

Tropical Journal of Pharmaceutical Research August 2011; 10 (4): 421-429

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Available online at <http://www.tjpr.org>
<http://dx.doi.org/10.4314/tjpr.v10i4.7>

Research Article

Lobeline Attenuates the Locomotor-Activating Properties of Repeated Morphine Treatment in Rats

Dennis K Miller*, James E Polston, Kelli R Rodvelt and Matthew J Will

Department of Psychological Sciences, Translational Neuroscience Center and Bond Life Sciences Center,
University of Missouri, Columbia MO 65210, United States

Abstract

Purpose: Lobeline perturbs intra- and extracellular neurotransmitter levels and diminishes the *in vitro* and *in vivo* effects of psychostimulants. More recently, lobeline was shown to bind to μ opiate receptors, block the effects of opiate receptor agonists, and decrease heroin self-administration in rats. The present study determined the effect of lobeline on morphine-induced changes in locomotor behavior in rats.

Methods: For 12 consecutive days (Days 1 - 12), male rats were administered lobeline (0.3 or 1 mg/kg) followed by morphine (5 or 10 mg/kg) and locomotor activity was measured. On Day 13, the effect of lobeline on the expression of morphine-induced increases in activity was determined.

Results: With repeated morphine treatment, an increase in locomotor activity was observed. In a dose-dependent manner, lobeline decreased the morphine-induced increase in activity. Acute lobeline challenge on Day 13 also attenuated the expression of this morphine-induced increase in activity.

Conclusion: These results are consistent with previous work where lobeline blocks the locomotor-activating properties of psychostimulants, and these findings support an emerging literature suggesting that lobeline produces its behavioral effects through an interaction with μ opiate receptors.

Keywords: Behavior, Morphine, Locomotor activity, Behavioural sensitization, μ Opiate receptors

Received: 14 December 2010.

Revised accepted: 7 June 2011

*Corresponding author: **E-mail:** millerden@missouri.edu; **Tel:** +1-573-874-3502

INTRODUCTION

Lobeline is an alkaloid that is found in *Lobelia inflata*, a herbaceous plant that grows throughout the eastern and southern United States. It has long been used in a variety of medicinal preparations, including programs aimed at tobacco smoking cessation [1,2]. Lobeline and its analogs are currently being investigated as a treatment for methamphetamine addiction [3]. Historically, lobeline has been characterized as a cholinergic ligand as it binds to nicotinic acetylcholine receptors with relatively high (K_i value $\approx 0.005 - 0.04 \mu\text{M}$) affinity [4-6]. However, lobeline also binds to (K_i value $\approx 0.7 - 50 \mu\text{M}$) and inhibits the activity of the vesicular monoamine transporter (VMAT2) and the plasmalemmal dopamine transporter (DAT) [7,8].

Several preclinical studies have shown that lobeline reduces or blocks the *in vitro* and *in vivo* effects of abused psychostimulants [9-14]. In rat striatum, lobeline attenuated α -amphetamine- and nicotine-evoked dopamine release [12,13] and diminished methamphetamine-induced changes in dopamine storage [9]. In behavioral studies, lobeline attenuates psychostimulant-induced activation in several rodent models of drug addiction (e.g., locomotor activity, drug discrimination and self-administration) [10,11,13,14]. For example, lobeline (0.3 – 3 mg/kg) decreased methamphetamine self-administration [10]. In locomotor activity studies in rats, lobeline (0.3 – 3 mg/kg) attenuated cocaine- and nicotine induced hyperactivity after acute and repeated (12 days) stimulant treatment [11,14].

Recently, our laboratory demonstrated that lobeline diminishes the effects of μ opiate receptor agonists, possibly *via* an interaction with μ opiate receptors in brain [15]. In that study, lobeline displaced D-Ala₂, NME-Phe₄, Gly₅-ol-enkephalin (DAMGO) binding (K_i value $\approx 0.74 \mu\text{M}$) in guinea pig brain homogenates, inhibited a DAMGO-induced potassium current in μ opiate receptors expressed in oocytes, and attenuated

morphine-evoked dopamine release in rat striatum. Overall, these *in vitro* results [15] suggest that lobeline functions as a μ opiate receptor antagonist. Regarding the impact of lobeline on the behavioral effects of μ opiate receptor agonists, Hart and colleagues recently showed that lobeline (1 – 3 mg/kg) decreased heroin (18 $\mu\text{g}/\text{kg}/\text{infusion}$) self-administration in rats [16].

