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Lobeline attenuates locomotor stimulation induced by repeated nicotine administration in rats

Dennis K. Miller[a,1], Steven B. Harrod[b], Thomas A. Green[b], Mei-Yee Wong[a], Michael T. Bardo[b], Linda P. Dwoskin[a,*]

[a]College of Pharmacy, University of Kentucky, Rose Street, Lexington, KY 40536-0082, USA
[b]Department of Psychology, University of Kentucky, Lexington, KY 40506, USA

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Abstract

Lobeline inhibits [^{3}H]nicotine binding to rat brain membranes and nicotine-induced [^{3}H]dopamine release from superfused rat striatal slices, indicating that lobeline acts as a nicotinic receptor antagonist. To determine whether lobeline also inhibits the effects of nicotine in vivo, the present study assessed the effect of lobeline pretreatment on nicotine-induced hyperactivity and sensitization. For 12 consecutive days, rats were injected subcutaneously with lobeline (3 mg/kg) or saline, followed 10 min later by nicotine (0.3 mg/kg) or saline injection, and activity was monitored. To determine if lobeline inhibits induction of sensitization to nicotine, 1 or 28 days later, rats were pretreated with saline followed by nicotine or saline. Lobeline attenuated nicotine-induced hyperactivity when both drugs were administered repeatedly. Although an initial injection of lobeline produced hypoactivity, tolerance to this effect developed. Importantly, tolerance did not develop to the lobeline-induced attenuation of nicotine hyperactivity. Lobeline attenuated the induction of sensitization to nicotine 1 day, but not 28 days, after the cessation of lobeline treatment. These results demonstrate that systemic administration of lobeline attenuates the locomotor-activating effects of repeated nicotine injection and the sensitization to nicotine, consistent with lobeline inhibition of nicotinic receptors and/or neurotransmitter transporters.

Keywords: Lobeline; Locomotor activity; Nicotine; Rat; Behavioral sensitization

1. Introduction

Recent studies indicate that α-lobeline (lobeline), a major alkaloidal constituent of Indian tobacco (*Lobelia inflata*), inhibits the neurochemical effects of nicotine. Both lobeline and nicotine have high affinity (K_i values = 4–30 nM) for nicotinic receptor binding sites (Abood et al., 1988; Reavill et al., 1990; Bhat et al., 1991; Court et al., 1994), and lobeline has been reported to inhibit nicotine-evoked [^{3}H]dopamine overflow from rat striatal slices (Miller et al., 2000), nicotine-evoked [^{3}H]norepinephrine release from rat locus coeruleus cells in culture (Gallardo and Leslie, 1998), and nicotine-evoked $^{86}Rb^+$ efflux from rat thalamic synaptosomes (Miller et al., 2000). In addition to the interaction with nicotinic receptors, lobeline also has been shown to inhibit [^{3}H]dopamine uptake into rat striatal synaptosomes and synaptic vesicles (Teng et al., 1997, 1998), thereby altering presynaptic dopamine storage and release. Thus, lobeline interacts with several pharmacological targets that have been associated with the stimulant properties of nicotine and other drugs of abuse.

Animal behavior studies have extended the neurochemical research by demonstrating that lobeline decreases the locomotor-activating and rewarding properties of psychostimulant drugs of abuse. For example, lobeline (1 mg/kg) attenuated amphetamine-induced hyperactivity in rats following acute administration (Miller et al., 2001a). Furthermore, lobeline (3 mg/kg) attenuated methamphetamine self-administration in rats following acute and repeated administration (Harrod et al., 2001). Considering the interaction of lobeline with nicotinic receptors, it is of interest to determine if lobeline can also alter the behavioral effects of

* Corresponding author. Tel.: +1-859-257-4743; fax: +1-859-257-7564.
E-mail address: ldwoskin@uky.edu (L.P. Dwoskin).
[1] Current address: Department of Psychology, McAlester Hall, University of Missouri, Columbia, MO 65211, USA.

