Lobeline Inhibits the Neurochemical and Behavioral Effects of Amphetamine

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α-Lobeline (lobeline), a major alkaloidal constituent of Indian tobacco (Lobelia inflata), has pharmacological effects in common with nicotine (Teng et al., 1997) and until only recently was thought to be an agonist at nicotinic receptors (Abood et al., 1988; Grady et al., 1992; Stolerman et al., 1995; Lecca et al., 2000). Nicotine and lobeline have high affinity (Kᵢ = 4–30 nM) for nicotinic receptor binding sites (Abood et al., 1988; Reavill et al., 1990; Bhat et al., 1991; Court et al., 1994). Whereas repeated nicotine administration up-regulates nicotinic receptors in brain, repeated administration with lobeline does not result in nicotinic receptor up-regulation (Bhat et al., 1991). No obvious structural resemblance between lobeline and nicotine is apparent; and structure-activity studies do not suggest a common pharmacophore (Barlow and Johnson, 1989).

Similar to nicotine, lobeline evokes ³H overflow from rat striatal slices preloaded with [³H]dopamine ([³H]DA) (Teng et al., 1997). However, lobeline does not appear to act as an agonist at nicotinic receptors on dopaminergic presynaptic terminals, since lobeline-induced ³H overflow is calcium-independent and mecamylamine-insensitive (Teng et al., 1997). Recently, lobeline-evoked [³H]norepinephrine overflow from the sympathetic nerve terminal innervating the vas deferens was also shown to be calcium-independent and mecamylamine-insensitive (Santha et al., 2000), suggesting that this effect of lobeline was not mediated by nicotinic receptors, consistent with Teng et al. (1997). Recently, administration of nicotine or lobeline (1 or 3.5 mM) via a dialysis probe was reported to increase endogenous DA levels in striatal microdialysate in rats (Lecca et al., 2000). These results are also consistent with in vitro findings that high concentrations (100 μM) of lobeline evoke endogenous DA overflow from rat striatal slices (Teng et al., 1997). Lecca et al. (2000) interpreted their results as indicative of lobeline-induced nicotinic receptor activation, since the increase in DA levels was partially inhibited by mecamylamine (also

ABSTRACT

Lobeline interacts with the dopamine transporter and vesicular monoamine transporter, presynaptic proteins involved in dopamine storage and release. This study used rodent models to assess lobeline-induced inhibition of the neurochemical and behavioral effects of amphetamine. Rat striatal slices were preloaded with [³H]dopamine and superfused with lobeline for 30 min, and then with d-amphetamine (0.03–3.00 μM) plus lobeline for 60 min. As predicted, lobeline (1–3 μM) intrinsically increased ³H overflow but did not inhibit d-amphetamine-evoked ³H overflow. Consequently, the effect of lobeline on d-amphetamine-evoked endogenous dopamine and dihydroxyphenylacetic acid overflow was assessed. Lobeline (0.1–1 μM) inhibited d-amphetamine (1 μM)-evoked dopamine overflow but did not inhibit electrically evoked ³H overflow, indicating a selective inhibition of this effect of d-amphetamine. To determine whether the in vitro results translated into in vivo inhibition, the effect of lobeline (0.3–10.0 mg/kg) pretreatment on d-amphetamine (0.1–1.0 mg/kg)-induced hyperactivity in rats and on d-methamphetamine (0.1–3.0 mg/kg)-induced hyperactivity in mice was determined. Doses of lobeline that produced no effect alone attenuated the stimulant-induced hyperactivity. Lobeline also attenuated the discriminative stimulus properties of d-methamphetamine in rats. Acute, intermittent, or continuous in vivo administration of lobeline (1–30 mg/kg) did not deplete striatal dopamine content. Thus, lobeline inhibits amphetamine-induced neurochemical and behavioral effects, and is not toxic to dopamine neurons. These results support the hypothesis that lobeline redistributes dopamine pools within the presynaptic terminal, reducing pools available for amphetamine-induced release. Collectively, the results support a role for lobeline as a potential pharmacotherapy for psychostimulant abuse.
administered in high concentration via the dialysis probe). However, pretreatment with lobeline before nicotine did not result in nicotinic receptor desensitization, as would be anticipated if lobeline were acting as a nicotinic receptor agonist. On the other hand, the lack of lobeline-induced receptor desensitization is consistent with a nicotinic receptor antagonist mechanism of action for lobeline.

Nevertheless, lobeline has high affinity for nicotinic receptors in brain (Abood et al., 1988; Reavill et al., 1990; Bhat et al., 1991; Court et al., 1994), and therefore, interacts with these sites. Lobeline does not evoke rubidium efflux from preloaded striatal and thalamic synaptosomes, as would be expected from a nicotinic receptor agonist (Terry et al., 1998; Miller et al., 2000). Moreover, lobeline recently has been shown to inhibit $S(-)-$nicotine-evoked $^3$H overflow from $[^3]$HIDA-preloaded rat striatal synaptosomes (Miller et al., 2000), to inhibit $S(-)-$nicotine-evoked dopamine overflow in nucleus accumbens microdialysis (Benwell and Balfour, 1998), to inhibit $S(-)-$nicotine-evoked rubidium efflux from preloaded rat thalamic synaptosomes (Miller et al., 2000), to inhibit nicotine-evoked $[^3]$Hnorepinephrine release from cultured rat locus coeruleus cells (Gallardo and Leslie, 1998), and to inhibit stimulation of $a_4b_2$ nicotinic receptors expressed in Xenopus oocytes (Damaj et al., 1997). Thus, based on these converging lines of evidence, lobeline more likely acts as an antagonist, rather than as an agonist, at both $a_3b_2$- and $a_4b_2$-containing nicotinic receptors.

The molecular mechanism by which amphetamine enhances DA release has been extensively investigated. $d$-Amphetamine evokes endogenous DA overflow from superfused striatal slices (Parker and Cubeddu, 1986; Dwoskin et al., 1988) via reversal of the DA transporter (DAT) (Fischer and Cho, 1979; Liang and Rutledge, 1982), via an interaction with the reserpin site on the vesicular monoamine transporter (VMAT2) (Peter et al., 1994; Pifl et al., 1995; Sulzer et al., 1995), and via disruption of the vesicular proton gradient as a consequence of its weak basicity and high lipophilicity (Barlow and Johnson, 1989). These $d$-amphetamine effects result in increased DA release from the vesicle into the cytosol, redistributing DA stores and ultimately enhancing the cytosolic DA concentration available for reverse transport via DAT (Philippu and Beyer, 1973; Ary and Komiskey, 1980; Liang and Rutledge, 1982). In addition, $d$-amphetamine inhibits monoamine oxidase (MAO) (Mantle et al., 1976; Miller et al., 1980), preventing metabolism of cytosolic DA to dihydroxyphenylacetic acid (DOPAC), further augmenting the cytosolic DA pool.

