sucrose reinforcement, nor did it maintain responding in rats trained previously to self-administer METH. Moreover, additional experiments demonstrated that lobeline (1.0 or 3.0 mg/kg) did not reinstate METH-seeking behavior. Taken together, these experiments suggest that lobeline decreases METH self-administration by decreasing reward, not by acting as a substitute reinforcer (Harrod et al., 2001, 2003). From a clinical perspective, the ability of lobeline to decrease METH self-administration, without producing reward itself, suggests that lobeline may serve as a useful pharmacotherapy lacking abuse liability (Harrod et al., 2001, 2003; Dwoskin and Crooks, 2002).

Despite these encouraging findings, previous research also indicates that lobeline produces emesis and nausea (Miller and Ruggiero, 1994; Dwoskin and Crooks, 2002). To further evaluate lobeline as a candidate pharmacotherapy,

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the present experiments determined the dose-dependent ability of lobeline to produce conditioned taste avoidance (CTA). To date, there have been no reports assessing lobeline-induced CTA in rats.

CTA is typically measured using a Pavlovian conditioning procedure in which the conditioned stimulus (CS), which is the flavor of a novel solution, becomes associated with the unconditioned stimulus (US), a novel effect of a drug. When the CS is presented a second time, the rat avoids the CS, thus demonstrating a conditioned avoidance response, i.e., CTA. Although some drug-unconditioned stimuli require multiple pairings with the CS before the CTA is demonstrated (Kumar et al., 1983; Shoaib et al., 2002; Grigson, 1997; Bevins et al., 1997), CTA can be learned in a single trial. With single-trial CTA, the influence of potential tolerance or sensitization to the effect of the drug US is obviated. The present study used a single-trial learning procedure, in which rats consumed a novel milk or sodium chloride (salt) solution and were injected immediately with lobeline (0.3–3.0 mg/kg). Two different CS types were utilized in order to assess the generality of the lobeline-induced CTA obtained. Additionally, separate groups of rats received exposure to the novel milk or salt solutions, but were injected with METH (0.3–3.0 mg/kg) instead of lobeline, as a positive control employing a US previously shown to produce CTA in rats (Parker, 1995). Thus, the current study provides a more comprehensive evaluation of the pharmacological effects of lobeline.

2. Methods

2.1. Subjects

Adult male, Sprague–Dawley rats (200–225 g body weight, $N = 124$) were obtained from Harlan (Indianapolis, IN) and were housed individually in standard polyurethane cages. The colony room was maintained at 24 °C and 45% humidity, with lights on 0700–1900 h. Prior to the start of the experiment, rats were handled and acclimated to the colony for 1 week with free access to food and water. Behavioral testing was conducted during the light phase. Procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee and conformed to the 1996 NIH Guide for the Care and Use of Laboratory Animals.

2.2. Apparatus/materials

The experiments were conducted in polyurethane home cages located in the colony room. Water, milk, and salt solutions were delivered to rats via plastic-graduated sipper tubes (volume of 50 ml) fitted with a rubber stopper and a stainless-steel drinking spout. The milk solution was 50% tap water and 50% evaporated milk (Nestle, Solon, OH). Salt was dissolved in tap water to provide a 0.6% w/v salt solution. Solutions were prepared fresh daily.

2.3. Procedure

On Day 1, water bottles were removed from the home cages. On Day 2, rats were given 15-min access to water in plastic-graduated sipper tubes in the home cage. On Day 3, rats were given access to the novel CS, either milk (Experiment 1) or salt solution (Experiment 2) for 15 min. Rats that did not drink from the sipper tube during the 15-min presentation of the CS were excluded from the experiment. Following consumption, intake of the CS (ml) was measured. Within 10 min following CS consumption, rats were injected with lobeline, METH, lithium, or saline. Rats were given access to water for 15 min on Day 4. On Day 5, all rats were tested for CTA. The CTA testing procedure utilized a one-bottle test in which rats were allowed to drink the CS solution for 15 min, to determine if different doses of lobeline or METH produced CTA (Batsell and Best, 1992). The amount of solution consumed (ml) was recorded immediately following testing.

