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Lobeline Attenuates *d*-Methamphetamine Self-Administration in Rats

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ABSTRACT

α-Lobeline inhibits *d*-amphetamine-evoked dopamine release from striatal slices in vitro, appearing to reduce the cytosolic pool of dopamine available for reverse transport by the dopamine transporter. Based on this neurochemical mechanism of action, the present study determined if lobeline decreases *d*-methamphetamine self-administration. Rats were surgically implanted with jugular catheters and were trained to lever press on a fixed ratio 5 schedule for intravenous *d*-methamphetamine (0.05 mg/kg/infusion). To assess the specificity of the effect of lobeline, another group of rats was trained to lever press on a fixed ratio 5 schedule for sucrose reinforcement. Pretreatment of rats with lobeline (0.3–3.0 mg/kg, 15 min prior to the session) decreased responding for both *d*-methamphetamine and sucrose reinforcement. Following repeated lobeline (3.0 mg/kg) administration, tolerance developed to the decrease in responding for sucrose; however, the lobeline-induced decrease in responding for *d*-methamphetamine persisted. Furthermore, the lobeline-induced decrease in responding for *d*-methamphetamine was not surmounted by increasing the unit dose of *d*-methamphetamine. These results suggest that lobeline produces a nonspecific rate suppressant effect following acute administration, to which tolerance develops following repeated administration. Importantly, the results also suggest that repeated administration of lobeline specifically decreases responding for *d*-methamphetamine in a noncompetitive manner. Thus, lobeline may be an effective, novel pharmacotherapy for *d*-methamphetamine abuse.

α-Lobeline, a lipophilic, alkaloidal constituent of Indian tobacco (*Lobelia inflata)*, interacts at nicotinic receptor sites with high affinity (Abood et al., 1989; Damaj et al., 1997; Miller et al., 2000a). Lobeline has been generally categorized as a nicotinic receptor agonist and is purported to exert its effects on the central nervous system via a mechanism similar to nicotine (Decker et al., 1995). However, results from neuropharmacological studies indicate that nicotine and lobeline do not share a common mechanism of action. Chronic nicotine administration has been shown to produce nicotinic receptor up-regulation, whereas chronic lobeline administration does not (Bhat et al., 1991). Additionally, nicotine evokes $^{86}Rb^{+}$ efflux from rat striatal synaptosomes, whereas lobeline was observed to only slightly increase $^{86}Rb^{+}$ efflux in a nicotinic and muscarinic antagonist-insensitive manner (Terry et al., 1998). Moreover, lobeline inhibited nicotine-evoked $^{86}Rb^{+}$ efflux from rat thalamic synaptosomes and inhibited nicotine-evoked ^{3}H overflow from rat striatal slices preloaded with [^{3}H]dopamine (Miller et al., 2000a). The latter in vitro results suggest that lobeline acts as a nicotinic receptor antagonist. Furthermore, administration of lobeline to rats inhibited nicotine-induced increases in dopamine overflow in microdialysate from nucleus accumbens (Benwell and Balfour, 1998), also suggesting a nicotinic antagonist action in vivo.

Similarly, behavioral studies provide corroborative evidence that lobeline and nicotine act via different mechanisms of action. In rats, an acute injection of nicotine produces a biphasic effect on locomotor behavior, characterized by an initial decrease in activity and a subsequent period of rebound hyperactivity (Clarke and Kumar, 1983). With repeated injections, tolerance develops to the initial depressant effect, while the hyperactivity becomes enhanced (Stolerman et al., 1973). In contrast, lobeline produces only hypoactivity following acute administration, and locomotor sensitization does not develop with repeated injections (Stolerman et al., 1995). Interestingly, nicotine-induced hypoactivity is attenuated by pretreatment with mecamylamine, a classical noncompetitive nicotinic receptor antagonist, whereas lobeline-induced hypoactivity is not inhibited by mecamylamine pretreatment (Stolerman et al., 1995). In drug discrimination studies, lobeline was initially shown to generalize to nicotine (Geller et al., 1971); however, this was not observed in subsequent studies (Romano and Goldstein, 1980; Reavill et al.,

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ABBREVIATIONS: DTBZ, dihydrotetrabenazine; VMAT2, vesicular monoamine transporter; HPLC-EC, high-performance liquid chromatography with electrochemical detection; ANOVA, analysis of variance.

1990). Furthermore, nicotine produces conditioned place preference and is readily self-administered in rats (Donny et al., 1995; Risinger and Oakes, 1995; Shoaib et al., 1997; Bardo et al., 1999), consistent with its rewarding properties. In contrast, lobeline does not produce conditioned place preference (Fudala and Iwamoto, 1986) and does not engender robust self-administration in mice (Rasmussen and Swedburg, 1998). Thus, the behavioral effects of lobeline differ from those produced by nicotine.

