Behavioral and pharmacokinetic interactions between monoamine oxidase inhibitors and the hallucinogen 5-methoxy-N,N-dimethyltryptamine

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Monoamine oxidase inhibitors (MAOIs) are often ingested together with tryptamine hallucinogens, but relatively little is known about the consequences of their combined use. We have shown previously that monoamine oxidase-A (MAO-A) inhibitors alter the locomotor profile of the hallucinogen 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) in rats, and enhance its interaction with 5-HT2A receptors. The goal of the present study was to investigate the mechanism for the interaction between 5-MeO-DMT and MAOIs, and to determine whether other behavioral responses to 5-MeO-DMT are similarly affected. Hallucinogens disrupt prepulse inhibition (PPI) in rats, an effect typically mediated by 5-HT2A activation. 5-MeO-DMT also disrupts PPI but the effect is primarily attributable to 5-HT1A activation. The present studies examined whether an MAOI can alter the respective contributions of 5-HT1A, and 5-HT2A receptors to the effects of 5-MeO-DMT on PPI. A series of interaction studies using the 5-HT1A antagonist WAY-100,635 and the 5-HT2A antagonist MDL 11,939 were performed to assess the respective contributions of these receptors to the behavioral effects of 5-MeO-DMT in rats pretreated with an MAOI. The effects of MAO-A inhibition on the pharmacokinetics of 5-MeO-DMT and its metabolism to bufotenine were assessed using liquid chromatography–electrospray ionization–selective reaction monitoring–tandem mass spectrometry (LC-ESI-SRM-MS/MS). 5-MeO-DMT (1 mg/kg) had no effect on PPI when tested 45-min post-injection but disrupted PPI in animals pretreated with the MAO-A inhibitor clorgyline or the MAO-A/B inhibitor pargyline. The combined effect of 5-MeO-DMT and pargyline on PPI was antagonized by pretreatment with either WAY-100,635 or MDL 11,939. Inhibition of MAO-A increased the level of 5-MeO-DMT in plasma and whole brain, but had no effect on the conversion of 5-MeO-DMT to bufotenine, which was found to be negligible. The present results confirm that 5-MeO-DMT can disrupt PPI by activating 5-HT2A, and indicate that MAOIs alter 5-MeO-DMT pharmacodynamics by increasing its accumulation in the central nervous system.

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1. Introduction

It is not uncommon for tryptamine hallucinogens to be administered in combination with drugs that inhibit monoamine oxidase-A (MAO-A). For example, the potent hallucinogenic beverage known as ayahuasca contains tryptamines such as N,N-dimethyltryptamine (DMT) and 5-methoxy-DMT (5-MeO-DMT) (Agurell et al., 1968; McKenna et al., 1984), as well as β-carbolines such as harmaline and harmine, which are reversible MAO-A inhibitors (MAOIs) (Buckholtz and Boggan, 1977; Kim et al., 1997). Although DMT is normally inactive orally due to first-pass metabolism (Turner and Merlis, 1959; Riba et al., 2015), the β-carbolines contribute to the activity of ayahuasca by blocking the catabolism of DMT in the gastrointestinal tract. Modern Brazilian syncretic religious groups such as União do Vegetal have adopted the use of ayahuasca as a sacrament, and this practice has now spread to Europe and North America (Labate and Feeney, 2012). DMT and 5-MeO-DMT are also used recreationally in Western societies, typically by smoking, but they can also be ingested orally in combination with an MAOI (Shulgin and Shulgin, 1997; Ott, 1999, 2001; Cakic et al., 2010). Indeed, there is evidence that tryptamine hallucinogens and MAOIs are abused together (Ott, 1996; Brush et al., 2004; Sklerov et al., 2005).

5-HT2A receptor activation is responsible for mediating the characteristic subjective and behavioral effects of hallucinogens in both humans and animals (Nichols, 2004; Halberstadt, 2015). All serotonergic hallucinogens bind to 5-HT2A receptors, but with differing selectivity. Phenethylalkylamines, including mescaline and 2,5-dimethoxy-4-methylamphetamine (DOM), are selective for 5-HT1 sites, whereas indoleamines such as N,N-dimethyltryptamine (DMT), 5-MeO-DMT,
psilocybin, and (+)-lysergic acid diethylamide (LSD), are non-selective for serotonin (5-HT) receptor subtypes.

Previous studies in this laboratory have shown that the behavioral effects of 5-MeO-DMT are modified in animals treated with an MAOI (Halberstadt et al., 2008, 2012). Rats treated with 5-MeO-DMT alone exhibit a brief reduction in locomotor activity that can be blocked by the 5-HT1A-selective antagonist WAY-100,635, but not by a 5-HT2A-selective antagonist (Krebs-Thomson et al., 2006). By contrast, when 5-MeO-DMT is administered to rats pretreated with a behaviorally inactive dose of an MAOI (e.g., clorglyline, pargyline, or harmaline), it produces biphasic effects, initially suppressing locomotor activity and then increasing activity at later time points. Administration of higher doses of 5-MeO-DMT alone did not reproduce this effect. The behavioral profile of 5-MeO-DMT is altered by the MAOA inhibitors harmaline and clorglyline, as well as by the MAOAβ inhibitor pargyline, whereas the selective MAOA inhibitor (−)-deprenyl is ineffective (Halberstadt et al., 2008, 2012). Given those findings, it is likely that MAOA inhibition is responsible for the interaction with 5-MeO-DMT. Importantly, the late hyperactivity produced by 5-MeO-DMT in combination with an MAOI can be blocked by the selective 5-HT2A antagonist MDL 11,939 but not by WAY-100,635 (Halberstadt et al., 2008). It appears MAOA inhibition markedly enhances the contribution that 5-HT2A receptors make to the behavioral effects of 5-MeO-DMT.