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nicotine following systemic administration. Acute nicotine administration to rats produces a dose-dependent depression in locomotor activity that is followed by an increase in locomotor activity (Morrison and Lee, 1967; Clarke, 1990; Clarke and Kumar, 1983; Ksir, 1994). With repeated nicotine injection, hyperactivity is displayed and behavioral sensitization develops (Stolerman et al., 1973; Clarke and Kumar, 1983; Benwell and Balfour, 1992; Ksir, 1994; Miller et al., 2001b). Sensitization is defined as an enhanced behavioral response following repeated drug injections. Sensitization to nicotine is long-lasting and has been reported to persist following a 3-week drug-free period (Miller et al., 2001b). Long-lasting behavioral sensitization is important from a clinical perspective, since the temporal change in animal behavior purportedly models the development of nicotine addiction, and the persistence of sensitization represents heightened drug sensitivity (Wise and Bozarth, 1987; Robinson and Berridge, 1993; Koob, 1994). The stimulating effects of nicotine on locomotor activity result from activation of nicotinic receptors, as pretreatment with mecamylamine, a classical noncompetitive antagonist at central nicotinic receptors (Takayama et al., 1989), inhibits this effect of nicotine (Clarke and Kumar, 1983; Miller et al., 2001b).

In contrast to nicotine, lobeline does not increase locomotor activity, and hypoactivity has been observed following acute injection (Stolerman et al., 1995; Miller et al., 2001a). However, the effect of repeated lobeline administration on locomotor activity has not been investigated. Interestingly, in a self-administration study using rats, acute lobeline (3 mg/kg) injection attenuated responding for *d*-methamphetamine and, in separate experiments, this dose of lobeline also attenuated responding for sucrose (Harrod et al., 2001). Following repeated lobeline administration, tolerance developed to the decrease in responding for sucrose; however, the lobeline-induced decrease in responding for *d*-methamphetamine persisted (Harrod et al., 2001). These results suggest that lobeline produced a nonspecific suppressant effect following acute administration, to which tolerance developed following repeated administration. However, the development of tolerance to the hypoactivity induced by lobeline following repeated injection has not been assessed directly and is important considering its potential contribution to the previously observed lobeline-induced attenuation in responding for psychostimulant drugs of abuse.

Based on neurochemical studies, which indicate that lobeline inhibits the effects of nicotine, the present study assessed the effect of lobeline on nicotine-induced changes in locomotor activity. Specifically, the present study determined if lobeline pretreatment inhibits the locomotor activation produced by repeated nicotine administration, when nicotine and lobeline were injected once daily for 12 consecutive days. To determine if lobeline disrupts the long-lasting adaptive changes associated with repeated nicotine administration, the effect of lobeline on the induction of sensitization to nicotine was determined. The present study also determined if tolerance develops to lobeline-induced hypoactivity, in order to ascertain if the lobeline attenuation of the effect of repeated nicotine was specific.

2. Materials and methods

2.1. Subjects

Seventy-two male Sprague–Dawley rats (200–225 g at the beginning of testing) from Harlan Laboratories (Indianapolis, IN) were housed two per cage with ad libitum access to food and water. All animal handling procedures were approved by the Institutional Animal Care and Use Committee at the University of Kentucky and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Apparatus

Locomotor activity was recorded automatically using an animal activity monitoring system with Digipro System software (AccuScan Instruments, Columbus, OH). The system consisted of six 42×42-cm and 30-cm-high clear acrylic chambers. Each chamber incorporated a horizontal 16×16 grid of photo beam sensors, with each beam 2.5 cm apart and 7.0 cm above the chamber floor. Horizontal activity was recorded for a 60-min period, comprised of twelve 5-min blocks. Activity was measured as photo beam interruptions and was expressed as total distance traveled (cm).

2.3. Drugs

S(−)-Nicotine di-*d*-tartrate (Research Biochemicals, Natick, MA) and lobeline hemisulfate (Sigma, St. Louis, MO) were dissolved in 0.9% wt/vol saline. The pH of the nicotine solution was adjusted to 7.4 with a sodium hydroxide solution (1 N). Lobeline dose represents salt weight, and nicotine dose represents the free base. Injection volume was 1 ml/kg body weight.

2.4. Effect of repeated lobeline and nicotine administration

Prior to the start of testing, rats were randomly assigned to four treatment groups (Saline–Saline, Lobeline–Saline, Saline–Nicotine, and Lobeline–Nicotine; $n = 12$/group). To habituate the rats to the testing procedure, on the first 2 days of the experiment, rats were weighed, injected subcutaneously with saline, and placed in the locomotor activity chamber for 10 min, followed by a second saline injection and placement in the chamber for 50 min. On the next 12 days (Days 1–12), rats received a subcutaneous injection, were placed in the chamber for 10 min, injected subcuta-

neously again, and returned to the chamber for 50 min. Rats in the Saline–Nicotine and Saline–Saline groups were injected with saline followed by nicotine (0.3 mg/kg) or saline, respectively. Rats in the Lobeline–Saline and Lobeline–Nicotine groups were injected with lobeline (3 mg/kg) followed by nicotine or saline, respectively.

