Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 83 (2006) 122 – 129
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Hallucinogen-like actions of 5-methoxy-*N*,*N*-diisopropyltryptamine in mice and rats

W.E. Fantegrossi [a,b,*], A.W. Harrington [b], C.L. Kiessel [b], J.R. Eckler [c], R.A. Rabin [c], J.C. Winter [c], A. Coop [d], K.C. Rice [e], J.H. Woods [b]

[a] *Division of Neuroscience, Yerkes National Primate Research Center, Emory University, 954 Gatewood Road, Atlanta, GA 30322, United States*
[b] *Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI, United States*
[c] *Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, NY, United States*
[d] *Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, MD, United States*
[e] *Laboratory of Medicinal Chemistry, NIDDK, National Institutes of Health, Bethesda, MD*

Received 2 June 2005; received in revised form 7 December 2005; accepted 29 December 2005
Available online 3 February 2006

Abstract

Few studies have examined the effects of 5-methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT) in vivo. In these studies, 5-MeO-DIPT was tested in a drug-elicited head twitch assay in mice where it was compared to the structurally similar hallucinogen *N*,*N*-dimethyltryptamine (*N*,*N*-DMT) and challenged with the selective serotonin $(5\text{-HT})_{2A}$ antagonist M100907, and in a lysergic acid diethylamide (LSD) discrimination assay in rats where its subjective effects were challenged with M100907 or the 5-HT_{1A} selective antagonist WAY-100635. Finally, the affinity of 5-MeO-DIPT for three distinct 5-HT receptors was determined in rat brain. 5-MeO-DIPT, but not *N*,*N*-DMT, induced the head twitch responses in the mouse, and this effect was potently antagonized by prior administration of M100907. In rats trained with LSD as a discriminative stimulus, there was an intermediate degree (75%) of generalization to 5-MeO-DIPT and a dose-dependent suppression of response rates. These interoceptive effects were abolished by M100907, but were not significantly attenuated by WAY-100635. Finally, 5-MeO-DIPT had micromolar affinity for 5-HT_{2A} and 5-HT_{2C} receptors, but much higher affinity for 5-HT_{1A} receptors. 5-MeO-DIPT is thus effective in two rodent models of 5-HT_2 agonist activity, and has affinity at receptors relevant to hallucinogen effects. The effectiveness with which M100907 antagonizes the behavioral actions of this compound, coupled with the lack of significant antagonist effects of WAY-100635, strongly suggests that the 5-HT_{2A} receptor is an important site of action for 5-MeO-DIPT, despite its apparent in vitro selectivity for the 5-HT_{1A} receptor.

Keywords: Hallucinogens; Drug-discrimination; Head twitch response; Serotonin receptors

1. Introduction

5-methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT, Fig. 1A) is a synthetic orally active hallucinogenic tryptamine analogue known by the street names "foxy" and "foxy methoxy." The synthesis and hallucinogen-like subjective effects of this compound were first described in the scientific literature (Shulgin and Carter, 1980), and expanded upon ten years later in a book which subsequently gained widespread dissemination via the internet (Shulgin and Shulgin, 1991). Since these initial descriptions, the United States Drug Enforcement Administration (DEA) has documented 5-MeO-DIPT seizures and reports of abuse in at least nine states, as well as the District of Columbia (US DEA, 2002). More recent case reports of 5-MeO-DIPT intoxication (Meatherall and Sharma, 2003; Smolinske et al., 2004; Wilson et al., 2005) suggest that abuse of this compound may be spreading beyond the geographic areas initially implicated. Similarly, a search of The American Association of Poison Control Centers Toxic Exposure Surveillance System database revealed 41 cases of 5-MeO-DIPT exposure reports to poison centers over a 15-month

* Corresponding author. Division of Neuroscience, Yerkes National Primate Research Center, Emory University, 954 Gatewood Road, Atlanta, GA 30322, United States. Tel.: +1 404 727 8512; fax: +1 404 727 1266.
E-mail address: wfanteg@emory.edu (W.E. Fantegrossi).

0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.12.015

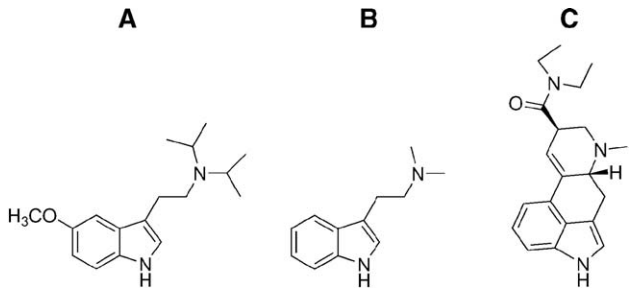

Fig. 1. Chemical structures of 5-MeO-DIPT (A. left), *N*,*N*-DMT (B. middle) and LSD (C. right). Fantegrossi et al.

period from April 2002 to the end of June 2003 (Smolinske et al., 2004).