In further investigating alternative neurochemical mechanisms of lobeline action, recent evidence demonstrates that lobeline alters vesicular storage by potently inhibiting [^{3}H]dopamine uptake into, and enhancing, [^{3}H]dopamine release from rat striatal synaptic vesicles (Teng et al., 1997). Furthermore, lobeline inhibits binding of [^{3}H]dihydrotetrabenazine ([^{3}H]DTBZ), a structural analog of tetrabenazine that binds to a single class of high-affinity sites on the vesicular monoamine transporter (VMAT2). Recent evidence suggests that VMAT2 is critically involved in the dopamine-releasing and rewarding effects of stimulant drugs such as *d*-amphetamine and *d*-methamphetamine. VMAT2 knockout mice show reduced *d*-amphetamine-conditioned reward (Takahashi et al., 1997) and reduced *d*-methamphetamine-induced dopamine release assessed by in vivo microdialysis (Wang et al., 1997; Fumagalli et al., 1999). *d*-Amphetamine has also been reported to inhibit [^{3}H]DTBZ binding to vesicle membranes, although it does so with a potency 1 to 2 orders of magnitude less than that reported for inhibition of monoamine uptake (Ary and Komiskey, 1980; Erickson et al., 1996; Teng et al., 1998). *d*-Methamphetamine, a drug structurally similar to *d*-amphetamine, more potently inhibits binding to the reserpine site on VMAT2 (Peter et al., 1994), suggesting that *d*-amphetamine-induced inhibition of vesicular monoamine uptake is via an interaction at the reserpine site. In contrast, lobeline potently inhibits [^{3}H]DTBZ binding to rat vesicle membranes, at concentrations consistent with its inhibition of [^{3}H]dopamine uptake into synaptic vesicles (Teng et al., 1998), suggesting that lobeline-induced inhibition of vesicular monoamine uptake is via an interaction with the [^{3}H]DTBZ site. Thus, although *d*-amphetamine is equipotent in inhibiting dopamine uptake and promoting release from synaptic vesicles, lobeline more potently (28-fold) inhibits dopamine uptake than it evokes vesicular dopamine release (Teng et al., 1998). Taken together, these results suggest that *d*-amphetamine and lobeline inhibit dopamine uptake by binding to different sites on VMAT2. Most recently, lobeline has been reported to inhibit *d*-amphetamine-evoked dopamine release from superfused rat striatal slices (Miller et al., 2001). Thus, the ability of lobeline to inhibit *d*-amphetamine's neurochemical effects prompted the investigation of lobeline to alter *d*-methamphetamine self-administration.

Experimental Procedures

Materials. The following drugs and chemicals were purchased from Sigma (St. Louis, MO): α-lobeline hemisulfate, *d*-methamphetamine HCl, perchloric acid, dopamine HCl, methanol, sodium octyl sulfate, sodium phosphate, citric acid, EDTA, and sodium chloride.

Animals. Adult male, Sprague-Dawley rats (200–225 g body weight) were obtained from Harlan Industries (Indianapolis, IN) and were caged individually with free access to food and water in the home cage. The colony room was maintained at 24°C and 45% relative humidity, with lights on from 7:00 AM to 7:00 PM. Prior to the start of each experiment, rats were acclimated to the colony room for 1 week and were handled for 2 days. Behavioral testing was conducted during the light phase. All procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee and conformed to the 1996 edition of the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health).

Surgery. Rats were anesthetized (80 mg/kg ketamine, 5 mg/kg diazepam, i.p.) and implanted with a catheter into the jugular vein that exited through a dental acrylic head mount. The head mount was affixed to the skull with metal screws (Peltier and Schenk, 1993). Daily infusions of heparinized saline (0.2 mg/0.1 ml/rat/day) containing streptokinase (250,000 IU) were given to maintain catheter patency. At the end of each experiment, catheter patency was verified with 15 mg/kg morphine (i.v.), which induced a rapid cataleptic response.

Apparatus. For *d*-methamphetamine and sucrose self-administration, operant chambers (ENV-001, Med Associates, St. Albans, VT) were enclosed in sound-attenuating compartments and were operated by computer interface equipment. Located on the front panel of each operant chamber was a 5 × 4.2 cm opening that allowed access to a recessed food tray. Two metal response levers on either side of the food tray were located 7.3 cm above a metal-grid floor. A 28 V white cue light, 3 cm in diameter, was centered 6 cm above each response lever. Drug infusions were delivered via a syringe pump (Med Associates, PHM-100). A water-tight swivel allowed attachment of the catheter tubing from a 10-ml syringe to the head mount of the rat within the operant chamber.

***d*-Methamphetamine Self-Administration Procedure.** Rats were reduced to 85% of free-feeding body weight by restricting food access and were shaped to press a lever for contingent sucrose pellet reinforcement. Only one lever was available during the shaping procedure. Lever position was counterbalanced among rats. The schedule of reinforcement during 15-min sessions was gradually increased across 3 days from a fixed ratio 1 to a fixed ratio 5 schedule of reinforcement. After training, rats were allowed free access to food for the remainder of the experiment. One week after training, rats were surgically implanted with a chronic indwelling jugular catheter and were allowed to recover for 1 week before commencing *d*-methamphetamine self-administration sessions.

Rats were first allowed to self-administer *d*-methamphetamine (0.05 mg/kg/infusion) on a fixed ratio 1/20-s signaled time out schedule of reinforcement during daily 1-h sessions. Drug was infused (60 μl, 3.5 s) following depression of one lever (active lever); responding on the second lever (inactive lever) was recorded, but was not reinforced. Each drug infusion was followed by a 20-s time out interval during which responding was not reinforced on either lever. The time out occurred immediately after the lever press and was signaled by turning on the lights above the response levers. The schedule of reinforcement was incremented from fixed ratio 1 to fixed ratio 2, and then to fixed ratio 5. Stable responding was operationally defined by less than 15% variability in the number of infusions earned across three consecutive sessions, a greater than 2:1 ratio of active to inactive responses and at least 10 infusions obtained per session.