The dose of nicotine was selected based on previous studies (Stolerman et al., 1973; Clarke and Kumar, 1983; Benwell and Balfour, 1992; Ksir, 1994; Miller et al., 2001b) that demonstrated hyperactivity in rats following repeated administration of nicotine (free base dose range 0.2–0.4 mg/kg). Thirty minutes following acute injection of nicotine (0.3 mg/kg, free base), concentrations of ~2 pmol/ml blood and ~1100 pmol/mg brain have been observed (Ghosheh et al., 2001). The dose of lobeline was selected based on our previous dose–response studies that demonstrated attenuation of *d*-amphetamine (0.1–1 mg/kg)-induced hyperactivity following acute lobeline (1–10 mg/kg) injection in rats (Miller et al., 2001a). Additionally, the dose (3 mg/kg) of lobeline selected was shown to attenuate *d*-methamphetamine self-administration in rats (Harrod et al., 2001).

To determine if lobeline inhibits the induction of sensitization to nicotine following a drug-free period, half of the rats ($n=6$) from each of the four groups were tested on Day 13. The other half of the rats from each of the four groups were not tested on Day 13, but remained in the animal colony for 28 days, and were subsequently tested on Day 40. On Days 13 and 40, the rats that were tested were administered only saline (no lobeline) and placed in the chamber for 10 min. Subsequently, rats in the Saline–Saline and Lobeline–Saline groups were administered saline, and rats in the Saline–Nicotine and Lobeline–Nicotine groups were administered nicotine, and then returned to the chamber for 50 min.

Contextual cues contribute to the sensitization associated with repeated drug administration (Post et al., 1981; Reid et al., 1998). Insofar as the administration of injections, placement of the rats in the test chambers, and other preinjection events may serve as conditional stimuli, it is reasonable to assume that reinstatement of such stimuli could play a contributory role in behavioral sensitization to nicotine. To assess the extent of conditioning and to determine if lobeline inhibits conditioned hyperactivity resulting from repeated nicotine injection, on Day 14 or 41, two subcutaneous injections of saline were administered to rats tested previously on Days 13 and 40, respectively, and locomotor activity was monitored.

2.5. Tolerance to lobeline-induced hypoactivity

To determine if tolerance developed to the hypoactivity induced by lobeline, separate groups of rats ($n=6$/group) were administered lobeline (1, 3, or 10 mg/kg sc) or saline in the animal colony once daily for 11 days. On the following day, rats were injected subcutaneously with lobeline or saline and placed in the locomotor activity chamber for 60 min. Thus, lobeline or saline was administered repeatedly in the home cage prior to the first exposure to the locomotor activity chamber. Lobeline doses were chosen based on the results from previous experiments, in which high doses (3–10 mg/kg) of lobeline produced hypoactivity following acute injection, whereas low doses (0.3–1 mg/kg) did not alter locomotor activity (Miller et al., 2001a).

2.6. Data analysis

Data collected on Days 1–12 were analyzed using a four-way repeated-measures analysis of variance (ANOVA) with Day and Time as within-subject factors and Lobeline and Nicotine injection as between-group factors. Data from each day were analyzed using three-way repeated-measures ANOVA with Time as a within-subject factor and Lobeline and Nicotine injection as between-group factors. The effect of lobeline on the induction of sensitization to nicotine following a drug-free period was assessed in separate groups of rats, with one group tested on Day 13 and the other group tested on Day 40. As such, data from Days 1 to 13 were analyzed using a four-way repeated-measures ANOVA with Time and Day as a within-subject factors and Lobeline and Nicotine injection as between-group factors, and a separate analysis was performed on data from Days 1 to 12 with data from Day 40. Conditioned hyperactivity to nicotine also was assessed in separate groups of rats, with one group tested on Day 14 and the other group tested on Day 41. As such, data from Days 1 to 14 were analyzed using a four-way repeated-measures ANOVA with Time and Day as within-subject factors and Lobeline and Nicotine injection as between-group factors, and a separate analysis was performed on data from Days 1 to 12 with data from Days 40 and 41. To determine if tolerance developed to repeated lobeline administration, locomotor activity data from the rats previously administered lobeline in the animal colony room were analyzed via two-way repeated-measures ANOVA with Lobeline injection as a between-groups factor and Time as a within-subjects factor. Where appropriate, main effect and Tukey post hoc analyses were performed ($P<.05$).

