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Tetrabenazine inhibition of monoamine uptake and methamphetamine behavioral effects: Locomotor activity, drug discrimination and self-administration

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ABSTRACT

Tetrabenazine (TBZ), a benzoquinolizine derivative, binds with high affinity to the vesicular monoamine transporter-2 (VMAT2), inhibiting uptake of cytosolic monoamines. The current study aimed to provide preclinical evidence supporting the potential use of TBZ as a treatment for methamphetamine abuse. Effects of TBZ on function of the dopamine transporter (DAT) and serotonin transporter (SERT) in striatal and hippocampal synaptosomes, respectively, and on VMAT2 function in isolated striatal synaptic vesicles were determined. Effect of TBZ (acute, 0.1–3.0 mg/kg, s.c.; repeated, 1.0 mg/kg for 7 days) on locomotor activity in methamphetamine-sensitized rats was assessed. Ability of TBZ (0.1–3.0 mg/kg; s.c.) or vehicle to decrease the discriminative effect of methamphetamine also was determined. Ability of TBZ (acute, 0.1–1.0 mg/kg, s.c.; repeated, 0.1 or 1.0 mg/kg for 7 days) to specifically decrease methamphetamine self-administration was determined; for comparison, a separate group of rats was assessed for effects of TBZ on food-maintained responding. Results show that TBZ was 11-fold more potent inhibiting DAT than SERT, and 2.5-fold more potent inhibiting VMAT2 than DAT. Results from behavioral studies showed that the lowest dose of TBZ transiently increased methamphetamine self-administration, whereas higher TBZ doses decreased methamphetamine self-administration. Also, TBZ at high doses decreased methamphetamine locomotor sensitization and discriminative stimulus effects, as well as food-maintained responding. Thus, despite acting as a potent VMAT2 inhibitor, these preclinical results indicate that TBZ lacks behavioral specificity as an inhibitor of methamphetamine-induced reinforcement, diminishing its viability as a suitable treatment for methamphetamine abuse.

1. Introduction

Methamphetamine continues to be a prominent drug of abuse. According to the 2008 National Survey on Drug Use and Health, 850,000 Americans age 12 and older used methamphetamine at least once in the year prior to being surveyed, with past month users reaching 314,000 (Substance Abuse and Mental Health Services Administration, 2009). Between 1 and 2% of high school youth reported using methamphetamine at least once in the year prior to being surveyed (Johnston et al., 2009). As such, the discovery and development of pharmacotherapies for methamphetamine abuse remains critical, with current treatment strategies to promote abstinence relying mainly on behavioral interventions such as contingency management (Prendergast et al., 2006; Roll et al., 2006; Shoptaw et al., 2006). However, a pharmacological treatment for methamphetamine abuse would be highly beneficial to augment current treatment strategies, and thus, medication development remains an active research area.

Pharmacological actions of methamphetamine include: (1) augmentation of vesicular dopamine (DA) release from the vesicles and inhibition of the vesicular monoamine transporter-2 (VMAT2), resulting in decreased accumulation of DA into synaptic vesicles and increased cytosolic DA; (2) inhibition of monoamine oxidase (MAO), preventing the metabolism of cytosolic DA; and (3) reversal of the dopamine transporter (DAT), contributing to increased extracellular DA concentrations (Mantle et al., 1976; Sulzer et al., 1995; Brown et al., 2000, 2001; Fleckenstein et al., 2007). In concert, these actions of methamphetamine produce an increase in extracellular DA, which has been shown to be critical for its rewarding effects (Vollm et al., 2004).

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Based on the mechanism of action of methamphetamine, VMAT2 has been identified as a potential pharmacological target for the treatment of methamphetamine abuse (Dwoskin and Crooks, 2002; Zheng et al., 2006). Inhibition of VMAT2 would be expected to redistribute DA from the vesicular to cytosolic pool, where it is metabolized by intracellular MAO, which may reduce the concentration of cytosolic DA available for methamphetamine-induced reverse transport of DAT, and thereby, attenuate the increase in extracellular DA mediating reward. Consistent with this possibility, heterozygous VMAT2 knockout mice display decreased amphetamine-evoked striatal DA release (Wang et al., 1997) and diminished amphetamine conditioned place preference (Takahashi et al., 1997). The VMAT2 inhibitor, lobeline, decreases both methamphetamine-evoked DA release and methamphetamine self-administration in outbred rats (Harrod et al., 2001, 2003; Miller et al., 2001). However, lobeline also acts as a potent antagonist at nicotinic receptors, and less potently inhibits DAT function (Miller et al., 2000; Zheng et al., 2005; Wilhelm et al., 2008). Lobelane, a des-oxy lobeline analog, inhibits VMAT2 more potently and selectively than its parent compound (Miller et al., 2004; Zheng et al., 2005), and similarly decreases methamphetamine-evoked DA release and methamphetamine self-administration (Neugebauer et al., 2007; Nickell et al., 2010). The latter findings provide preclinical support for VMAT2 as a pharmacotherapeutic target for the treatment of methamphetamine abuse. Unfortunately, tolerance develops rapidly to the lobelane-induced decrease in methamphetamine self-administration (Neugebauer et al., 2007), revealing a pharmacological profile not suitable for clinical use.

Since the benzoquinolizine derivative, tetrabenazine (TBZ; Xenazine®) potently and reversibly binds to VMAT2 (Scherman et al., 1983; Erickson et al., 1996) and has been approved recently by the FDA for the treatment of chorea and other symptoms associated with Huntington's disease (Yero and Rey, 2008), the current study assessed the preclinical effects of tetrabenazine on DAT, serotonin transporter (SERT) and VMAT2 function and on methamphetamine-induced behavioral effects, including hyperactivity, discriminative stimulus effects and self-administration. The aim was to evaluate its preclinical profile as a potential treatment for methamphetamine abuse.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (250–275 g) were obtained from Harlan Industries (Indianapolis, IN, USA) and housed individually with *ad libitum* access to food (2018 Teklad Global 18% Protein Rodent Diet, Harlan; Madison, WI) and water in their home cage, except where noted, and were maintained in a temperature-controlled colony room on a 12:12-h light/dark cycle (lights on at 0700 h). Rats were handled and acclimated to the colony room for at least 1 week prior to the start of the behavioral experiments. Behavioral testing was conducted during the light cycle. Experimental protocols were in accordance with the 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.