Due to the apparent abuse and toxicity of this compound, 5-MeO-DIPT was placed temporarily into Schedule I under the Controlled Substances Act in April of 2003 (Brown, 2003), and this placement was made permanent in September of 2004 (Leonhart, 2004). Despite these aggressive regulatory measures, 5-MeO-DIPT remains available for purchase from foreign sources via the internet. Anecdotal reports from human users posted to internet sites specializing in the dissemination of drug information (for example, erowid.org and lycaeum.org) further suggest that 5-MeO-DIPT has profound psychedelic actions in man, but few studies regarding the effects of this compound in laboratory animals have been published. However, 5-MeO-DIPT has been shown to generalize to the interoceptive cue induced by *R*-(−)-1-(2,5-dimethoxy-4-methylphenyl)-2-amino-propane (DOM) in rats (Glennon et al., 1983), although the pharmacological mechanism for this effect was not explored using antagonist challenges. A later study reported that related tryptamine analogues have affinity for 5-HT_2 receptors, and suggests that these compound are likely to function as agonists due to their higher affinity for [^{3}H]4-bromo-2,5-dimethoxy-phenylisopropylamine (DOB)-labelled receptors than for [^{3}H] ketanserin-labelled sites (Lyon et al., 1988). These findings, coupled with the chemical structure of 5-MeO-DIPT and anecdotal reports of its hallucinogenic activity in man, strongly suggest that serotonin systems, specifically 5-HT_{2A} receptors (Sadzot et al., 1989), may be involved in the mediation of the behavioral and subjective effects of this compound.

In this regard, the drug-elicited head twitch response (Corne et al., 1963; Corne and Pickering, 1967) is a selective behavioral model for 5-HT_2 agonist activity in the rodent, and several previous studies have established that direct and indirect 5-HT agonists induce this effect (Peroutka et al., 1981; Colpaert and Janssen, 1983; Green et al., 1983; Goodwin and Green, 1985; Darmani et al., 1990a,b, 1992; Fantegrossi et al., 2004). Further, 5-HT_2 receptor antagonists selectively block head twitch behavior (Lucki et al., 1984; Handley and Singh, 1986; Fantegrossi et al., 2004), and the potency with which they do so is highly correlated with the antagonist's affinity for 5-HT_2 receptors (Peroutka et al., 1981; Ortmann et al., 1982). Similarly, the strong correlation between discriminative stimuli in nonverbal species and subjective effects reported by humans (Schuster and Johanson, 1988; Sanger et al., 1994; Brauer et al., 1997) allows for a useful characterization of the interoceptive cues produced by psychedelic drugs using drug discrimination procedures in laboratory rodents. The discriminative stimulus properties of hallucinogens such as mescaline, DOM and lysergic acid diethylamide (LSD, Fig. 1C) have been extensively investigated in several different animal species and it has been shown that, in agreement with studies in humans, these drugs generalize with one another (Winter, 1978; Glennon et al., 1983; Fiorella et al., 1995a). Furthermore, antagonist correlation analysis has determined that the stimulus effects of phenylisopropylamine and indolealkylamine hallucinogens are mediated by agonist activity at 5-HT_{2A} receptors (Fiorella et al., 1995b) and possibly modulated by agonist activity at 5-HT_{2C} receptors (Fiorella et al., 1995c).

Thus, in order to compare potency and effectiveness of 5-MeO-DIPT with the more familiar tryptamine hallucinogens, we established dose–effect functions for 5-MeO-DIPT and the structurally similar psychedelic *N*,*N*-dimethyltryptamine (DMT, Fig. 1B) in the head twitch assay in mice. Antagonist studies were then conducted with the selective 5-HT_{2A} antagonist (+)-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol (M100907, formerly MDL100907) in order to gauge the involvement of 5-HT_{2A} receptors in the induction of this behavior. A parallel series of drug discrimination experiments was conducted in rats in order to characterize the similarity of the discriminative stimulus effects of 5-MeO-DIPT with those of LSD. The effects of M100907 and the selective 5-HT_{1A} antagonist *N*-(2-(1-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridyl) cyclohexane-carboxamide (WAY-100635) on LSD-appropriate responding were also tested in rats receiving an active dose of 5-MeO-DIPT. Finally, binding of 5-MeO-DIPT to 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors was characterized in rat brain using a competition binding technique.

2. Methods

All studies were carried out in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory animals as adopted and promulgated by the National Institutes of Health. Experimental protocols were approved by the Animal Care and Use Committees at the University of Michigan and the State University of New York at Buffalo.

2.1. Animals — drug-elicited head twitch response

Male NIH Swiss mice (Harlan Sprague Dawley Inc., Indianapolis, IN) weighing approximately 20–30 g were housed 12 animals per 44.5×22.3×12.7 cm Plexiglas cage and used in drug-elicited head twitch experiments. Mice were housed in a temperature-controlled room at the University of Michigan that was maintained at an ambient temperature of 22±2 °C at 45–50% humidity. Lights were set to a 12-h light/dark cycle. Animals were fed Lab Diet rodent chow (Laboratory Rodent Diet #5001, PMI Feeds, Inc., St. Louis, MO) and water ad

libitum until immediately before testing. Animals were not used in experiments until at least 2 days after arrival in the laboratory. Each animal was used only once, and was sacrificed immediately after use.

2.2. Animals — drug discrimination experiments

Male Fischer-344 rats obtained from Harlan Sprague–Dawley Inc. (Indianapolis, IN, USA) at an age of approximately 6 weeks were used in LSD discrimination experiments. Rats were housed in pairs with free access to food and water in a temperature-controlled room at the State University of New York at Buffalo under a constant 12-h light/dark cycle (all experiments were conducted during the light phase.) Caloric intake was controlled to yield a mean body weight of approximately 300 g; supplemental feedings of standard rat chow were provided following experimental sessions.

2.3. Procedure

2.3.1. Drug-elicited head-twitch response in mice

On experimental days, mice were weighed, marked, and returned to the home cage. Doses were then calculated and prepared for injection. Individual animals were subsequently removed from the home cage, injected intraperitoneally (i.p.) with saline or 0.01 mg/kg M100907, then placed into a 15.24×25.40×12.70 cm Plexiglas mouse cage. Ten minutes after the initial injection, mice were injected i.p. with various doses of 5-MeO-DIPT, *N*,*N*-DMT or saline and returned to the small observation cage. Five minutes after this second injection, a camera mounted above the observation cage began recording behavior, and continued to do so for 10-min. Videotapes were later scored by two blind observers for incidence of the head twitch response, here defined as a rapid rotational jerk of the head that is not contiguous with any grooming or scratching behaviors. All experiments were conducted in the colony room at an ambient temperature of 22±2 °C, and neither food nor water were available during the tests.