3. Results

3.1. Acute administration of lobeline and nicotine

Analysis of data from Day 1 (Fig. 1) revealed a significant Time × Lobeline × Nicotine interaction [$F(4,240)=3.76$, $P<.01$]. Acute nicotine injection significantly decreased locomotor activity in the Saline–Nicotine group. At the 20-, 35-, 40-, and 45-min time points, locomotor activity was less for the Saline–Nicotine group than for the Saline–Saline group. Acute lobeline injection also significantly decreased locomotion. Activity for the Lobeline–Saline group was decreased relative to activity for the

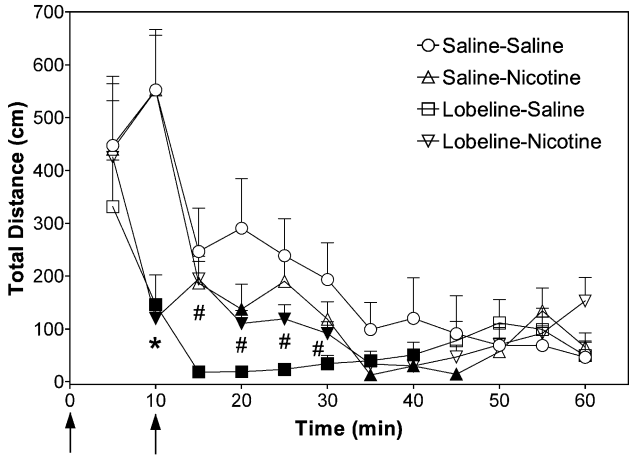

Fig. 1. Time course of the effect of lobeline, nicotine, and/or saline on locomotor activity on Day 1. Locomotor activity (mean ± S.E.M.) is expressed as total distance traveled (cm) during 5-min blocks across a 60-min session. Legend designation indicates first and second injection. Rats were administered lobeline (3 mg/kg sc) or saline, placed in the locomotor activity chamber for 10 min, and then injected with nicotine (0.3 mg/kg sc) or saline and returned to the chamber for 50 min. The left arrow designates the first injection (lobeline or saline) and the right arrow designates the second injection (nicotine or saline). Filled symbols indicate difference ($P < .05$) from the Saline–Saline group at each time point. $^{*}P < .05$ indicates Lobeline–Nicotine group different from Saline–Nicotine group at each time point. $^{\#}P < .05$ indicates Lobeline–Nicotine group different from Lobeline–Saline group at each time point.

Saline–Saline group at the 10- to 40-min time points. Prior to nicotine injection at the 10-min time point, activity for the Lobeline–Saline and Lobeline–Nicotine groups was less than activity for the Saline–Saline and Saline–Nicotine groups. After nicotine injection at the 15- to 30-min time points, activity for the Lobeline–Nicotine group was greater than that for the Lobeline–Saline group. From 15 to 60 min, activity for the Lobeline–Nicotine group did not differ from that for the Saline–Nicotine group. Thus, acute injection of either nicotine or lobeline alone produced hypoactivity, but these effects were not additive, as the combination of lobeline and nicotine produced less hypoactivity than lobeline alone. Moreover, acute lobeline did not alter the effect of nicotine on locomotor activity at the doses examined.

3.2. Repeated administration of lobeline and nicotine

Data from Days 1 to 12 are presented in Fig. 2 as total distance traveled during the 50-min period following nicotine injection. Analysis revealed a significant Day × Lobeline × Nicotine interaction [$F(11,660) = 2.09$, $P < .05$]. On Day 1, locomotor activity was decreased in the Saline–Nicotine group compared to the Saline–Saline group; however, on Days 2–12, activity was greater for the Saline–Nicotine group compared to the Saline–Saline group. Thus, acute administration of nicotine on Day 1 produced hypoactivity, but hyperactivity was evident following repeated nicotine injection on the subsequent days. Activity for the Lobeline–Nicotine group was greater on Days 3–12 than on Day 1, and was greater on Days 6–12 than on Days 1–5 [$F(11,165) = 27.58$, $P < .001$]. Thus, sensitization to nicotine was evident following repeated nicotine administration.

Post hoc analysis also revealed that locomotor activity was lower in the Lobeline–Saline group than in the Saline–Saline group on Days 1–2 and 5–7 (Fig. 2). However, on Days 3–4 and 8–12, no difference in locomotor activity was evident between these groups. Thus, lobeline produced hypoactivity, but tolerance appeared to develop to the decrease in activity following repeated lobeline administration.