2.2. Materials

D-Methamphetamine HCl was purchased from Sigma (St. Louis, MO) and was prepared in 0.9% NaCl (saline). Tetrabenazine and (2*R*,3*S*,11b*S*)-2-ethyl-3-isobutyl-9,10-dimethoxy-2,2,4,6,7,11b-hexahydro-1*H*-pyrido[2,1-a]isoquinolin-2-ol (Ro4-1284) were generous gifts from Hoffman-LaRoche Inc. (Nutley, NJ). TBZ was prepared in vehicle (20 mM HCl, adjusted to pH 4 with phosphoric acid). Ketamine and diazepam were purchased from N.L.S. Animal Health (Pittsburgh, PA). [^{3}H]DA (specific activity, 28.0 Ci/mmol), and [^{3}H]5-hydroxytryptamine (5-HT; specific activity, 30.0 Ci/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). [^{3}H]Dihydrotetrabenazine (DTBZ; specific activity, 20.0 Ci/mmol) was obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Bovine serum albumin (BSA), L-ascorbic acid, disodiumethylenediamine tetraacetate (EDTA), ethylene glycol tetraacetate (EGTA), L-(+) tartaric acid, N-[2-hydroxyethyl] piperazine-N′-[2-ethanesulfonic acid] (HEPES), 3-hydroxytyramine (DA), sucrose, magnesium sulfate ($MgSO_4$), D-glucose, sodium bicarbonate ($NaHCO_3$), pargyline, polyethyleneimine (PEI), fluoxetine HCl, 1-(2-(bis-(4-fluorophenyl)methoxy)ethyl)-4-(3-phenylpropyl)piperazine (GBR 12909), catechol and adenosine 5′-triphosphate magnesium salt (ATP-Mg^{2+}) were purchased from Sigma-Aldrich (St. Louis, MO). All other commercial chemicals were purchased from Fisher Scientific Co. (Pittsburgh, PA).

2.3. Synaptosomal [^{3}H]DA and [^{3}H]5-HT uptake

TBZ-induced inhibition of [^{3}H]DA and [^{3}H]5-HT uptake into rat striatal and hippocampal synaptosomes, respectively, was determined using modifications of a previously described method (Teng et al., 1997). Brain regions were homogenized in 20 ml of ice-cold 0.32 M sucrose solution containing 5 mM $NaHCO_3$ (pH 7.4) with 16 up-and-down strokes of a Teflon pestle homogenizer ("clearance, 0.005"). Homogenates were centrifuged (2000*g* for 10 min at 4 °C), and resulting supernatants centrifuged (20,000*g* for 17 min at 4 °C). Pellets were resuspended in 1.5 ml of Krebs' buffer (125 mM NaCl, 5 mM KCl, 1.5 mM $MgSO_4$, 1.25 mM $CaCl_2$, 1.5 mM KH_2PO_4, 10 mM α-D-glucose, 25 mM HEPES, 0.1 mM EDTA, with 0.1 mM pargyline and 0.1 mM ascorbic acid, saturated with 95% O_2/5% CO_2, pH 7.4). Synaptosomal suspensions (20 μg protein/50 μl) were added to duplicate tubes containing 50 μl TBZ (9 concentrations, 1 nM–0.1 mM, final concentration) and 350 μl of buffer and incubated at 34 °C for 5 min in a total volume of 450 μl. Samples were placed on ice and 50 μl of [^{3}H]DA or [^{3}H]5-HT (10 nM; final concentration), was added to each tube for a final total volume of 500 μl. Reactions proceeded for 10 min at 34 °C and were terminated by the addition of 3 ml of ice-cold Krebs' buffer. Nonspecific [^{3}H]DA and [^{3}H]5-HT uptake were determined in the presence of 10 μM GBR 12909 and 10 μM fluoxetine, respectively. Samples were rapidly filtered through Whatman GF/B filters using a cell harvester (MP-43RS; Brandel Inc.). Filters were washed 3 times with 4 ml of ice-cold Krebs' buffer containing catechol (1 μM). Complete counting cocktail was added to the filters and radioactivity determined by liquid scintillation spectrometry (B1600 TR scintillation counter; PerkinElmer, Inc.).

2.4. [^{3}H]DA uptake into synaptic vesicles

TBZ-induced inhibition of [^{3}H]DA uptake into isolated rat striatal vesicle preparations was determined using modifications of a previously described method (Teng et al., 1997). Briefly, rat striata were homogenized with 10 up-and-down strokes of a Teflon pestle homogenizer (clearance, 0.008″) in 14 ml of 0.32 M sucrose solution. Homogenates were centrifuged (2000*g* for 10 min at 4 °C), and the resulting supernatants were centrifuged again (10,000*g* for 30 min at 4 °C). Pellets were resuspended in 2 ml of 0.32 M sucrose solution and subjected to osmotic shock by adding 7 ml of ice-cold water, followed by immediate restoration of osmolarity by adding 900 μl of 0.25 M HEPES buffer and 900 μl of 1.0 M potassium tartrate solution. Samples were centrifuged (20,000*g* for 20 min at 4 °C), and the resulting supernatants centrifuged again (55,000*g* for 1 h at 4 °C), followed by addition of 100 μl of 10 mM MgSO4, 100 μl of 0.25 M HEPES and 100 μl of 1.0 M potassium tartrate solution prior to the final centrifugation (100,000*g* for 45 min at 4 °C). Final pellets were resuspended in 2.4 ml of assay buffer (25 mM HEPES, 100 mM potassium tartrate, 50 μM EGTA, 100 μM EDTA, 1.7 mM ascorbic acid, 2 mM ATP-Mg^{2+}, pH 7.4). Aliquots of the vesicular suspension (100 μl) were added to tubes containing assay buffer, various concentrations of TBZ (1 nM–100 μM) and 0.1 μM [^{3}H]DA for a final volume of 500 μl. Nonspecific uptake was determined in the presence of Ro4-1284 (10 μM). Reactions were processed as previously described.

To determine the mechanism of inhibition of [^{3}H]DA uptake for TBZ, kinetic analyses were performed. The concentration (35 nM) of TBZ utilized for the kinetic analysis approximated the K_i concentration previously determined in the inhibition assays. Nonspecific uptake was determined in the presence of Ro4-1284 (10 μM). Incubations were initiated by the addition of 50 μl of the vesicular suspension to 150 μl assay buffer, 25 μl of TBZ or Ro4-1284 and 25 μl of a range of concentrations of [^{3}H]DA (0.001–5.0 μM). Following an 8-min incubation period uptake was terminated by filtration, and radioactivity retained by the filters was determined as described previously.

2.5. Behavioral apparatus

Locomotor activity was recorded automatically using an animal activity monitoring system with Versamax System software (AccuScan Instruments Inc., Columbus, OH). Rats were placed in monitoring chambers (42 × 42 × 30 cm) made of clear acrylic walls and floor. Each chamber incorporated a horizontal 16 × 16 grid of photo beam sensors, with each beam 2.5 cm apart and 7 cm above the chamber floor. Horizontal activity was expressed as total distance traveled (cm).