One possible explanation for the interaction between 5-MeO-DMT and MAOIs is that MAOA inhibition alters the pharmacokinetics of 5-MeO-DMT. The primary route of metabolism for 5-MeO-DMT is oxidative deamination to 5-methoxyindoleacetic acid by monoamine oxidase-A (MAOA). Small amounts of 5-MeO-DMT are O-demethylated to bufotenine by cytochrome P450 2D6 (see Fig. 1). Inhibition of MAOA has been shown to enhance the conversion of 5-MeO-DMT to bufotenine in mice (Shen et al., 2010a, 2010b). Since bufotenine is a potent and highly efficacious 5-HT2A agonist (Roth et al., 1997; Egan et al., 2000), we have theorized that the delayed hyperactive phase induced by 5-MeO-DMT in the presence of an MAOI may occur as a consequence of the formation of bufotenine, which could potentially accumulate in the brain and induce hyperactivity by activating 5-HT2A (Halberstadt et al., 2012).

The goal of the present studies was to investigate the mechanism for the interaction between 5-MeO-DMT and MAOIs, and to determine whether other behavioral responses to 5-MeO-DMT are similarly affected. Hallucinogens, including LSD and DOI, are known to inhibit prepulse inhibition (PPI) in rats by activating 5-HT2A (Sipes and Geyer, 1995; Padich et al., 1996; Ouagazzal et al., 2001; Halberstadt and Geyer, 2010). 5-MeO-DMT also disrupts PPI in rats (Rigdon and Weatherspoon, 1992), but the effect of 5-MeO-DMT on PPI is mediated by 5-HT1A and not by 5-HT2A (Krebs-Thomson et al., 2006). Because MAOA inhibition can enhance the behavioral relevance of 5-MeO-DMT interactions with 5-HT2A, the present studies assessed whether MAOI pretreatment alters the respective contributions of 5-HT1A and 5-HT2A receptors to the effects of 5-MeO-DMT on PPI. Additional studies were conducted to determine whether inhibition of MAOA alters the pharmacokinetics of 5-MeO-DMT and its biotransformation to bufotenine.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN, USA; initial weight 250–275 g) were housed in pairs in a temperature- and humidity-controlled vivarium under a 12-h reverse light–dark cycle (lights off at 0700 h). Food and water were available ad libitum (except during behavioral testing). Animals were allowed to acclimatize for approximately 1 week after arrival prior to behavioral testing and maintained in AALAC-approved facilities that meet all federal and state

![Fig. 1. Biotransformation of 5-MeO-DMT. The primary route of 5-MeO-DMT metabolism is oxidative deamination to 5-methoxyindoleacetic acid by monoamine oxidase-A (MAOA). Small amounts of 5-MeO-DMT are O-demethylated to bufotenine by cytochrome P450 2D6 (CYP2D6).](image-url)
guidelines. Procedures were approved by the University of California San Diego (UCSD) institutional animal care and use committee. Principles of laboratory animal care were followed as well as specific laws of the United States.

2.2. Materials

5-Methoxy-N,N-dimethyltryptamine (5-MeO-DMT), N-methylserotonin (NMS), N-methyl-N-propargyl-3-(2,4-dichlorophenoxo)-propylamine HCl (clorgyline), pargyline HCl, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridylcyanoclohexanecarboxamide maleate (WAY-100,635), ammonium acetate, sodium phosphate monobasic, and sodium phosphate dibasic were purchased from Sigma-Aldrich (St. Louis, MO). Bufotenine was purchased from Cerilliant (Round Rock, TX). α-Phenyl-1-(2-phenylethyl)-4-piperidinemethanol (MDL 11,939) was purchased from Tocris Bioscience (Ellisville, MO). LC-MS grade methanol and ethyl acetate were obtained from Honeywell-Burdick & Jackson (Muskegon, MI). Blank rat plasma used to prepare the calibrators and controls was purchased from Biochemed (Winchester, VA).

For the in vivo experiments, drug doses are expressed as the salt form of the drug, with the exception of 5-MeO-DMT and MDL 11,939, which refer to the freebase weight. All drugs were administered subcutaneously (SC) in a volume of 1 mL/kg. 5-MeO-DMT, WAY-100,635, clorgyline, and pargyline were dissolved in isotonic saline. MDL 11,939 was dissolved in saline (pH 5.0) containing 0.75% Tween 80.

2.3. Apparatus

2.3.1. Behavioral pattern monitor

Activity was measured in the Behavioral Pattern Monitor (BPM), which assesses spatiotemporal patterns of exploratory and investigatory behavior (for details, see: Geyer et al., 1986). The rat BPM is a 30.5 × 61.0 × 28.0 cm black Plexiglas chamber equipped with 2.5 cm holes in the walls and floor. A 4 × 8 grid of infrared photobeams is used to detect the animal’s position in an X−Y plane. Infrared photobeams in each hole are used to detect investigatory nosepokes (holepokes). Rearings are detected by touchplates on the walls. Each chamber is illuminated by a 15-W red incandescent light located above the center. The status of the photobeams and the touchplate is sampled every 55 ms, digitized, and the data stored on a PC for offline analysis.