Sucrose Reinforcement Procedure. Rats were reduced to 85% of free-feeding body weight by restricting food access and were shaped to press a lever for contingent sucrose pellet reinforcement (45 mg pellets, NOYES Co., Inc., Lancaster, NH). Only one lever was available during the shaping procedure. Lever position was counterbalanced among rats. In contrast to the *d*-methamphetamine self-administration sessions, these sessions were only 15 min in duration to ensure that responding for sucrose was maintained at a constant high rate. The schedule of reinforcement was gradually increased across 3 days from a fixed ratio 1 to a fixed ratio 5 schedule of reinforcement. The schedule of reinforcement was incremented from fixed ratio 1 to fixed ratio 2, and then to fixed ratio 5. Stable responding was operationally defined by less than 15% variability in the number of pellets earned across three consecutive sessions, a

greater than 2:1 ratio of active to inactive responses and at least 10 sucrose pellets obtained per session.

Acute Lobeline Pretreatment. To determine whether lobeline altered *d*-methamphetamine self-administration, rats were pretreated with saline or lobeline (0.3, 1.0, or 3.0 mg/kg s.c.) 15 min prior to the operant session using a within-subject Latin square design. Each lobeline dose was tested twice. Pretreatments were separated by 2 intervening maintenance days of *d*-methamphetamine (0.05 mg/kg/infusion) self-administration to maintain stable responding for *d*-methamphetamine in the absence of lobeline.

To determine whether lobeline altered lever pressing for sucrose reinforcement, rats were pretreated with saline or lobeline (0.3, 1.0, or 3.0 mg/kg s.c.) 15 min prior to the operant session using a within-subject Latin square design. Each lobeline dose was tested twice. Pretreatments were separated by 2 intervening maintenance days of sucrose responding to maintain stable responding for sucrose in the absence of lobeline.

Repeated Lobeline Pretreatment. To determine whether lobeline altered *d*-methamphetamine (0.05 mg/kg/infusion) self-administration across repeated pretreatments, a separate group of rats was treated with lobeline (3.0 mg/kg) prior to seven 60-min sessions. To determine whether repeated lobeline would alter responding for sucrose reinforcement, another group of rats was pretreated with lobeline (3.0 mg/kg) prior to seven 15-min sessions. To maintain stable responding in both the *d*-methamphetamine and sucrose experiments, two intervening maintenance sessions occurred between each pretreatment session, in which no lobeline pretreatment was administered prior to the session.

Surmountability of Lobeline Pretreatment. To determine whether the lobeline-induced decrease in *d*-methamphetamine self-administration was surmountable, a separate group of rats was administered lobeline (3.0 mg/kg) prior to responding for one of the varying doses of *d*-methamphetamine (both above and below the training dose of 0.05 mg/kg/infusion). After rats were first trained to lever press for *d*-methamphetamine, the unit dose (0.0005–0.1 mg/kg/infusion) was varied across consecutive sessions to establish the dose response for *d*-methamphetamine in the absence of lobeline. A randomized Latin square design determined the order of doses. Subsequently, rats were pretreated with lobeline (3.0 mg/kg s.c., 15 min prior to session) prior to redetermination of the dose-response curve for *d*-methamphetamine using a higher range of doses (0.0005–0.4 mg/kg/infusion). Each *d*-methamphetamine dose was tested on two sessions. To maintain stable responding during determination of the dose-response curve in the presence of lobeline, two intervening maintenance sessions occurred between each pretreatment session; on these sessions, no lobeline pretreatment was administered prior to self-administration of a maintenance dose of *d*-methamphetamine (0.05 mg/kg/infusion).

Assay for Dopamine Content. To determine whether sustained *d*-methamphetamine self-administration reduced dopamine tissue content, rats from the experiment determining the surmountability of lobeline were killed by decapitation between 3 to 7 days following the last experimental session. Striatum and nucleus accumbens were dissected rapidly on an ice-cold dissection plate according to the methods described in Pierce et al. (1990). A control group of drug-naïve rats was killed concomitantly. The control group was obtained at the same time as the *d*-methamphetamine self-administration rats and maintained in the colony across the period of experimentation, but without any experimental manipulation. Striatum and nucleus accumbens were stored in 10 and 20 volumes, respectively, of 0.1 N perchloric acid at −70°C until assay. Upon assay, samples were thawed on ice, sonicated, and centrifuged for 15 min at 30,000*g* at 4°C. A 20-μl aliquot part of the supernatant was assayed for dopamine using high-performance liquid chromatography with electrochemical detection (HPLC-EC; working electrode maintained at 700 mV relative to the reference electrode). The mobile phase consisted of 5% methanol, with 0.02% sodium octyl sulfate, 50 mM sodium phosphate, 124 mM citric acid, 0.1 mM EDTA, and 10 mM sodium chloride (pH 3.0). Retention times of standards were used to identify all peaks, and calibration standards were run to determine the linearity and sensitivity of the HPLC-EC system. Peak height measurements and calibration factors based on standards were obtained daily and used to calculate the detected amount of dopamine.

Statistics. One-way repeated measures analyses of variance (ANOVAs) were performed to determine the effect of acute lobeline pretreatment on *d*-methamphetamine self-administration, the effect of acute lobeline pretreatment on sucrose reinforced responding, the effect of repeated lobeline pretreatment on *d*-methamphetamine self-administration, and the effect of repeated lobeline pretreatment on sucrose reinforced responding. Three-way repeated measures ANOVA, with pretreatment, day, and time block as within-subject factors were performed to analyze the time course of the effect of repeated lobeline pretreatment on *d*-methamphetamine self-administration over the entire 60-min session. A two-way repeated measures ANOVA with dose and pretreatment as within-subject factors determined the ability of increasing the *d*-methamphetamine unit dose to surmount the effect of lobeline. A two-way mixed-factor ANOVA, with treatment as a between-groups factor and brain region as a within-groups factor, determined if rats pretreated with lobeline and self-administering *d*-methamphetamine had altered dopamine levels in striatum and nucleus accumbens. Planned *t* tests incorporating Bonferroni's correction were used to compare pairs of means. To compare the effect of acute and repeated lobeline in the *d*-methamphetamine self-administration experiments with that in the sucrose self-administration experiments, data from the first 15 min of the 60-min *d*-methamphetamine self-administration session were also analyzed separately. A Pearson *r* correlational analysis also determined if the average daily total intake of *d*-methamphetamine for each animal was correlated with dopamine content in either striatum or nucleus accumbens.