Importantly, between-group comparisons revealed that lobeline pretreatment attenuated the hyperactivity produced by nicotine injection (Fig. 2). On Days 2–12, locomotor activity was lower in the Lobeline–Nicotine group than in the Saline–Nicotine group. Thus, although tolerance appeared to develop to the hypoactivity produced by lobeline, tolerance did not develop to the lobeline-induced attenuation of the nicotine-induced hyperactivity. It is notable, however, that lobeline did not inhibit nicotine-induced hyperactivity completely, or that it did not prevent sensitization to nicotine. On each of the 12 days, locomotor activity was greater for the Lobeline–Nicotine group than for the Lobeline–Saline group, and on Days 3–12, locomotor activity was greater for the Lobeline–Nicotine group than for the Saline–Saline group. Furthermore, for the Lobeline–Nicotine group, locomotor activity was greater on Days 3–12 than on Days 1–2. Thus, lobeline attenuated,

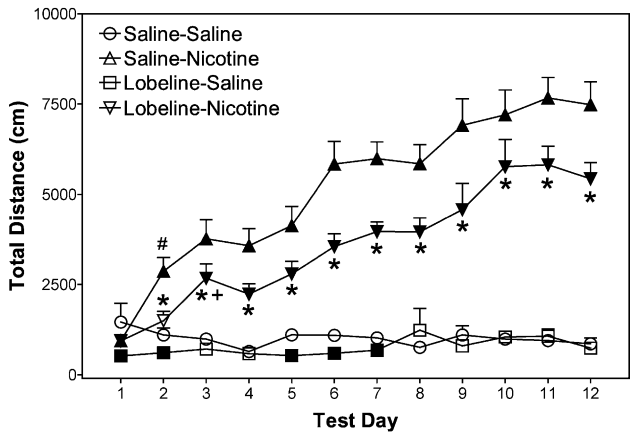

Fig. 2. Lobeline attenuates nicotine-induced hyperactivity following repeated injection. Locomotor activity (mean ± S.E.M.) is expressed as total cumulative distance traveled (cm) across a 50-min period after nicotine or saline injection. Legend designation indicates first and second injections. Rats were administered lobeline (3 mg/kg sc) or saline, placed in the locomotor activity chamber for 10 min, injected with nicotine (0.3 mg/kg sc) or saline, and returned to the chamber for 50 min. Filled symbols indicate difference ($P < .05$) from the Saline–Saline group at each time point. $^{*}P < .05$ indicates Lobeline–Nicotine group different from Saline–Nicotine group on each day. $^{\#}P < .05$ indicates activity on Days 2–12 different from Day 1 for the Saline–Nicotine group. $^{+}P < .05$ indicates activity on Days 3–12 different from Day 1 for the Lobeline–Nicotine group.

but did not completely inhibit, nicotine-induced hyperactivity across repeated injections.

3.3. Induction of sensitization to nicotine

The effect of lobeline on the induction of sensitization to nicotine was assessed on Days 13 and 40. Analysis of data from Days 1 to 12 and Day 13 revealed a significant Day × Time × Lobeline × Nicotine interaction [$F(4,112) = 3.38$, $P < .05$]. Simple main effect analyses and post hoc tests revealed that rats injected with nicotine on Day 13 showed greater locomotor activity than rats injected with saline on Day 13 (data not shown). No significant difference in activity on Day 13 was evident between the groups that were administered two injections of saline on Day 13 (Saline–Saline and Lobeline–Saline groups, data not shown). Furthermore, for rats administered nicotine, locomotor activity on Day 13 was greater than activity on Day 1 (compare Figs. 1 and 3). Importantly, although lobeline was not administered on Day 13, locomotor activity on Day 13 was less in the Lobeline–Nicotine group pretreated with lobeline on Days 1–12 than in the Saline–Nicotine group pretreated with saline on Days 1–12. Thus, previous repeated pretreatment with lobeline attenuated the locomotor response to an injection of nicotine, even in the absence of lobeline pretreatment, indicating that lobeline attenuated the induction of sensitization to nicotine.

Analysis of locomotor data from Days 1 to 12 and Day 40 revealed that for the Saline–Nicotine group, locomotor activity was greater on Day 40 than on Day 1, and activity on Day 40 was not significantly different from activity on Day 12, indicating that nicotine-induced hyperactivity and sensitization to nicotine persisted through a 28-day drug-free period (data not shown). Analysis of data from Day 40 revealed a significant main effect of Nicotine [$F(1,28) = 12.51$, $P < .01$] and a Time × Nicotine interaction [$F(4,112) = 9.30$, $P < .001$], but neither the main effect of Lobeline [$F(1,28) = 0.29$, $P = .59$] nor the Lobeline × Nicotine interaction [$F(4,112) = 1.87$, $P = .12$] was found to be significant. Administration of nicotine on Day 40 resulted in hyperactivity for both the Saline–Nicotine and Lobeline–Nicotine groups, but the Saline–Nicotine and Lobeline–Nicotine groups did not differ significantly. Thus, sensitization to nicotine persisted across a 28-day drug-free period, but the inhibitory effect of lobeline evident 1 day following the last injection was not evident after the prolonged drug-free period.