2.3.2. Acoustic startle

Startle chambers (SR-LAB system, San Diego Instruments, San Diego, CA) were used to measure startle reactivity (Mansbach et al., 1988). Each startle test chamber consists of a sound-attenuated, lighted, and ventilated enclosure holding a clear nonrestrictive cylindrical Plexiglas stabiilitmer, 8.2 cm in diameter. The acoustic stimuli were generated by a high-frequency loudspeaker mounted 24 cm above the Plexiglas cylinder. The peak and average amplitude of the startle response were detected by a piezoelectric accelerometer, digitized, and stored on a PC. At the onset of the startling stimulus, 100 1-ms samples were recorded, and the average amplitude was used to determine the startle response. Dynamic calibration system was used to ensure comparable stabiilitmer sensitivity across test chambers, and sound levels were measured using the dB(A) scale, as described previously (Mansbach et al., 1988).

Acoustic startle test sessions consisted of startle trials (PULSE-ALONE) and prepulse trials (PREPULSE + PULSE). The PULSE-ALONE trial consisted of a 40-ms 120-dB pulse of broadband white noise. PREPULSE + PULSE trials consisted of a 20-ms acoustic prepulse, an 80-ms delay, and then a 40-ms 120-dB startle pulse (100 ms onset). There was an average of 15 s (range, 9–21 s) between trials. During each inter-trial interval, the movements of the rats were recorded once to measure responding when no stimulus was present (data not shown). Each startle session began with a 5-min acclimation period to a 65-dB broadband noise that was present continuously throughout the session. The startle test session included three blocks. The first and last block consisted of 5 PULSE-ALONE trials that were not used in the calculation of PPI values. The second block was designed to assess PPI; it contained 12 PULSE-ALONE trials and 30 PREPULSE + PULSE trials [10 prepulses each of 68, 71, and 77 dB (3, 6, and 12 dB above background)] presented in a pseudo-randomized order. One week after arrival, animals were tested in a brief startle/PPI session to create treatment groups matched for baseline levels of startle and PPI.

2.4. Experimental design

2.4.1. Acoustic startle

Animals were placed in the startle chambers 45 min after treatment with 5-MeO-DMT. The animals were tested 45 min after drug administration because the experiments were designed to assess acoustic startle and PPI during the period of time when animals treated with clorgyline and 5-MeO-DMT display locomotor hyperactivity in the BPM (i.e., 40–70 min after injection of 5-MeO-DMT; see Halberstadt et al., 2012). In experiment 1, rats (n = 10/group, 60 total) were treated with the nonselective MAO inhibitor pargyline (0 or 10 mg/kg) 20 min before administration of 5-MeO-DMT (0.1, 0.1, or 1.0 mg/kg). In experiment 2, rats (n = 12/group, 48 total) were treated with the MAOa inhibitor clorgyline (0 or 0.3 mg/kg) 20 min before administration of 5-MeO-DMT (0 or 1.0 mg/kg). In experiment 3, rats (n = 10/group, 60 total) were pretreated with vehicle, the selective 5-HT1A antagonist WAY-100,635 (1.0 mg/kg), or the selective 5-HT2A antagonist MDL 11,939 (0.3 mg/kg), 20 min before treatment with vehicle or 1.0 mg/kg 5-MeO-DMT. For experiment 3, all the animals that were injected with 5-MeO-DMT were pretreated (20 min) with 10 mg/kg pargyline. WAY-100,635 and MDL 11,939 were tested at doses previously shown to block 5-HT1A and 5-HT2A-mediated behavioral responses in Sprague–Dawley rats (Krebs-Thomson et al., 2006; Halberstadt et al., 2008; Halberstadt and Geyer, 2010).

2.4.2. Behavioral pattern monitor

One day before the study, animals were taken to the BPM testing room, weighed, handled briefly, placed in a clear Plexiglas box (24 × 46 cm) for approximately 30 s, and then returned to their cages in the vivarium. On the testing day, rats were brought to the testing room and allowed to sit for 60 min before receiving injections. Injections were administered under red lights in the testing room. Animals were tested during the dark phase in darkness. Rats were divided into two groups (n = 30/group) and pretreated with vehicle or clorgyline (0.3 mg/kg) 20 min before administration of 1.0 mg/kg 5-MeO-DMT. The animals were placed in the BPM chambers 10 min after 5-MeO-DMT treatment, and locomotor activity was monitored for 10, 20, 30, 40, 50, or 60 min (n = 5/time point/group). The rats were immediately removed from the BPM chambers, anesthetized (isoflurane), decapitated, and trunk blood collected in cooled heparinized tubes. Plasma was isolated by centrifugation (3200 × g) and stored at −40 °C. The brains were removed, frozen in isopentane at −80 °C, and stored at −40 °C.

2.5. Procedures for quantification of 5-MeO-DMT and bufotenine in plasma and brain tissue

2.5.1. Preparation of calibrators and controls

The calibrators and controls were prepared in silanized, 16 × 100 mm glass culture tubes containing 1 mL blank rat plasma. Working solutions containing 0.01, 0.1, 1.0, and 10 ng/μL of 5-MeO-DMT or bufotenine were prepared in methanol for spiking the analytes. Separate sets of working solutions were used for the calibrators and controls. The calibrators were prepared at 1, 2, 5, 10, 20, 50, 100, and 200 ng/mL and were run in duplicate with one set at the beginning...
A total of 60 rats were used for this experiment (n = 10/group). Tissue was weighed, transferred to a 50-mL conical polypropylene tube, and nine volumes of tissue homogenate sample was prepared for each brain. Brain tissue, a 10-fold dilution of tissue homogenate, was prepared for each sample, standard, and control tube. The tubes were vortexed, and 3 mL of 0.1 M sodium phosphate buffer (pH 6.0) was added to each tube. The tubes were briefly vortexed and then centrifuged (1200×g). The supernatants from each tube were then applied to their respective conditioned solid-phase extraction column as described below.