2.3.2. LSD-like discriminative stimulus effects in rats

Six small animal test chambers (Med-Associates Model ENV-008), each equipped with a house light and an exhaust fan, and housed in larger lightproof Malaguard sound attenuating cubicles (Med-Associates Model ENV-022M) were used for these experiments. The chamber contained two levers mounted on opposite sides of one wall. Centered between the levers was a dipper that delivered 0.1 ml of sweetened condensed milk diluted 2:1 with tap water.

Eleven subjects were trained to discriminate LSD (0.1 mg/kg, 15 min pretreatment time, i.p. injection) from saline, as described previously (Fiorella et al., 1995a). A non-resetting fixed ratio 10 (FR10) schedule of reinforcement was employed using the MED-PC version IV behavioral programming application. Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, 83% or more of all responses prior to the delivery of the first reinforcer were on the appropriate lever. The LSD training dose produced approximately 99.5% drug-appropriate responding. After stimulus control was established with the training agents, tests with 5-MeO-DIPT were conducted once per week in each animal so long as performance did not fall below the criterion level of 83% correct responding in any one of the previous three training sessions. Half of the test sessions was conducted the day after saline training sessions with the remainder following LSD training sessions. During test sessions, no responses were reinforced and the session was terminated after the emission of ten responses on either lever. The distribution of responses between the two levers was expressed as a percentage of total responses emitted on the drug-appropriate lever. Response rate was calculated for each session by dividing the total number of responses emitted on both levers by the elapsed time prior to 10 responses on either lever.

To establish the role of 5-HT_{2A} and 5-HT_{1A} receptors in the stimulus effects of 5-MeO-DIPT, either 0.05 mg/kg M100907 or 0.3 mg/kg WAY-100635 was injected 45 min before 5-MeO-DIPT, i.e., 60 min before testing. Complete generalization of a training drug to a test drug is said to be present when (a) a mean of 80% or more of all test responses occurs on the drug-appropriate lever; (b) there is no statistically significant difference between the response distributions of the training drug and the test drug; and (c) there is a statistically significant difference between the response distributions of the test drug and saline control sessions. An intermediate degree of generalization is defined as being present when response distributions after a test drug are less than 80% drug-appropriate, and are significantly different from both training conditions. Finally, when the response distribution after a test drug is not statistically significantly different from that in saline control sessions, an absence of generalization of the training drug to the test drug is assumed. Similar criteria are applied to the definitions of full, partial, and no antagonism. Thus, full antagonism is assumed to be present when (a) less than 20% of all test responses are on the training drug-appropriate lever; (b) there is no significant difference between the response distributions in the test of antagonism and the saline control, and (c) there is a statistically significant difference between the response distributions of the test drug alone and in combination with the antagonist.

2.3.3. Competition binding in rat brain

Frontal cortex (5-HT_{2A} receptors), hippocampus (5-HT_{1A} receptors), and brain stem (5-HT_{2C} receptors) were harvested from male CDF rats (Charles Rivers Laboratories) and homogenized (Dounce tissue grinder) in 50 mM Tris–HCl (pH 7.4). Homogenates were centrifuged at 40,000 ×*g* for 15 min at 4 °C, and the resulting pellets were resuspended in the Tris buffer and stored at −80 °C. On the day of the assays, tissue samples were thawed and centrifuged at 40,000 ×*g* for 15 min at 4 °C. The resulting pellets were resuspended in 30 ml warm 50 mM Tris–HCl (pH 7.4) and incubated for 10 min at 37 °C to remove endogenous serotonin. Samples were again centrifuged at 40,000 ×*g* for 15 min at 4 °C. Final resuspension of the pellets (frontal cortex: 6.7 mg/ml; hippocampus: 5 mg/ml; brain stem 13.3 mg/ml) was carried

out in Tris assay buffer (50 mM Tris–HCl, pH 7.4, containing 4 mM $MgCl_2$, 10 μM pargyline and 0.1% ascorbate).

[^{3}H]8-OH-DPAT (8-hydroxy-(2-di-*n*-propylamino)tetralin) binding assays were carried out for 30 min at 37 °C in a final volume of 0.5 ml Tris assay buffer containing 1 nM radioligand (129 Ci/mmole; Perkin-Elmer, Boston MA), appropriate drugs and hippocampal membranes (2 mg wet weight/tube). [^{3}H] ketanserin binding assays were carried out for 30 min at 30 °C in a final volume of 0.5 ml Tris assay buffer containing 1.5 nM radioligand (88 Ci/mmole; Perkin-Elmer, Boston MA), 100 nM prazosin to prevent binding to α_1-adrenergic receptors, appropriate drugs and frontal cortical membranes (2 mg wet weight/tube). [^{3}H]mesulergine binding assays were carried out for 45 min at 37 °C in a final volume of 0.5 ml Tris assay buffer containing 2 nM radioligand (77 Ci/mmole; Amersham Biosciences), 100 nM spiperone to prevent binding to 5-HT_{2A} and dopamine D_2 receptors, appropriate drugs and membranes from the brain stem (4 mg wet weight/tube). Reactions were terminated by rapid vacuum filtration (Brandel harvester) through GF/B glass fiber filters presoaked in 0.1% polyethylenimine. Filters were washed twice with cold 50 mM Tris–HCl (pH 7.4), and the amount of bound radioactivity measured by scintillation spectrophotometry. Nonspecific binding was defined as the difference in the amount of radioligand binding in the absence and presence of either 10 μM 5-HT ([^{3}H]8-OH-DPAT binding), 20 μM 5-HT ([^{3}H]mesulergine binding) or 100 μM cinanserin ([^{3}H]ketanserin binding).