Drug discrimination and self-administration were conducted in operant conditioning chambers (ENV-001; Med Associates, St Albans, VT), housed in sound-attenuated outer chambers, and using a Med Associates Interface model SG-503 with MED-IV software. The end walls of each operant conditioning chamber were aluminum, the front and back walls were made of clear Plexiglas and the floor consisted of 18 stainless steel rods (4.8 mm in diameter and placed 1.6 cm apart). Located in the bottom center of one of the end walls was an opening (5 × 4.2 cm) to a recessed food tray. Located on either side of the food tray was a response lever. A 28-V white cue light was located 6 cm above each response lever. A 28-V white house light was centered 20 cm above the floor on the wall opposite the response levers. An infusion pump (Med Associates) delivered drug via a silastic tube attached to a swivel mounted on the outside of the back wall.

2.6. Behavior

2.6.1. Methamphetamine-sensitized locomotor activity

On 10 consecutive days (sessions 1–10), rats were injected with methamphetamine (1.0 mg/kg, s.c.) and placed immediately in locomotor activity chambers for 90 min in order to induce sensitization.

2.6.2. Methamphetamine discrimination

Rats were trained initially to lever press for sugar-based 45 mg pellets (F0021 dustless precision pellet, Bio-Serve, Frenchtown, NJ) under a fixed ratio (FR) 1 schedule with both levers present in the operant chambers during a single 1-h session. Then, the FR requirement was increased subsequently to a terminal FR10 over the next 12 daily sessions, which were 15 min in duration. In addition, and in order to enhance acquisition of the methamphetamine-saline discrimination, only one lever (the saline-appropriate lever; counterbalanced across rats) was presented during these sessions following the initial lever-press training session. Once rats responded for 2 sessions (one with each lever present) under the FR10 schedule, methamphetamine discrimination training began. In this phase, methamphetamine (1.0 mg/kg, i.p.) or saline was administered 15 min prior to each daily 15-min session. Rats were then placed in the operant conditioning chambers, and the cue lights were illuminated to signal the beginning of the session. When methamphetamine was administered, only the methamphetamine-appropriate lever was presented and the saline-appropriate lever was retracted. When saline was administered, only the saline-appropriate lever was presented and the methamphetamine-appropriate lever was retracted. Again, responding was reinforced according to the FR10 schedule on these single-lever acquisition sessions. For half of the rats, the left lever was designated the methamphetamine-appropriate lever and the right lever was designated the saline lever; the reverse was true for the remaining rats. Methamphetamine and saline were administered according to a double-alternation sequence (i.e., MMSSMMSS or SSMMSSMM, counterbalanced across rats) for 8 consecutive sessions in which only the injection-appropriate lever was presented. Subsequently, and for the remainder of the experiment, both levers were presented during each session, and only responding on the injection-appropriate lever was reinforced with a food pellet in accordance with the FR10 schedule; responses on the incorrect lever were recorded but had no programmed consequence. Training continued until the following criteria were met on 7 of 8 consecutive sessions: 1) no more than 13 total responses were emitted prior to earning the first reinforcer; and 2) ≥85% of the total session responses occurred on the injection-appropriate lever.

Test sessions were initiated once acquisition criteria were met. Test sessions were similar to the 15-min discrimination training sessions, except that they were only 3 min in duration and completion of an FR10 on either lever resulted in food pellet delivery; further, a minimum of 2 daily training sessions (1 methamphetamine and 1 saline) were conducted between each test session (see below). To determine if TBZ inhibited the discriminative stimulus effects of methamphetamine, the methamphetamine dose-effect curve was determined following TBZ (0.1, 1.0, and 3.0 mg/kg, s.c.) or vehicle administration. For each rat, TBZ was administered according to a Latin Square design, and testing of each TBZ dose was completed prior to testing a new TBZ pretreatment dose. Also, methamphetamine doses were administered according to a Latin Square design. TBZ was administered 15 min prior to methamphetamine; methamphetamine was administered 15 min prior to the test session. Between each test session, a minimum of two daily 15-min training sessions occurred, in which either the methamphetamine training dose or saline was administered in a random order. Test sessions were conducted only if baseline performance during these intervening training sessions remained stable, defined as: 1) no more than 13 total responses emitted prior to earning the first reinforcer; and 2) ≥85% of the total session responses occurred on the injection-appropriate lever. Two dependent measures were collected during each test session: 1) percentage of total responses occurring on the methamphetamine-appropriate lever (calculated as the number of responses on the methamphetamine-appropriate lever divided by the total number of responses on each lever); and 2) rate of responding in sec (calculated as the total number of responses on each lever divided by 180). Lever selection data from rats failing to complete at least 10 responses were excluded from the statistical analyses of response rate.

2.6.3. Methamphetamine self-administration

The current methods were chosen to be consistent with previous methods examining the effect of lobeline or lobelane on methamphetamine self-administration (Harrod et al., 2001; Neugebauer et al., 2007). Three days prior to commencement of the study, rats were food restricted to intake of 15 g per day. Rats were trained briefly to respond for food reinforcement (45 mg Precision Pellets, Bio-Serv). On day 1 of training, rats were shaped to lever press for contingent food pellet reinforcement during a 60-min session. Only one lever was available, and lever positions were counterbalanced across rats. On the following consecutive days, both levers were available and rats experienced three sessions on each of the following FR schedules of responding for food reinforcement: FR1, FR3 and FR5. After training, rats were allowed free access to food and water for the remainder of the experiment. Within one week after training, rats were surgically implanted with a chronic indwelling jugular catheter. Rats were anesthetized with ketamine (80 mg/kg i.p.) and diazepam (5 mg/kg i.p.) and a silastic catheter (0.2 mm inner diameter; Fisher Scientific, Hampton, NH) was threaded subcutaneously to exit from a piece of stainless steel hypodermic tubing (22 gauge) embedded in a dental acrylic head cap mounted to the top of the skull with four stainless steel jeweler's screws. An infusion pump was attached to the head mount via a silastic leash during the self-administration sessions. Rats were given 7 days to recover from surgery before initiation of methamphetamine self-administration.

Rats self-administered methamphetamine during daily 60-min sessions for the remainder of the study. Rats began on an FR1 schedule with a 20-s time out signaled by the illumination of both lights above the levers. When the active lever was pressed, rats received an immediate infusion of methamphetamine (0.05 mg/kg/0.1 ml over 5.9 s). During the time out period, which began immediately with drug infusion, there were no programmed consequences of active lever response. Rats experienced three sessions responding on each of the following FR schedules: FR1, FR3 and FR5. Rats were trained up to an FR5 to engender a greater number of responses in order to provide enhanced sensitivity to pharmacological manipulation. Once training was completed at FR5, rats were monitored for stability criteria. Criteria consisted of <15% variability in infusions across three consecutive sessions, at least a 2:1 ratio of active to inactive lever responses, and at least 10 infusions of methamphetamine obtained during each session.