Lobeline also alters DA storage and release, but in a manner different from $d$-amphetamine. In contrast to $d$-amphetamine, lobeline evokes DOPAC overflow, rather than DA overflow (Teng et al., 1997, 1998), suggesting that lobeline does not inhibit MAO, but nevertheless redistributes DA storage within the presynaptic terminal. Lobeline only weakly inhibits $[^3]$HIDA uptake into rat striatal synaptosomes (Teng et al., 1997), but potently interacts with the tetrahydrobenazine site on VMAT2 to inhibit vesicular DA uptake (Peter et al., 1994; Erickson et al., 1996; Teng et al., 1998). $d$-Amphetamine is equipotent in inhibiting DA uptake and promoting DA release from synaptic vesicles (Philippu and Beyer, 1973; Liang and Rutledge, 1982; Pifl et al., 1995; Sulzer et al., 1995; Erickson et al., 1996). In contrast, lobeline more potently inhibits DA uptake than it evokes DA release from the vesicle to redistribute presynaptic DA storage (Teng et al., 1997, 1998). Thus, lobeline is predicted to inhibit the neurochemical and behavioral effects of amphetamine.

The reinforcing effects of psychomotor stimulants are thought to result from enhanced $d$-amphetamine-induced DA release (Lyness et al., 1979; Carr and White, 1983; Hoebel et al., 1983; Wise and Bozarth, 1987; Hiroi and White, 1991). Amphetamine-induced hyperactivity is a reliable assay reflecting the rewarding properties and addictive potential of psychostimulants (Wise and Bozarth, 1987; Koob, 1992; Robinson and Berridge, 1993). Furthermore, the subjective effects of stimulants are important determinants of their abuse potential (Evans and Johanson, 1987; Childress et al., 1988). Drugs that block the discriminative stimulus effects of stimulants in rats are predicted to attenuate the subjective effects of stimulants in humans (Holtzman, 1990).

The present study determined whether lobeline selectively inhibits $d$-amphetamine-evoked DA release from rat striatal slices, $d$-amphetamine- and $d$-methamphetamine-induced hyperactivity, and the discriminative stimulus effects of $d$-methamphetamine. $d$-Amphetamine and $d$-methamphetamine produce similar psychostimulant effects (Kuczenski et al., 1995). To provide an initial assessment of nigrostriatal toxicity, the effect of lobeline administration on striatal DA content was also determined.

**Materials and Methods**

**Drugs.** Lobeline [25,6R,8S-(+)-lobeline] was purchased from ICN (Costa Mesa, CA) as the sulfate salt. $d$-Amphetamine sulfate, $d$-methamphetamine hydrochloride, and pargyline hydrochloride were purchased from Sigma (St. Louis, MO). L-Ascorbic acid, D-glucose, and TS-2 tissue solubilizer were purchased from AnaRA (BHD Ltd., Poole, UK), Aldrich Chemical Company (Milwaukee, WI), and Research Products International (Mount Prospect, IL), respectively. $[^3]$HIDA (3,4-ethyl-2-[N$^3$H]dihydroxyphenylethylamine; specific activity 25.6 Ci/mmol) was purchased from NEN Life Science Products (Boston, MA). All other chemicals were purchased from Fisher Scientific (Pittsburgh, PA). For in vivo administration, drug doses refer to salt weight.

**Subjects.** For neurochemical and locomotor activity assays, male Sprague-Dawley rats (225–250 g; Harlan, Indianapolis, IN) were housed two per cage with ad libitum access to food and water. Male Swiss-Webster mice (32–39 g; Taconic Farms, Germantown, NY) were housed five per cage with ad libitum access to food and water, and were also used in locomotor activity experiments. For the drug discrimination experiments, male Sprague-Dawley rats (280–350 g; Charles River, Wilmington, MA) were housed individually, and their body weights were gradually reduced to ~80% of free-feeding weight by limiting daily access to food. Water was available ad libitum. All animals were housed in a temperature- and humidity-controlled room and were maintained on a 12:12 h light/dark cycle (lights on at 7:00 AM) in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care. Experiments were conducted during the light phase. Experimental protocols involving the animals were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by respective Institutional Animal Care and Use Committees.

**$^3$H Overflow Superfusion Assay.** The inhibitory effect of lobeline on $d$-amphetamine-evoked $^3$H overflow from rat striatal slices preloaded with $[^3]$HIDA was determined using previously published methods (Dwoskin and Zahniser, 1986). Briefly, rats were killed by rapid decapitation. Striatum was dissected and within 2 min placed into ice-cold Krebs’ buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl$_2$, 1 mM NaH$_2$PO$_4$, 1.3 mM CaCl$_2$, 11.1 mM glucose, 25 mM
NaHCO₃, 0.11 mM l-ascorbic acid, and 0.004 mM EDTA, pH 7.4, saturated with 95% O₂, 5% CO₂). Coronal striatal slices (500 μm, 6–8 mg) were incubated for 30 min in Krebs' buffer at 34°C in a metabolic shaker. Slices were incubated in fresh buffer containing 0.1 μM [³H]DA (6–8 slices/3 ml) for an additional 30 min. After rinsing, each slice was transferred to a glass superfusion chamber maintained at 34°C and was superfused at 1 ml/min with oxygenated Krebs' buffer containing pargyline (10 μM), which remained in the buffer until the end of the experiment. Concentrations of lobeline were chosen based on our previous results from DA release and uptake studies (Teng et al., 1997, 1998). After 30 min, a concentration of d-amphetamine (0.03–1.0 μM) was added to the buffer of individual chambers, and superfusion continued for an additional 60 min. Using a repeated-measures design, striata from a single rat were used to determine the concentration effect of lobeline in the absence and presence of d-amphetamine, and the concentration effect of d-amphetamine in the absence and presence of lobeline. Due to the limited number of superfusion chambers and of striatal slices that can be obtained from a single rat, the effect of lobeline on d-amphetamine-evoked ³H overflow was determined in three series of experiments. The first series assessed the ability of low concentrations (0.1–0.3 μM) of lobeline to inhibit d-amphetamine (0.1–1.0 μM)-evoked ³H overflow. The second series assessed the inhibitory effect of higher concentrations (1.0–3.0 μM) of lobeline to inhibit d-amphetamine (0.1–3.0 μM)-evoked ³H overflow. Each superfusion chamber was exposed to only one concentration of lobeline and one concentration of d-amphetamine. Additionally, one striatal slice in each experiment was superfused in the absence of lobeline and field stimulated at 60, 300, or 600 pulses, and served as the control condition. Each slice was transferred to a glass superfusion chamber maintained at 34°C and was superfused at 1 ml/min with oxygenated Krebs' buffer containing pargyline (10 μM). After 60 min of superfusion, two 5-min samples (5 ml) were collected to determine basal ³H outflow. After collection of the second basal sample, striatal slices from an individual rat were superfused for 30 min in the absence or presence of a single concentration of lobeline (1–30 μM), which remained in the buffer until the end of the experiment. Subsequently, electrical stimulation was applied and superfusate samples were collected for an additional 60 min. For each of the two series of experiments, the number of pulses was a between-group factor, and lobeline concentration was a within-subjects factor.

