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Dose Dependent Attenuation of Heroin Self-administration with Lobeline

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Abstract

Behavioral studies have yielded results that show lobeline has the ability to attenuate *d*-methamphetamine self-administration. Further in vivo and in vitro studies have demonstrated the blockade of μ opioid receptors with lobeline. The present investigation examined the ability of lobeline to attenuate heroin intravenous (i.v.) self-administration when administered prior to testing. Male Sprague-Dawley rats were surgically implanted with jugular catheters and trained to lever press for i.v. heroin infusions (18 μ g/kg) on an FR-2 schedule. Rats then were tested for heroin self-administration after pretreatment with subcutaneous lobeline injections (0.3, 1.0, or 3.0 mg/kg, 15 min prior to testing sessions). At doses of 1.0 and 3.0 (mg/kg), lobeline was shown to attenuate self-administration of heroin. The results suggest a potential for lobeline to be used in pharmacotherapy for opioid abuse.

Lobeline (LOB) is the most biologically active alkaloidal component synthesized from Indian Tobacco (*Lobelia inflata*). In addition to its high affinity for $\alpha_4\beta_2$ subtypes of the nicotinic acetylcholine receptor (nAChR), LOB is also believed to alter dopaminergic neurotransmission by interrupting the activity of vesicular monoamine transporter-2s (VMAT2) (Felpin and Lebreton, 2004), theoretically inhibiting vesicular dopamine (DA) transport within the pre-synaptic terminal.

Recent studies have focused on LOB's ability to alter behavioral effects of drugs of abuse, particularly psychostimulants. Pretreatment with a low dose of LOB that would cause no behavioral effect on its own has been shown to attenuate *d*-methamphetamine induced hyperactivity in mice (Miller et al., 2001). A dose dependent effect of LOB on the development of sensitization to cocaine-induced hyperactivity also has been demonstrated (Polston et al., 2006). Specifically, at a dose of .3 mg/kg, LOB was found to augment cocaine-induced hyperactivity brought about by repeated administration of 10–20 mg/kg of cocaine in rats, but at the 1.0 mg/kg dose of LOB, the development of cocaine-induced hyperactivity with repeated administration was prevented. Neither dose was effective when the cocaine dose was 30 mg/kg. Elsewhere, pretreatment with LOB at doses ranging from 3.0–30mg/kg, 15 min prior to administration of *d*-methamphetamine has been shown to decrease the intensity of stereotypy in imprinting control region (ICR) mice (Tatsuta et al., 2006). In a study that examined whether LOB would be self-administered by rats, it was demonstrated that rats would not self-administer for LOB either alone, or in place of *d*-methamphetamine (Harrod et al, 2003). Based on evidence from in vitro studies that demonstrated LOB's ability to interrupt *d*-methamphetamine dopaminergic neurotransmission, Harrod et al. (2001) conducted a *d*-methamphetamine self-administration study that revealed how pretreatments with LOB (0.3–

3.0 mg/kg, 15 min prior to testing) attenuated lever pressing for intravenous (i.v.) d-methamphetamine in rats, thus demonstrating LOB's potential use in pharmacotherapy for methamphetamine addiction. Dopamine release is a common factor with numerous drugs of abuse (Wise and Bozarth, 1987), and is thought to be responsible for the "wanting" of a drug that develops with repeated administrations (Berridge, 2007; Salamore et al., 2007), consequently the ability of LOB to interrupt dopaminergic neurotransmission may implicate a practical application in treatment for drug addiction that extends beyond psychostimulants.

A recent study found that LOB has a high affinity for opioid receptors. That is, LOB pretreatment attenuates opiate-induced hyperactivity, and it prevents the development of sensitization to morphine (Miller et al., 2007). Also, LOB has been shown to bind to and inhibit VMAT2's (Teng et al., 1998; Eyerman and Yamamoto, 2005). This inhibition of vesicular DA transport within the pre-synaptic terminal may affect opiate sensitivity, similar to the manner in which it affects psychostimulant sensitivity, as demonstrated by the reduced psychostimulant-conditioned reward seen in VMAT2 knock-out mice (Takahashi et al., 1997). At this juncture the effects of LOB on i.v. opiate self-administration are not known. Certainly, the ability of LOB to inhibit opiate-induced DA release in a manner similar to its inhibition of d-methamphetamine-induced DA release necessitates an examination of its ability to attenuate heroin self-administration, inasmuch as DA is involved in defining the rewarding properties of heroin (Bozarth and Wise, 1981; White and Kalivas, 1998; Koob and Le Moal, 2001; Le Foll et al., 2005). Accordingly, the purpose of the present investigation was to test the hypothesis that pretreatment with LOB would attenuate heroin self administration in rats. In this investigation, animals shaped to self-administer heroin were pretreated with LOB administered subcutaneously (s.c.) and subsequently tested for differential heroin self-administration.