Results

Effect of Acute Lobeline Pretreatment on *d*-Methamphetamine Self-Administration and Sucrose Reinforced Responding. When responding stabilized in both the *d*-methamphetamine and sucrose experiments, the number of lever presses on the active lever was greater than that on the inactive lever. During *d*-methamphetamine self-administration, the average number of responses on the active and inactive levers during the first 15 min of the session was 59 and 6, respectively. When responding for sucrose was assessed, the average number of responses on the active and inactive levers was 162 and 9, respectively. These results indicate that subjects discriminated between the active and inactive levers in both series of experiments.

The effect of lobeline pretreatment on *d*-methamphetamine self-administration is illustrated in Fig. 1. A repeated measure one-way ANOVA revealed that lobeline pretreatment decreased the number of *d*-methamphetamine infusions during the first 15 min of the session [$F_{(3,18)} = 6.86, p < 0.05$]. Planned comparisons indicated that the highest dose of lobeline (3.0 mg/kg) significantly reduced the number of *d*-methamphetamine infusions when compared with saline pretreatment, whereas there was no significant effect of lower doses (0.3 or 1.0 mg/kg). The effect of lobeline was specific to the active lever, since no significant change in responding was observed on the inactive lever (data not shown). However, since the number of responses on the inactive lever was considerably lower than on the active lever, a floor effect may have obscured detection of a lobeline-induced decrease in responding on the inactive lever.

The effect of acute lobeline pretreatment on sucrose rein-

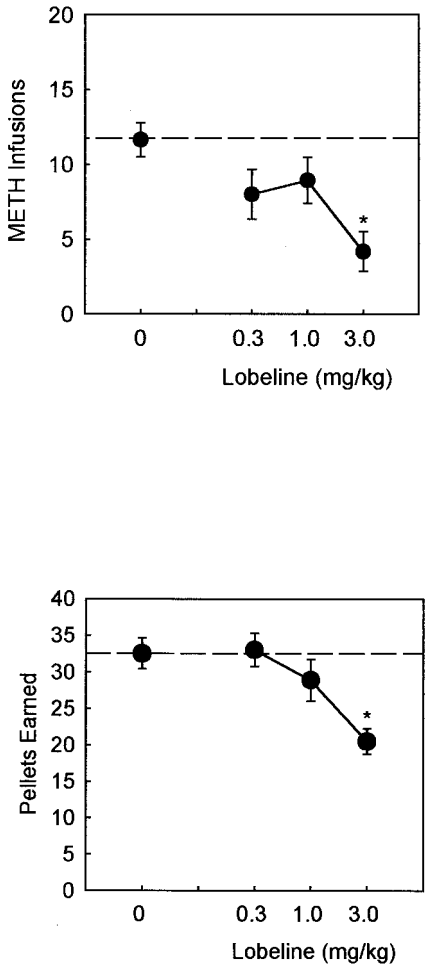


Fig. 1. Lobeline-induced attenuation of responding for *d*-methamphetamine (METH) and sucrose. Data are presented as the mean number (±S.E.M.) of *d*-methamphetamine infusions (0.05 mg/kg/infusion) earned during the first 15 min of the 60-min operant session (top), and as mean number (±S.E.M.) of sucrose pellets earned during the entire 15-min operant session (bottom). Lobeline (0.3, 1.0, and 3.0 mg/kg) or saline (0 mg/kg) was administered 15 min prior to the beginning of the operant session. $^*p < 0.05$, different from saline control, $n = 5$ to 6 rats.

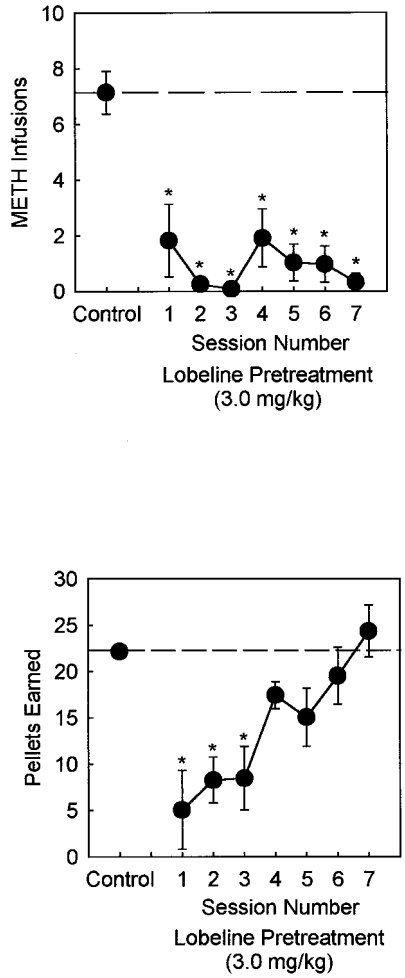


Fig. 2. Repeated pretreatment with lobeline persistently decreases *d*-methamphetamine (METH) self-administration, but not sucrose reinforced responding. Data are presented as mean number (±S.E.M.) of *d*-methamphetamine infusions (0.05 mg/kg/infusion) earned during the first 15 min of 60-min operant sessions, and as mean number (±S.E.M.) of sucrose pellets earned during 15-min sessions (bottom) as a function of lobeline pretreatment session. Rats were pretreated with lobeline (3.0 mg/kg) 15 min prior to operant sessions. Control indicates mean number of *d*-methamphetamine infusions or sucrose pellets in top and bottom panels, respectively, obtained on maintenance days prior to lobeline pretreatment sessions. $^*p < 0.05$, different from control, $n = 5$ to 6 rats.