2.4. Data analysis

Data from the head twitch response experiments are presented as mean±SEM and were compared to values obtained from equivolume saline controls using one way ANOVA and Tukey's post hoc tests. Drug discrimination data are expressed as percent drug-appropriate responding, which is the number of responses emitted on the drug-appropriate lever as a percentage of the total number of responses emitted. Response rates are expressed as the number of responses per minute, calculated for each session by dividing the total number of responses emitted (prior to the emission of 10 responses on either lever) by elapsed time. Data for any subjects failing to emit 10 responses within the constraints of the 10-min test session were not considered in the calculation of the percent drug-appropriate responding but were included in the analysis of response rates. Generalization was said to occur if 80% or more of the responses were on the drug-appropriate lever. The statistical significance of the generalization of LSD to 5-MeO-DIPT in rats trained with LSD and the antagonism of these effects by M100907 and WAY-100635 were determined using one-way ANOVA to compare the two training conditions with 5-MeO-DIPT and with 5-MeO-DIPT in the presence of an antagonist, respectively. Subsequent multiple comparisons were made by the method of Student–Newman–Keuls. Binding data were analyzed by nonlinear regression using the program EBDA/LIGAND (Elsevier BIOSOFT). All differences were considered to be statistically significant if the probability of their having arisen by chance was <0.05, and all statistical analyses were conducted using SigmaStat 2.03 for Windows™ (Jandel Scientific Software, San Rafael, CA).

2.5. Drugs

(+)-LSD, *N*,*N*-DMT and 5-MeO-DIPT were supplied by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC) and dissolved in 0.9% physiological saline solution. M100907 was synthesized at Laboratory of Medicinal Chemistry at the National Institutes of Diabetes, Digestive and Kidney Disorders at the National Institutes of Health (Bethesda, MD), and dissolved in sterile water and 0.5 *N* HCl. WAY-100635 was purchased from Tocris (Ellisville, MO). With the exception of WAY-100635, which was administered subcutaneously, all injections were administered i.p. at a volume of 1.0 ml/kg (rats) or 1.0 ml/100 g (mice).

3. Results

3.1. Drug-elicited head-twitch response in mice

5-MeO-DIPT induced a dose-dependent HTR in mice, producing a maximum of approximately 9 twitches during the 10-min observation period at a dose of 1.0 mg/kg (Fig. 2, closed circles). Doses of 1.0 and 3.0 mg/kg 5-MeO-DIPT elicited significantly more head twitch behavior than did saline ($P<0.05$ for both doses). Pretreatment with 0.01 mg/kg M100907 produced a ten-fold rightward shift in the 5-MeO-DIPT HTR dose–response curve (Fig. 2, open circles). Following antagonist pretreatment, 10 mg/kg 5-MeO-DIPT induced significantly more head twitch behavior than did saline ($P<0.05$). At the highest dose tested (30.0 mg/kg, in the presence of M100907), 5-MeO-DIPT induced convulsions in 3/6 mice; no such evidence of toxicity was observed at 10.0 mg/kg, either with or without the antagonist. Interestingly, *N*,*N*-DMT did not elicit

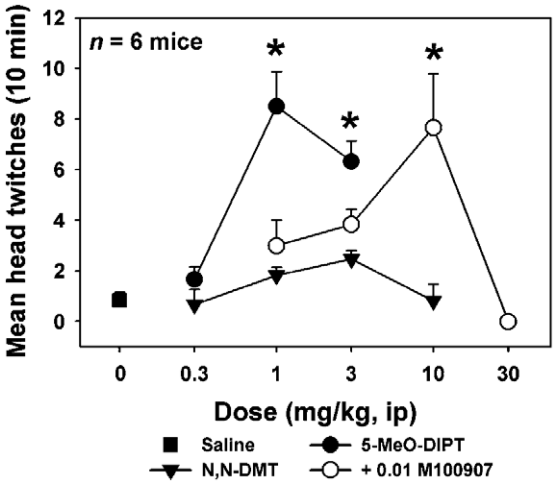

Fig. 2. Effects of 5-MeO-DIPT (circles) and *N*,*N*-DMT (triangles) on head twitch behavior in mice treated with M100907 (open symbols) or saline (filled symbols). Each point represents the mean±SEM ($n=6$ mice per dose). Ordinate: mean head twitches/10 min. Abscissa: 5-MeO-DIPT or DMT dose (mg/kg, i.p.). Asterisks indicate significant differences from saline controls (filled square) ($P<0.05$). Fantegrossi et al.

significantly more head twitches than saline at any dose tested (Fig. 2, filled triangles).

3.2. LSD-like discriminative stimulus effects in rats

An intermediate degree of generalization of LSD to 5-MeO-DIPT was observed with approximately 52% and 75% LSD-appropriate responding at doses of 1.0 and 3.0 mg/kg, respectively ($P<0.05$ for both doses) (Fig. 3, left panel, closed circles); higher doses were not tested due to the greater than 50% reduction in response rates observed at a dose of 3.0 mg/kg (Fig. 3, right panel, closed circles) — indeed, only 6/11 rats responded at this dose. The discriminative stimulus effects of 1.0 and 3.0 mg/kg 5-MeO-DIPT were completely blocked ($P<0.05$ for both doses) by the 5-HT_{2A} selective antagonist M100907 (Fig. 3, left panel, open circles). In contrast, the discriminative stimulus effects of 1.0 mg/kg 5-MeO-DIPT were non-significantly attenuated ($P=0.106$) by the 5-HT_{1A} selective antagonist WAY-100635, and this effect was overcome at the 3.0 mg/kg dose of 5-MeO-DIPT (Fig. 3, left panel, open hexagons), although only 5/11 rats responded at this dose.