The goal of the present study was to further research on the interaction of lobeline with μ opiate receptors by determining if lobeline diminishes morphine-induced changes in locomotor activity after acute and repeated drug treatment to rats. Previous studies have shown that morphine produces a slight decrease, a slight increase or no change in locomotor activity with acute injection [17-19], while increased locomotor activity is observed with repeated morphine treatment [20-22].

EXPERIMENTAL

Animals

The University of Missouri Institutional Animal Care and Use Committee approved the study, which was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* [23]. Male Sprague-Dawley rats (Harlan, Indianapolis IN, USA) were housed two per cage in a room controlled for temperature (maintained at $\sim 21^\circ\text{C}$) and humidity (maintained at 40-70%). Rats were allowed *ad libitum* access to standard chow (Teklad, Harlan, Indianapolis IN, USA) and tap water. The animal facility was maintained on a 12-h/12-h light/dark cycle and behavioral testing was conducted during the cycle's light phase.

Drugs

Lobeline sulfate was purchased from Acros Organics (Geel, Belgium) and morphine sulfate pentahydrate was purchased from Sigma (St. Louis MO, USA). Both were prepared in saline (0.9 %w/v) and the

Table 1: Group assignment and injection regimen for Days 1 - 12

Group	First Injection	Second Injection	n
Saline-Saline	Saline	Saline	14
Saline-Morphine	Saline	Morphine (5 mg/kg)	16
	Saline	Morphine (10 mg/kg)	15
Lobeline-Saline	Lobeline (0.3 mg/kg)	Saline	13
	Lobeline (1.0 mg/kg)	Saline	10
Lobeline-Morphine	Lobeline (0.3 mg/kg)	Morphine (5 mg/kg)	8
	Lobeline (0.3 mg/kg)	Morphine (10 mg/kg)	8
	Lobeline (1.0 mg/kg)	Morphine (5 mg/kg)	9
	Lobeline (1.0 mg/kg)	Morphine (10 mg/kg)	8

injection volume was 1 ml solution/kg body weight. Drug doses represent free base weight.

Procedures

Locomotor activity was monitored automatically using Med Associates' (Georgia VT, USA) Open Field Test Environment (ENV-515) monitors. Each monitor surrounded an acrylic cage (43.2 x 43.2 x 30.5 cm), and each monitor and cage were housed in a sound-resistant cubicle (ENV-017M). Data were collected using Med Associates' Open Field Activity Software (SOF-811) that recorded the number of monitor sensor breaks. The software computed sensor break data to a measure of distance traveled (in cm). This type of equipment was used in previous studies of lobeline's effects on locomotor behavior [14]. On two days prior to beginning of drug treatment, rats were injected (s.c.) with saline, placed in the monitor for 20 min, injected (i.p.) with saline, and returned to the monitor for 60 min. This was done to acclimatize the rats to experimental procedures.

On the next 12 consecutive days (Days 1 - 12), rats received their first injection (saline or lobeline, s.c.), were placed in the monitor for 20 min, received their second injection (saline or morphine, i.p.), and were returned to the monitor for 60 min. Table 1 presents the group assignments that designated the first

(i.e., saline or lobeline) and second (i.e., saline or morphine) injection received on Days 1 - 12 and the drug doses.

On Days 1– 12, rats in the Saline-Saline group received saline for both the first and the second injection. For rats in the Saline-Morphine groups, the first injection was saline and the second injection was morphine (5 or 10 mg/kg). For rats in the Lobeline-Saline groups, the first injection was lobeline (0.3 or 1 mg/kg) and the second injection was saline. For rats in the Lobeline-Morphine groups, the first injection was lobeline (0.3 or 1 mg/kg) and the second injection was morphine (5 or 10 mg/kg). Rats received the same injection combination on Days 1 – 12.