**Endogenous Overflow Assay.** Striata were prepared and superfused as described previously (Gerhardt et al., 1989). To assess the role of MAO on endogenous DA overflow, Krebs' buffer was prepared in the absence or presence of pargyline (10 μM), and slices were superfused for 60 min before the collection of the basal samples. To determine the ability of lobeline to inhibit d-amphetamine, lobeline (0.1–1.0 μM) was added to the superfusion buffer of individual chambers after collection of the third basal superfusate sample (1-min sample collected at 5-min intervals). Superfusion with lobeline for 30 min was followed by superfusion for an additional 30-min period either in the absence of presence of d-amphetamine (1 μM), which was added to the buffer containing lobeline. One-minute samples were collected at 5-min intervals during the period of d-amphetamine exposure. The absence or presence of pargyline was a between-groups factor and lobeline concentration was a within-subjects factor. An aliquot part (50 μl) of each 1-ml superfusate sample was then injected onto the high pressure liquid chromatography with electrochemical detection system, which consisted of a Beckman model 116 high pressure liquid chromatography pump (Beckman, Fullerton CA), a Beckman model 504 autosampler, an ESA ODS ultrasphere C₁₈ reverse-phase column (4.6 cm × 75 mm, 3-μm particle size; ESA, Bedford, MA), and an ESA 5100A coulometric electrochemical detector with a model 5011 detector cell (E₁ = +0.05 V, E₂ = +0.32 V). The eluent was 0.07 M citrate/0.1 M acetate buffer (pH 4) containing 50 mg/dl disodium EDTA, 100 mg/l octylsulfonic acid-sodium salt, and 7% methanol. All separations were performed at room temperature at a flow rate of 2 ml/min. Complete separation of DA and DOPAC and re-equilibration of the system required 5 min. Retention times of DA and DOPAC standards were used to identify relevant peaks. Peak heights were used to calculate detected amounts on the basis of standard curves. The detection limits were 1 and 2 pg/50 μl for DA and DOPAC, respectively.

**Locomotor Activity Assay.** To assess lobeline inhibition of d-amphetamine-induced hyperactivity, an Omitech Digiscan Animal Activity Monitoring System with Digipro software (AccuScan Instruments Inc., Columbus, OH) was used to automatically record activity. This system used acrylic chambers measuring 42 × 42 × 30 cm high and incorporated horizontal sensors in a 16 × 16 horizontal photoemitter grid. Photobeams were placed 2.5 cm apart and 7.0 cm above the floor of the chamber. On the first 2 days of the experiment, rats were habituated to transport from the colony room to the test room, and placed in the apparatus for 50 min. At the end of each session, rats were removed from the activity chamber and returned to the colony room, and the apparatus was washed thoroughly with a mild soap solution. On the third day following transport to the test room, rats were injected with lobeline (s.c.), returned to the housing cage, 15 min later injected with d-amphetamine (s.c.), and immediately placed in the activity monitor for 50 min. Each rat was administered either vehicle (0.9% w/v saline, 1 ml/kg) or one of four doses of lobeline (0.3–10.0 mg/kg) followed by vehicle or one of three doses of d-amphetamine (0.1–1.0 mg/kg). Rats were as-
signed randomly to 16 groups comprised of 4 lobeline doses (0.0, 0.3, 1.0, 3.0 mg/kg) × 4 d-amphetamine doses (0.0, 0.1, 0.3, 1.0 mg/kg). To assess the effects of a higher dose of lobeline on d-amphetamine-induced hyperactivity, three additional groups were administered lobeline (10.0 mg/kg) followed by d-amphetamine (0.0 or 1.0 mg/kg). In these experiments, both lobeline and d-amphetamine doses were between-group factors.

The ability of lobeline to inhibit d-methamphetamine-induced hyperactivity in mice was also assessed. In these experiments, mice were injected with lobeline (s.c.) and returned immediately to the housing cage. Mice were administered d-methamphetamine (i.p.) 45 min later and immediately placed in the activity monitor for 60 min. Mice were assigned randomly to 20 groups. Each mouse was administered either vehicle (0.9% w/v saline, 1 ml/kg) or one of three doses of lobeline (1.0–10.0 mg/kg) followed by vehicle or one of four doses of d-methamphetamine (0.1–3.0 mg/kg). In these experiments, both lobeline and d-methamphetamine doses were between-group factors.

**Drug Discrimination Assay.** Rats were trained to discriminate d-methamphetamine (1.0 mg/kg) from saline using standard operant chambers (Coulbourn Instruments, Lehigh Valley, PA) as previously described (Munzar and Goldberg, 1999). Each chamber contained two levers, separated by a recessed tray in which a dispenser delivered 45-mg food pellets (F0021; Bioserv, Frenchtown, NJ). Each press of a lever (force 0.4 N through 1 mm) was recorded as a response and was accompanied by the audible click of a relay. Behavioral response and event presentations were controlled by MED-PC software (Med Associates Inc., East Fairfield, VT). The rats were administered (i.p.) d-methamphetamine or saline and returned to the home cage. Fifteen minutes following injection, rats were placed in the operant chamber, and a white house light was illuminated. Responses on one lever after an injection of the training dose of d-methamphetamine (1.0 mg/kg) and responses on the other lever after an injection of saline vehicle (1.0 ml/kg) resulted in food pellet delivery under a fixed-ratio 10 schedule. Food pellet delivery initiated a 45-s time-out period, during which the chamber was dark and responding had no programmed consequence. Responses on the incorrect lever had no programmed consequence, other than to reset the response number requirement on the correct lever. After each time-out, the house light was again illuminated and the next trial began. Each session ended after completion of 20 trials or 30 min, whichever occurred first. The right lever was assigned as correct following d-methamphetamine for half of the rats, and the left lever was assigned as correct for the other half of the rats. Discrimination-training sessions were conducted 5 days each week under a double alternation schedule (i.e., DDSSDDSS, etc., with D, drug, and S, saline). Training continued until eight consecutive sessions occurred in which 90% of the responses were emitted on the correct lever, and no more than four responses were emitted on the incorrect lever during the first trial.

Generalizations induced by d-methamphetamine (0.1–1.0 mg/kg i.p.), lobeline (1.8–10.0 mg/kg s.c.), and the combination of d-methamphetamine and lobeline were assessed. Lobeline was injected 45 min before d-methamphetamine, which was injected 15 min before the session. Test sessions were identical to training sessions, with the exception that 10 consecutive responses on either lever ended a trial. Responding on one lever and then the other reset the response requirement. Experiments were conducted on Tuesdays and Fridays, if the requirement of the discrimination criteria were met on the two preceding training sessions. Generalization data were expressed as the percentage of the total responses emitted on the d-methamphetamine-appropriate lever. Response-rate data were expressed as response per second averaged over the session, with responding during time-out periods not included in the analysis.