2.6.4. Food-maintained responding

Three days prior to commencement of the study, rats were food restricted to 15 g per day. On day 1 of training, rats were shaped to lever press for contingent food pellet reinforcement (45 mg Precision Pellets, Bio-Serv) during a 60-min session. Only one lever was available and the lever position was counterbalanced across rats. On the following consecutive days, both levers were available and rats experienced three sessions on each of the following FR schedules of reinforcement responding for food: FR1, FR3, FR5. After the second FR3 session, rats were allowed free access to food and water for the remainder of the experiment. Subsequently, rats were monitored for stability criteria consisting of <15% variability in food pellets earned across three consecutive sessions, at least a 2:1 ratio of active to inactive lever responses, and at least 10 food pellets earned during each session.

2.6.5. Assessment of acute TBZ pretreatment

The acute dose effect of TBZ on locomotor activity was assessed in rats sensitized previously to methamphetamine for 10 days. TBZ (0.1, 0.3, 1.0 or 3.0 mg/kg; s.c.) or vehicle pretreatments were given 15 min prior to either methamphetamine (1.0 mg/kg, s.c.) or saline. Rats were placed immediately in the locomotor activity apparatus for 90 min. All rats received each TBZ dose paired with either methamphetamine or saline in a randomized order. Between each pretreatment day, rats received only methamphetamine for two days to allow activity to return to baseline. Using similar procedures, the acute dose effect of TBZ (0.1, 0.3 and 1.0 mg/kg, s.c.; administered in a random order 15 min prior to the start of the session) was assessed on methamphetamine self-administration and food-maintained responding.

2.6.6. Assessment of repeated TBZ pretreatment

The effect of repeated TBZ on locomotor activity was assessed in rats sensitized to methamphetamine for 10 days. TBZ (1.0 mg/kg, s.c.) or vehicle pretreatments were given 15 min prior to methamphetamine (1.0 mg/kg; s.c.) in separate groups. Methamphetamine (1.0 mg/kg; s.c.) was administered immediately prior to the start of 7 consecutive sessions, followed by one day in which no pretreatment was administered in order to determine if the effect of TBZ was prolonged. Similarly, the effect of TBZ (0.1 or 1.0 mg/kg, s.c.) following repeated administration was assessed on methamphetamine self-administration and food-maintained responding in separate groups.

2.7. Statistical analyses

Specific [^{3}H]DA and [^{3}H]5-HT uptake were determined by subtracting nonspecific uptake from total uptake. TBZ concentrations that produced 50% inhibition of specific uptake (IC_{50} values) were determined from the concentration-effect curves via an iterative curve-fitting program (Prism 5.0; GraphPad Software Inc., San Diego, CA). Inhibition constants (K_i values) were determined using the Cheng–Prusoff equation [$K_i = IC_{50}/(1 + L/K_D)$]. Unpaired Student t-test using the log K_i values determined differences between TBZ inhibition of the transporters. K_m and V_{max} values were determined from concentration-effect curves using nonlinear regression (Prism 5.0). Paired two-tailed t tests were performed on the log K_m and V_{max} values to determine differences ($p < 0.05$) in kinetic parameters in the absence (control condition) and presence of TBZ. For drug discrimination data, ED_{50} (±95% confidence interval [CI]) values were calculated for the linear portion of each dose–response curve (i.e., the portion of the dose-effect curve above 25% and below 75% methamphetamine-appropriate responding, with no more than one data point below or above those boundaries), using the formula [effect = slope × log(dose) + intercept] (Prism 5.0). Shifts in the methamphetamine dose-effect curve following TBZ pretreatment were considered significant when the 95% confidence intervals did not overlap; in all other cases, statistical significance was declared at $p < 0.05$. For locomotor activity, methamphetamine self-administration and food-maintained responding experiments, only the first 15 min of each session were analyzed and graphically illustrated to allow comparison to previous work (Harrod et al., 2001; Neugebauer et al., 2007). Dose-effect curves and repeated pretreatments were analyzed by one- or two-way