### 2.5.3. Extraction

Clean Screen ZSDAU020 solid phase extraction columns (United Chemical Technologies, Bristol, PA) were used for the extraction procedure. A separate column was utilized for each sample, standard, and control. A glass manifold device was employed for the extraction process. First, the extraction columns were conditioned by sequential addition of 3 mL of methanol, 3 mL of water, and 2 mL of 0.1 M sodium phosphate (pH 6.0), and then the supernatants from sample preparation were added. Next, the columns were sequentially washed with 3 mL of water and 3 mL of methanol. With care taken to prevent drying of the columns, the columns were eluted by the addition of 3 mL of 2% ammonium hydroxide in ethyl acetate and 4% ammonium hydroxide in methanol. The eluates from each column were collected into clean 16 × 100 mm glass culture tubes. The extracts were reconstituted with 100 μL of 10 mM ammonium acetate (pH 5.0)/methanol (80:20) and then transferred to separate 300 μL vials. The extracts were reconstituted with 100 μL of 10 mM ammonium acetate (pH 5.0)/methanol (80:20) and then transferred to separate 300 μL vials.

### 2.5.4. LC–MS/MS analysis

The liquid chromatography–tandem mass spectrometry (LC–MS/MS) system consisted of an Agilent (Santa Clara, CA) 1100 liquid chromatograph interfaced with a Thermo-Finnigan (San Jose, CA) TSQ Quantum Access triple quadrupole mass spectrometer. The system was operated by an Xcalibur® 2.0 SR2 and Tune Master version 1.4.1 operating systems (Thermo-Finnigan). A LUNA C18(2), 150 × 4.6 mm LC column (Phenomenex, Torrance, CA) at 35 °C was used. The mobile phase was 10 mM ammonium acetate (pH 5.0)/methanol (80:20) at a flow rate of 0.2 mL/min. For the MS/MS analysis, positive ion electrospray was used for ionization. The capillary temperature was 270 °C. Nitrogen was used as the sheath gas (60 units) and the aux gas (20 units). Selected reaction monitoring was used for the analysis. Ultra high purity argon was used as the collision gas for collision-induced dissociation. The following transitions were monitored: 5-MeODMT 270 → 255 and 5-MeODMT 270 → 230.
DMT = 219 → 174; bufotenine = 205 → 166; NMS = 191 → 160. For all compounds, the precursor ions were the protonated molecular ions. A calibration curve generated from the analysis of the calibration standards was used to extrapolate the 5-MeO-DMT and bufotenine concentrations in the analytical quality controls and the extracts prepared from the study samples. LCquan 2.5 data analysis software (ThermoFinnigan) was used for the quantification.

2.6. Data analysis

2.6.1. Acoustic startle

The amount of PPI was calculated as a percentage score for each PREPULSE + PULSE trial type: \( \% \text{PPI} = 100 - \left( \frac{\text{startle response for PREPULSE + PULSE trial})}{\text{startle response for PULSE-ALONE trial}} \right) \times 100 \). Startle magnitude was calculated as the average response to all of the PULSE-ALONE trials. The level of spontaneous motor activity was assessed as the average response to all NOSTIM trials. PPI data were analyzed with two- or three-factor analysis of variance (ANOVA) with pretreatment and treatment as between-subjects factors and trial type (prepulse intensity) as a repeated measure. For experiments in which there was no significant interaction between drug and prepulse intensity, PPI data were collapsed across prepulse intensity and the average PPI was used as the main dependent measure. Startle magnitude and NOSTIM data were analyzed with two-factor ANOVA. Post hoc analyses were carried out using Tukey’s studentized range method. Significance was demonstrated for these and other experiments by surpassing an alpha level of 0.05.

2.6.2. Behavioral pattern monitor

The raw data were reduced to the X and Y coordinates of the rat in the chamber. Locomotor activity was quantified by the number of crossings between any of eight equal square sectors within the BPM. Data were examined in 10-min time blocks. For each rat, only the behavioral data from the last 10-min time block (i.e., the test block immediately prior to collection of plasma and brain samples) were analyzed. Data were analyzed using two-way ANOVA with clorgyline pretreatment to all of the PULSE-ALONE trials.
and time block as between-subject factors. Specific post hoc comparisons were made using Bonferroni’s multiple comparisons test.

2.6.3. Analysis of 5-MeO-DMT and bufotenine in plasma and brain

To facilitate data reporting in cases where 5-MeO-DMT or bufotenine concentrations fell below the limit of detection (<LOD), Helsel’s Robust Method (implemented using the UNCENSOR program: Newmann and Evans, 2005) was used to estimate group means and variance. However, simple substitution (null values) was used for groups with ≥4 censored values. The trapezoidal rule was used to calculate the area under the curve up to the last measured time point (AUC₂₀→₇₀ min). The density of brain tissue in rats was assumed to be 1.04 g/mL (Smith, 1930; Johansson and Linder, 1982; Başkaya et al., 1997). Statistical comparisons of raw analyte levels were made using Kaplan–Meier survival analysis; the nonparametric Tarone–Ware test (SPSS Ver. 22) was used to assess equivalence between groups. AUC and Cₘₐₓ values were compared using one-way ANOVA.

