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GZ-793A inhibits the neurochemical effects of methamphetamine via a selective interaction with the vesicular monoamine transporter-2

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ABSTRACT

Lobeline and lobelane inhibit the behavioral and neurochemical effects of methamphetamine via an interaction with the vesicular monoamine transporter-2 (VMAT2). However, lobeline has high affinity for nicotinic receptors, and tolerance develops to the behavioral effects of lobelane. A water-soluble analog of lobelane, *R-N*-(1,2–dihydroxypropyl)–2,6-*cis*-di-(4-methoxyphenethyl)piperidine hydrochloride (GZ-793A), also interacts selectively with VMAT2 to inhibit the effects of methamphetamine, but does not produce behavioral tolerance. The current study further evaluated the mechanism underlying the GZ-793A-mediated inhibition of the neurochemical effects of methamphetamine. In contrast to lobeline, GZ-793A does not interact with the agonist recognition site on α4β2* and α7* nicotinic receptors. GZ-793A (0.3–100 μM) inhibited methamphetamine (5 μM)-evoked fractional dopamine release from rat striatal slices, and did not evoke dopamine release in the absence of methamphetamine. Furthermore, GZ-793A (1–100 μM) inhibited neither nicotine (30 μM)-evoked nor electrical field-stimulation-evoked (100 Hz/1 min) fractional dopamine release. Unfortunately, GZ-793A inhibited [^{3}H]dofetilide binding to human-ether-a-go-go related gene channels expressed on human embryonic kidney cells, and further, prolonged action potentials in rabbit cardiac Purkinje fibers, suggesting the potential for GZ-793A to induce ventricular arrhythmias. Thus, GZ-793A selectively inhibits the neurochemical effects of methamphetamine and lacks nicotinic receptor interactions; however, development as a pharmacotherapy for methamphetamine use disorders will not be pursued due to its potential cardiac liabilities.

1. Introduction

Methamphetamine use disorders negatively impact global health, and approved medications to treat these disorders are not available. The euphoria and high abuse liability of methamphetamine are consequences of dopamine release in brain reward pathways (Rawson et al., 2007). A lipophilic weak base, methamphetamine diffuses across presynaptic plasmalemmal and vesicular membranes, and interacts with the vesicular monoamine transporter-2 (VMAT2), dopamine transporter and monoamine oxidase, leading to increased extracellular dopamine concentrations (Sulzer et al., 1992, 1993, 2005). Considering the integral role that VMAT2 plays in the mechanism of action of methamphetamine to increase extracellular dopamine, VMAT2 represents an important target for novel therapeutics designed to treat methamphetamine use disorders.

Lobeline, a lipophilic non-pyridino alkaloid of *Lobelia inflata* (Indian tobacco), has demonstrated efficacy in attenuating the neurochemical effects of methamphetamine. Lobeline inhibits dopamine uptake at VMAT2 and increases extracellular concentrations of the dopamine metabolite, 3,4 dihydroxyphenylacetic acid (DOPAC), indicating that lobeline does not inhibit monoamine oxidase (Nickell et al., 2010; Teng et al., 1997). Importantly, lobeline also inhibits methamphetamine-evoked dopamine release at both synaptic vesicle and terminal levels (Meyer et al., 2013). Through interactions with VMAT2, lobeline reduces the pool of cytosolic dopamine available for methamphetamine-induced reverse-transport via the dopamine transporter, thereby decreasing dopamine release into the extracellular compartment (Dwoskin and Crooks, 2002). Thus, VMAT2 is implicated as a primary target for the lobeline-mediated inhibition of the pharmacological effects of methamphetamine.

Although VMAT2 is the likely site of action for the lobeline-mediated inhibition of methamphetamine, lobeline also exhibits high affinity for α4β2* nicotinic receptors (Nickell et al., 2014). Lobelane, a chemically defunctionalized analog of lobeline, exhibited low affinity

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for the predominant nicotinic receptor subtypes, while retaining high affinity for VMAT2 (Miller et al., 2004). Furthermore, lobelane decreased methamphetamine-evoked dopamine release (Nickell et al., 2010) and decreased methamphetamine self-administration in rats (Neugebauer et al., 2007). Unfortunately, tolerance developed to the behavioral effects of lobelane.

Another promising lobelane analog emerged from our iterative drug discovery approach. *R-N*-(1,2−Dihydroxypropyl)−2,6-*cis*-di-(4-methoxyphenethyl)piperidine hydrochloride (GZ-793A), a novel water-soluble analog of lobelane, exhibits high affinity for VMAT2 and is > 50-fold more selective for VMAT2 versus the dopamine and serotonin transporters (Horton et al., 2011b). Following both parenteral and oral administration, GZ-793A specifically decreases methamphetamine self-administration in rats (Beckmann et al., 2012; Wilmouth et al., 2013). However, GZ-793A interaction with nicotinic receptors has not been evaluated previously. Thus, the current study determined GZ-793A affinity for the predominant nicotinic receptor subtypes using both radioligand binding and nicotine-evoked dopamine release assays. To further evaluate the selectivity of GZ-793A with respect to inhibition of methamphetamine-evoked dopamine release, inhibition of electrical field-stimulation-evoked dopamine release was determined. In support of securing an investigational new drug application for GZ-793A, its potential to inhibit cardiac function also was evaluated by assessing affinity for the human-ether-a-go-go related gene (hERG) channel expressed in human embryonic kidney (HEK) cells and by determining action potential duration in rabbit cardiac Purkinje cells following GZ-793A exposure.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (200–250 g upon arrival) were purchased from Harlan (Indianapolis, IN) and housed two per cage with ad libitum access to food and water in the Division of Laboratory Animal Resources in the Biomedical Biological Sciences Research Building at the University of Kentucky (Lexington, KY). Following arrival, rats acclimated to the environment for 1 week prior to the start of experiments, at which time rats were approximately 9 weeks of age and weighed 250–275 g. Experimental protocols involving the animals were in accord with the 2011 *NIH Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky. Also, female New Zealand white rabbits (2.6–3.0 kg upon arrival) were purchased from Myrtle's Rabbitry, Inc. (Thompsons Station, TN), and were housed in an AALAC accredited facility. Studies involving the rabbits were performed in accordance with procedures published in peer-reviewed journals and according to standard operating procedures of ChanTest Corporation (Cleveland, OH).