**DA and DOPAC Content Assay.** To assess the potential for toxicity to DA neurons, DA and DOPAC content was determined following acute, intermittent, or continuous lobeline administration to rats. For acute experiments, lobeline (1–30 mg/kg) or vehicle was administered (s.c.), and 1 h later, striata were obtained for determination of endogenous DA and DOPAC content. For intermittent administration, lobeline (3 and 10 mg/kg) or vehicle was administered (s.c.) once daily for 10 days. Twenty-four hours after the final injection, striata were obtained for determination of endogenous DA and DOPAC content. For continuous administration, an osmotic minipump (ALZET 2 ml4 model; ALZA Corporation, Palo Alto, CA) containing 152 mg/ml lobeline or vehicle was implanted (s.c.) under the scapula during ether anesthesia. A flow rate of 2.5 µl/h delivered lobeline (30 mg/kg/day) or vehicle continuously for a 21-day period. Striata were obtained for endogenous DA and DOPAC content determination 21 days after osmotic minipump implantation.

**Data Analysis.** Three series of experiments were conducted to assess the effect of lobeline on d-amphetamine-evoked [3H]overflow from rat striatal slices preloaded with [3H]DA. For each series of experiments, lobeline-evoked [3H]overflow was determined in the absence of d-amphetamine via one-way repeated measures ANOVA. Lobeline inhibition of d-amphetamine-evoked [3H]overflow was analyzed for each series of experiments via two-way repeated measures ANOVA, with d-amphetamine concentration and lobeline concentration as within-subject factors. For each series of experiments, lobeline inhibition of electrically evoked [3H]overflow was analyzed via two-way repeated measures ANOVA, with lobeline concentration as a within-subject factor and number of pulses as a between-group factor. Endogenous DA and DOPAC overflow was analyzed via three-way repeated measures ANOVA, with lobeline concentration and d-amphetamine as within-subject factors and pargyline as a between-group factor. The effect of lobeline on d-amphetamine- or d-methamphetamine-induced hyperactivity was analyzed via two-way ANOVA, with d-amphetamine dose and lobeline dose as between-group factors. To assess generalization of lobeline to d-methamphetamine, drug discrimination data were analyzed using one-way repeated measures ANOVA. To assess lobeline-induced inhibition of d-methamphetamine generalization, discrimination data were analyzed by two-way repeated measures ANOVA. To determine the effects of lobeline on striatal content, separate one-way repeated measures ANOVAs were performed for DA and DOPAC data, and for acute, intermittent, and continuous administration, with lobeline concentration as a within-subject factor. Where appropriate, Tukey’s post hoc tests and simple main effect analyses were performed (p < 0.05).

**Results**

**Interaction of Lobeline and d-Amphetamine in the [3H]Overflow Assay.** Striatal slices were superfused for 30 min with lobeline (0.1–3.0 µM) followed by 60 min with lobeline plus d-amphetamine (0.03–1.0 µM), and [3H]overflow was determined. Data presented in Fig. 1 and Table 1 illustrate [3H]overflow during the first and second periods of superfusion, respectively. Thus, Fig. 1 illustrates that lobeline increased [3H]overflow from rat striatal slices preloaded with [3H]DA. In the first series of experiments in which low concentrations (0.1–0.3 µM) of lobeline were assessed, a significant main effect of lobeline concentration was not evident (p > 0.05). However, in the second series of experiments in which higher concentrations (1–3 µM) of lobeline were assessed, a significant main effect of lobeline concentration was found (F(2,14) = 9.09, p < 0.01). Post hoc tests indicated that 1 and 3 µM lobeline increased [3H]overflow above control (0 µM lobeline). In the third series of experiments, in which intermediate concentrations of lobeline (0.1–1.0 µM) were assessed, a significant main effect of lobeline concentration was observed (F(3,20) = 4.30, p < 0.05), and post hoc tests revealed
that 1 μM lobeline increased 3H overflow above control. Thus, the higher concentrations of lobeline (1–3 μM) intrinsically increased 3H overflow from [3H]DA-preloaded striatal slices.

Table 1 shows that lobeline (0.1–3.0 μM) did not inhibit d-amphetamine-evoked 3H overflow from rat striatal slices preloaded with [3H]DA. In the first series of experiments, low concentrations (0.1–0.3 μM) of lobeline were assessed for their ability to inhibit d-amphetamine (0.1–1.0 μM)-evoked 3H overflow. A significant main effect of d-amphetamine concentration was found ($F = 142.36, p < 0.001$), and Tukey’s post hoc tests revealed that d-amphetamine increased 3H overflow in a concentration-dependent manner. However, neither the main effect of lobeline concentration nor the d-amphetamine × lobeline interaction was significant ($p > 0.05$). In the second series of experiments in which the ability of lobeline concentration to inhibit d-amphetamine-evoked 3H overflow was assessed, a significant main effect of d-amphetamine concentration was again found ($F_{3,42} = 63.91, p < 0.001$), and post hoc tests revealed that d-amphetamine (0.1–1.0 μM) increased 3H overflow in a concentration-dependent manner. However, neither the main effect of lobeline concentration nor the d-amphetamine × lobeline interaction was significant ($p > 0.05$). In the third series of experiments, intermediate concentrations of lobeline (0.1–1.0 μM) and a low concentration of d-amphetamine (0.03 μM) were assessed. A significant main effect of d-amphetamine was found ($F_{1,20} = 17.62, p < 0.001$), indicating that 0.03 μM d-amphetamine increased 3H overflow relative to buffer alone. However, neither the main effect of lobeline concentration nor the d-amphetamine × lobeline interaction was significant ($p > 0.05$). Thus, lobeline did not inhibit d-amphetamine-evoked 3H overflow from rat striatal slices preloaded with [3H]DA. However, the inhibitory properties of lobeline may have been masked due to the intrinsic activity of lobeline, to the possibility that [3H]DA incompletely compartmentalizes within the DA presynaptic terminal, or that 3H overflow does not accurately reflect endogenous DA overflow.