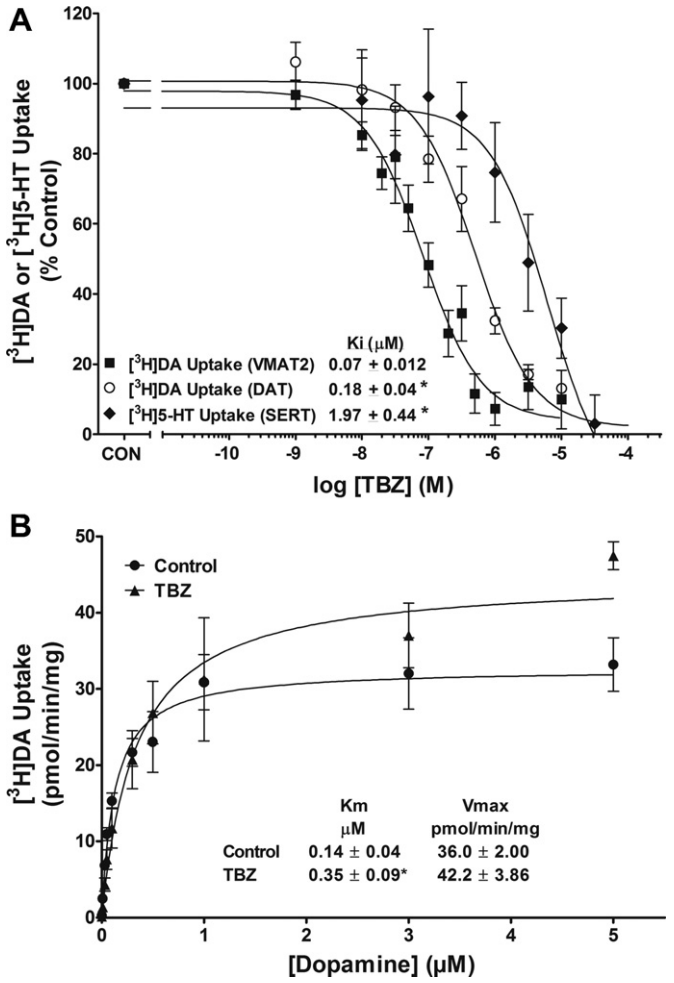

Fig. 1. Panel A: TBZ (1 nM–100 μM) inhibits [^{3}H]DA (○)and [^{3}H]5-HT (◆)uptake into rat striatal and hippocampal synaptosomes and [^{3}H]DA (■) uptake into rat striatal synaptic vesicle preparations. Nonspecific [^{3}H]DA and [^{3}H]5-HT uptake at DAT and SERT, respectively, were determined in the presence of 10 μM nomifensine and 10 μM fluoxetine, respectively. Nonspecific uptake at VMAT2 was determined in the presence of Ro4-1284 (10 μM). Data are presented as pmol/min/mg protein (mean ± SEM) specific [^{3}H]DA and [^{3}H]5-HT uptake as a percentage of control (CON; in the absence of TBZ). Control values for [^{3}H]DA (DAT), [^{3}H]5-HT (SERT), and [^{3}H]DA (VMAT2) uptake were 24.2 ± 1.08, 0.63 ± 0.11, and 49.3 ± 3.81 pmol/min/mg protein, respectively. $^*p < 0.05$ different from K_i value for VMAT2; $n = 4$–9 rats/assay. Panel B: Kinetic analysis of TBZ inhibition of [^{3}H]DA uptake into rat striatal synaptic vesicles. The concentration of TBZ utilized for the kinetic analysis was the K_i concentration from the inhibition curve illustrated in Panel A. Data are presented as pmol/min/mg (mean ± SEM) [^{3}H]DA uptake. Control represents [^{3}H]DA uptake in the absence of TBZ. Nonspecific uptake was determined in the presence of Ro4-1284 (10 μM). K_m and V_{max} values (mean ± SEM) are provided in the inset. $^*p < 0.05$ different from control; $n = 4$–7 rats/group.

analysis of variance (ANOVA). Self-administration data are presented as a percent of the vehicle control in order to compare results from both methamphetamine self-administration and food-maintained responding experiments. Post-hoc comparisons were conducted when ANOVA revealed significant main effects or interactions. Bonferroni's corrections were made for multiple comparisons.

3. Results

3.1. Effect of TBZ on [^{3}H]DA and [^{3}H]5-HT uptake into synaptosomes

Inhibition of [^{3}H]DA and [^{3}H]5-HT uptake into synaptosomes by TBZ is illustrated in Fig. 1A. TBZ inhibited DAT and SERT function with K_i values of 0.18 μM and 1.97 μM, respectively.

3.2. Effect of TBZ on [^{3}H]DA uptake into synaptic vesicles

Inhibition of [^{3}H]DA uptake into synaptic vesicles by TBZ is illustrated in Fig. 1A. TBZ inhibited [^{3}H]DA uptake with a K_i value of 0.07 μM. To elucidate the mechanism of TBZ-mediated inhibition of VMAT2, kinetic analyses were performed (Fig. 1B). TBZ increased Km compared to control (K_m = 0.35 and 0.14 μM, respectively, $p < 0.05$), without altering V_{max}, indicating that TBZ inhibits VMAT2 functioning in a competitive manner.

3.3. Effect of acute and repeated TBZ on methamphetamine-sensitized locomotor activity

The effect of acute TBZ on locomotor activity in methamphetamine-sensitized rats is shown in Fig. 2A. A two-way ANOVA revealed significant main effects of TBZ dose [$F(4,40) = 23.60$, $p < 0.001$] and methamphetamine treatment [$F(1,10) = 9.21$, $p < 0.05$] on locomotor activity. However, no TBZ dose × methamphetamine treatment interaction was found. Post-hoc *t*-tests, collapsed across methamphetamine and saline treatments, revealed differences in the amount of locomotor activity following TBZ (at doses of 0.3, 1.0, and 3.0 mg/kg) compared to vehicle [$t(11) = 4.49$; $t(11) = 6.47$; $t(11) = 6.71$,

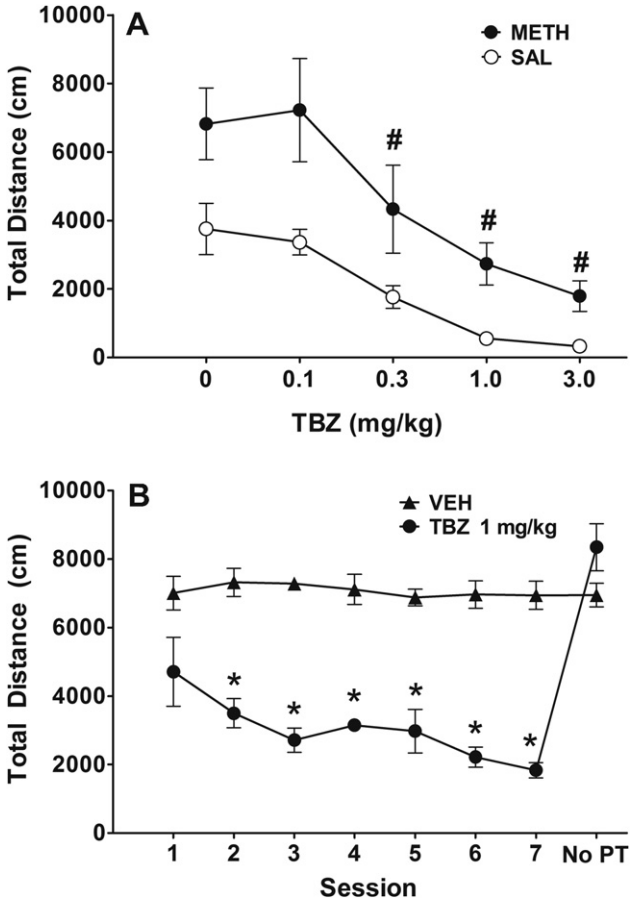

Fig. 2. Acute and repeated TBZ decreases locomotor activity in methamphetamine-sensitized rats. Data are presented as total distance traveled (cm; mean ± SEM) for the first 15 min of the 90-min locomotor session. Panel A: TBZ (0.1–3.0 mg/kg) or vehicle (VEH; 0) was administered 15 min prior to methamphetamine (METH, 1.0 mg/kg) or saline (SAL). # represents difference between TBZ dose and VEH (0), when collapsed across METH and SAL treatment, $p < 0.05$; $n = 6$ rats/group. Panel B: TBZ (1.0 mg/kg) or VEH was administered 15 min prior to methamphetamine (1.0 mg/kg) for 7 consecutive sessions. Prior to the 8th session, TBZ was not administered prior to methamphetamine (No PT). * represents a difference from VEH, $p < 0.05$; $n = 6$ rats/group.