3.3. Competition binding in rat brain

Because the present behavioral results strongly suggest an involvement of 5-HT receptors in the effects of 5-MeO-DIPT, the affinity of this compound at 5-HT_{2A}, 5-HT_{2C}, and 5-HT_{1A} receptors was determined. 5-MeO-DIPT has micromolar affinity for 5-HT_{2A} and 5-HT_{2C} receptors, and higher affinity for 5-HT_{1A} receptors. The equilibrium dissociation constant (K_I) for 5-MeO-DIPT at the 5-HT_{2A} receptor, as measured using [^{3}H]ketanserin, was 5620 nM ($pK_I=5.25$). By way of comparison, the K_I for $R(-)$-DOI at the 5-HT_{2A} receptor was 141 nM ($pK_I=6.85$), while the K_I for $R(-)$-DOM at this site was 513 nM ($pK_I=6.29$) (previously reported in Fantegrossi et al., in press). The K_I for 5-MeO-DIPT at 5-HT_{2C} receptors, which was determined using [^{3}H]mesulergine, was 1700 nM ($pK_I=5.77$). Binding affinity of 5-MeO-DIPT at the 5-HT_{1A} receptor, as measured using [^{3}H]8-OH-DPAT, was appreciably higher with a K_I of 35 nM ($pK_I=7.44$). Binding affinities for 5-MeO-DIPT at these three 5-HT receptors are summarized in Table 1.

Table 1
Affinity of 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors for 5-MeO-DIPT

Compound	pK_I (K_I) [^{3}H]8-OH-DPAT	pK_I (K_I) [^{3}H]ketanserin	pK_I (K_I) [^{3}H]mesulergine
5-MeO-DIPT	7.44±0.04 (35 nM)	5.25±0.04 (5620 nM)	5.77±0.36 (1700 nM)

Binding of 5-MeO-DIPT to the various serotonin receptors was carried out as described in the Methods. Data are expressed as the negative log of the equilibrium dissociation constant (pK_I) and are presented as mean±S.E.M. of 3-8 separate experiments. Equilibrium dissociation constants, K_I, are presented in the parenthesis. For comparative purposes, see the Results section for a discussion of the previously determined affinity of the 5-HT_{2A} receptor for the phenylisopropylamines $R(-)$-DOI, and $R(-)$-DOM.
Fantegrossi et al.

4. Discussion

The presently reported results suggest that 5-MeO-DIPT is behaviorally active in two rodent assays which model hallucinogen effects. The capacity of this compound to induce the head twitch response in the mouse, and the potent antagonism of this effect by prior injection of M100907, suggests that a primary site of action for 5-MeO-DIPT is the 5-HT_{2A} receptor. Similarly, the LSD-like stimulus effects elicited by 5-MeO-DIPT in the rat, the antagonism by M100907, and an absence of antagonism by WAY-100635, are also consistent with a 5-HT_{2A}-mediated mechanism of action for this compound. This receptor has previously been implicated in the mediation of hallucinogen effects for the ergoline (LSD-like), indolealkylamine (DMT-like), and phenylisopropylamine (DOI-like) hallucinogens (Sadzot et al., 1989; Aghajanian and Marek, 1999; Nichols, 2004). It should be noted that the dose of M100907 employed in the present discrimination studies have previously been shown to completely antagonize LSD-induced stimulus control (Winter et al., 2004) while the dose

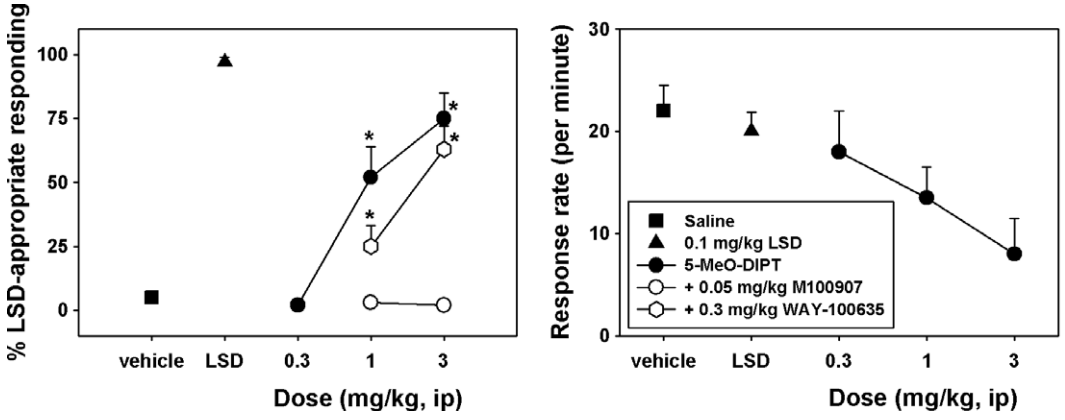

Fig. 3. Left panels — discriminative stimulus effects of 5-MeO-DIPT (closed circles) in rats discriminating between 0.1 mg/kg LSD (closed triangle) and saline (closed square), and the effects of pretreatment with 0.05 mg/kg M100907 (open circles) and 0.3 mg/kg WAY-100635 (open hexagons). Each point represents the mean±SEM. Ordinate: average percentage of responses on the LSD-associated lever. Abscissa: dose in mg/kg body weight. Right panel — response rate altering effects of 5-MeO-DIPT. Symbols remain as described for the left panel. Ordinate: average rate of response on either lever. Abscissa: dose in mg/kg body weight. Asterisks indicate significant differences from saline controls ($P<0.05$). Fantegrossi et al.

of WAY-100635 chosen for use in the present studies has previously been shown to completely antagonize stimulus control by the prototypic 5-HT_{1A} agonist, 8-OH-DPAT (Reissig et al., 2005).