**Lobeline Inhibits d-Amphetamine-Evoked Endogenous DA and DOPAC Overflow.** To assess the latter possibilities, endogenous DA and DOPAC overflow were assessed during a 30-min period of superfusion with lobeline (0.1–1.0 μM) in the absence or presence of pargyline (10 μM), followed by a 30-min period of superfusion in the absence and presence of d-amphetamine (1.0 μM), which was added to the buffer. Experiments were conducted in the absence or presence of pargyline to assess the role of MAO in the lobeline-induced inhibition. Table 2 and Fig. 2 illustrate DA and DOPAC overflow during the latter 30-min period of superfusion. Regarding DA overflow, the overall analysis of the data revealed a significant main effect of d-amphetamine ($F_{1,8} = 7.08, p < 0.05$), a lobeline concentration × pargyline interaction ($F_{3,24} = 3.99, p < 0.05$), a lobeline concentration × d-amphetamine interaction ($F_{3,24} = 4.13, p < 0.05$), and a lobeline concentration × d-amphetamine × pargyline interaction ($F_{3,24} = 3.65, p < 0.05$). Regarding DOPAC overflow, the overall analysis of the data revealed significant main effects of d-amphetamine ($F_{1,9} = 5.60, p < 0.05$), lobeline ($F_{3,27} = 3.45, p < 0.05$), and pargyline ($F_{1,9} = 6.21, p < 0.05$), and a d-amphetamine × pargyline interaction ($F_{1,9} = 7.18, p < 0.05$), a lobeline concentration × pargyline interaction ($F_{3,27} = 3.56, p < 0.05$), a lobeline concentration × d-amphet-

**TABLE 1**

<table>
<thead>
<tr>
<th>d-Amphetamine (μM)</th>
<th>0</th>
<th>0.1</th>
<th>0.3</th>
<th>1.0</th>
<th>3.0</th>
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<td>0.03</td>
<td>0.48 ± 0.16a</td>
<td>1.20 ± 0.91b</td>
<td>1.91 ± 0.52b</td>
<td>3.52 ± 0.97b</td>
<td>10.6 ± 6.67b</td>
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<tr>
<td>0.3</td>
<td>4.69 ± 1.09a</td>
<td>8.68 ± 0.89a</td>
<td>7.38 ± 1.39a</td>
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<tr>
<td>1.0</td>
<td>19.4 ± 1.73a</td>
<td>23.9 ± 2.85a</td>
<td>23.9 ± 4.17a</td>
<td>27.0 ± 5.50a</td>
<td></td>
</tr>
</tbody>
</table>

N.D., not determined.

Data are mean ± S.E.M. total 3H overflow expressed as a percentage of tissue tritium. *p < 0.05, different from the 0 μM lobeline/0 μM d-amphetamine condition. N = 6–21 rats/condition.
TABLE 2

Lobeline produces a concentration-dependent effect on DA and DOPAC overflow in the presence of pargyline (10 μM), but not in its absence.

Data represents results from superfusion with lobeline (0.1–1.0 μM) for 30 min in the absence or presence of pargyline (10 μM). Subsequently, slices were superfused for an additional 30 min in the absence or presence of d-amphetamine (1 μM), which was added to buffer containing lobeline in the absence or presence of pargyline. The present data represent the results from slices superfused in the absence of d-amphetamine. Lobeline inhibition of d-amphetamine-evoked overflow is shown in Fig. 2.

<table>
<thead>
<tr>
<th>Pargyline (μM)</th>
<th>Lobeline (μM)</th>
<th>DA</th>
<th>DOPAC</th>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>19.1 ± 8.1*</td>
<td>73.4 ± 26.3</td>
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<tr>
<td>0</td>
<td>0.1</td>
<td>4.2 ± 4.2</td>
<td>61.3 ± 43.0</td>
</tr>
<tr>
<td>0</td>
<td>0.3</td>
<td>0.0 ± 0.0</td>
<td>76.8 ± 62.6</td>
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<tr>
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<td>24.0 ± 24.0</td>
<td>280.7 ± 92.1*</td>
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<tr>
<td>10</td>
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<td>21.2 ± 14.8</td>
<td>161.1 ± 12.9*</td>
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<tr>
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<td>10.8 ± 7.9</td>
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<td>16.8 ± 12.3</td>
</tr>
<tr>
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<td>1.0</td>
<td>22.1 ± 22.1</td>
<td>7.3 ± 7.3</td>
</tr>
</tbody>
</table>

* Data are mean ± S.E.M. total overflow (pg/mg). *p < 0.05, different from 0 μM pargyline/0 μM lobeline condition. † p < 0.05, different from the 0 μM pargyline/0 μM lobeline and the 0.1–0.3 μM lobeline conditions. N = 5–7 rats/condition.

amine interaction ($F_{3,27} = 5.75, p < 0.01$), and a lobeline concentration × d-amphetamine × pargyline interaction ($F_{3,27} = 6.35, p < 0.01$). Subsequent simple main effect analyses and post hoc tests were conducted to elucidate significant between-group and within-subject differences.

As presented in Table 2, in the absence of d-amphetamine, lobeline (0.1–1.0 μM) did not evoke significant ($p > 0.05$) amounts of DA overflow in either the absence or presence of pargyline ($p > 0.05$). However, for DOPAC overflow, significant main effects of lobeline concentration ($F_{3,27} = 5.42, p < 0.05$) and pargyline ($F_{3,9} = 7.01, p < 0.05$), and a significant lobeline concentration × pargyline interaction ($F_{3,27} = 6.18, p < 0.01$) were found in the absence of d-amphetamine (Table 2). As expected, in the absence of pargyline, lobeline significantly decreased DOPAC overflow. In the absence of pargyline, lobeline (1.0 μM) increased DOPAC overflow relative to control and to 0.1 to 0.3 μM lobeline. However, in the presence of pargyline, lobeline did not increase DOPAC overflow. Thus, superfusion with pargyline prevented the increase in DOPAC overflow evoked by 1.0 μM lobeline.

Figure 2 illustrates DA and DOPAC overflow following addition of d-amphetamine (1.0 μM) to the buffer, and the lobeline (0.1–1.0 μM)-induced inhibition of the effect of d-amphetamine in the absence or presence of pargyline. Regarding DA overflow, a significant main effect of lobeline concentration ($F_{3,24} = 4.90, p < 0.01$) and a significant lobeline concentration × pargyline interaction ($F_{3,24} = 5.19, p < 0.01$) were found; however, a main effect of pargyline was not observed ($p > 0.05$). In the absence of lobeline, d-amphetamine (1.0 μM) increased DA overflow by ~5-fold either in the absence or presence of pargyline (compare Fig. 2 with Table 2). In the absence of pargyline, 0.3 and 1.0 μM lobeline inhibited (~53%) d-amphetamine-evoked DA overflow. In the presence pargyline, 0.1 and 0.3 μM lobeline inhibited d-amphetamine-evoked endogenous DA overflow by ~90%. Interestingly, when d-amphetamine was added to the buffer containing 0.1 μM lobeline, DA overflow was greater in the absence than in the presence of pargyline. Regarding DOPAC overflow (Fig. 2, bottom), neither the main effect of lobeline concentration nor the main effect of lobeline concentration × pargyline interaction were significant ($p > 0.05$). In the absence of lobeline, this concentration (1.0 μM) of d-amphetamine did not alter DOPAC overflow compared with control (compare Fig. 2 with Table 2).

Taken together, the highest concentration (1.0 μM) of lobeline examined increased DOPAC overflow only in the absence of pargyline, but otherwise had no effect alone (Table 2). The concentration (1.0 μM) of d-amphetamine tested significantly increased DA overflow, but did not alter DOPAC overflow (Fig. 2; Table 2). Importantly, low concentrations of lobeline (0.1–0.3 μM) that had no effect alone (Table 2) significantly inhibited d-amphetamine-evoked endogenous DA overflow, either in the absence or presence of pargyline (Fig. 2).