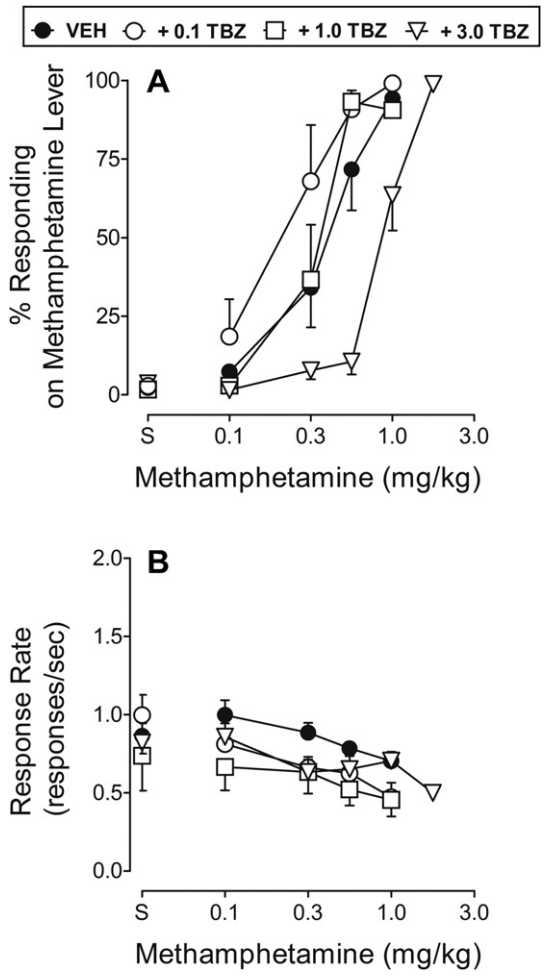

Fig. 3. TBZ (3.0 mg/kg) decreases the discriminative stimulus effects of methamphetamine in rats trained to discriminate methamphetamine (1.0 mg/kg) from saline. Panel A: Percent of responses on the methamphetamine-appropriate lever following methamphetamine (0.1–1.0 or 1.7 mg/kg) in combination with TBZ (0.1–3.0 mg/kg, open symbols) or vehicle (closed circles). $n = 6$ rats/group. Panel B: Response rate following methamphetamine (0.1–1.0 or 1.7 mg/kg) in combination with TBZ (0.1–3.0 mg/kg, open symbols) or vehicle (closed circles). $n = 6$ rats/group.

respectively; ps < 0.001]. Thus, TBZ dose-dependently decreased locomotor activity regardless of whether rats received methamphetamine or saline.

The effect of repeated TBZ (1.0 mg/kg) on methamphetamine-sensitized locomotor activity is shown in Fig. 2B. A two-way ANOVA revealed significant main effects of TBZ treatment [$F(1,10) = 132.45, p < 0.001$], and session [$F(7,70) = 10.52, p < 0.001$], as well as a significant TBZ treatment × session interaction [$F(7,70) = 11.09$, $p < 0.001$]. Post-hoc t-tests revealed significant differences between TBZ and vehicle on sessions 2–7 [$t(10) = 6.50$; $t(10) = 11.55$; $t(10) = 8.28$; $t(10) = 5.74$; $t(10) = 9.62$; $t(10) = 11.06$, respectively; ps < 0.001], indicating that TBZ decreased the effect of methamphetamine across repeated treatment.

3.4. Effect of TBZ on discriminative stimulus effects of methamphetamine

The effect of TBZ on the methamphetamine discriminative stimulus is shown in Fig. 3A. Across the dose range evaluated, TBZ alone did not substitute for methamphetamine (data not shown). However, pretreatment with TBZ (3.0 mg/kg) produced a significant rightward shift in the ED_{50} value (0.92 mg/kg; CI = 0.82–1.05 mg/kg) compared to vehicle control (0.44 mg/kg; CI = 0.23–0.75 mg/kg). A methamphetamine dose of 1.7 mg/kg was required to completely overcome the inhibition produced by TBZ (3.0 mg/kg). No dose of TBZ altered response rate (Fig. 3B).

3.5. Effect of acute and repeated TBZ on methamphetamine self-administration

The effect of acute TBZ on methamphetamine self-administration is shown in Fig. 4A. A one-way repeated measures ANOVA revealed an effect of TBZ dose [$F(3,12) = 12.66, p < 0.001$]. Post-hoc t-tests revealed differences in the number of methamphetamine infusions following 0.1 and 1.0 mg/kg doses of TBZ compared to vehicle [$t(4) = 3.65, p < 0.05$; $t(6) = 6.06, p < 0.001$; respectively], with the 0.1 mg/kg dose increasing infusions and the 1 mg/kg dose decreasing infusions.

The effect of repeated TBZ (0.1 or 1.0 mg/kg) on methamphetamine self-administration is shown in Fig. 4B. A two-way ANOVA revealed main effects of TBZ dose [$F(2,17) = 10.51, p < 0.001$] and session [$F(7,119) = 4.58$, $p < 0.001$], and a TBZ dose × session interaction [$F(14,119) = 3.58, p < 0.001$]. Post-hoc t-tests revealed

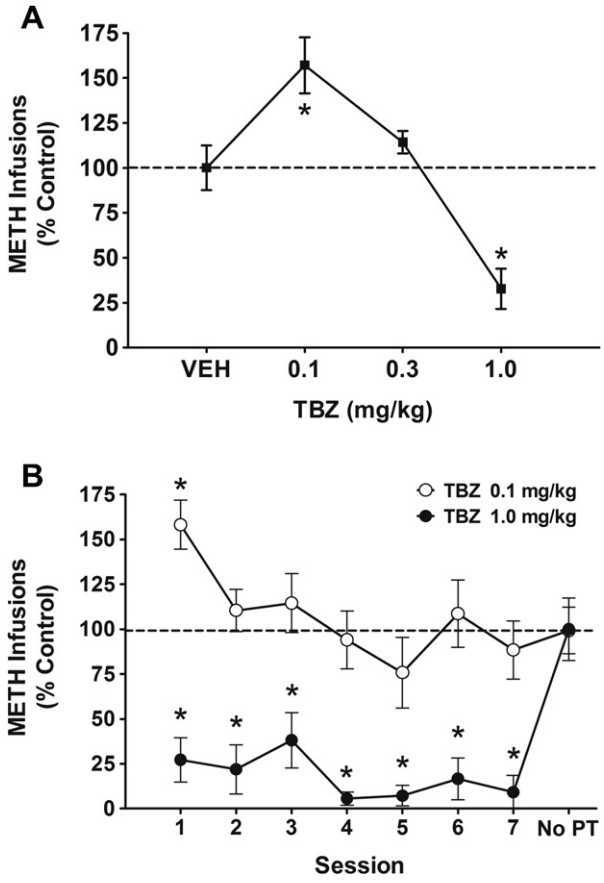

Fig. 4. TBZ produces a biphasic dose effect on methamphetamine (METH) self-administration, with a low dose (0.1 mg/kg) increasing responding acutely and a high dose (1.0 mg/kg) decreasing responding across repeated pretreatments. Panel A: Number of METH infusions during the first 15 min of the session following TBZ (0.1–1.0 mg/kg) or vehicle (VEH), expressed as a percentage of VEH control (7.0 ± 0.8 infusions; dashed line). * indicates a difference from VEH control, $p < 0.05$; $n = 5–8$ rats/group. Panel B: Number of METH infusions during the first 15 min of the 7 consecutive sessions following repeated pretreatment with TBZ (0.1 or 1.0 mg/kg), expressed as a percentage of VEH control (7.0 ± 1.0 infusions; dashed line). Prior to the 8th session, TBZ was not administered as a pretreatment (No PT). * indicates a difference from VEH control, $p < 0.05$; $n = 5–8$ rats/group.