It is notable that effects of 5-MeO-DIPT on head twitch behavior were less profound than we have previously observed with the phenylisopropylamine hallucinogens 2,5-dimethoxy-4-(*n*)-propylthiophenethylamine (2C-T-7) and DOM (see Fantegrossi et al., in press). Indeed, the most effective dose of 5-MeO-DIPT elicited approximately half as much head twitch behavior as was observed with 2C-T-7 and DOM. While the pK_I values reported herein suggest that the affinity of 5-MeO-DIPT for the 5-HT_{2A} receptor is appreciably less than that of the phenylisopropylamines, one might expect that this would be reflected in lower potency, but not necessarily effectiveness, in the head twitch assay. However, the observed range of active doses for 5-MeO-DIPT was identical to those previously reported for 2C-T-7 and DOM in the head twitch assay. Indeed, aside from the effectiveness difference noted above, the dose–effect curves for all three compounds are quite similar in shape. Interestingly, 5-MeO-DIPT is *more* susceptible (shifted further to the right) to the antagonist actions of M100907 than either of the phenylisopropylamines previously studied in the head twitch assay.

Another significant finding of these studies is the failure of *N*,*N*-DMT to elicit a significant head twitch response, particularly given previous reports of its 5-methoxy congener to induce this behavior in the mouse (e.g., Matsumoto et al., 1997). The presently reported data, as well as the lack of any literature reports documenting induction of the head twitch response by *N*,*N*-DMT or *N*,*N*-DIPT, suggest that methoxy substituents might be functionally relevant groups for the molecular pharmacology of the tryptamine hallucinogens. Alternatively, this structural modification may impact pharmacokinetic variables, resulting in greater bioavailability or brain penetrance. However, it has previously been shown that i.p. administration of 10 mg/kg *N*,*N*-DMT to rats resulted in greater and longer lasting brain concentrations of drug than were observed following an equivalent dose of its 5-methoxy congener (Sitaram and McLeod, 1990). Clearly, further behavioral and pharmacokinetic studies of the tryptamine hallucinogens and their 5-methoxy congeners are warranted.

Of necessity, much of our information regarding the effects of newer hallucinogens is largely anecdotal in nature. Nonetheless, accounts of the effects of 5-MeO-DIPT in human subjects by Shulgin and Shulgin (1991), as well as those posted online (for example, erowid.org and lycaeum.org), leave little doubt as to the psychoactive properties of this drug. Based upon those reports and the previously described generalization of LSD in LSD-trained rats to *R*(−)-DOM (Winter and Rabin, 1988) and vice versa (Glennon et al., 1983), we would expect 5-MeO-DIPT to substitute for LSD. The data of Fig. 3 only partially fulfill that prediction. It is seen that a maximum of 75% LSD-appropriate responding followed the administration of 3.0 mg/kg 5-MeO-DIPT, with all doses of this compound producing significant suppression of the rate of responding, thus precluding the testing of higher doses.

The preferential affinity of 5-MeO-DIPT for 5-HT_{1A} receptors is not unexpected given the structural similarity of this compound to other high affinity tryptamines such as 5-MeO-DMT and psilocin (McKenna et al., 1990). The role of 5-HT_{1A} receptors in drug-elicited head twitch behavior is complex, but previous studies have shown that 8-OH-DPAT inhibits DOI-induced head twitch behavior in the rat (Arnt and Hyttel, 1989; Schreiber et al., 1995). In comparison to the phenylisopropylamines (e.g., Fantegrossi et al., 2005) the decreased effectiveness of 5-MeO-DIPT presently observed in the head twitch assay may therefore be a consequence of 5-HT_{1A} receptor activation, which can function to inhibit effects mediated by 5-HT_{2A} receptors (Darmani et al., 1990b; Yocca et al., 1990). Similarly, 8-OH-DPAT has previously been shown to occasion LSD-appropriate responding in LSD-trained rats (Winter and Rabin, 1988), although the reciprocal generalization is only partial (Cunningham and Appel, 1987). It may be the case that 5-HT_{1A}-mediated components of a compound's stimulus effects are more salient for LSD than for 5-MeO-DIPT. Furthermore, the present results suggest that, for 5-MeO-DIPT, these 5-HT_{1A}-mediated interoceptive effects are more salient at lower doses (i.e., the partial attenuation of LSD-appropriate responding by WAY-100635 at a dose of 1.0 mg/kg 5-MEO-DIPT), while the stimulus effects of higher doses are more likely to be dependent on 5-HT_{2A} receptors (i.e., no effect on LSD-appropriate responding at a dose of 3.0 mg/kg 5-MeO-DIPT by WAY-100635, but complete abolition by M100907).

