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Lobeline, a potential pharmacotherapy for drug addiction, binds to μ opioid receptors and diminishes the effects of opioid receptor agonists

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Abstract

Lobeline diminishes the behavioral and neurochemical effects of nicotine and amphetamines, and is considered a potential pharmacotherapy for drug abuse and addiction. Lobeline has high affinity for nicotinic acetylcholine receptors and inhibits the function of vesicular monoamine and dopamine transporters. The present study investigated the less-explored interaction of lobeline and the endogenous opioid system. In guinea pig brain homogenates, lobeline displaced ($K_i = 0.74\ \mu M$) the binding of [^{3}H]DAMGO [(D-Ala^2, *N*-ME-Phe^4, Gly^5-ol)-enkephalin]. In a functional assay system comprised of MOR-1 μ opioid receptors and GIRK2 potassium channels expressed in *Xenopus* oocytes, lobeline had no effect on the resting current, but maximally inhibited ($IC_{50} = 1.1\ \mu M$) morphine- and DAMGO-activated potassium current in a concentration-dependent manner. In a second functional assay, lobeline-evoked [^{3}H]overflow from rat striatal slices preloaded with [^{3}H]dopamine was not blocked by naltrexone. Importantly, concentrations of lobeline (0.1–0.3 μM) that did not have intrinsic activity attenuated (∼50%) morphine-evoked [^{3}H]overflow. Overall, the results suggest that lobeline functions as a μ opioid receptor antagonist. The ability of lobeline to block psychostimulant effects may be mediated by opioid receptor antagonism, and lobeline could be investigated as a treatment for opiate addiction.

Keywords: DAMGO; Dopamine; Dopamine transporter; Lobeline morphine; Opioid receptor; Striatum

1. Introduction

The dried flowers and seeds of *lobelia inflata* (lobeline) have been used in a variety of natural products as a treatment for asthma, bronchitis, and pneumonia. Lobeline also has been used, with modest success, as a treatment for tobacco smoking addiction (Davison and Rosen, 1972; Nunn-Thompson and Simon, 1989). More-recent findings suggest that lobeline could be a pharmacotherapy for drug addiction and neurotoxicity (Dwoskin and Crooks, 2002; Eyerman and Yamamoto, 2005; Miller, 2006; Tatsuta et al., 2006), as lobeline inhibits the effect of psychostimulants in behavioral and neurochemical assays. Lobeline attenuated *d*-amphetamine-evoked endogenous dopamine release from rat striatal slices (Miller et al., 2001) and inhibited nicotine-evoked [^{3}H]overflow from rat striatal slices preloaded with [^{3}H]dopamine (Miller et al., 2000). In rodent behavioral assays, lobeline blocked the discriminative-stimulus properties of methamphetamine and cocaine (Miller et al., 2001; Cunningham et al., 2006) and decreased methamphetamine self-administration (Harrod et al., 2001).

The efficacy of lobeline to inhibit psychostimulant pharmacology is likely mediated in the central nervous system by its activity at nicotinic acetylcholine receptors and/or its ability to alter presynaptic dopamine storage and release. Lobeline

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has high affinity (K_i value ≈ 10–20 nM) for both heteromeric ($\alpha4\beta2^*$, probed by [^{3}H]nicotine) and homomeric ($\alpha7^*$, probed by [^{3}H]methyllycaconitine) nicotinic acetylcholine receptors (Flammia et al., 1999; Miller et al., 2004), where it functions as both an antagonist and an agonist (Miller et al., 2000; Damaj et al., 1997; Gallardo and Leslie, 1998). Lobeline also binds to and inhibits (IC_{50} value ≈ 1 μM) the function of the vesicular monoamine transporter (VMAT) (Teng et al., 1998; Wilhelm et al., 2004; Eyerman and Yamamoto, 2005). Finally, lobeline binds to and inhibits (IC_{50} value ≈ 40–100 μM) the function of the dopamine transporter (DAT) (Teng et al., 1997; Miller et al., 2004).

While lobeline diminishes the behavioral and neurochemical effects of psychostimulants, the influence of lobeline on the effects of opiates has only minimally been investigated. Aceto et al. (2001) demonstrated that lobeline (designated as NIH-11034) attenuated withdrawal from morphine in opiate-dependent monkeys, although the magnitude of the attenuation did not achieve statistical significance. These initial findings suggest that lobeline is an opioid receptor agonist, although follow-up research has not been reported to understand clearly the nature of the potential interaction between lobeline and opioid receptors. This is an important area because an interaction with opioid receptors may contribute to lobeline's efficacy to diminish the behavioral effects of psychostimulants, as well as provide a potential pharmacotherapy for opiate abuse.

Morphine and [D-Ala^2, *N*-Me-Phe^4, Gly^5-ol]-enkephalin (DAMGO) are examples of selective mu (μ) opioid receptor agonists. Such agonists bind to and activate μ opioid receptors, which leads to membrane hyperpolarization (K^+ efflux) and inhibition of adenyl cyclase activity (Childers, 1991). This process subsequently leads to heightened activity of postsynaptic dopamine neurons, and is generally responsible for the reinforcing effects of morphine (Herz, 1998; Pierce and Kumaresan, 2006). A number of opioid receptor antagonists, such as naloxone and naltrexone, bind to opioid receptors and prevent the activity of receptor agonists. Opioid receptor antagonists and mixed agonist–antagonists, such as naltrexone and buprenorphine, respectively, have been investigated as pharmacotherapies for opiate psychostimulant abuse and addiction (Ling et al., 1994; Vocci and Ling, 2005).