differences in the number of methamphetamine infusions following 0.1 mg/kg of TBZ compared to vehicle on session 1 [$t(6) = 2.15$, $p < 0.05$, one tailed]. Differences were also found between 1.0 mg/kg of TBZ and vehicle on sessions 1–7 [$t(7) = 5.88$, $t(7) = 5.69$, $t(7) = 4.01$, $t(7) = 25.53$, $t(7) = 15.96$, $t(7) = 7.18$, $t(7) = 9.78$, ps < 0.001, respectively]. No between-groups difference was found when TBZ pretreatment was terminated on session 8.

3.6. Effect of acute and repeated TBZ on food-maintained responding

The effect of acute TBZ on food-maintained responding is shown in Fig. 5A. A one-way repeated measures ANOVA revealed an effect of TBZ dose [$F(3,15) = 43.88$, $p < 0.001$]. Post-hoc t-tests revealed that the number of pellets earned was different following TBZ (1.0 mg/kg) compared to vehicle [$t(5) = 6.26$, $p < 0.005$].

The effect of repeated TBZ (0.1 or 1.0 mg/kg) on food-maintained responding is shown in Fig. 5B. A two-way ANOVA revealed main effects of TBZ dose [$F(2,15) = 44.21$, $p < 0.001$] and session [$F(7,105) = 4.38$, $p < 0.001$], and a TBZ dose × session interaction [$F(14,105) = 4.42$, $p < 0.001$]. Post-hoc t-tests revealed differences in the number of pellets earned between 1.0 mg/kg of TBZ and vehicle on sessions 2–7 [$t(5) = 41.22$, $t(5) = 16.47$, $t(5) = 128.07$, $t(5) = 32.04$, $t(5) = 32.01$, $t(5) = 42.56$, ps < 0.001, respectively]. No between-groups difference was found when TBZ pretreatment was terminated on session 8.

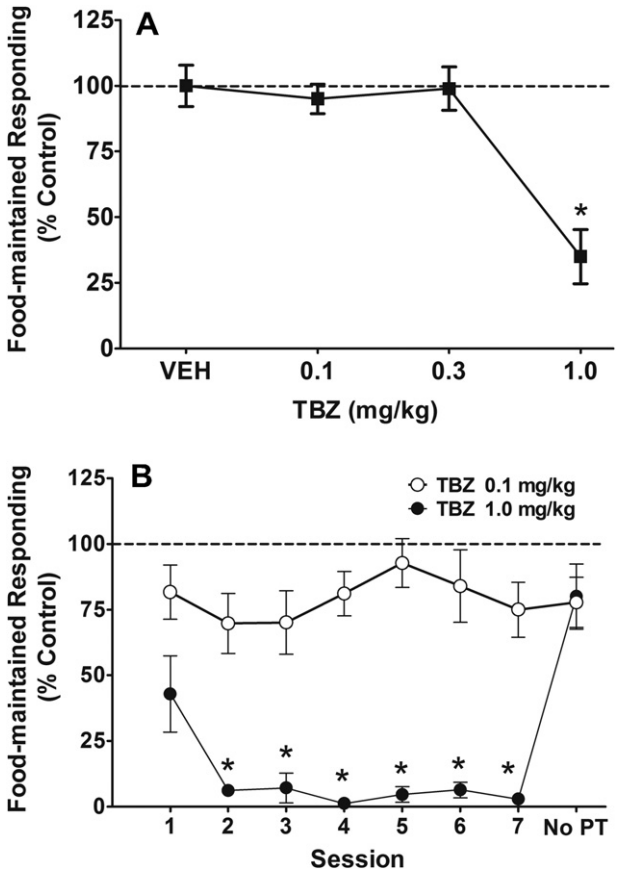

Fig. 5. TBZ produces a dose-dependent decrease in food-maintained responding, both acutely and repeatedly. Panel A: Number of food pellets earned during the first 15 min of the session following TBZ (0.1–1.0 mg/kg) or vehicle (VEH), expressed as a percentage of VEH control (30.5 ± 2.4 food pellets; dashed line). * indicates a difference from VEH control, $p < 0.05$; $n = 6$ rats/group. Panel B: Number of food pellets earned during the first 15 min of the 7 consecutive sessions following repeated pretreatment with TBZ (0.1 or 1.0 mg/kg), expressed as a percentage of VEH control (25.8 ± 2.4 food pellets; dashed line). Prior to the 8th session, TBZ was not administered as a pretreatment (No PT). * indicates a difference from VEH control, $p < 0.05$; $n = 6$ rats/group.

4. Discussion

The current study sought to evaluate TBZ, which has been approved for the treatment of chorea associated with Huntington's disease, as a potential treatment for methamphetamine abuse. Analogs of TBZ have been used extensively as radioligand probes for VMAT2 in neurological studies evaluating neurodegeneration due to its specific high affinity binding to this presynaptic protein (Frey et al, 1996; Bohnen et al., 2000). Also, TBZ has been reported to potently inhibit monoamine transport at VMAT2 (Scherman, 1986; Erickson et al., 1996). The current study extends these latter findings by showing that TBZ inhibits VMAT2 function with high affinity and in a competitive manner. Lobelane, an analog of lobeline, also competitively inhibits VMAT2 function and decreases methamphetamine-evoked DA release and methamphetamine self-administration (Neugebauer et al., 2007; Nickell et al., 2010). Further, TBZ was found to be only 2.5-fold selective for VMAT2 over DAT, demonstrating some inhibition of DAT function across the range of concentrations which inhibit VMAT2. Thus, the current study extends previous findings by showing that TBZ inhibits DAT function at concentrations similar to those for cocaine, methylphenidate and methamphetamine that also inhibit DAT function (Han and Gu, 2006; Nickell et al., 2010). The interaction of TBZ at DAT may be challenging in terms of drug development, as the potential for abuse liability has been associated with this pharmacological profile (Ritz et al., 1987). TBZ also exhibited 11-fold lower potency for inhibiting SERT compared with DAT. Thus, TBZ is 2.5-fold selective for VMAT2 over DAT and 56-fold selective for VMAT2 over SERT. Of further note, previous research has shown that TBZ inhibits the norepinephrine transporter (NET) across a concentration range similar to that inhibiting DAT and SERT ($IC_{50} = 0.5$ μM; Ross and Renyi, 1966). In comparison, lobelane is 35-fold selective for VMAT2 over DAT and 80-fold selective for VMAT2 over SERT. Taken together, TBZ and lobelane are equipotent competitive inhibitors of VMAT2, with TBZ exhibiting lower selectivity as an inhibitor of VMAT2 than lobelane.