On Day 13, a follow-up experiment was performed to determine if an acute lobeline injection (challenge) alters the increased activity observed with repeated morphine treatment. On Day 13 half (n = 7 - 8 rats) of the rats in the Saline-Morphine groups received saline followed by their regular morphine (5 or 10 mg/kg) injection. The other half (n = 8 rats) of the rats in the Saline-Morphine groups received lobeline (0.3 mg/kg) followed by their regular morphine (5 or 10 mg/kg) treatment. On Day 13 half (n = 7 rats) of the rats in the Saline-Saline group received two saline injections, and the other half (n = 7 rats) received lobeline (0.3 mg/kg) injection followed by a saline injection.

Data analyses

The dependent measure for all analyses was distance traveled (in cm), which was collected in 5-min intervals. For all analyses, significance was established *a priori* as $p < 0.05$. Data from the entire 80-min period on each day (Days 1 - 12) were analyzed *via* 4-way repeated measures analysis of variance (ANOVA). In this analysis Day (Days 1 - 12) and Time (sixteen 5-min intervals, 80 min) were within-subject factors and Lobeline Dose (0 [saline], 0.3 and 1 mg/kg) and Morphine Dose (0 [saline], 5 and 10 mg/kg) were between-group factors. A second ANOVA was performed on data from the 60-min period after the second injection (morphine or saline) with Day and Time (twelve 5-min intervals, 60 min) as within-subject factors and Lobeline Dose and Morphine Dose as between-group factors. Main effect analyses and Tukey post hoc analyses were then performed to determine between-group differences on each day and to examine within-subject differences across days. To determine if lobeline challenge alters morphine-induced hyperactivity on Day 13, data between saline and lobeline treatment conditions were compared *via* t-tests.

RESULTS

Analysis of distance traveled data from the entire 80-min period on Day 1 revealed a significant main effect of Lobeline Dose ($F(1,38) = 5.67, p < 0.05$) and a Lobeline Dose x Time interaction ($F(15,570) = 7.30, p < 0.001$). Figure 1 presents distance traveled for rats in the Saline-Saline and Lobeline (0.3 and 1 mg/kg)-Saline groups on Day 1. Post hoc tests showed that there was less activity for the Lobeline (1 mg/kg)-Saline group than for the Saline-Saline group at the 5 and 10 min time points. There were no differences between the Lobeline (0.3 mg/kg)-Saline group and the Saline-Saline group at any time point. Thus, the high--but not the low--lobeline dose briefly decreased locomotor activity after acute injection.

Neither the main effect of Morphine Dose, the Morphine Dose x Time, nor the Lobeline Dose x Morphine Dose x Time interaction,

were significant on Day 1. Thus, these morphine doses did not alter activity after acute administration.

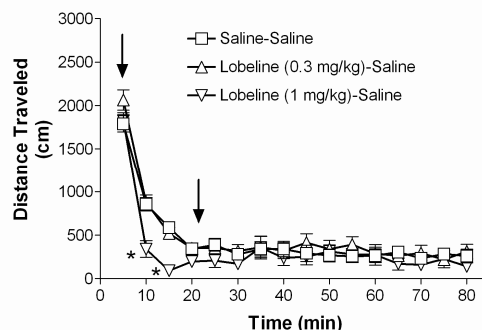


Figure 1: Data represent mean (\pm S.E.M) distance traveled. The left arrow indicates the first (saline or lobeline) injection and the right arrow indicates the second (saline) injection. Asterisks indicate a significant ($p < 0.05$) difference from the Saline-Saline group at the respective time point.