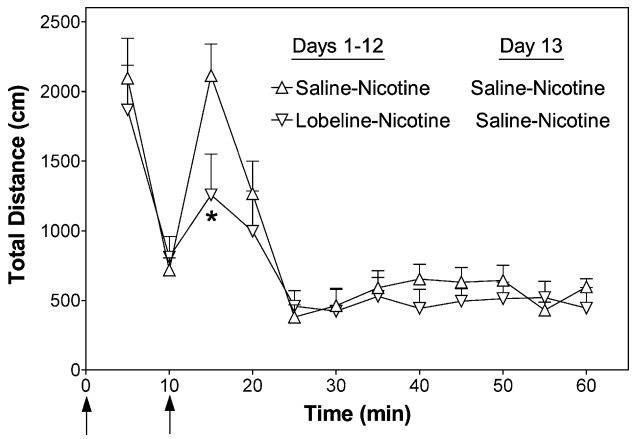

Fig. 3. Lobeline inhibits the induction of sensitization to nicotine following repeated nicotine injection (Day 13). Locomotor activity (mean ± S.E.M.) is expressed as total distance traveled (cm) during 5-min blocks across a 60-min session. Legend designation indicates the first and second injection on Days 1–12. On Day 13, following the first subcutaneous injection of saline, rats were placed in the locomotor activity chamber for 10 min, and following a second subcutaneous injection of nicotine (0.3 mg/kg sc) were returned to the chamber. The left arrow designates the first injection (saline) and the right arrow designates the second injection (nicotine). * $P < .05$ indicates difference between the Lobeline–Nicotine and Saline–Nicotine groups at the 15-min time point.

3.4. Conditioned hyperactivity

Conditioned hyperactivity was assessed on Days 14 and 41 following two injections of saline to each treatment group (data not shown). Analysis of data from Day 14 revealed a significant main effect of Nicotine [$F(1,28) = 32.64$, $P < .001$]. However, the main effect of Lobeline [$F(1,28) = 0.96$, $P = .34$], the Lobeline × Nicotine interaction [$F(1,28) = 0.014$, $P = .91$], and the Time × Lobeline × Nicotine interaction [$F(4,112) = 1.56$, $P = .19$] were not significant. Thus, when only saline was administered to rats on Day 14, greater activity was observed in rats that were administered nicotine on Days 1–13; however, lobeline pretreatment did not inhibit this conditioned hyperactivity.

Analysis of data from Day 41 also revealed a significant main effect of Nicotine [$F(1,28) = 6.29$, $P < .05$], although the main effect of Lobeline [$F(1,28) = 0.30$, $P = .59$], the Lobeline × Nicotine interaction [$F(1,28) = 0.10$, $P = .76$], and the Time × Lobeline × Nicotine interaction [$F(4,112) = 0.74$, $P = .56$] were not found to be significant. Thus, following a 28-day drug-free period, saline injection resulted in greater locomotor activity in rats previously administered nicotine than in rats previously administered saline. Lobeline pretreatment on Days 1–12 did not inhibit the conditioned hyperactivity to nicotine on Day 41.

3.5. Tolerance to lobeline-induced hypoactivity

Lobeline produced hypoactivity on Days 1–2 and 5–7 (Fig. 2). Tolerance appeared to develop to this effect of lobeline across days of administration, as locomotor activities for the Lobeline–Saline and Saline–Saline groups were not significantly different on Days 3–4 and 8–12 (Fig. 2). However, activity in the Saline–Saline group was low (less than 1000 cm traveled during a 60-min session). As such, lobeline-induced hypoactivity may have been obscured due to the low activity in the control group.