In Experiment 1, rats ($n = 51$) were assigned randomly to seven experimental groups. The SAL group ($n = 11$) was injected with saline following milk consumption. Rats in the lobeline 0.3, 1.0, and 3.0 mg/kg groups ($n = 7$/group) consumed the milk CS; lobeline was the US. Rats in the METH 0.3 mg/kg ($n = 6$), 1.0 mg/kg ($n = 6$), and 3.0 mg/kg ($n = 7$) groups consumed the milk CS; METH was the US. The procedure for Experiment 2 ($n = 73$; $n = 8–9$/group) was similar to Experiment 1, except that a 0.6% salt solution was used as the CS. Additionally, two lithium carbonate 19 mg/kg ($n = 8$) and 55 mg/kg ($n = 8$) groups were added as positive control groups, because lithium was shown to produce CTA in rats (e.g., Parker, 1995). The carbonate form of lithium was used rather than the chloride form because previous results from our laboratory using lithium carbonate were helpful for selecting the doses used in the current study (Bevins et al., 1996).

Table 1
Mean milk and salt solution intake (± S.E.M.) for the conditioning trial for each group in Experiments 1 and 2

Group	Experiment 1		Experiment 2	
	Mean milk consumed (ml/15-min; ± S.E.M.)	*n*	Mean salt solution consumed (ml/15-min; ± S.E.M.)	*n*
METH (mg/kg)				
SAL	5.8 (± 0.35)	11	15.0 (± 1.4)	9
0.3	6.3 (± 0.21)	6	13.6 (± 0.8)	8
1.0	5.8 (± 0.61)	6	15.0 (± 0.9)	8
3.0	6.9 (± 0.51)	7	16.0 (± 0.7)	8
LOB (mg/kg)				
0.3	6.6 (± 0.65)	7	15.6 (± 1.0)	8
1.0	6.9 (± 0.26)	7	16.0 (± 0.9)	8
3.0	5.4 (± 0.95)	7	16.4 (± 1.0)	8
Lithium (mg/kg)				
19.0	–	–	17.3 (± 0.9)	8
55.0	–	–	15.0 (± 0.7)	8

2.4. Drugs

Drugs included lithium carbonate (Li_2CO_3), α-lobeline hemisulfate (lobeline; Sigma, St. Louis, MO) and METH HCl. METH HCl was a gift from the National Institute on Drug Abuse (Bethesda, MD). Lithium was dissolved in water as 0.25 and 0.75 M solutions. Doses of lobeline and METH were calculated as salt weight and were dissolved in saline (0.9%, w/v). Rats were injected at a volume of 1 ml/kg with lobeline, METH, or saline subcutaneously, or with lithium intraperitoneally.

2.5. Statistics

Similar statistical tests were conducted on data from Experiments 1 and 2, in which milk and salt solution were used as the CS, respectively. A between-groups one-way analysis of variance (ANOVA) determined whether groups drank different amounts of the milk or salt solutions during the 15-min conditioning session, prior to drug administration. A two-way ANOVA, with drug and dose as between-subjects factors, determined if there were significant differences in the amount of the milk or salt solutions consumed during the 15-min testing session. When appropriate, Dunnett's tests were used to compare treatment groups to the saline control group in both experiments.

3. Results

3.1. Milk CS

The data for milk consumption during the first exposure to the CS are presented in Table 1. Milk intake among the different treatment groups on the first exposure (i.e., conditioning day) was not significant [$F(6,44) = 1.06$, $P > .05$], indicating that initial milk consumption was not different across all groups prior to drug exposure.

Data for milk consumption during the second exposure (i.e., conditioning test day which occurred 24 h following drug exposure) are presented in Fig. 1A. For visual comparison purposes, the data from the saline control group are illustrated with both the lobeline and METH data in Fig. 1A. ANOVA revealed a main effect of drug [$F(1,34) = 54.05$, $P < .001$] and dose [$F(2,34) = 37.61$, $P < .001$], and a significant Drug × Dose interaction [$F(2,34) = 7.56$, $P < .01$]. Dunnett's tests revealed that only the high dose of lobeline (3.0 mg/kg) decreased milk intake significantly compared to the saline control group; whereas at every dose tested, METH significantly decreased milk consumption compared to the saline control group.

3.2. Salt solution CS

Salt solution consumptions during the first exposure (i.e., conditioning day) are presented in Table 1. Intake of salt solution among the treatment groups on the first exposure (i.e., conditioning day) was not significant [$F(8,44) = 1.13$, $P > .05$], indicating that initial consumption of the salt solution was not different across all the groups prior to drug exposure.