The next series of analyses examined the effect of lobeline across days. Figure 2 depicts total distance traveled during the entire 80-min session on Days 1 - 12 for rats in the Saline-Saline and Lobeline (0.3 and 1 mg/kg)-Saline groups. Analysis determined that neither the main effect of Lobeline Dose nor the Lobeline Dose x Day interaction were significant. These results suggest that lobeline produced a decrease in activity on the first day (Day 1, Figure 1), it did not alter locomotor activity (i.e., produce an increase or decrease in activity) after repeated drug treatment.

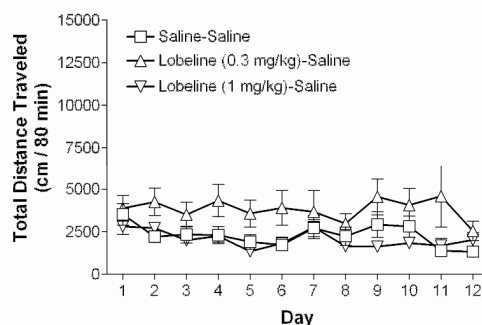


Figure 2: Data represent mean (\pm S.E.M) total distance traveled for the entire 80-min session.

To assess the interaction between lobeline and morphine with repeated administration, data from the 60-min period after the second injection (morphine or saline) on Days 1 - 12 were analyzed. A significant Lobeline Dose x Morphine Dose x Day interaction ($F(11,418) = 2.43, p < 0.01$) was found.

Figure 3 depicts total distance traveled during the 60-min period after the second (saline or morphine) injection for rats in the Saline-Saline and Saline-Morphine (5 and 10 mg/kg) groups. Between-group comparisons revealed that rats in the Saline-Morphine (5 mg/kg) group were more active than rats in the Saline-Saline group on Days 3 - 12. A within-group analysis for the Saline-Morphine (5 mg/kg) group revealed that activity was greater on Days 4 - 12 compared to Day 1. Between-group analyses revealed that rats in the Saline-Morphine (10 mg/kg) group were more active than rats in the Saline-Saline group on Days 2 - 12. Within-group analysis revealed greater activity for the Saline-Morphine (10 mg/kg) group on Days 5 and 8 - 12 than on Day 1. Thus, repeated morphine treatment resulted in increased locomotor activity.

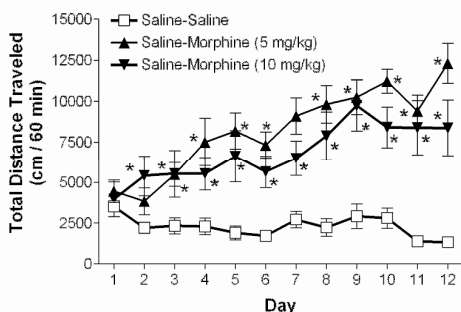


Figure 3: Data represent mean (\pm S.E.M) total distance traveled for the 60-min period after the second (saline or morphine) injection. Asterisks designate a significant ($p < 0.05$) difference from the Saline-Saline group at the respective day.

Figure 4 depicts total distance traveled during the 60-min period after the second (morphine) injection for rats that received the lower (5 mg/kg) morphine dose. Rats in the Lobeline (0.3 mg/kg)-Morphine (5 mg/kg) group were less active than rats in the Saline-

Morphine (5 mg/kg) group on Days 7 and 9 - 12. Rats in the Lobeline (1 mg/kg)-Morphine (5 mg/kg) group were less active than rats in the Saline-Morphine (5 mg/kg) group on Days 5, 7 and 9 - 12. As such, lobeline attenuated the increased activity observed with repeated morphine (5 mg/kg) treatment.

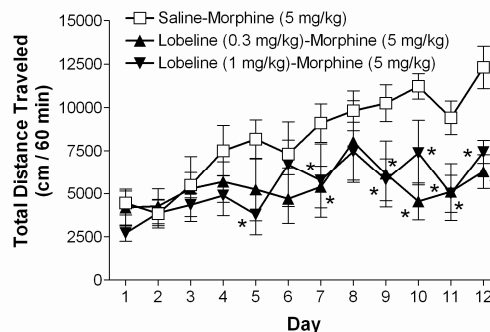


Figure 4: Data represent mean (\pm S.E.M) total distance traveled for the 60-min period after the second (morphine) injection. Asterisks designate a significant ($p < 0.05$) difference from the Saline-Morphine group at the respective day.