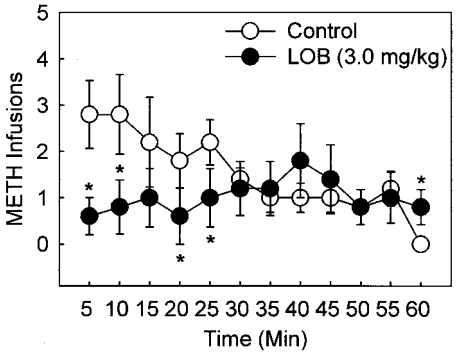
forced responding during 15-min sessions is also illustrated in Fig. 1. Repeated measures one-way ANOVA indicated that lobeline attenuated sucrose reinforced responding [$F_{(3,21)} = 9.07$, $p < 0.05$]. Planned comparisons showed that the highest dose of lobeline (3.0 mg/kg) significantly reduced the number of sucrose pellets earned, whereas lower doses produced no significant effect. Furthermore, lobeline did not alter responding on the inactive lever in these experiments (data not shown).

Effects of Repeated Lobeline Pretreatment on *d*-Methamphetamine Self-Administration and Sucrose Reinforced Responding. Repeated lobeline (3.0 mg/kg) pretreatment decreased *d*-methamphetamine Self-Administration (Fig. 2). Planned comparisons revealed that the number of *d*-methamphetamine infusions was significantly reduced following each lobeline pretreatment when compared with the mean number of infusions earned on the maintenance days preceding each lobeline pretreatment. A repeated measure ANOVA revealed that the number of *d*-methamphetamine infusions did not differ across the operant sessions in which rats were pretreated with lobeline [$F_{(6,24)} = 1.02$, $p > 0.05$]. These results indicate that tolerance did not develop to lobeline's effect, such that lobeline pretreatment consistently reduced the number of *d*-methamphetamine infusions across repeated sessions. Furthermore, pretreatment did not decrease body weight in rats administered repeated lobeline (data not shown).

The effect of repeated lobeline pretreatment on sucrose reinforced responding is also illustrated in Fig. 2. Repeated measures ANOVA revealed a significant alteration in the number of pellets earned across sessions [$F_{(6,24)} = 5.39$, $p < 0.05$]. Planned comparisons revealed that the number of sucrose pellets earned was different from baseline control on sessions 1 to 3, but not on sessions 4 to 7. Thus, in contrast to the persistent attenuation of *d*-methamphetamine self-administration observed following repeated lobeline pretreatment, tolerance developed to the decrease in responding for sucrose following repeated lobeline pretreatments.

Time Course Analysis of the Effect of Repeated Lobeline Pretreatment on *d*-Methamphetamine Self-Administration. To determine the time course of the effect of lobeline on *d*-methamphetamine self-administration, analyses were conducted on the number of *d*-methamphetamine infusions obtained across 5-min time blocks during 60-min sessions. Repeated measures ANOVA revealed main effects of pretreatment [$F_{(1,4)} = 23.47$, $p < 0.05$] and time block [$F_{(11,44)} = 2.45$, $p < 0.05$]; however, the main effect of day was not significant [$F_{(6,24)} = 0.39$, $p > 0.05$]. Furthermore, the pretreatment × time block interaction was significant [$F_{(11,44)} = 14.36$, $p < 0.05$], whereas interactions of pretreatment × day [$F_{(6,24)} = 1.35$, $p > 0.05$], day × time block [$F_{(66,264)} = 0.90$, $p > 0.05$], and pretreatment × day × time block [$F_{(66,264)} = 0.90$, $p > 0.05$] were not significant.

The number of *d*-methamphetamine infusions obtained during each 5-min block during the 60-min sessions for the lobeline pretreated and control conditions on sessions 1 and 7 are illustrated in Fig. 3. Lobeline decreased *d*-methamphetamine self-administration for about 25 min on session 1; subsequently, responding was not decreased compared with control for the remainder of the session. Lobeline continued to decrease *d*-methamphetamine self-administration for 15 min on session 7. Furthermore, rats pretreated with lobeline exhibited increased *d*-methamphetamine self-administration during the last 5-min block of session 1 and at several of the blocks during the latter part of session 7.



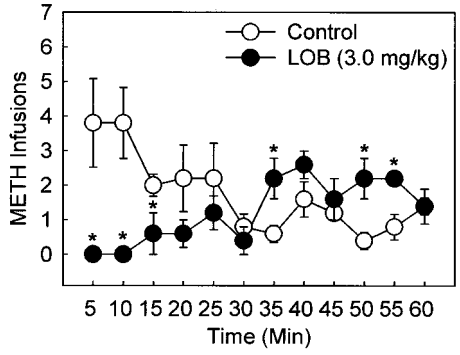


Fig. 3. Time course for the effect of lobeline (LOB) on *d*-methamphetamine (METH) self-administration. Top and bottom panels illustrate the effect of lobeline pretreatment on *d*-methamphetamine self-administration (0.05 mg/kg/infusion) on sessions 1 and 7, respectively. Rats were pretreated with lobeline (3.0 mg/kg) 15 min prior to the beginning of the session. Data are presented as the mean number (±S.E.M.) of *d*-methamphetamine infusions obtained in 5-min time blocks during the 60-min operant sessions. Control indicates mean number of *d*-methamphetamine infusions per 5-min time block obtained on maintenance days prior to lobeline pretreatment sessions. *$p < 0.05$ (one-tailed), different from control, $n = 5$ to 6 rats.