With respect to the current behavioral studies, acute TBZ (0.3–3.0 mg/kg) dose-dependently decreased locomotor activity following either methamphetamine or saline challenge in rats presensitized to methamphetamine, indicating that TBZ attenuated general locomotor activity nonspecifically. This general attenuation of activity may be attributed to TBZ-induced depletion of monoamines, as this is the primary mechanism thought to be responsible for its beneficial treatment of chorea associated with Huntington's disease (Reches et al., 1983). Alternatively, TBZ may have decreased locomotor activity by inhibiting NET function (Moran-Gates et al., 2005). In any case, these results support previous work showing that TBZ decreases locomotor activity in drug naive mice, and profoundly reduces methamphetamine-induced hyperactivity (Kuribara, 1997). However, in the current study, rats were first sensitized to methamphetamine to allow for comparison to the results from the methamphetamine self-administration experiments and to predict the utility of TBZ as a pharmacotherapeutic in human methamphetamine abusers. As an alternative explanation, it is also possible that TBZ specifically attenuated the expression of conditioned hyperactivity following the methamphetamine or saline challenge. Regardless of the interpretation, repeated TBZ decreased locomotor activity across 7 consecutive sessions. Although tolerance did not develop, the effect of repeated TBZ treatment was reversible, since methamphetamine-induced activity returned to baseline after termination of TBZ treatment.

TBZ did not substitute for methamphetamine in the current drug discrimination studies and did not alter response rates in this behavioral assay. However, the low dose of TBZ (0.1 mg/kg) tended to produce a leftward shift in the methamphetamine generalization dose–response curve, suggesting a modest enhancement in the discriminative stimulus effect of methamphetamine. In contrast, as the TBZ dose increased, the methamphetamine generalization curve shifted to the right, indicating that TBZ attenuates the discriminative stimulus effect of methamphetamine. However, the TBZ-induced attenuation of methamphetamine discriminative stimulus effects was overcome by administration of a higher dose of methamphetamine. Importantly, within the dose range tested, TBZ did not produce rate suppression effects in this behavioral assay.

When TBZ was administered acutely prior to methamphetamine self-administration, a biphasic effect was found such that the low dose of TBZ (0.1 mg/kg) increased responding, while the high dose (1.0 mg/kg) decreased responding for methamphetamine. In contrast, acute lobelane produced a monophasic decrease in methamphetamine self-administration (Neugebauer et al., 2007). With repeated TBZ administration in the current study, tolerance developed to the TBZ-induced increase in responding, whereas no tolerance developed to the TBZ-induced decrease in responding for methamphetamine across the 7 consecutive sessions. In contrast, tolerance developed to the lobelane-induced attenuation of responding for methamphetamine (Neugebauer et al., 2007). Taken together, TBZ may have benefits over lobelane as a pharmacotherapeutic treatment for methamphetamine abuse, since tolerance does not develop to the decrease in methamphetamine self-administration.

To evaluate the specificity of TBZ-induced decreases in responding for methamphetamine, the effect of TBZ to decrease responding maintained by food also was evaluated. Food-maintained responding was not altered by acute or repeated administration of the low doses of TBZ, whereas responding was decreased by both acute and repeated administration of the highest dose (1.0 mg/kg), indicating that the high dose of TBZ did not specifically alter responding for methamphetamine. An ideal treatment for methamphetamine abuse would be specific for methamphetamine reinforcement and have little effect on food reinforcement. In contrast to TBZ, lobelane produced no effect on food-maintained responding (Neugebauer et al., 2007). The persistent decrease in food-maintained responding produced by TBZ likely reflects either a decrease in the appetitive property of food or a nonspecific decrease in lever pressing. In either case, when repeated TBZ administration ceased, responding for food returned rapidly to baseline, indicating that TBZ did not have prolonged effects.

Unexpectedly, while TBZ decreased methamphetamine self-administration, locomotor activity and food-maintained responding, there was no rate suppressant effect of TBZ in the drug discrimination test. One possible explanation for these contrasting findings across behavioral assays is that methamphetamine self-administration, locomotor activity, and food-maintained responding may depend more on DA-mediated mechanisms disrupted by TBZ, whereas the discriminative stimulus effects from methamphetamine may involve non-DA mechanisms. Alternatively, the relative insensitivity of response rates to TBZ in the drug discrimination assay may simply reflect a procedure difference in training history compared with the other assays.

The most surprising finding from the current study is the acute increase in methamphetamine self-administration at the low dose of TBZ (0.1 mg/kg), as well as a trend toward a leftward shift in the generalization dose-effect curve in the methamphetamine discrimination assay. These results suggest that low doses of TBZ may increase the rewarding effect, and perhaps the discriminative stimulus effect, of methamphetamine. A possible explanation for this increased sensitivity is that, in addition to inhibiting VMAT2, TBZ also inhibits DAT function (current findings) and/or pre- and post-synaptic DA receptors (Login et al., 1982; Reches et al., 1983). If low doses of TBZ inhibit the D2 autoreceptor preferentially, DA release would be enhanced in the presence of methamphetamine, which could increase methamphetamine self-administration. Alternatively, inhibition of post-synaptic DA receptors may result in a compensatory increase in responding for methamphetamine in an attempt to surmount the inhibition. Regardless of the mechanism, however, tolerance to the TBZ-induced increase in methamphetamine self-administration occurs rapidly, with responding returning to baseline following repeated TBZ administration.

Although TBZ potently and competitively inhibits VMAT2 and decreases the behavioral effects of methamphetamine, the potential abuse liability and nonspecific behavioral effects may limit its therapeutic potential as a treatment for methamphetamine abuse. These results also bring forth a note of caution and controversy in the development of VMAT2 inhibitors as pharmacotherapies for methamphetamine abuse. Previous research using heterozygous VMAT2 knockout mice has shown increased methamphetamine-induced neurotoxicity (Fumagalli et al., 1999). In contrast, the VMAT2 inhibitor lobeline protects against methamphetamine neurotoxicity (Eyerman and Yamamoto, 2005), and is currently in clinical trials (Jones, 2007). More research is necessary to further understand the value of VMAT2 as a potential target for methamphetamine pharmacotherapies and future work may discover more selective novel analogs derived from lobelane as potential treatments for methamphetamine abuse.

Disclosure statement

The University of Kentucky holds patents on lobeline, lobelane, and novel analogs of these compounds. A potential royalty stream to Dwoskin may occur consistent with University of Kentucky policy.

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