3. Results

3.1. Effect of pargyline and 5-MeO-DMT on acoustic startle and sensorimotor gating

5-MeO-DMT was previously shown to disrupt PPI when administered 10 min prior to startle testing (Krebs-Thomson et al., 2006). The current studies tested whether 5-MeO-DMT alters PPI when administered 45 min prior to testing, and examined whether the response to 5-MeO-DMT is altered in animals pretreated with 10 mg/kg pargyline. As shown in Fig. 2, 5-MeO-DMT did not alter PPI in saline-pretreated animals but significantly reduced PPI in animals pretreated with pargyline, resulting in an interaction between pretreatment and treatment (F(2,54) = 17.59, p < 0.0001) and a main effect of 5-MeO-DMT (F(2,54) = 18.87, p < 0.0001). There was a significant main effect of prepulse intensity (F(2,108) = 162.27, p < 0.0001), and pairwise comparisons revealed that 1 mg/kg 5-MeO-DMT significantly reduced PPI at all three prepulse intensities in animals pretreated with pargyline (p < 0.01, Tukey’s test). There was also significant interaction between prepulse pretreatment and prepulse intensity (F(2,108) = 5.05, p = 0.008), but pargyline did not significantly reduce PPI at any prepulse intensity compared with vehicle pretreatment.

For the startle response, there was a main effect of 5-MeO-DMT (F(2,54) = 3.97, p < 0.03) and an interaction between pargyline and 5-MeO-DMT (F(2,54) = 5.07, p < 0.01). Pretreatment with pargyline significantly reduced the amplitude of the startle response (p < 0.05, Tukey’s test; see Table 1), but the startle response was unaffected by treatment with 5-MeO-DMT. In order to confirm that the effect of pargyline and 5-MeO-DMT on PPI is independent of changes in startle magnitude, we examined the effect on PPI in subgroups of animals matched for startle level (see: Geyer and Swerdlow, 1998). We picked 7 animals from each group that had overlapping startle responses (mean ± SEM = 192.5 ± 26.3, 230.0 ± 33.0, 232.4 ± 37.0, 232.5 ± 39.7, 236.2 ± 45.4, and 304.9 ± 29.5). Even though the magnitude of the startle response in those subgroups was unaffected by pretreatment (F(1,36) = 0.09, NS), treatment (F(2,36) = 1.21, NS), or pretreatment × treatment interactions (F(2,36) = 1.36, NS), PPI was still significantly reduced by the combination of pargyline and 5-MeO-DMT (Pretreatment × Treatment interaction: F(2,36) = 10.44, p = 0.0003). This shows that the PPI disruption produced by pargyline and 5-MeO-DMT did not occur because of changes in startle magnitude.

3.2. Effect of clorgyline and 5-MeO-DMT on acoustic startle and sensorimotor gating

We also examined whether the response to 5-MeO-DMT is altered in animals pretreated with 0.3 mg/kg clorgyline. Similar to the experiment with pargyline, the animals were tested 45 min after administration of 5-MeO-DMT. 5-MeO-DMT did not alter PPI in saline-pretreated animals but significantly reduced PPI in animals pretreated with clorgyline (Pretreatment × Treatment: F(1,36) = 24.74, p < 0.0001). 5-MeO-DMT significantly reduced PPI at all three prepulse intensities in animals pretreated with clorgyline (p < 0.01, Tukey’s test; Fig. 3). As shown in Table 1, the startle response was not altered by treatment with 5-MeO-DMT (Main effect: F(1,44) = 0.79, NS) or by pretreatment with clorgyline (Main effect: F(1,44) = 0.63, NS), and there was no interaction between clorgyline and 5-MeO-DMT (F(1,44) = 0.69, NS).

3.3. Involvement of 5-HT₁₄ and 5-HT₂₅ receptors in the effects of pargyline and 5-MeO-DMT on sensorimotor gating

To determine whether the PPI reduction produced by 10 mg/kg pargyline and 1 mg/kg 5-MeO-DMT is mediated by interactions with 5-HT₁₄ or 5-HT₂₅ receptors, antagonist blockade studies were conducted with WAY-100,635 and MDL 11,939, respectively. As expected, there was a main effect of treatment (F(1,54) = 92.13, p < 0.0001), as well as an interaction between treatment and prepulse intensity (F(2,108) = 7.23, p < 0.002), and the combination of pargyline and 5-MeO-DMT significantly reduced PPI at all three prepulse intensities in vehicle-pretreated animals (p < 0.01, Tukey’s test). The magnitude of the startle response was unaffected by treatment with pargyline and 5-MeO-DMT (F(1,44) = 0.26, NS; Table 1). The effects of pretreatment with WAY-100,635 and MDL 11,939 were assessed in a single experiment, with a shared control group, but the effect of each antagonist was analyzed independently (see below).

3.3.1. WAY-100,635

For PPI, there was an interaction between pretreatment and treatment (F(1,36) = 73.11, p < 0.0001). The reduction of PPI produced by 10 mg/kg pargyline and 1 mg/kg 5-MeO-DMT was significantly antagonized by pretreatment with 1 mg/kg WAY-100,635 (p < 0.01, Tukey’s test; Fig. 4A). There was also a trend toward a main effect of pretreatment (F(1,36) = 8.17, p < 0.08), but this was not confirmed by pairwise comparisons. Although there was a main effect of pretreatment on startle magnitude (F(1,36) = 5.91, p < 0.03), the reduction of startle amplitude produced by 1 mg/kg WAY-100,635 did not achieve significance (see Table 1).

3.3.2. MDL 11,939

The ability of pargyline and 5-MeO-DMT to reduce PPI was significantly attenuated by pretreatment with MDL 11,939 (Pretreatment × Treatment: F(1,36) = 23.96, p < 0.0001), and this was confirmed by post-hoc analysis (p < 0.01, Tukey’s test; Fig. 4B). Pretreatment with MDL 11,939 did not alter startle amplitude (F(1,36) = 0.25, NS).

### Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>% CV</th>
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<td>Bufotenine</td>
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<tr>
<td>160 ng/mL</td>
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<td>12.7</td>
</tr>
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</table>

a For 5-methoxy-DMT, 3 low and 1 medium QC’s were treated as outliers and not in calculation of precision and accuracy.

b For bufotenine, 1 medium QC was treated as an outlier and not used in calculation of precision and accuracy.
Values are listed as mean ± SEM.