Lobeline Decreases $^{3}$H Overflow Evoked by Electrical-Field Stimulation. $^{3}$H]-DA-preloaded striatal slices were superfused for 30 min in the absence or presence of
lobeline (1–30 μM), and were subsequently field stimulated with 60, 300, or 600 electrical pulses (1 Hz stimulation for 1, 5, or 10 min, respectively) (Table 3). In the first series of experiments, we determined the effect of lobeline (1–30 μM) on $^3$H overflow evoked by 60 or 300 pulses. A main effect of pulse number was found indicating that $^3$H overflow was greater following 300-pulse compared with 60-pulse stimulation ($F_{2,8} = 42.15, p < 0.001$). However, neither the main effect of lobeline concentration ($p > 0.05$) nor the lobeline concentration $\times$ pulse number interaction ($p > 0.05$) was significant. During application of the electrical-field stimulation, current (0.16–0.24 mA) did not differ significantly among the groups ($p > 0.05$). The effect of lobeline at 600 pulses was analyzed using simple main effect analyses. A significant main effect of lobeline concentration was found ($F_{4,17} = 5.72, p < 0.01$). Tukey’s post hoc tests revealed that 10 and 30 μM lobeline significantly decreased electrically evoked $^3$H overflow, relative to the 0 to 3 μM lobeline conditions. Thus, low concentrations of lobeline (0.1–3.0 μM) did not inhibit electrically evoked $^3$H overflow after stimulation with a range of applied pulses.

**Lobeline Attenuates d-Amphetamine and d-Methamphetamine-Induced Hyperactivity.** The ability of lobeline (0.3–10.0 mg/kg s.c.) to inhibit d-amphetamine (0.1–1.0 mg/kg s.c.)-induced hyperactivity was assessed in rats by administering lobeline 15 min before d-amphetamine and immediate placement in the activity monitor for 50 min (Fig. 3). Significant main effects of d-amphetamine dose ($F_{3,85} = 91.84, p < 0.001$) and lobeline dose ($F_{3,85} = 17.44, p < 0.001$), and a significant lobeline dose $\times$ d-amphetamine dose interaction ($F_{10,85} = 2.93, p < 0.01$) were obtained. In the absence of lobeline pretreatment, d-amphetamine (0.3–3.0 mg/kg) produced a significant increase in activity relative to rats administered vehicle ($F_{3,22} = 20.47, p < 0.001$). Further-
more, in the absence of d-amphetamine, pretreatment with high doses of lobeline (3.0–10.0 mg/kg) produced a significant decrease in activity relative to low doses of lobeline (0.3 and 1.0 mg/kg) ($F_{2,23} = 7.89, p < 0.001$). Regarding the lobeline \times d-amphetamine interaction, lobeline pretreatment resulted in an overall attenuation (~30%) of d-amphetamine-induced hyperactivity. High doses of lobeline (3 and 10 mg/kg) attenuated (~80%) the hyperactivity induced by d-amphetamine (0.3 and 1.0 mg/kg, respectively). However, these high doses of lobeline produced hypoactivity when administered alone, suggesting that the inhibition of d-amphetamine-induced hyperactivity may have been the result of the general depressant effect produced by high doses of lobeline. Importantly, a dose of lobeline (1.0 mg/kg), which did not alter activity, attenuated the hyperactivity produced by d-amphetamine (0.3 mg/kg, $F_{3,20} = 12.38, p < 0.001$). The time course of the lobeline-induced inhibition of hyperactivity produced by d-amphetamine is shown in Fig. 3 (insert). A dose of lobeline (1.0 mg/kg) that alone had no effect on activity (not different from saline control group) clearly inhibited the hyperactivity induced by d-amphetamine (0.3 mg/kg) ($F_{4,96} = 3.19, p < 0.05$). Thus, lobeline attenuated d-amphetamine-induced hyperactivity at a dose of lobeline that was behaviorally inactive.

To assess the ability of lobeline (1.0–10.0 mg/kg s.c.) to inhibit d-methamphetamine (0.1–3.0 mg/kg i.p.-induced hyperactivity, mice were administered lobeline 45 min before injection of d-methamphetamine and were placed immediately in the activity monitor for 60 min. Significant main effects of lobeline dose ($F_{3,138} = 4.51, p < 0.01$) and d-methamphetamine dose ($F_{4,138} = 80.96, p < 0.001$), and a significant lobeline dose \times d-methamphetamine dose interaction ($F_{12,138} = 2.48, p < 0.05$) were found. In the absence of lobeline pretreatment, d-methamphetamine (1.0–3.0 mg/kg) produced a significant increase in activity relative to mice administered vehicle ($F_{4,34} = 37.28, p < 0.001$). In contrast to the data observed using rats, lobeline did not produce hypoactivity at any dose tested ($p > 0.05$). Lobeline (10.0 mg/kg) pretreatment attenuated (~35%) the hyperactivity induced by d-methamphetamine (1.0 mg/kg) ($F_{2,28} = 3.14, p < 0.05$). The time course of the lobeline-induced inhibition of hyperactivity produced by d-methamphetamine is shown in Fig. 4 (insert). A dose of lobeline (10.0 mg/kg) that alone had no effect on activity (not different from saline control group) significantly inhibited the hyperactivity induced by d-methamphetamine (1.0 mg/kg) ($F_{4,102} = 5.06, p < 0.05$). Thus, similar to the locomotor activity experiment using rats, lobeline attenuated d-methamphetamine-induced hyperactivity, at a dose of lobeline that was behaviorally inactive in mice.

**Lobeline Attenuates the Discriminative-Stimulus Properties of d-Methamphetamine**

Initially, the ability of lobeline to substitute for d-methamphetamine was determined in rats. When administered in the absence of d-methamphetamine, lobeline (1.8–10.0 mg/kg) did not produce generalization to the discriminative stimulus of d-methamphetamine (1.0 mg/kg). Thus, lobeline produced predominantly saline-appropriate responding when administered alone. Furthermore, only administration of a high dose (10.0 mg/kg) of lobeline significantly inhibited response rates when given alone in these generalization experiments ($F_{4,32} = 6.18, p < 0.001$) (data not shown).