Surmountability of the Lobeline-Induced Decrease in *d*-Methamphetamine Self-Administration. Dose-response curves for *d*-methamphetamine self-administration in the absence and presence of lobeline (3.0 mg/kg) pretreatment are illustrated in Fig. 4. A repeated measures ANOVA revealed a main effect of *d*-methamphetamine dose [$F_{(5,20)} = 6.26$, $p < 0.05$], a main effect of lobeline pretreatment [$F_{(1,4)} = 9.42$, $p < 0.05$], and a *d*-methamphetamine dose × pretreatment interaction [$F_{(5,20)} = 2.70$, $p < 0.05$]. Peak responding for *d*-methamphetamine occurred at a dose of 0.0025 mg/kg/infusion, both in the absence and presence of lobeline pretreatment. Planned comparisons at each *d*-methamphetamine dose revealed a significant reduction in the number of infusions following lobeline pretreatment when the unit dose of *d*-methamphetamine was 0.005 to 0.1 mg/kg/infusion; however, there was no difference in the number of infusions obtained at lower doses of *d*-methamphetamine (0.0005–0.0025 mg/kg/infusion). There was no evidence that lobeline produced a left or rightward shift in the *d*-methamphetamine dose-response curve, but rather an overall flattening of the curve was observed (Fig. 4). Importantly, lobeline pretreatment continued to decrease responding as the unit dose of *d*-methamphetamine was increased up to 8-fold higher than the training dose (i.e., up to 0.4 mg/kg/infusion). Since these high unit doses did not increase responding in the within-subject dose-response function following lobeline pretreatment, these results demonstrate that the lobeline-induced decrease in responding for *d*-methamphetamine was not surmounted.

The high variability in the group data observed at the *d*-methamphetamine dose that produced peak responding in both the absence and presence of lobeline (0.0025 mg/kg/infusion; see Fig. 4) prompted an examination of the data from individual rats. Although the group data represents 10 rats in the no pretreatment condition, only five

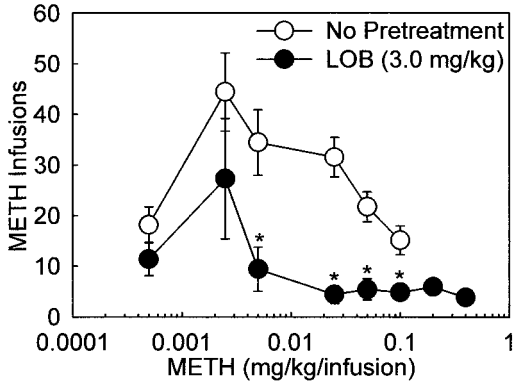


Fig. 4. Increasing the unit dose of *d*-methamphetamine (METH) does not surmount the lobeline (LOB)-induced decrease in *d*-methamphetamine self-administration. Data are presented as the mean number (±S.E.M.) of *d*-methamphetamine infusions obtained during 60-min operant sessions. The training dose of *d*-methamphetamine was 0.05 mg/kg/infusion. Rats were pretreated with lobeline (3.0 mg/kg) 15 min prior to the session. *$p < 0.05$, different from no pretreatment condition; $n = 10$ for the no pretreatment condition and $n = 5$ for the lobeline pretreatment condition.

rats completed the lobeline pretreatment condition at each unit dose of *d*-methamphetamine due to the loss of catheter patency during the course of dose-response analysis. The pattern of response for these five rats revealed an inverted U-shaped dose-response curve for each animal, although there were differences among animals in the magnitude of the lobeline effect, and the dose at which peak responding was observed (Fig. 5). Except for one rat (rat 3), lobeline produced a flattening of the dose-response curve for *d*-methamphetamine self-administration. Increasing the unit dose of *d*-methamphetamine did not surmount the effect of lobeline in any individual rat tested.

Effect of Lobeline Pretreatment and *d*-Methamphetamine Self-Administration on Dopamine Levels in Striatum and Nucleus Accumbens. A mixed-factor ANOVA revealed no differences in dopamine content in striatum or nucleus accumbens in the rats from the surmountability experiments compared with drug-naïve control rats. Mean (±S.E.M.) striatal dopamine levels for drug-treated and control rats were 9.12 (±0.28) and 8.97 (±0.22) μg/g of tissue wet weight, respectively; mean (±S.E.M.) accumbal dopamine levels for drug-treated and control rats were 6.50 (±0.35) and 6.99 (±0.28) μg/g of tissue wet weight, respectively. The main effect of drug treatment [$F_{(1,11)} = 0.27, p > 0.05$] and the brain region × drug treatment interaction [$F_{(1,11)} = 1.94, p > 0.05$] were not significant, whereas the main effect of brain region was significant [$F_{(1,11)} = 99.26, p < 0.05$]. Furthermore, the average total amount of *d*-methamphetamine intake per session for each rat was not correlated with striatal ($r = -0.46, p > 0.05$) or accumbal ($r = 0.15, p > 0.05$) dopamine content. These results indicate that drug treatment did not result in toxicity to dopaminergic neurons. However, it should be noted that the control group and the drug-