3.4. Effect of clorgyline pretreatment on 5-MeO-DMT and bufotenine levels in plasma and brain

3.4.1. Analysis method

Several different extraction procedures were investigated, including liquid–liquid extraction and precipitation with methanol, but were associated with poor recovery and sensitivity (data not shown). Previous studies measuring 5-MeO-DMT and bufotenine levels in biological fluids used solid phase extraction to prepare the samples (Forssstrom et al., 2001; Martin et al., 2013). We used a different solid phase extraction method for the simultaneous detection of 5-MeO-DMT and bufotenine in plasma and brain, using liquid chromatography–electrospray ionization–selective reaction monitoring–tandem mass spectrometry (LC-ESI-SRM-MS/MS), with N-methylserotonin (NMS) as the internal standard. Performance characteristics were collected over the course of three pre-study and four study sample analytical runs. The method was selective as determined from examination of analytical results for 7 blank plasma samples fortified with internal standard; the mean detectable signal (peak area ratio) at the retention time of analytes did not exceed 0.25% and 14.7% of mean LLOQ peak area heights for 5-MeO-DMT and bufotenine, respectively. Chromatography of 5-MeO-DMT, bufotenine, and NMS was subject to ion suppression, with little variation in the chromatographic effusion window of the analytes (3.5–4.5 min). Retention times were not affected by ion suppression, with little variation within or between runs (mean 5-MeO-DMT, 4.11; bufotenine, 2.60; and NMS, 2.64 min); the coefficient of variation (CV) for retention times relative to the NMS was 3.5% and 0.95% for 5-MeO-DMT and bufotenine, respectively. There was relatively high extraction recovery (52.7–88.0%) for all analytes. Calibration was reproducible with the use of eight calibration points in duplicate; back-calculated concentrations were within 5% of target with %CVs within 12.9% for both analytes; mean r² values were 0.990 for 5-MeO-DMT and 0.994 for bufotenine. With the accuracy (93.3–104.7% for 5-MeO-DMT and 96.3–107.9% for bufotenine) and precision (%CV not exceeding 17.8 for 5-MeO-DMT and 17.6 for bufotenine) achieved (see Table 2; data specific for study sample runs), the conditions were adequate for reliable analysis.

Table 3
Effect of pretreatment with vehicle or clorgyline (0.3 mg/kg) on the concentration of 5-MeO-DMT in plasma.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Vehicleb</th>
<th>Clorgylineb</th>
<th>Tarone–Ware test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/mL</td>
<td>nM</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>52.7 ± 11.8 (0)</td>
<td>241.6 ± 54.1</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>29.2 ± 6.4 (0)</td>
<td>133.8 ± 29.4</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>12.6 ± 4.8 (0)</td>
<td>57.6 ± 22.1</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>3.5 ± 1.7 (1)</td>
<td>15.8 ± 7.6</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.6 ± 0.5 (4)</td>
<td>2.7 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>0 ± 0 (0)</td>
<td>0 ± 0</td>
<td></td>
</tr>
</tbody>
</table>

Values are listed as mean ± SEM.

Table 4
Effect of pretreatment with vehicle or clorgyline (0.3 mg/kg) on the concentration of 5-MeO-DMT in whole brain.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Vehicleb</th>
<th>Clorgylineb</th>
<th>Tarone–Ware test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/g</td>
<td>nM</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>30.3 ± 11.2 (1)</td>
<td>144.3 ± 53.2</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>16.9 ± 3.6 (3)</td>
<td>80.6 ± 16.9</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>3.7 ± 3.3 (4)</td>
<td>17.8 ± 15.7</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0 ± 0 (0)</td>
<td>0 ± 0</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0 ± 0 (0)</td>
<td>0 ± 0</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>0 ± 0 (0)</td>
<td>0 ± 0</td>
<td></td>
</tr>
</tbody>
</table>

Values are listed as mean ± SEM.

Effect of clorgyline pretreatment on the concentration of 5-MeO-DMT in plasma and brain

To characterize the effect of MAOβ inhibition on the pharmacokinetics and metabolism of 5-MeO-DMT, rats were administered 1 mg/kg 5-MeO-DMT after pretreatment with vehicle or clorgyline (0.3 mg/kg), and 5-MeO-DMT and bufotenine levels in plasma and whole brain were quantified. The concentration of 5-MeO-DMT in plasma is shown in Table 3; the concentration in brain is shown in Table 4. The level of 5-MeO-DMT in plasma and brain peaked within the first 20 min after SC administration and then rapidly declined. Clorgyline did not significantly increase the plasma Cmax of 5-MeO-DMT (vehicle = 52.7 ± 11.8 ng/mL; clorgyline = 78.1 ± 9.5 ng/mL), but the clearance of 5-MeO-DMT from plasma was slowed by clorgyline, as evidenced by the fact that the concentration of 5-MeO-DMT in plasma was significantly elevated 30–70 min after administration in animals pretreated with clorgyline (see Table 3). Furthermore, clorgyline significantly increased the plasma AUCinf–70 min for 5-MeO-DMT (vehicle = 12.2 ± 1.7 ng·h/mL; clorgyline = 31.4 ± 2.3 ng·h/mL; F(1,59) = 45.6, p < 0.0001). In contrast to the negligible effect of clorgyline on the plasma Cmax, clorgyline significantly increased the Cmax of 5-MeO-DMT in whole brain (vehicle: 30.3 ± 11.2 ng/g; clorgyline: 633.8 ± 146.5 ng/g; F(1,9) = 16.8, p < 0.004). As shown in Table 4, the concentration of 5-MeO-DMT in whole brain was markedly elevated in animals pretreated with clorgyline, and 5-MeO-DMT was also detectable for a longer period of time in the presence of an MAO inhibitor. It does not appear that clorgyline pretreatment significantly altered the concentration of bufotenine in plasma or brain because most of the samples were ≤LOD (plasma = 1–2 ng/mL; brain = 10 ng/g). The plasma Cmax for bufotenine was 1.3 ± 0.1 ng·h/mL in rats pretreated with vehicle, and 1.8 ± 0.3 ng·h/mL in rats pretreated with clorgyline (data not shown).