To ascertain if tolerance developed to lobeline-induced hypoactivity following repeated injection, rats were admin-

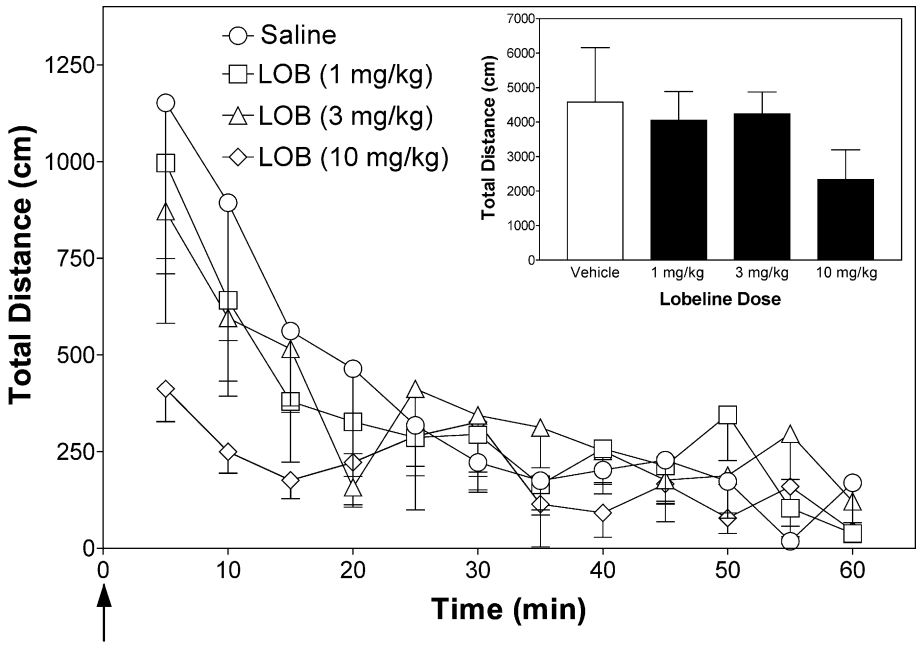

Fig. 4. Tolerance develops to the hypoactivity induced by lobeline (LOB). For 11 consecutive days, rats were administered once daily with LOB (1, 3, or 10 mg/kg sc) or saline in the colony room. On Day 12, the respective LOB dose was injected, and rats were immediately placed in the locomotor activity chamber for 60 min. Locomotor activity (mean ± S.E.M.) is expressed as total distance traveled (cm) during 5-min blocks across a 60-min period after LOB or saline injection. The arrow designates injection of LOB or saline. Inset illustrates locomotor activity (mean ± S.E.M.) expressed as total cumulative distance traveled (cm) across a 60-min period after LOB or saline injection.

istered lobeline (1, 3, or 10 mg/kg) or saline in the animal colony once daily for 11 days. On Day 12, lobeline was administered and locomotor activity was measured (Fig. 4). Although it appears that activity was lower in rats administered the high (10 mg/kg) lobeline dose than in rats administered either saline or the lower (1–3 mg/kg) doses of lobeline, neither the main effect of Lobeline injection [Fig. 4, inset; $F(3,20) = 0.932$, $P = .44$] nor the Lobeline × Time interaction [Fig. 4; $F(33,220) = 1.06$, $P = .39$] was found to be significant. Thus, tolerance developed to the hypoactivity induced by repeated lobeline injection.

4. Discussion

The present study demonstrates that following acute administration, lobeline produced hypoactivity and tolerance developed to this effect following repeated lobeline administration. Moreover, lobeline attenuated the hyperactivity induced by nicotine when both drugs were administered repeatedly. Importantly, tolerance did not develop to the lobeline-induced attenuation of hyperactivity induced by repeated nicotine administration. Thus, the lobeline-induced attenuation of nicotine-induced hyperactivity is a specific effect of lobeline.

In the present study, acute administration of nicotine resulted in hypoactivity, but hyperactivity was not evident during the 60-min session. It is possible that if behavior were monitored for a longer time period in the present study, hyperactivity would have been evident, although previous studies have shown hypoactivity followed by hyperactivity within a 60-min time course (Clarke and Kumar, 1983; Ksir, 1994; Miller et al., 2001b). The present study showed clearly that repeated nicotine injection produced hyperactivity, and sensitization was evident across consecutive days of nicotine injection. The hyperactivity to nicotine was long-lasting, as it persisted across a 28-day drug-free period, indicating that repeated nicotine administration produces enduring changes in behavior.

Similar to nicotine, lobeline produces hypoactivity in rats following acute administration (Stolerman et al., 1995; Miller et al., 2001a). The present study shows that tolerance developed to the hypoactivity induced by lobeline. Additionally, repeated lobeline administration did not produce hyperactivity, in contrast to nicotine. These results extend the results of previous studies that demonstrate that although lobeline and nicotine bind with high affinity to nicotinic receptors (Abood et al., 1988; Reavill et al., 1990; Bhat et al., 1991; Court et al., 1994), these two alkaloids do not have similar pharmacological profiles in vivo. For example, repeated nicotine treatment increases the number of nicotinic receptors in brain, whereas repeated treatment with lobeline does not (Bhat et al., 1991). Furthermore, in contrast to nicotine, lobeline does not produce conditioned place preference (Shoaib et al., 1994), does not generalize to nicotine in drug discrimination assays (Reavill et al., 1990), and is not readily self-administered (Rasmussen and Swedberg, 1998; Harrod et al., in press).