Materials and Method

Animals

The animals used in this study were seven adult male Sprague-Dawley rats (Harlan; Houston, TX USA) that were approximately 60 days old at the time of their arrival at the laboratory. Body weights ranged from 250 to 300 g. All animals were individually housed in standard hanging plastic cages in a temperature and humidity controlled room with a 12/12 hr light/dark cycle. Animals had continuous access to standard rat chow (Teklad; Madison, WI USA) and tap water throughout the experiment.

Surgical procedures

Details of the surgical procedures have been presented earlier (Rocha et al., 2005; Valles et al., 2006). Briefly, after allowing the animals to acclimate to the vivarium for 7 days, chronic indwelling jugular catheters were implanted in rats. Rats were anesthetized with separate injections of 50 mg/kg ketamine and 50 mg/kg sodium pentobarbital. A catheter consisting of 0.25 mm interior diameter (ID)/0.76 mm outside diameter (OD) Silastic tubing (Dow Corning; Midland, MI USA) was inserted into the right jugular vein and sutured to muscle tissue in the area of the vein. Using an 11 ga stainless steel tube as a guide, the catheter was passed s.c. through the body of the animal and exited the back between the scapulae. A backplate consisting of two stainless steel ovals separated by polypropylene mesh (Ethicon, Inc.; Sommerville, N.J., USA) accommodated a spring leash, through which the catheter was threaded. Connecting to the backplate at one end, the other end of the leash was connected to a single fluid channel swivel. Swivels were made in-house using 20-ga hypodermic tubing, a 20-ga needle, a 1 cc syringe, a 3 cc syringe, and a rubber stopper. The swivel design permitted an interlock with separate connecting arms located in the home cage and operant conditioning chambers (see below). The movable arm allowed for free movement and delivery of appropriate

solutions in either the home cage or test chamber. A 0.51 mm ID/1.53 mm OD catheter continued from the top of the swivel to an infusion pump that controlled solution delivery. The rats were allowed 5 days to recover from surgery before commencing self-administration testing. During this recovery period, each rat received in the home cage automated hourly intravenous (i.v.) infusions (8-s; .25 ml) of a sterile saline solution containing heparin (1.25 IU/ml). On self-administration test days, the cannulae were flushed with this solution daily following the test, and this solution was cleared with a subsequent application of .10 ml heparinized saline. Catheter patency was verified throughout the experiment by administering an i.v. infusion of 7.50 mg/kg sodium pentobarbital and checking for rapid onset of brief anesthesia.

Apparatus

Seven operant conditioning chambers (Model E10-10; Coulbourn, Allentown, Pa., USA) in sound attenuating cubicles served as the test apparatus. Each chamber had two levers (left/inactive, right/active) and a stimulus light located above each lever. Infusion pumps (Razel Scientific Instruments; Stamford, Conn., USA) controlled drug delivery to each of the boxes. A 20-ml syringe delivered .16 mL i.v. infusions over a 6-s time frame. The system was interfaced with an IBM computer controlling drug delivery and recording data from the seven chambers. Testing occurred during the light phase of the cycle.

Testing procedures

Baseline training commenced five days after catheter surgeries. Rats initially were shaped to lever press for a 6-s infusion of .50 mg/kg cocaine HCl on a fixed ratio (FR1), where a depression of the active (right) lever resulted in drug delivery and illumination of the stimulus light above the lever. After 5 consistent days of cocaine self-administration (>25 presses in 2-hr session), rats were shaped to lever press for an infusion of 18 μ g/kg heroin on an FR2 schedule, where two depressions of the active (right) lever were required for drug deliver. After 5 consistent days of heroin self-administration (>25 presses in 2-hr session), rats received 5 days of s.c. saline injections 15 min prior to being placed in testing chambers for a 2-hr session of heroin self-administration. These last 5 days of saline injections were carried out to prevent the animals from associating the s.c. injections with a reduced drug effect for heroin.

Dose-effect testing began the day immediately following the final day of baseline training. Rats were tested daily (one day at each dose) for 1 hr with escalating pretreatment doses of LOB (0.3, 1.0, and 3.0 mg/kg) administered SC 15 min prior to testing sessions, with two days of 2-hr baseline sessions between escalating doses.

Drugs

The Research Technology Branch of the National Institute of Drug Abuse generously supplied the 3,6 diacetyl morphine HCl (heroin), and the drug was administered as the salt. Lobeline sulfate was purchased from Fisher Scientific (Pittsburg, PA USA) and the drug was administered as the base.