To confirm that the expected behavioral interaction occurred between 0.3 mg/kg clorgyline and 1 mg/kg 5-MeO-DMT, locomotor activity was assessed in the BMPD prior to collection of analytical samples (see Fig. 5). There was a significant main effect of clorgyline (F(1,48) = 7.25, p < 0.01) and a significant interaction between clorgyline and time block (F(5,48) = 3.64, p < 0.008). Importantly, compared with vehicle pretreatment, rats pretreated with clorgyline made significantly more crossings (a measure of locomotor activity) during the fifth 10-min interval.
time block (p < 0.05, Bonferroni’s test), confirming the animals were hyperactive.

4. Discussion

These studies demonstrate that pretreatment with an MAOI markedly prolongs the behavioral response to 5-MeO-DMT. Although 5-MeO-DMT has previously been shown to disrupt PPI by activating 5-HT1A receptors (Krebs-Thomson et al., 2006), the effect on PPI has a relatively short duration, and the present results confirm that 5-MeO-DMT does not alter PPI 45 min post-injection. By contrast, 5-MeO-DMT disrupted PPI at that time point in rats pretreated with an MAOI. The effect of combined treatment with 5-MeO-DMT and the MAOI pargyline on PPI was antagonized by pretreatment with the 5-HT1A antagonist WAY-100,635 and the 5-HT2A antagonist MDL 11,939. Based on the findings with WAY-100,635 and MDL 11,939, it appears that the PPI disruption produced by 5-MeO-DMT in animals pretreated and an MAOI is dependent on activation of 5-HT1A and 5-HT2A receptors. These findings confirm that 5-MeO-DMT, like other hallucinogens, can alter PPI in rats by activating 5-HT2A receptors. Analysis of 5-MeO-DMT pharmacokinetics revealed that systemic and central exposure to the drug is increased and prolonged after inhibition of MAO, and showed that there is minimal conversion of 5-MeO-DMT to bufotenine in rats.

Pretreatment with the MAO inhibitor clorgyline dramatically altered the pharmacokinetics of 5-MeO-DMT. These effects probably occurred because clorgyline blocked the metabolism of 5-MeO-DMT. 5-MeO-DMT is metabolized by deamination and clorgyline was administered at a dose that is known to inhibit MAO. Using 5-HT deamination as a measure of MAO activity ex vivo, Felner and Waldmeier (1979) determined that clorgyline inhibits MAO A in liver and brain by approximately 60% and 90%, respectively, when administered to rats at 0.3 mg/kg SC. Compared to the present results, previous studies in rats and mice have yielded similar findings regarding the effects of MAOIs on 5-MeO-DMT pharmacokinetics (Narasimhachari et al., 1979; Sitaram et al., 1987; Shen et al., 2010b; Jiang et al., 2013). Sitaram and coworkers (Sitaram et al., 1987) examined the effect of pretreatment with the non-selective MAOI iproniazid phosphate on tissue levels of 5-MeO-DMT in Sprague–Dawley rats. After treatment with 5-MeO-DMT (10 mg/kg IP), blood and brain levels averaged 0.03 nmol/g (6.9 ng/mL) and 0.08 nmol/g (17.5 ng/g), respectively, when samples were collected 45 min post-injection. By contrast, in rats pretreated with iproniazid, the concentration of 5-MeO-DMT in blood was 1.2 nmol/g (275 ng/mL) and the concentration in brain was 7.8 nmol/g (1703 ng/g). In summary, iproniazid increased 5-MeO-DMT blood levels 40-fold and brain levels almost 100-fold.

One of the more interesting findings with 5-MeO-DMT is that its pharmacodynamics can be altered by inhibition of MAO. Normally, 5-MeO-DMT produces brief alterations of locomotor activity and PPI that are mediated by 5-HT1A and not by 5-HT2A receptors (Krebs-Thomson et al., 2006). Conversely, in rats pretreated with an MAO inhibitor, the action of 5-MeO-DMT is prolonged and involves 5-HT2A activation (Halberstadt et al., 2008, 2012: Fig. 4). Yu and colleagues have shown (Shen et al., 2010b) that there is a marked increase in the O-demethylation of 5-MeO-DMT in mice pretreated with an MAOI, resulting in elevated and prolonged systemic exposure to bufotenine. Based on their findings, we hypothesized that if MAOIs produce similar effects on the metabolism of 5-MeO-DMT in rats, the behavioral interaction between MAOIs and 5-MeO-DMT could be a consequence of increased formation of bufotenine, a potent and highly efficacious 5-HT2A agonist. The present results, however, reveal that there is negligible conversion of 5-MeO-DMT to bufotenine in rats, even in the presence of an MAOI. Similarly, a previous study failed to detect any bufotenine in the brains of rats treated with 5-MeO-DMT and iproniazid (Sitaram et al., 1987). Importantly, we found that inhibition of MAO-A did produce a significant increase in the central accumulation of 5-MeO-DMT, and micromolar concentrations were present in the brain for at least 60 min post-injection. Therefore, it appears that MAOIs alter the behavioral response to 5-MeO-DMT by blocking its deamination, which allows 5-MeO-DMT to accumulate in the brain at concentrations sufficient to activate 5-HT2A receptors for an extended period of time. The fact that α,α,β,β-tetradeutero-5-MeO-DMT, a 5-MeO-DMT isotopologue that is resistant to metabolism by MAO, produces a behavioral profile identical to that of 5-MeO-DMT in combination with an MAO-A inhibitor (Halberstadt et al., 2012) supports the conclusion that MAOIs alter the response to 5-MeO-DMT by blocking its deamination.