2.2. Chemicals

[^{3}H]Nicotine (specific activity, 66.9 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA), while [^{3}H] methyllycaconitine (specific activity, 100 Ci/mmol) and [^{3}H]dofetilide (specific activity 80 Ci/mmol) were purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). (+)-Methamphetamine hydrochloride, sodium chloride, perchloric acid, disodium ethylenediamine tetra acetate (EDTA), ethyleneglycol tetra acetate (EGTA), L-(+) tartaric acid, *N*-[2-hydroxyethyl] piperazine-*N*'-[2-ethanesulfonic acid] (HEPES), dopamine hydrochloride, magnesium sulfate, D-glucose, 1-octanesulfonic acid sodium salt, amitriptyline, polyethyleneimine, ascorbic acid, potassium aspartate, adenosine triphosphate, and ascorbate oxidase were purchased from Sigma-Aldrich (St. Louis, MO). Citric acid, sodium bicarbonate, sodium phosphate, potassium chloride, calcium chloride, potassium phosphate and magnesium chloride were purchased from Fisher Scientific Co. (Pittsburgh, PA).

2.3. Drugs

Lobeline hemisulfate was purchased from ICN Biomedicals Inc. (Costa Mesa, CA). Lobelane, a defunctionalized, saturated *meso*-analog of lobeline, was synthesized as previously described (Zheng et al., 2005). The water-soluble analog of lobelane, GZ-793A, was synthesized as previously described (Horton et al., 2011b). Amitriptyline hydrochloride, ketamine hydrochloride, sodium heparin, and xylazine were purchased from Sigma-Aldrich.

2.4. Dopamine release assay

Rat coronal striatal slices (0.5 mm thick) were prepared and incubated in Krebs' buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgCl_2$, 1.0 mM NaH_2PO_4, 1.3 mM $CaCl_2$, 11.1 mM α-D-glucose, 25 mM $NaHCO_3$, 0.11 mM L-ascorbic acid and 0.004 mM EDTA, pH 7.4, saturated with 95% O_2/5% CO_2) at 34 °C in a metabolic shaker for 60 min (Teng et al., 1997). Each slice was then transferred to a glass superfusion chamber and superfused at 1 ml/min for 60 min with Krebs' buffer before sample collection. After 1 h, two 5-min basal samples (1 ml into 50 µl of 0.1 M perchloric acid) were collected. To determine the ability of GZ-793A to evoke dopamine release in the absence of methamphetamine, each slice was superfused for 30 min in the absence and presence of a single concentration of GZ-793A (1–100 µM), samples collected and dopamine and DOPAC concentrations in superfusate were determined. To determine the ability of GZ-793A to inhibit methamphetamine-evoked dopamine release, methamphetamine (5 µM) was added to the superfusion buffer, and slices were superfused for 15 min in the absence and presence of a range of GZ-793A concentrations. Following removal of methamphetamine from the buffer, superfusion continued for 25 min in the absence and presence of GZ-793A. Thus, in each experiment, duplicate slices were superfused with methamphetamine in the absence of GZ-793A, serving as the methamphetamine control condition. Also, in each experiment, slices were superfused in the absence of methamphetamine and GZ-793A, serving as the buffer control condition.

To determine the ability of GZ-793A to inhibit nicotine-evoked dopamine release, slices were prepared and superfused in the absence and presence of GZ-793A (3–100 µM) as described above. Nicotine (30 µM) was included in the superfusion buffer for 45 min in the absence and presence of GZ-793A. In each experiment, slices were superfused with nicotine in the absence of GZ-793A, serving as the nicotine control, as well as in the absence of nicotine and GZ-793A, serving as the buffer control.

To determine the ability of GZ-793A to inhibit electrical field stimulation-evoked dopamine release, slices were prepared and superfused in the absence and presence of GZ-793A (3–100 µM) as previously described. After 30 min, an electrical field stimulus (100 Hz, 1 min, 60 unipolar rectangular pulses; SD9 stimulator; Grass instruments, Quincy, MA) was applied. Stimulation parameters were chosen to obtain dopamine release comparable to that evoked by nicotine, as determined in pilot studies (data not shown).

Superfusate samples were processed immediately after collection. To each sample (500 µl), 20 µl of ascorbate oxidase (168 U/mg; reconstituted to 81 U/1 ml) was added, and samples vortexed for 30 s. An aliquot (100 µl) of each sample was injected onto the HPLC-EC, equipped with pump and auto-sampler (model 508, Beckman Coulter, Inc., Fullerton, CA), an ODS ultrasphere C18 reverse-phase column (80×4.6 mm, 3-µm ESA Inc., Chelmsford, MA) and a coulometric-II detector with guard cell (model 5020, ESA, Inc., Chelmsford, MA) maintained at +0.60 V and an analytical cell (model 5011) maintained at E1=−0.05 V and E2=+0.35 V. Mobile phase was 0.07 M citrate/0.1 M acetate buffer (175 mg/L octanesulfonic acid-sodium salt, NaCl 650 mg/L, and 7% methanol; pH=4). HPLC was