The appreciable affinities for 5-HT_{1A} receptors displayed by the tryptamine-like hallucinogens have long distinguished them from the phenylisopropylamine hallucinogens (Nichols, 1999; Winter et al., 2000). In light of the present binding data, one might argue that 5-MeO-DIPT does not act directly upon 5-HT_{2A} receptors to partially mimic LSD but instead acts indirectly via 5-HT_{1A} receptors to influence 5-HT_{2A} receptors. That this is not the case is indicated by the results presented in Fig. 3, in which it was seen that the selective 5-HT_{1A} antagonist, WAY-100635, did not significantly antagonize the effects of 5-MeO-DIPT in LSD-trained rats. These findings are complete in keeping with an earlier study of the related tryptamine hallucinogen, 5-MeO-DMT, in which it was observed that the intermediate mimicry of *R*(−)-DOM by 5-MeO-DMT was not antagonized by WAY-100635 but was blocked by pirenperone (Winter et al., 2000). Further research into these intriguing effects, particularly as they may relate to subtle differences in the subjective effects induced by chemically distinct hallucinogens in man, would be informative.

Acknowledgements

This research was supported by by USPHS grants DA 03385 (JRE, RAR, JCW), DA09161 and DA05923 (WEF and JHW), as well as by the College on Problems of Drug Dependence. The authors express their gratitude to the University of Michigan Undergraduate Research Opportunity Program, and for the expert technical assistance provided by the University of Michigan Unit for Laboratory Animal Medicine staff.

References

Aghajanian GK, Marek GJ. Serotonin and hallucinogens. Neuropsychopharmacology 1999;21(2S):16S–23S.

Arnt J, Hyttel J. Facilitation of 8-OH-DPAT-induced forepaw treading of rats by the 5-HT_2 agonist DOI. Eur J Pharmacol 1989;161:45–51.

Brauer LH, Goudie AJ, de Wit H. Dopamine ligands and the stimulus effects of amphetamine: animal models versus human laboratory data. Psychopharmacology 1997;130(1):2-13.

Brown JB. Schedules of controlled substances:temporary placement of alpha-methyltryptamine and 5-methoxy-*N*,*N*-diisopropyltryptamine into schedule I. Fed Regist 2003;68(18):4127–30.

Colpaert FC, Janssen PAJ. The head twitch response to intraperitoneal injection of 5-hydroxytryptophan in the rat: antagonist effects of purported 5-hydroxytryptamine antagonists and of pirenperone, an LSD antagonist. Neuropharmacology 1983;22(8):993-1000.

Corne SJ, Pickering RW. A possible correlation between drug-induced hallucinations in man and a behavioral response in mice. Psychopharmacologia 1967;11:65–78.

Corne SJ, Pickering RW, Warner BT. A method for assessing the effects of drugs on the central actions of 5-hydroxytryptamine. Brit J Pharmacol 1963;20:106–20.

Cunningham KA, Appel JB. Neuropharmacological reassessment of the discriminative stimulus properties of D-lysergic acid diethylamide (LSD). Psychopharmacology 1987;91:67–73.

Darmani NA, Martin BR, Glennon RA. Withdrawal from chronic treatment with (±)-DOI causes supersensitivity to 5-HT_2 receptor-induced head-twitch behavior in mice. Eur J Pharmacol 1990a;186:115–8.

Darmani NR, Martin bR, Pandey U, Glennon RA. Do functional relationships exist between 5-HT_{1A} and 5-HT_2 receptors? Pharmacol Biochem Behav 1990b;36:901–6.

Darmani NA, Martin BR, Glennon RA. Behavioral evidence for differential adaptation of the serotonergic system after acute and chronic treatment with (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) or ketanserin. J Pharmacol Exp Ther 1992;262(2):692–8.

Fantegrossi WE, Kiessel CL, Leach PT, Martin CV, Karabenick RL, Chen X, et al. Nantenine: an antagonist of the behavioral and physiological effects of MDMA in mice. Psychopharmacology 2004;173(3–4):270–7.

Fantegrossi WE, Harrington AW, Eckler JR, Arshad S, Rabin RA, Winter JC, Coop A, Rice KC, Woods JH. Hallucinogen-like actions of 2,5-dimethoxy-4-(*n*)-propylthiophenethylamine (2C-T-7) in mice and rats. Psychopharmacology 2005;181(3):496–503.

Fiorella D, Rabin RA, Winter JC. Role of 5-HT_{2A} and 5-HT_{2C} receptors in the stimulus effects of hallucinogenic drugs: II. Reassessment of LSD false positives. Psychopharmacology 1995a;121(3):357–63.

Fiorella D, Rabin RA, Winter JC. The role of the 5-HT_{2A} and 5-HT_{2C} receptors in the stimulus effects of hallucinogenic drugs: I. Antagonist correlation analysis. Psychopharmacology 1995b;121(3):347–56.

Fiorella D, Helsley SE, Lorrain DS, Palumbo PA, Rabin RA, Winter JC. The role of the 5-HT_{2A} and 5-HT_{2C} receptors in the stimulus effects of hallucinogenic drugs: III. The mechanistic basis for supersensitivity to the LSD stimulus following serotonin depletion. Psychopharmacology 1995c;121(3):364–72.

Glennon RA, Young R, Jacyno JM, Slusher M, Rosecrans JA. DOM-stimulus generalization to LSD and other hallucinogenic indolealkylamines. Eur J Pharmacol 1983;86(3–4):453–9.

Goodwin GM, Green AR. A behavioural and biochemical study in mice and rats of putative selective agonists and antagonists for 5-HT1 and 5-HT2 receptors. Br J Pharmacol 1985;84(3):743–53.

Green AR, O'Shaughnessy K, Hammond M, Schachter M, Grahame-Smith DG. Inhibition of 5-hydroxytryptamine-mediated behaviours by the putative 5-HT_2 receptor antagonist pirenperone. Neuropharmacology 1983;22:573–8.

Handley SL, Singh L. Neurotransmitters and shaking behavior: more than a "gut bath" for the brain. Trends Pharmacol Sci 1986;7:324–8.