Figure 5 depicts total distance traveled during the 60-min period after the second (morphine) injection for rats that received the higher (10 mg/kg) morphine dose. Rats in the lobeline (0.3 mg/kg)-Morphine (10 mg/kg) group were less active than rats in the Saline-Morphine (10 mg/kg) group on Day 9. Rats in the Lobeline (1 mg/kg)-Morphine (10 mg/kg) group were less active than rats in the Saline-Morphine (10 mg/kg) group on Days 8, 9 and 11. Overall, these data indicate lobeline attenuated the increased activity observed with repeated morphine (10 mg/kg) treatment.

The effect of acute lobeline challenge on morphine-induced increases in locomotor activity was determined on Day 13. Figure 6 depicts total distance traveled during the 60-min period after the second (saline or morphine) injection. There were no significant differences in activity between rats in the Saline-Saline group that were administered saline twice and those challenged with 0.3 mg/kg lobeline followed by saline. Regarding the Saline-Morphine (5

mg/kg) group, rats challenged with lobeline (0.3 mg/kg) followed by 5 mg/kg morphine were less active than rats administered saline followed by morphine (5 mg/kg, $t(15) = 4.11$, $p < 0.05$). Regarding the higher morphine dose (10 mg/kg), there were no significant differences between the group of rats administered saline followed by morphine (10 mg/kg) and those challenged lobeline (0.3 mg/kg) followed by morphine (10 mg/kg, data not shown). Thus, acute lobeline challenge attenuated morphine (5 mg/kg)-induced hyperactivity.

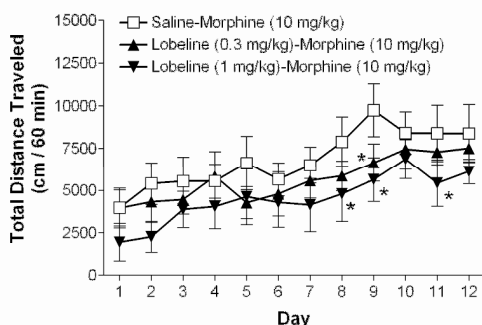


Figure 5: Data represent mean (\pm S.E.M) total distance traveled for the 60-min period after the second (morphine) injection. Asterisks designate a significant ($p < 0.05$) difference from the Saline-Morphine group at the respective day.

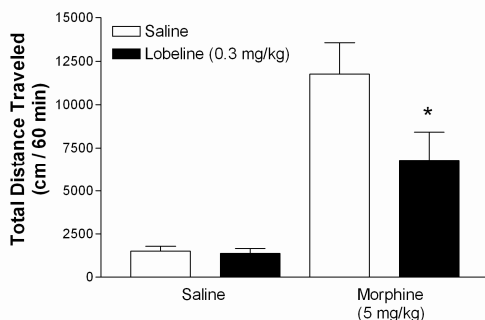


Figure 6: Data represent mean (\pm S.E.M) distance for the 60-min period after the second (saline or morphine) injection. Asterisks designate a significant ($p < 0.05$) difference from the Saline-Morphine group

DISCUSSION

The present study determined the effect of lobeline on changes in locomotor activity after acute and repeated morphine treatment. It follows previous experiments where lobeline blocked the effects of opiate receptor agonists *in vitro* [15] and diminished heroin self-administration [16].

Recently, Harrod and Van Horn reported a lobeline-induced decrease in locomotor activity in rats, but tolerance developed to this inhibition with repeated lobeline treatment [24]. Similar findings were observed presently, where the 1 mg/kg lobeline dose decreased activity, relative to the saline-treated group, during the first 15 min on Day 1, but no other differences were observed.