Data for salt solution consumption during the second exposure (conditioning test day, which occurred 24 h following drug exposure) are presented in Fig. 1B. For visual comparison purposes, the data from the saline control group are illustrated with the lobeline, METH, and lithium data in Fig. 1B. ANOVA revealed a main effect of drug [$F(1,36) = 17.42$, $P < .001$] and dose [$F(2,36) = 14.44$, $P < .001$], but no Drug × Dose interaction [$F(2,36) = 0.39$, $P > .05$]. Dunnett's tests revealed that the 1.0- and 3.0-mg/kg

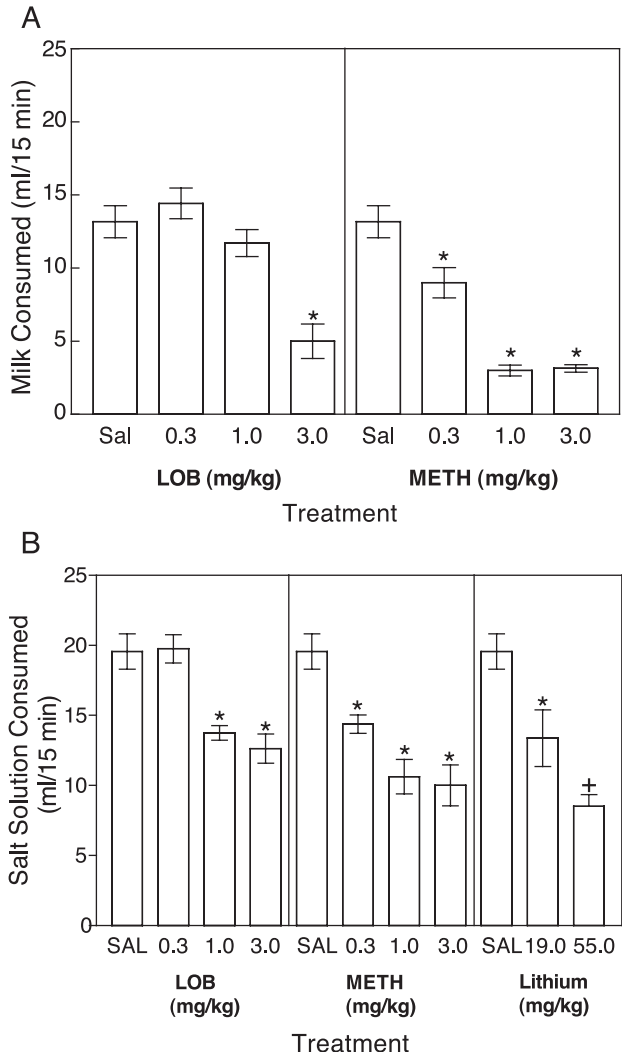

Fig. 1. (A) Mean milk intake (± S.E.M.) during the testing session in Experiment 1. The duration of the testing procedure was 15 min. $n = 7–11$ rats per group. METH, methamphetamine; LOB, lobeline; SAL, saline; * different from saline control, $P < .05$. (B) Mean salt solution intake (± S.E.M.) during the testing session in Experiment 2. The duration of the testing procedure was 15 min. $n = 8–9$ rats per group. METH, methamphetamine; LOB, lobeline; SAL, saline; * different from saline control, $P < .05$; $^{+}$ different from saline control and 19.0 mg/kg lithium group, $P < .05$.

doses of lobeline decreased intake of salt solution compared to the saline control group. METH significantly decreased intake of salt solution compared to the saline control group at every dose tested. Dunnett's tests also revealed that lithium dose-dependently decreased intake of salt solution when compared to the saline control group. Interestingly, injection of these doses of lobeline (1.0 and 3.0 mg/kg) and METH (0.3–3.0 mg/kg), following salt solution consumption, resulted in comparable levels of CTA relative to that produced by lithium (19 and 55 mg/kg).

4. Discussion

Previous findings have demonstrated that lobeline pretreatment decreased METH-induced hyperactivity, METH discriminative stimulus effects, and METH self-administration in rats (Miller et al., 2001, 2003; Harrod et al., 2001). Based on those preclinical data, lobeline appears to have potential as a novel pharmacotherapy for psychostimulant abuse. As such, full evaluation of the pharmacological effects of lobeline is warranted. The present results demonstrate a conditioned avoidance response to lobeline at the highest dose (3.0 mg/kg) tested. Specifically, rats which had previously consumed a novel milk or salt solution and injected with lobeline, subsequently avoided consuming the novel solution during testing. In addition, when salt was the CS, a significant CTA was also observed following 1 mg/kg lobeline. Thus, the present report is the first to demonstrate that lobeline induces CTA in rats.