The most important finding from this study is that lobeline attenuated nicotine-induced hyperactivity across repeated injections and tolerance did not develop to this effect of lobeline. The underlying mechanism responsible for the attenuation of the effects of nicotine may be inhibition of nicotinic receptor function, as previous neurochemical research indicates that lobeline is a nicotinic receptor antagonist (Gallardo and Leslie, 1998; Miller et al., 2000). Other potential targets of lobeline action are the dopamine transporter and vesicular monoamine transporter (VMAT2), as lobeline inhibits [^{3}H]dopamine uptake into rat striatal synaptosomes and into rat striatal synaptic vesicles, as well as inhibits binding of [^{3}H]dihydroxytetrabenazine to VMAT2 vesicular preparations (Teng et al., 1997, 1998).

Alternatively, the attenuation of nicotine-induced hyperactivity may have been the result of a nonspecific decrease in activity produced by lobeline, and not due to an interaction with either nicotinic receptors or neurotransmitter transporters. However, in the experiment in which lobeline and nicotine were administered once daily and activity monitored subsequently, lobeline attenuated nicotine-induced hyperactivity on days when lobeline-induced hypoactivity was not observed. In the latter experiment, lobeline-induced hypoactivity may not have been detected due to a potential floor effect, since locomotor activity in the Saline–Saline control group was low across repeated test days. Nevertheless, in a subsequent experiment, tolerance to lobeline-induced hypoactivity was clearly shown to develop following repeated injection in the colony room, indicating that the attenuation of nicotine-induced hyperactivity was not due to locomotor-depressant effects of lobeline. Furthermore, the lobeline-induced attenuation of the induction of nicotine sensitization also does not support the explanation that the effect of lobeline is the result of a nonspecific lobeline-induced hypoactivity. Thus, the lobeline-induced attenuation of the locomotor-activating effect of nicotine is a specific effect, associated with alterations in the neural systems responsible for nicotine-induced hyperactivity.

When lobeline pretreatment was terminated and rats were injected with nicotine 1 day—but not 28 days—later, the attenuation of the locomotor response to nicotine was maintained. That is, on Day 13, when all rats were pretreated with saline, nicotine-induced hyperactivity was less in rats that were pretreated with lobeline on Days 1–12 compared to rats that were pretreated with saline on Days 1–12. The latter results suggest that lobeline attenuated the induction of sensitization to nicotine, but the long-lived adaptive response to repeated nicotine persisted longer than the inhibition resulting from lobeline pretreatment. Alternatively, the lobeline-induced attenuation of the induction of sensitization to nicotine observed on Day 13 may have been the result of a carryover effect of the lobeline injection on the preceding day. However, the alternative explanation is not supported by the $\sim$50-min plasma half-life of lobeline (F.H. Schneider, personal communication) and by the previously reported, short-lived decrease ($\sim$30 min) in *d*-methamphetamine self-administration in rats (Harrod et al., 2001). Thus, the present results support the hypothesis that lobeline attenuates the induction of sensitization to nicotine.

Previous studies have shown that contextual cues contribute to drug sensitization, such that administering injections, the test chamber environment, and other preinjection events serve as conditional stimuli (Post et al., 1981; Reid et al., 1998). In the present study, when conditioned hyperactivity was assessed, rats that were administered nicotine previously displayed greater activity than rats administered saline previously. Thus, a component of the hyperactivity and sensitization following repeated drug injection is the result of classical conditioning. Lobeline pretreatment did not significantly alter the conditioned hyperactivity, suggesting that the inhibition of nicotine by lobeline in the current study is not due to an inhibition of conditioning effects.

In summary, the present results demonstrate that lobeline attenuated the behavioral effects of nicotine following repeated administration. The lobeline-induced attenuation of nicotine-induced hyperactivity and sensitization may be due to inhibition of nicotinic receptors (Gallardo and Leslie, 1998; Miller et al., 2000) and/or alteration of presynaptic dopamine storage and release (Teng et al., 1997, 1998). Taken together, results from the current in vivo and previous in vitro studies suggest that lobeline may have utility as a novel pharmacotherapy for the treatment of psychostimulant abuse.

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