Interactions between serotonergic and dopaminergic signaling may contribute to the delayed 5-HT2A-mediated behavioral response induced by 5-MeO-DMT and an MAOI. 5-HT2A receptors are expressed as heteroreceptors on dopaminergic terminals; evidence indicates that 5-HT2A can facilitate phasic dopamine release (Gobert and Millan, 1999). Activation of 5-HT2A has been observed to produce a delayed sensitization of the dopaminergic system (Marona-Lewicka and Nichols, 1997; Marona-Lewicka et al., 2009). It is possible that the high level of 5-HT2A activation produced by 5-MeO-DMT in the presence of an MAOI could induce a delayed sensitization of dopamine release, resulting in hyperactivity and disruption of PPI. The ability of 5-HT2A agonists to produce delayed effects on dopaminergic transmission would explain why 5-HT2A antagonists block the behavioral response to 5-MeO-DMT at ~45 min post-treatment, but not at earlier time-points, despite the fact that 5-MeO-DMT would occupy 5-HT2A receptors in the central nervous system immediately after administration. Future studies will examine whether the dopaminergic system contributes to the behavioral interactions between 5-MeO-DMT and MAOI.

The present findings confirm that 5-MeO-DMT can activate 5-HT2A receptors in vivo. We found the combined effect of 5-MeO-DMT and pargyline on PPI was partially dependent on 5-HT2A receptor activation. Along the same lines, we had previously shown that in the presence of clorgyline, 5-MeO-DMT induces delayed hyperactivity via 5-HT2A receptor activation (Halberstadt et al., 2008). It has also been reported that 5-MeO-DMT can elicit the head twitch response (HTR), a 5-HT2A-mediated behavior, in rats at doses ranging from 0.2–1 mg/kg (Bedard and Pycock, 1977; Matthews and Smith, 1980). Furthermore, although the discriminative stimulus effects of 5-MeO-DMT are primarily mediated by 5-HT1A receptors (Spencer et al., 1987; Winter et al., 2000), 5-MeO-DMT can produce full substitution in rats trained to discriminate...
the 5-HT2 agonist DOM (Young et al., 1980). Given that 5-MeO-DMT activates 5-HT1A receptors in rats in vivo, it is surprising that the receptor normally does not contribute to the effects of 5-MeO-DMT on PPI and locomotor activity. One possible explanation is that activation of the 5-HT1A receptor by 5-MeO-DMT may attenuate its ability to induce behavioral responses via 5-HT2A. It is well known that the 5-HT1A receptor inhibits 5-HT2A-mediated behavioral responses (Arnt and Hyttel, 1989; Darmani et al., 1990; Schreiber et al., 1995). Reports indicate that the concentration of 5-MeO-DMT required to activate 5-HT2A is on the order of several hundred nanomolar (Newton et al., 1996; Rabin et al., 2002; Kurrasch-Orbaugh et al., 2003; Strachan et al., 2010) or in the low micromolar range (Blair et al., 2000; Parrish and Nichols, 2006). Therefore, the peak level of 5-MeO-DMT in the brain (144.3 nM) may not produce sufficient 5-HT1A receptor activation to overcome the relatively potent countervailing effects mediated by 5-HT1A. It would appear, however, that in the presence of an MAO inhibitor 5-MeO-DMT accumulates in the brain at levels that are adequate to increase locomotor activity and disrupt PPI via 5-HT2A.

5-MeO-DMT can induce lateral head-weaving, tremor, and other symptoms of serotonin syndrome in rats (Graham-Smith, 1971; Sloviter et al., 1978; Lucki et al., 1984). It has been reported that MAOIs can markedly intensify and prolong the serotonin syndrome induced by 5-MeO-DMT (Graham-Smith, 1971; Ortmann et al., 1980). It is unlikely, however, that 5-MeO-DMT–induced serotonin syndrome interfered with the assessment of the startle response in the present studies. First, the duration of the serotonin syndrome after combined administration of 5-MeO-DMT (1 mg/kg IP) and an MAOI is only 25–30 min (Ortmann et al., 1980), which is shorter than the interval between drug treatment and startle testing in the present studies. Second, we have confirmed that the magnitude of the startle response is not significantly altered by treatment with pargyline and 5-MeO-DMT, demonstrating that the serotonin syndrome did not interfere with expression of the startle response.

The interaction between MAOIs and tryptamine hallucinogens is important from a toxicological perspective because these substances are used concurrently by humans. Use of ayahuasca, a hallucinogenic beverage containing DMT and MAOIs such harmaline and harmine, is currently increasing worldwide (Labate and Feeney, 2012; Lanaro et al., 2015). These beverages are legally available in some countries and are made from the Banisteriopsis caapi complex mechanism at serotonin 5-HT2A and 5-HT2C receptors. Synapse 35, 82-92.

A recent study (Nagai et al., 2007) demonstrated that the serotonin syndrome did not interfere with expression of the startle response. Therefore, the present study confirms that MAOIs can markedly intensify and prolong the serotonin syndrome in rats by activating the 5-HT2A receptor. Psychopharmacology 208, 179-189.

Acknowledgments

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References