Leonhart MM. Schedules of controlled substances: placement of alpha-methyltryptamine and 5-methoxy-*N*,*N*-diisopropyltryptamine into schedule I of the controlled substances act. Final rule. Fed Regist 2004;69 (188):58050–3.

Lucki I, Nobler MS, Frazer A. Differential actions of serotonin antagonists on two behavioral models of serotonin receptor activation in the rat. J Pharmacol Exp Ther 1984;228:133–9.

Lyon RA, Titeler M, Seggel MR, Glennon RA. Indolealkylamine analogs share 5-HT_2 binding characteristics with phenylalkylamine hallucinogens. Eur J Pharmacol 1988;145(3):291–7.

Matsumoto K, Mizowaki M, Takayama H, Sakai S-I, Aimi N, Watanabe H. Suppressive effects of mitragynine on the 5-methoxy-*N*,*N*-dimethyltryptamine-induced head-twitch response in mice. Pharmacol Biochem Behav 1997;57(1/2):319–23.

McKenna DJ, Repke DB, Peroutka SJ. Differential interactions of indolealkylamines with 5-hydroxytryptamine receptor subtypes. Neuropsychopharmacology 1990;29:193–8.

Meatherall R, Sharma P. Foxy, a designer tryptamine hallucinogen. J Anal Toxicol 2003;27(5):313–7.

Nichols DE. Role of serotonergic neurons and 5-HT receptors in the action of hallucinogens. In: Baumgarten HG, Gothert M, editors. Serotonergic neurons and 5-HT receptors in the CNS. Berlin: Springer; 1999. p. 563–85.

Nichols DE. Hallucinogens. Pharmacol Ther 2004;101:131–81.

Ortmann R, Biscoff S, Radeke E, Bueche O, Delini-Stula A. Correlation between different measures of antiserotonin activity of drugs. Naunyn Schmiedeberg's Arch Pharmacol 1982;321:265–70.

Peroutka SJ, Lebovitz RM, Snyder SH. Two distinct central serotonin receptors with different physiological functions. Science (Wash. DC) 1981;212: 827-829.

Reissig CJ, Eckler JR, Rabin RA, Winter JC. The 5-HT_{1A} receptor and the stimulus effects of LSD in the rat. Psychopharmacology 2005;182 (2):197–204.

Sadzot B, Baraban JM, Glennon RA, Lyon RA. Hallucinogenic drug interactions at human brain 5-HT_2 receptors: implications for treating LSD-induced hallucinogenesis. Psychopharmacology 1989;98 (4):495–9.

Sanger DJ, Benavides J, Perrault G, Morel E, Cohen C, Joly D, et al. Recent developments in the behavioral pharmacology of benzodiazepine (omega) receptors: evidence for functional significance of receptor subtypes. Neurosci Biobehav Rev 1994;18:355–72.

Schreiber R, Brocco M, Audinot V, Gobert A, Veiga S, Millan MJ. (1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane-induced head-twitches in the rat are mediated by 5-Hydroxytryptamine (5-$HT)_{2A}$ receptors: modulation by novel 5-$HT_{2A/2C}$ antagonists, D_1 antagonists and 5-HT_{1A} agonists. J Pharmacol Exp Ther 1995;273:101–12.

Schuster C, Johanson C. Relationship between the discriminative stimulus properties and subjective effects of drugs. In: Colpaert F, Balster R, editors. Transduction mechanisms of drug stimuli. Berlin: Springer; 1988. p. 161–75.

Shulgin AT, Carter MF. *N*,*N*-diisopropyltryptamine (DIPT) and 5-methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT). Two orally active tryptamine analogs with CNS activity. Commun Psychopharmacol 1980;4:363–9.

Shulgin A, Shulgin A. TIHKAL: the continuation. Berkley: Transform Press; 1991. p. 527–31.

Sitaram BR, McLeod WR. Observations on the metabolism of the psychotomimetic indolealkylamines: implications for future clinical studies. Biol Psychiatry 1990;28(10):841–8.

Smolinske SC, Rastogi R, Schenkel S. Foxy methoxy: a new drug of abuse. Int J Med Toxicol 2004;7(1):3 [accessed on 26 May 2005 at http://www.acmt.net/ijmt/page.asp?PageID=183].

United States Department of Justice / Drug Enforcement Administration. Drug intelligence brief 02052. Trippin' on tryptamines: the emergence of Foxy and AMT as drugs of abuse. Microgram Bull 2002;35 (12):264–8.

Wilson JM, McGeorge F, Smolinske S, Meatherall R. A foxy intoxication. Forensic Sci Int 2005;148(1):31–6.

Winter JC. Stimulus properties of phenethylamine hallucinogens and lysergic acid diethylamide: the role of 5-hydroxytryptamine. J Pharmacol Exp Ther 1978;204:416–23.

Winter JC, Rabin RA. Interactions between serotonergic agonists and antagonists in rats trained with LSD as a discriminative stimulus. Pharmacol Biochem Behav 1988;30:617–24.

Winter JC, Filipink RF, Timineri D, Helsley SE, Rabin RA. The paradox of 5-methoxy-*N*,*N*-dimethyltryptamine: a hallucinogen which induces stimulus control via 5-HT_{1A} receptors. Pharmacol Biochem Behav 2000;65:75–82.

Winter JC, Eckler JR, Rabin RA. Serotonergic/glutamatergic interactions: the effects of mGlu2/3 receptor ligands in rats trained with phencyclidine and LSD as discriminative stimuli. Psychopharmacology 2004;172:233–40.

Yocca FD, Wright RN, Margraf RR, Eison AS. 8-OH-DPAT and buspirone analogs inhibit the ketanserin-sensitive quipazine-induced head shakes response in rats. Pharmacol Biochem Behav 1990;35:251–4.