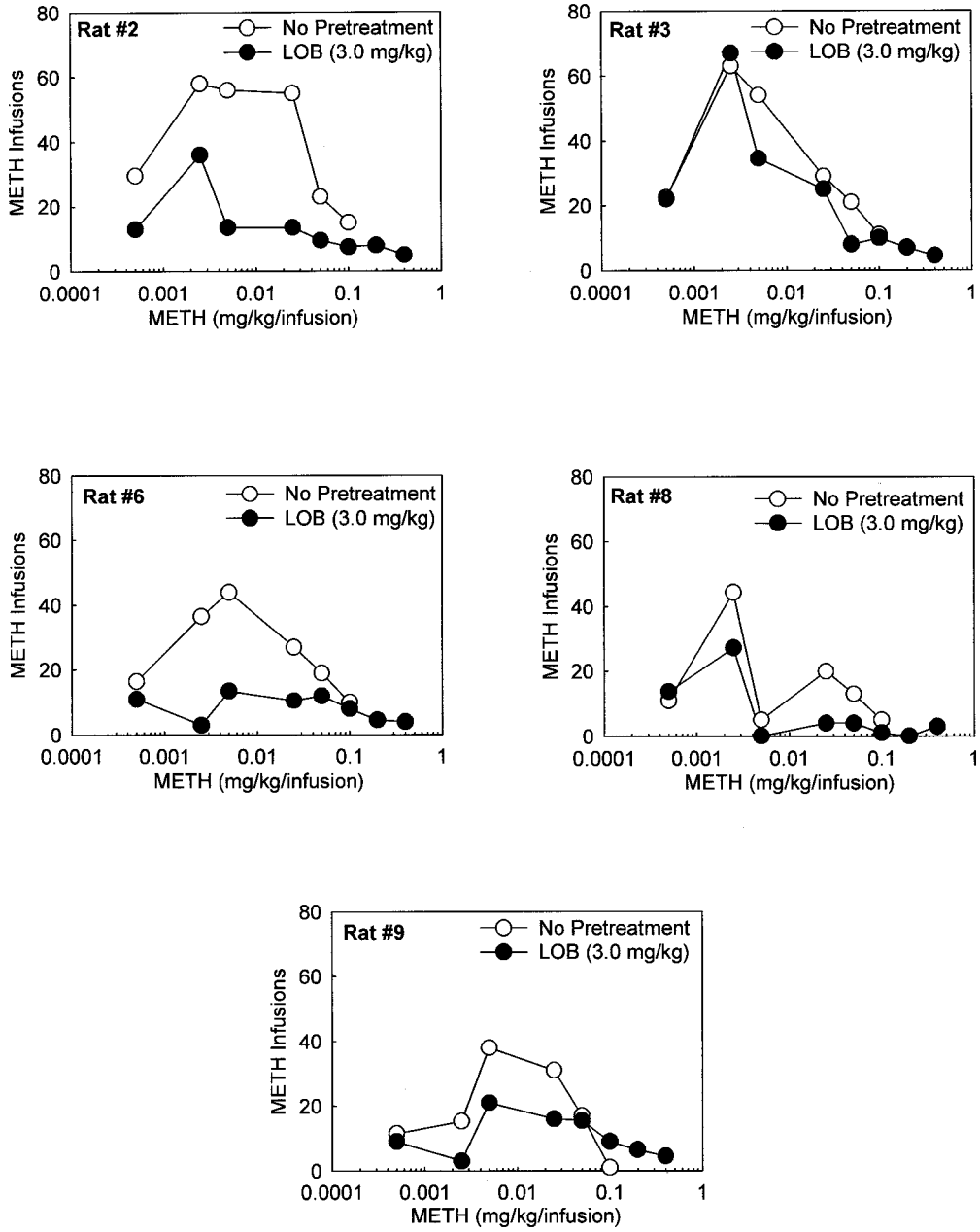


Fig. 5. Individual dose-response curves for the effect of lobeline (LOB) on *d*-methamphetamine (METH) self-administration. Data are presented as the number of *d*-methamphetamine infusions obtained for five individual rats. The training dose of *d*-methamphetamine was 0.05 mg/kg/infusion. Rats were pretreated with lobeline (3.0 mg/kg) 15 min prior to 60-min operant sessions, in which METH was available across a range of doses.

treated group were not matched for handling, which may also alter neurochemistry.

Discussion

The present study demonstrates that lobeline pretreatment attenuated *d*-methamphetamine self-administration in rats and that tolerance did not develop rapidly, if at all, to this effect. When administered acutely, lobeline decreased responding for both *d*-methamphetamine and sucrose. When administered repeatedly, however, lobeline consistently decreased *d*-methamphetamine self-administration, with no evidence for development of tolerance in the first 15 min of the session across seven repeated injections. However, examination of the time course revealed that the duration of the lobeline-induced attenuation was longer on day 1 (~25 min) than on day 7 (~15 min), suggesting some evidence for the development of tolerance. In contrast, tolerance clearly developed to the lobeline-induced decrease of sucrose reinforced responding. Taken together, these results suggest a specific decrease in *d*-methamphetamine self-administration following repeated lobeline pretreatment. Furthermore, the lobeline-induced decrease in *d*-methamphetamine self-administration was not surmounted by increasing the unit dose of *d*-methamphetamine by 8-fold, suggesting that lobeline noncompetitively attenuated responding for *d*-methamphetamine.