Previous research utilizing the taste reactivity test (Grill and Norgren, 1978) has demonstrated that rats injected with lithium chloride not only avoid the CS, but also exhibit a gaping response indicative of emesis and nausea after tasting the solution (Parker, 1995, 2003). The present results corroborate these latter findings. The results of the current study also corroborate previous findings (Parker, 1995) showing that administration of METH (0.3–3.0 mg/kg), following the consumption of a novel milk, or a salt solution, produced CTA. Related research has demonstrated that drugs which are reinforcing, such as amphetamine, METH, nicotine, and morphine, produce conditioned avoidance, but not conditioned gaping responses that are observed with drugs that induce emesis (Parker, 1995). Rats avoid the CS because the drug changes the physiological state of the animal, not because the drug produced emesis or nausea (Parker, 2002). Thus, drugs that are readily self-administered (e.g., METH) share the ability to produce a conditioned avoidance response in rats in the absence of emesis or nausea. Although lobeline reportedly produced emesis (Dwoskin and Crooks, 2002), the present study did not utilize a taste reactivity test (Grill and Norgren, 1978), and thus, it cannot be determined if lobeline-induced CTA was the result of emesis upon tasting the CS during testing.

Interestingly, the present experiments showed that rats more readily acquired lobeline-induced CTA if the CS was a salt solution rather than milk. That is, two doses of lobeline (1.0 and 3.0 mg/kg) produced CTA when the salt solution was the CS, whereas CTA was only observed at the highest dose (3.0 mg/kg) when milk was the CS. Differential conditioning between two types of CS is not uncommon in CTA studies (Bevins et al., 1996, 1997). Previous research examining the stimulus parameters in Pavlovian fear conditioning has shown that the magnitude of the CR increases as the salience of the CS or US increases (Kamin and Brimer, 1963). With respect to the present experiment, the ability of the 1.0-mg/kg dose of lobeline to produce a CTA with the salt solution, and not the milk solution, suggests that the salt CS was more salient than the milk CS. Because rats consumed more of the salt solution (16 ml) than the milk solution (6 ml) during the initial exposure to the CS (Table 1), the greater consumption of the salt solution may have made it more salient.

The finding that METH produced CTA using either CS type in the current study is inconsistent with the reward comparison hypothesis proposed by Grigson (1997, 2000). This hypothesis suggests that drugs of abuse produce CTA because they reduce the relative rewarding effect of a palatable CS. Importantly, this hypothesis assumes that drugs of abuse produce CTA only when a palatable CS is used, such as saccharin, but not when a nonpalatable or neutral CS is used, such as salt. Contrary to this hypothesis, the current work found that METH produced CTA regardless whether the CS type was milk or salt. However, caution is needed in making direct comparisons across experiments. In the work of Grigson (1997, 2000), rats were tested using multiple CS–US pairings of saccharin or salt with either cocaine or morphine. In contrast, the current study used only a single pairing of milk or salt with METH.

The present experiments used a "one-bottle," rather than a "two-bottle" testing procedure to determine if lobeline, METH, or lithium induced CTA in rats. The two-bottle test has been argued to be a more sensitive measure of CTA (Dragoin et al., 1971; Elkins, 1973). However, other research suggest that the choice between using a one- and a two-bottle test depends on the experimental procedure. Thus, if a research procedure utilizes a design in which different groups acquire aversions of differential strength, the one-bottle test is a more sensitive measure to detect CTA. In contrast, if it is important to detect a weak aversion, the two-bottle test may be more appropriate (Batsell and Best, 1992). In the present study, a range of lobeline (0.3–3.0 mg/kg) and METH (0.3–3.0 mg/kg) doses was administered following the consumption of the novel solution. Varying the dose of the US is assumed to have produced differential avoidance behavior in the present study. Although a dose-dependent effect was not observed in the lobeline groups, there was a dose-dependent effect of METH using the milk CS. A dose-dependent effect of lithium was also observed in the current study. Thus, the lack of dose dependency in the lobeline groups is likely due to the specific dose range examined, rather than the one-bottle testing procedure.

Preclinical results with lobeline suggest that lobeline may be an effective treatment for METH abuse (Harrod et al., 2001, 2003; Miller et al., 2001, 2003; Dwoskin and Crooks, 2002). In the present study, a dose of lobeline (3.0 mg/kg) that was found previously to attenuate METH self-administration (Harrod et al., 2001) produced robust CTA for novel milk and salt solutions. These findings suggest that although lobeline effectively decreases METH self-administration, lobeline also produces conditioned avoidance behavior. Current research is aimed at developing lobeline analogs which reduce psychostimulant self-administration while minimizing conditioned avoidance behavior.

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