To determine whether lobeline (3.0 and 5.6 mg/kg) attenuated the discriminative stimulus properties of d-methamphetamine (0.1–1.0 mg/kg), rats were pretreated with lobeline 45 min before administration of d-methamphetamine (Fig. 5). Pretreatment with lobeline (3.0 mg/kg) before d-methamphetamine did not shift the d-methamphetamine generalization dose-response curve ($p > 0.05$). However, following pretreatment with a higher lobeline dose (5.6 mg/kg), the d-methamphetamine generalization dose-response curve was significantly shifted down ($F_{1,16} = 7.85, p < 0.05$). Thus, lobeline inhibited the interoceptive effects of d-methamphet-

![Fig. 4. Lobeline attenuates d-methamphetamine-induced hyperactivity in mice.](https://jpet.aspetjournals.org/)

![Fig. 4. Lobeline attenuates d-methamphetamine-induced hyperactivity in mice.](https://jpet.aspetjournals.org/)
inhibitory effect was obscured in the $^3$H overflow experiments. The lobeline-induced inhibition of $d$-amphetamine-evoked DA overflow was selective, since these lobeline concentrations did not inhibit electrically evoked overflow across a range of applied pulses, and inhibition was observed at concentrations of lobeline that alone did not alter overflow. The lobeline-induced inhibition of the effect of amphetamine observed in vitro translated into an in vivo effect. At behaviorally inactive doses, lobeline pretreatment attenuated both $d$-amphetamine- and $d$-amphetamine-induced hyperactivity. Lobeline also attenuated the $d$-amphetamine discriminative stimulus at doses that did not generalize to the interoceptive cues of $d$-amphetamine and did not decrease response rates. Furthermore, in vivo administration of lobeline over a wide dose range did not deplete striatal DA or DOPAC content, indicating that lobeline does not inhibit amphetamine via dopaminergic toxicity.

Both $d$-amphetamine and lobeline alter DA storage and release; however, $d$-amphetamine releases DA from the presynaptic terminal via reversal of DAT (see Introduction). As expected, in the present study, $d$-amphetamine increased DA overflow, either in the absence or presence of pargyline. However, in the absence of pargyline, the low concentration of $d$-amphetamine examined did not significantly inhibit MAO, as evidenced by the continued presence of DOPAC in superfusate. On the other hand, lobeline evokes endogenous DOPAC overflow from superfused rat striatal slices in a concentration-dependent manner (Teng et al., 1997), indicating that lobeline and $d$-amphetamine have different mechanisms of action. Furthermore, lobeline-induced endogenous DA overflow was only detected when concentrations of lobeline approached 100 μM (Teng et al., 1997). This study was conducted in the absence of pargyline in the superfusion buffer, indicating that lobeline does not inhibit MAO. Similarly, in the present study, the highest concentration (1 μM) of lobeline evoked DOPAC overflow, but did not increase DA overflow. Taken together, these findings indicate that lobeline redistributes presynaptic DA stores to increase the availability of DA for metabolism, which results in an increased DOPAC overflow. Importantly, in the present study, lobeline did not evoke DA or DOPAC overflow when pargyline was included in the superfusion buffer. Thus, when MAO was inhibited, lobeline did not release DA into the extracellular space, suggesting that lobeline does not reverse the direction of DA transport, and that unlike amphetamine, lobeline does not appear to be a DAT substrate. However, lobeline does inhibit $[^3]$HIDA uptake into striatal synaptosomes, but with a low affinity ($IC_{50}$ value = 80 μM; Teng et al., 1997). The results suggest that the pool of redistributed DA resulting from lobeline exposure is vulnerable to metabolism in the absence of MAO inhibition; but when MAO is inhibited, the redistrib-

![Graph](https://via.placeholder.com/150)

**Fig. 5.** Lobeline attenuates the discriminative stimulus properties of $d$-methamphetamine in rats. Rats trained to discriminate $d$-methamphetamine (1.0 mg/kg) from saline were administered vehicle or lobeline (3.0–6.6 mg/kg s.c.) 45 min before $d$-methamphetamine (0.1–1.0 mg/kg i.p.). The percentage of $d$-methamphetamine-appropriate responding is shown as a function of $d$-methamphetamine dose (top), and response rates are expressed as responses per second (bottom). The $d$-methamphetamine dose-response curve after lobeline (5.6 mg/kg) pretreatment was significantly ($p < 0.05$) different from the $d$-methamphetamine dose-response curve after saline pretreatment. $N = 9$ rats.

Lack of Lobeline Toxicity. Lobeline or vehicle was administered acutely (1.0–30.0 mg/kg s.c.), intermittently (3.0–10.0 mg/kg/day for 10 days, s.c.) or continuously (30.0 mg/kg/day by osmotic minipump delivery for 21 days). Subsequently, striata were obtained for the determination of endogenous DA and DOPAC content (Table 4). For both analyses of DA and DOPAC content, the main effect of lobeline concentration was not significant ($p > 0.05$). Therefore, lobeline administration did not deplete striatal DA or DOPAC content at any dose of lobeline or following any treatment regimen.

Discussion

The present study assessed lobeline-induced inhibition of the neurochemical and behavioral effects of amphetamine. Lobeline intrinsically increased $^3$H overflow from superfused rat striatal slices preloaded with $[^3]$HIDA, but did not inhibit $d$-amphetamine-evoked $^3$H overflow. The inhibitory properties of lobeline may have been masked as a result of the intrinsic activity of lobeline, the incomplete compartmentalization of $[^3]$HIDA within the presynaptic terminal, and/or the possibility that DA overflow is not accurately reflected by measurement of $^3$H overflow. Moreover, lobeline inhibited $d$-amphetamine-evoked endogenous DA overflow, supporting the supposition that the inhibitory effect was obscured in the $^3$H overflow experiments. The lobeline-induced inhibition of $d$-amphetamine-evoked DA overflow was selective, since these lobeline concentrations did not inhibit electrically evoked overflow across a range of applied pulses, and inhibition was observed at concentrations of lobeline that alone did not alter overflow. The lobeline-induced inhibition of the effect of amphetamine observed in vitro translated into an in vivo effect. At behaviorally inactive doses, lobeline pretreatment attenuated both $d$-amphetamine- and $d$-amphetamine-induced hyperactivity. Lobeline also attenuated the $d$-amphetamine discriminative stimulus at doses that did not generalize to the interoceptive cues of $d$-amphetamine and did not decrease response rates. Furthermore, in vivo administration of lobeline over a wide dose range did not deplete striatal DA or DOPAC content, indicating that lobeline does not inhibit amphetamine via dopaminergic toxicity.
lobeline that inhibited both in the absence and presence of pargyline, suggesting significantly inhibited pool, and is not available for release. 

**TABLE 4**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DA (ng/mg protein)</th>
<th>DOPAC (ng/mg protein)</th>
</tr>
</thead>
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<tr>
<td>Acute DA</td>
<td>739 ± 64</td>
<td>87 ± 12</td>
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<td>Intermittent DA</td>
<td>743 ± 57</td>
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<tr>
<td>Chronic DA</td>
<td>840 ± 72</td>
<td>N.D.</td>
</tr>
<tr>
<td>Acute DOPAC</td>
<td>84 ± 12</td>
<td>81 ± 12</td>
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<tr>
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<td>63 ± 6</td>
<td>57 ± 12</td>
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<td>Chronic DOPAC</td>
<td>61 ± 4</td>
<td>N.D.</td>
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N.D., not determined.

* Data are mean ± S.E.M. content (ng/mg of protein). N = 6–8 rats/condition.

used DA accumulates in a compartmentalized cytosolic pool, and is not available for release.