The current results from the *d*-methamphetamine and sucrose experiments provide evidence for a specific effect of repeated lobeline. However, one must take into account that these results were obtained using somewhat different procedures. Specifically, in contrast to rats trained to respond for sucrose, the rats trained to self-administer *d*-methamphetamine underwent anesthesia followed by a surgical procedure to insert the catheter. Furthermore, rats responding for sucrose did not receive exposure to *d*-methamphetamine, had a shorter session length, and were food deprived in the home cage throughout the experiment; in contrast, rats responding for *d*-methamphetamine were given continuous access to food in the home cage. Another difference between experiments was that the baseline rate of responding for sucrose was higher than the rate of responding for *d*-methamphetamine. In general, high rates of responding are more readily disrupted by drugs, compared with low rates of responding (Dews, 1958; Kelleher and Morse, 1968). Based on rate dependence, one would predict that the relatively high rate of responding in the sucrose reinforced group, compared with the *d*-methamphetamine reinforced group, would be more susceptible to the effect of lobeline. Since this was not the case (i.e., the lobeline-induced decrease in responding for sucrose and *d*-methamphetamine did not differ), it is unlikely that the current findings can be explained by a rate dependence interpretation. However, baseline differences in the rate of responding for the two reinforcers may have influenced the rate of development of behavioral tolerance to lobeline.

The lobeline dose (3.0 mg/kg) that acutely decreased responding for both *d*-methamphetamine and sucrose was similar to the dose observed previously to decrease locomotor activity in an open field (Miller et al., 2000b, 2001). The latter locomotor results suggest that a nonspecific motor impairment may have contributed to the lobeline-induced attenuation of *d*-methamphetamine self-administration observed in the present study. However, while tolerance occurs to the lobeline-induced hypoactivity following 8 consecutive days of administration (Miller et al., 2000b), there was no change in the lobeline-induced decrease in *d*-methamphetamine self-administration across repeated injections in the present report. These results suggest that locomotor impairment does not readily explain the current findings with repeated lobeline.

In the present study, *d*-methamphetamine self-administration exhibited an inverted U-shaped dose-response curve when the range of unit dose varied by greater than 2 orders of magnitude. Increasing the unit dose of *d*-methamphetamine did not surmount the attenuation produced by lobeline, consistent with a noncompetitive mechanism of action for lobeline. Evidence for a competitive antagonism by lobeline would have been reflected by a rightward shift in the peak of the *d*-methamphetamine dose-response curve. However, this was not the case. Importantly, the dose of lobeline (3.0 mg/kg), which attenuated *d*-methamphetamine self-administration, did not shift the dose-response curve to the right, or the left, but generally flattened the curve, suggesting a noncompetitive mechanism of action. The latter findings are consistent with recent evidence indicating that lobeline noncompetitively inhibits the neurochemical effects of *d*-methamphetamine. Lobeline has been shown to inhibit *d*-amphetamine-evoked endogenous dopamine overflow from superfused rat striatal slices (Miller et al., 2001). The concentrations (0.1–1.0 μM) of lobeline that inhibited *d*-amphetamine-evoked dopamine overflow were in the same range as those that inhibited dopamine uptake into striatal vesicles (Teng et al., 1998). Taken together, the results suggest that VMAT2 may be the molecular target for the lobeline-induced inhibition of *d*-amphetamine-evoked dopamine release. Additional evidence suggesting the importance of VMAT2 in the action of *d*-amphetamine includes the observation of impaired *d*-amphetamine-conditioned place preference in heterozygous VMAT2 knockout mice compared with wild-type mice (Takahashi et al., 1997), suggesting that intact vesicle function is required for *d*-amphetamine-conditioned reward. Other studies using heterozygous VMAT2 knockout mice demonstrate that VMAT2 function is necessary for *d*-methamphetamine-evoked striatal dopamine release in vivo (Wang et al., 1997; Fumagalli et al., 1999). Moreover, *d*-amphetamine interacts with the reserpine site on VMAT2, whereas lobeline interacts with the tetrabenazine site on VMAT2 (Erickson et al., 1996; Teng et al., 1998). The latter observations suggest that lobeline inhibits the behavioral and neurochemical effects of lobeline via a noncompetitive mechanism of action, with VMAT2 as the molecular target.

High-dose methamphetamine administration has been reported to produce dopaminergic toxicity in rats (Brown et al., 2000; Hogan et al., 2000; Wallace et al., 2000) and humans (McCann et al., 1998; Ernst et al., 2000). However, recent evidence demonstrated that acute, repeated, or continuous lobeline administration (1.0–30.0 mg/kg) did not deplete striatal dopamine or dihydroxyphenylacetic acid content (Miller et al., 2001). Additionally, results from the current study indicate that repeated lobeline pretreatment prior to *d*-methamphetamine self-administration did not change body weight, and did not decrease dopamine content in striatum or nucleus accumbens. Thus, these initial results indicate that

the combination of lobeline with *d*-methamphetamine was not toxic to dopaminergic neurons.

The current preclinical results also provide evidence for the potential development of lobeline, or synthetic lobeline analogs, as a novel pharmacotherapy for the treatment of *d*-methamphetamine abuse. Even though the incidence of *d*-methamphetamine abuse is increasing in the United States (Office of Applied Studies, 1996), there is currently no widely efficacious pharmacotherapy. The results from the current study show that lobeline decreases *d*-methamphetamine self-administration, and moreover, that this effect of lobeline is not surmounted by increasing the unit dose of *d*-methamphetamine. Additionally, when lobeline was administered prior to *d*-methamphetamine self-administration, there was no initial indication for dopaminergic toxicity. Thus, the current results provide a preclinical rationale for investigating lobeline as a safe and effective pharmacotherapy for *d*-methamphetamine abuse.

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