Results

Statistical analysis

Data for the mean number of right lever presses for heroin infusions across test day are presented in Figure 1(left lever data are not presented), and data for the mean number of right lever presses for the three doses of LOB are presented in Figure 2. LOB doses of 1.0 and 3.0 mg/kg suppressed right lever pressing but the 0.3 mg/kg dose did not. A two-way (lever $\times$ day) within subjects repeated measures analysis of variance (ANOVA) of the right and left lever

data was computed (SPSS) using lever and test day as repeated measures. The analyses showed a main effect for the within group factor of lever [$F(1,6)=55.11$, $P<0.01$], as well as a main effect for test day [$F(8,48)=12.67$, $P<0.01$], and an interaction for lever and test day [$F(8,48)=12.56$, $P<0.01$].

Tukey's post hoc tests revealed statistically significant contrasts for means for right lever pressing with the LOB 3.0 mg/kg dose vs. baseline test days and against the LOB 0.3 mg/kg dose, but not against the LOB 1.0 mg/kg dose. Contrasts for the means for right lever pressing for the LOB 1.0 mg/kg dose were statistically significant between baseline test days and against the LOB 0.3 dose as well. The contrasts between the means for right lever pressing for the LOB 0.3 mg/kg dose vs. the relevant baseline test days were not significant.

Discussion

The present study tested the hypothesis that pretreatment with LOB would attenuate heroin self-administration in rats. The findings from this investigation demonstrated that LOB pretreatment effectively attenuates opiate self-administration in a dose dependent manner. The lowest dose of LOB (0.3 mg/kg) had no effect, while attenuation of heroin self-administration was demonstrated with LOB doses of 1.0 and 3.0 mg/kg. Our findings are in agreement with the findings of Miller et al. (2007) where lobeline was found to be a μ opioid receptor antagonist, due to having a high affinity for μ opioid receptors, thereby producing an inhibitory effect on opiate pharmacology.

A notable occurrence found in the data from the dose effect testing was the level of active (heroin) lever pressing on the subsequent baseline (saline) days (See Figure 1). After the ineffective dose of LOB (0.3 mg/kg), lever presses for heroin infusions on the following baseline days (3 and 4) stayed steady or slightly increased. Following the first effective dose of LOB (1.0 mg/kg) we observed a decrease in the average number of active lever presses on baseline days 5 and 6. This likely was due to carry-over effects and resulted from feed-forward (Pavlovian) conditioning cues that may have contributed to the antagonistic effects of LOB on heroin reinforcement.

Heroin, once it reaches the brain, is converted to morphine, which then binds to μ opiate receptors acutely expressed on GABAergic ventral tegmental area (VTA) interneurons and on nucleus accumbens (NAc) neurons (Hyman, Malenka, and Nestler, 2006). These inhibitory GABA neurons in the region of the VTA modulate tonic glutamate (Glu) activation of dopamine neurons projecting to the NAc, therein producing reward effects. In this cascade, when opiates bind to the μ receptors on GABA fibers they operate as antagonists. The resulting disinhibition of Glu stimulation permits elevated levels of DA to accrue in the NAc, thereby defining the rewarding properties of heroin. It is possible that LOB may competitively or noncompetitively antagonize opiate action at the μ receptor site (cf. Miller et al., 2007), thus interfering with the disinhibitory effects of heroin that would otherwise occur. Of course, LOB may act more directly on DA in the mesolimbic pathway of the brain. As discussed, LOB interrupts the activity of VMAT2, preventing vesicular DA transport within the pre-synaptic terminal (Felpin and Lebreton, 2004) and the attendant compromise in DA activity in the NAc may challenge the reward properties of heroin. This potential direct interference with the activation of the DA reward circuit may lessen heroin drug-seeking, and could account for the pattern of results observed in this study.

Whatever the mechanism(s), the results from the present study increase our understanding of potential chemical interventions and therein add opiates to the growing list of abused drugs for which LOB could potentially become part of an extensive pharmacotherapy regimen. The implications along these lines are profound. Currently, methadone is the treatment of choice

for heroin addiction (see Fugelstad et al., 2007), but it comes with established risks. Specifically, methadone overdose (death) is much more common than many people realize (Srivastava and Kahan, 2006; Zador, 2007), and the development of potentially safer, substitute pharmacotherapy's is desired. In this regard, the fact that LOB acts antagonistically on the opiate system may offer a reliable and less dangerous way to manage selective addiction profiles.

Acknowledgments

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Figure 1.
Mean right lever press rates and standard error values for dose effect testing days (0.3, 1.0, 3.0) with two days of baseline (Sal) preceding each dose.

Figure 2.
Mean right lever press rates and standard error values for dose effect testing days (0.3, 1.0, 3.0).