The goal of the present study was to evaluate the interaction of lobeline with the μ opioid receptor and drugs that are μ opioid receptor agonists. The affinity of lobeline for μ opioid receptors was determined by displacement of radiolabeled DAMGO binding in guinea pig brain. To determine if lobeline functions as a μ opioid receptor agonist or antagonist, the interaction of lobeline with morphine and DAMGO was investigated in a functional assay system comprised of μ opioid receptors and G-protein activated K^+ channels expressed in *Xenopus* oocytes. This combination of μ opioid receptors and GIRK2 channels mimics the native neuronal G-protein-coupled pathway for opioid analgesia (Blednov et al., 2003). Slice superfusion was used as a second functional assay system to investigate the activity of lobeline and its interaction with morphine in a relatively complex brain preparation.

2. Materials and methods

2.1. Subjects

The University of Missouri Institutional Animal Care and Use Committee approved the procedures involving the use of animal subjects and this research was conducted in accordance to the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the US National Institutes of Health. English Hartley guinea pigs (Rockland Immunochemicals, Gilbertsville, PA) were used for the radioligand binding experiments, *Xenopus laevis* frogs (Xenopus I, Dexter, MI) were used for the electrophysiological experiments, and male Sprague-Dawley rats (Harlan, Indianapolis, IN) were used for the [^{3}H]overflow assay.

2.2. Chemicals and drugs

Lobeline sulfate was purchased from Acros Organics (Geel, Belgium). Collagenase type II, DAMGO, mecamylamine hydrochloride, morphine sulfate pentahydrate, naloxone dihydrochloride, (−)-nicotine ditartrate, nomifensine maleate and pargyline hydrochloride were purchased from Sigma (St. Louis, MO). Naltrexone hydrochloride was purchased from Mallinckrodt (St. Louis, MO). [^{3}H]DAMGO (66 Ci/mmol) and [^{3}H]dopamine (1 Ci/mmol) were purchased from Amersham Biosciences (Piscataway, NJ) and Perkin-Elmer Life Sciences (Boston, MA), respectively. All other chemicals were purchased from Fisher Scientific (Fairlawn, NJ).

2.3. Radioligand binding experiment

The affinity of lobeline for μ opioid receptors was ascertained by measuring displacement of [^{3}H]DAMGO binding in guinea pig brain. This assay has been used in previous research to probe affinity for μ opioid receptors, and the present experiment was conducted using modifications of a previously-reported method (Heyl and Mosberg, 1992). The displacement curve for naltrexone was determined as a control, and the curves for nicotine and mecamylamine also were determined to compare lobeline to a traditional nicotinic acetylcholine receptor agonist and antagonist, respectively.

Crude P2 synaptosomal fractions were prepared from whole, fresh-frozen guinea pig brains as previously described (Lever et al., 2006). Protein concentrations were determined using a bicinchoninic acid assay kit (Pierce Biotechnology, Rockford, IL), and homogenates were stored at −82 °C. Thawed aliquots were suspended for use at 1.7 mg protein/ml by adding binding buffer (50 mM Tris–HCl, pH 7.4, 25 °C). Assays were run in glass tubes containing 0.34 mg protein (200 μl), [^{3}H]DAMGO (25 μl, final concentration 0.65 nM), and either vehicle (25 μl, total binding), DAMGO (25 μl, final concentration 5 μM, non-specific binding) or varying concentrations of competing ligands (25 μl) equally spaced on the log scale. Serial dilutions of naltrexone were prepared in distilled water. Dilutions of lobeline and mecamylamine were prepared in aqueous ethanol (0.1%) containing glacial acetic acid (0.01%). Incubations were conducted for 75 min at 25 °C to reach steady-state. Bound and free [^{3}H]DAMGO were separated by dilution with ice-cold assay buffer (5 ml) and rapid filtration through glass fiber filters (GF/B) pretreated with polyethyleneimine (0.5%) using a cell harvester (Brandel, Gaithersburg, MD). Tubes and filter papers were rinsed with cold assay buffer (3 × 5 ml), and the filter discs were dried under vacuum. Cocktail (OptiPhase HiSafe 2, Perkin-Elmer Life Sciences) was added, and the filters extracted for 24 h prior to liquid scintillation counting (LS 6500 Scintillation Counter, Beckman-Coulter, Fullerton, CA; counting efficiency ≈45–55%). Assays were run in duplicate.

Data were analyzed by non-linear regression using Radlig (Biosoft Inc., Ferguson, MO) and Prism (GraphPad Software, San Diego, CA). Apparent affinities (K_i) were calculated from IC_{50} values using the Cheng–Prusoff equation (Cheng and Prusoff, 1973). An experimentally determined K_d of 0.59 nM for [^{3}H]DAMGO was used as an input value, and agrees well with the K_d of 0.85 nM noted by Heyl and Mosberg (1992).