The major finding of the current study is that lobeline significantly inhibited *d*-amphetamine-evoked DA overflow, both in the absence and presence of pargyline, suggesting that MAO activity is not a factor in lobeline-induced inhibition of *d*-amphetamine. Importantly, the inhibitory effect of lobeline was selective, since concentrations (0.1–3.0 μM) of lobeline that inhibited *d*-amphetamine-evoked DA overflow did not alter field stimulation-evoked overflow. Moreover, the lobeline concentrations (0.1–1.0 μM) that inhibit *d*-amphetamine-evoked DA overflow are in the same range of lobeline concentration that inhibits DA uptake via VMAT2 (Teng et al., 1998), suggesting that VMAT2 may be the molecular target for lobeline-induced inhibition of this effect of *d*-amphetamine. In these latter studies, lobeline inhibited DA uptake at VMAT2 more potently (100 times greater) than it evoked DA overflow from synaptic vesicles (Teng et al., 1998). In contrast, *d*-amphetamine evoked DA overflow from synaptic vesicles more potently than it inhibited uptake into synaptic vesicles (Erickson et al., 1996; Teng et al., 1998). Furthermore, *d*-amphetamine interacts with the reseprine site on VMAT2, whereas lobeline interacts with the tetrabenazine site (Erickson et al., 1996; Teng et al., 1998). Taken together, these results suggest that both lobeline and *d*-amphetamine redistribute DA from the vesicular pool to a cytosolic pool. Thus, lobeline-induced inhibition of *d*-amphetamine-evoked DA release maybe due to a noncompetitive interaction at VMAT2 and redistribution of vesicular DA into a compartmentalized cytosolic pool not available for reverse transport via DAT. One other possible mechanism may be an interaction of lobeline with the DA substrate site on the intracellular face of the DAT protein, exposed following *d*-amphetamine dissociation from the transporter. Thus, lobeline inhibits the neurochemical effects of *d*-amphetamine either via an interaction with VMAT2, DAT, or a cytosolic protein involved in DA compartmentalization.

The behavioral studies extend the results of the neurochemical experiments and demonstrate inhibition by lobeline of several behavioral effects of amphetamine. Acute administration of *d*-amphetamine produces a transient increase in locomotor activity (Stolk and Rech, 1967) resulting from stimulation of DA release from presynaptic terminals (Wise and Bozarth, 1987). In the present study, high doses (3.0–10 mg/kg) of lobeline produced hypoactivity and inhibited *d*-amphetamine-induced hyperactivity. Due to the hypoactivity induced by lobeline at these doses, the mechanism of lobeline’s inhibition cannot be attributed to a specific alteration in dopaminergic DA pathways. However, an intermediate concentration of lobeline (1.0 mg/kg) that did not inherently produce hypoactivity, significantly attenuated the *d*-amphetamine-induced hyperactivity in rats. Furthermore, in studies with mice, lobeline did not produce hypoactivity, but inhibited *d*-methamphetamine-induced hyperactivity.

The results of the locomotor activity experiments demonstrate that in two rodent species, behaviorally inactive doses of lobeline attenuated the hyperactivity induced by amphetamine. There is a plethora of potential neurochemical mechanisms that may underlie the observed lobeline-induced inhibition of amphetamine effects in these locomotor activity studies. Lobeline may be acting as an antagonist at nicotinic receptors to alter dopaminergic function, and thereby, inhibit the locomotor activating effect of amphetamine via an indirect mechanism. Also, lobeline may inhibit the locomotor activating effects of amphetamine via an alteration of noradrenergic or serotonergic function. However, the results of the locomotor activity experiments are clearly consistent with those of the present neurochemical experiments, which demonstrate lobeline-induced inhibition of *d*-amphetamine-evoked DA release from striatal slices. Thus, from the results of the current study, inhibition of amphetamine-evoked DA release appears to be a likely neurochemical mechanism underlying the lobeline-induced inhibition of amphetamine’s locomotor activating effects.

The increase in locomotor activity that follows psycho-stimulant administration is mediated at least in part by activation of central dopaminergic fibers (Wise and Bozarth, 1987), and the discriminative stimulus effects of amphetamine are dependent largely upon DA receptor activation (Munzar et al., 1999). Furthermore, the subjective effects of psychomotor stimulants are believed to be important determinants of their abuse potential and maintenance of dependence in humans (Evans and Johanson, 1987; Childress et al., 1988). Lobeline significantly shifted the *d*-methamphetamine generalization dose-response relationship to the right, suggesting that lobeline alters the interoceptive properties of *d*-methamphetamine. Furthermore, this rightward shift resulting from lobeline treatment was concentration-dependent, and at doses that did not inherently inhibit response rates. It is also important to note that lobeline pretreatment, in the absence of *d*-methamphetamine, did not produce generalization to the *d*-methamphetamine discriminative stimulus, indicating that lobeline and *d*-methamphetamine did not produce similar interoceptive states. Lobeline may be acting on a number of neurochemical systems to alter the interoceptive properties of amphetamine; however, previous reports indicate that the discriminative stimulus effects of amphetamine are dependent largely upon DA re-
ceptron activation (Munzar et al., 1999). Taken together with the current neurochemical findings that lobeline inhibits amphetamine-evoked DA release in vitro, the results of the drug discrimination studies are consistent with the interpretation that the effect of lobeline on the dopaminergic system underlies the alteration in the interoperative properties of amphetamine.

Acute, intermittent, and continuous systemic administration of lobeline did not produce a significant depletion of striatal DA or DOPAC content in the present study. Although the present results are not consistent with lobeline-induced dopaminergic toxicity, the possibility remains that toxicity could occur as a result of decreased tyrosine hydroxylase, DAT, or VMAT2 protein. Future studies will be aimed at examining this possibility. However, a high dose of lobeline (30 mg/kg/day) was administered continuously for a prolonged period (21 days) and no decrease in DA content was observed in the current study. Thus, these initial results suggest that the inhibitory effect of lobeline on amphetamine neurochemistry and behavior is not likely due to neurotoxicity.

In summary, the results of the present study, demonstrating the ability of lobeline to inhibit the neurochemical and behavioral effects of amphetamine in both in vitro and in vivo studies using two different rodent species, provide compelling evidence that lobeline may constitute a novel pharmacotherapy for the treatment of psychostimulant abuse. Furthermore, the in vitro studies demonstrated that concentrations of lobeline, which inhibited amphetamine-evoked DA overflow, did not reduce the vesicular DA stores released upon electrical-field stimulation, indicating a selective inhibitory effect. Systemic administration of lobeline also did not produce a significant depletion of striatal DA and DOPAC content, suggesting that its inhibitory effect is not the result of a neurotoxic effect of the alkaloid. Since no effective therapies for dependence upon psychomotor stimulants are currently available, further preclinical and clinical evaluation of lobeline as a potential pharmacotherapy for psychostimulant abuse should be seriously considered.

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References


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