In previous studies, acute lobeline treatment diminished the drug-induced increase in locomotor activity observed after acute cocaine and *D*-amphetamine administration [13,14]. The interaction of acute lobeline and morphine treatment could not be determined presently because acute morphine treatment did not significantly change activity (i.e., it did not increase or decrease activity). Others have shown that acute morphine treatment in rats, within the range tested here (5 – 10 mg/kg), decreases activity during the first 60 – 90 min after parenteral administration [17], while others report that increased activity is observed after initial morphine injection [18,19]. An important factor in the initial response to morphine is the context in which the opiate is administered. Palone and colleagues [19] demonstrated a larger response to morphine when the drug was administered in a novel environment, than when the drug was administered in a familiar one [19]. Presently, rats were acclimated (i.e., habituated) to the monitors for two consecutive days prior to acute morphine treatment.

With repeated morphine treatment, a dose-dependent increase in activity was observed in morphine-treated rats, relative to saline-

treated rats, consistent with other morphine locomotor activity studies [18,21,22]. Most research investigating activity with repeated drug treatment focuses on the development of sensitization [25]. We are hesitant to define the present results in the realm of sensitization, as morphine was ineffective to increase locomotor behavior at the first presentation. However, the observation of increasing activity with repeated opiate treatment likely reflects the processes (e.g., change in glutamate and dopamine communication) described in the sensitization literature [26]. The lobeline challenge (Day 13) results suggest that lobeline attenuates the expression of morphine sensitization.

In a dose-dependent manner, lobeline attenuated the morphine-induced increases in activity observed after repeated (Day 1 - 12) drug treatment. With 5 mg/kg morphine, the 1 mg/kg lobeline dose attenuated morphine's effects to a greater degree than the 0.3 mg/kg lobeline dose. A similar lobeline dose-dependent inhibition was observed with 10 mg/kg morphine. However, lobeline's inhibitory effect was less pronounced with 10 mg/kg morphine than with 5 mg/kg morphine. This suggests that the lobeline (putative antagonist) inhibition was surmounted by increasing the morphine (agonist) dose, which is characteristic of pharmacological antagonism. This supports *in vitro* work suggesting lobeline is a μ opiate receptor antagonist [15].

Others have shown that opiate receptors modulate morphine's effects on activity [18]. Opiate receptor antagonism is one possible mechanism for lobeline to produce the present results. However, lobeline interacts with other neural targets associated with drug-induced behaviors within the same concentration range (K_i value \approx 0.7 – 50 μ M for DAT and VMAT2 [7, 8]) or at lower concentrations (K_i value \approx 0.005 – 0.05 μ M for nicotinic acetylcholine receptors [5, 6]) than where lobeline has affinity for μ opiate receptors (K_i value \approx 0.7 μ M [15]). Lobeline's affinity for nicotinic acetylcholine receptors is

a likely target, as these receptors have a modulatory role in morphine's effects on rodent behavior [27,28]. Biala and Staniak (2010) recently reported that nicotinic receptor agonists and antagonists attenuated locomotor cross-sensitization between nicotine and morphine in mice [27].

The present study and previous work with lobeline [3, 29] suggest that lobeline may have clinical potential as a treatment for managing narcotic abuse and dependence. Lobeline decreases heroin self-administration behavior [16], which models drug-taking behavior in humans. Various lobeline analogs have been reported in the literature [6] and these ligands may produce greater inhibition of opiate effects. More experiments are required--including studies on lobeline's interaction with the physical and psychological withdrawal symptoms that contribute to opiate dependence--to understand lobeline's potential as an addiction treatment.

CONCLUSION

Morphine produced a marked increase in locomotor activity with repeated treatment. Lobeline, a ligand with a multifaceted pharmacological profile, attenuated the morphine-induced increase in locomotor activity with repeated opiate treatment and in an acute lobeline challenge. These findings are consistent with lobeline's activity as a putative μ opiate receptor antagonist and its ability to diminish the effects of μ opiate receptor agonists *in vitro* and *in vivo*.

ACKNOWLEDGEMENT

This research was supported by a grant from the Missouri Research Board. The authors appreciate the contributions of Dr. James Kracke (University of Missouri Department of Anesthesiology) and Dr. John Lever (University of Missouri Department of Radiology) in the development of this research.

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