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Agonist Properties of *N,N*-Dimethyltryptamine at Serotonin 5-HT_{2A} and 5-HT_{2C} Receptors

RANDY L. SMITH, HERVÉ CANTON, ROBERT J. BARRETT AND ELAINE SANDERS-BUSH

Department of Pharmacology, Vanderbilt University School of Medicine, Veterans Administration Medical Center, and John F. Kennedy Center and Department of Psychiatry, Vanderbilt University, Nashville, TN 37232

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R. L. SMITH, H. CANTON, R. J. BARRETT AND E. SANDERS-BUSH. *Agonist properties of* N,N-*Dimethyltryptamine at 5-HT_{2A} and 5-HT_{2C} receptors*. PHARMACOL BIOCHEM BEHAV **61**(3) 323–330, 1998.—Extensive behavioral and biochemical evidence suggests an agonist role at the 5-HT_{2A} receptor, and perhaps the 5-HT_{2C} receptor, in the mechanism of action of hallucinogenic drugs. However the published in vitro pharmacological properties of *N,N*-dimethyltryptamine (DMT), an hallucinogenic tryptamine analog, are not consistent with this hypothesis. We, therefore, undertook an extensive investigation into the properties of DMT at 5-HT_{2A} and 5-HT_{2C} receptors. In fibroblasts transfected with the 5-HT_{2A} receptor or the 5-HT_{2C} receptor, DMT activated the major intracellular signaling pathway (phosphoinositide hydrolysis) to an extent comparable to that produced by serotonin. Because drug efficacy changes with receptor density and cellular microenvironment, we also examined the properties of DMT in native preparations using a behavioral and biochemical approach. Rats were trained to discriminate an antagonist ketanserin from an agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) in a two-lever choice paradigm. Pharmacological studies showed that responding on the DOI and ketanserin lever reflected agonist and antagonist activity at 5-HT_{2A} receptors, and hence, was a suitable model for evaluating the in vivo functional properties of DMT. Like other 5-HT_{2A} receptor agonists, DMT substituted fully for DOI. Intact choroid plexus was used to evaluate the agonist properties at endogenous 5-HT_{2C} receptors; DMT was a partial agonist at 5-HT_{2C} receptors in this native preparation. Thus, we conclude that DMT behaves as an agonist at both 5-HT_{2A} and 5-HT_{2A} receptors. One difference was evident in that the 5-HT_{2C}, but not the 5-HT_{2A}, receptor showed a profound desensitization to DMT over time. This difference is interesting in light of the recent report that the hallucinogenic activity of DMT does not tolerate in humans and suggests the 5-HT_{2C} receptor plays a less prominent role in the action of DMT. © 1998 Elsevier Science Inc.

N,N-Dimethyltryptamine Hallucinogen Serotonin Receptor Agonist Behavior Drug discrimination

N,N-DIMETHYLTRYPTAMINE (DMT) produces intense visual hallucinations, and a dissociated state reminiscent of lysergic acid diethylamide (LSD). Recent controlled clinical studies of the behavioral and physiological properties of DMT (18,19) have set the stage for evaluating theories of hallucinogenic drug action based on animal models. One of the most prominent hypotheses proposes that activation of 5-HT_{2A} receptors (formerly referred to as 5-HT_2 receptors) is an important component in the mechanism of action of hallucinogenic drugs (5). More recently, agonist properties at a closely-related receptor, the 5-HT_{2C} receptor (formerly 5-HT_{1C} receptor), have also been implicated (12). However, in vitro pharmacological studies of DMT have lead to the assertion that this drug acts as an antagonist rather than an agonist at 5-HT_{2A} receptors (3). These latter studies in slices of rat cerebral cortex reported that DMT fails to activate phospholipase C, the principal second messenger signaling pathway for the 5-HT_{2A} receptor (14). In addition, based on its interaction with 5-HT, Deliganis et al. (3) concluded that DMT functions as an antagonist at 5-HT_{2A} receptors. This conclusion is inconsistent with the behavioral studies showing that DMT substitutes for DOM in drug discrimination (6). Furthermore, other hallucinogenic drugs have been shown to activate the intracellular phosphoinositide hydrolysis signaling cascade by interacting with 5-HT_{2A} receptors as well as 5-HT_{2C} receptors (11). Considering these apparently conflicting results and the recent onset of clinical studies of DMT, we undertook a detailed analysis of the agonist/antagonist properties of DMT at 5-HT_{2A} and 5-HT_{2C} receptors using recombinant cell lines expressing cloned receptors, intact tissue naturally expressing 5-HT_{2C} receptors, and a behavioral model of 5-HT_{2A} receptors. In the latter studies, rats were trained in a drug–drug discrimination

Requests for reprints should be addressed to Elaine Sanders-Bush, Ph.D., Department of Pharmacology, Vanderbilt University School of Medicine, 459 Medical Research Building II, Nashville, TN 37232-6600. Phone: (615)322-2207

paradigm where the drugs to be discriminated were the agonist, DOI, and the antagonist, ketanserin. Previous studies using a similar paradigm have shown it to be uniquely sensitive to both the agonist and antagonist effects of 5-HT_{2A} compounds (17). Thus, training animals to discriminate between DOI and ketanserin provides a sensitive within-subject baseline for determining which interoceptive cue state, 5-HT_{2A} agonist or 5-HT_{2A} antagonist, DMT, best approximates.

METHOD

Drug and Cell Culture Supplies

5-HT_{2A} and 5-HT_{2C} receptor cDNAs were the gift of David Julius (UCSF, San Francisco, CA). Clonal cell lines were generated in NIH 3T3 fibroblasts (American Type Culture Collection, Rockville, MD) by calcium phosphate precipitation, selected in presence of G418, with screening by radioligand binding. Cells expressing the 5-HT_{2A} receptor ($\sim$ 10,000 fmol/mg protein) are referred to as 3T3/2A and cells expressing 5-HT_{2C} receptors ($\sim$10,000 fmol/mg protein), 3T3/2C. Mianserin hydrochloride, ketanserin hydrochloride, and DOI hydrochloride were purchased from Research Biochemicals, Inc. (Nattick, MA). LSD tartrate and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane hydrochloride (DOM) were provided by the National Institute of Drug Abuse. MDL 100,907, SB 206,553, and LY 53,857 were gifts from Marion Merrell Dow, SmithKline Beecham, and Eli Lilly & Company, respectively. DMT was purchased from Aldrich Chemical Company. [^{3}H]mesulergine (77 Ci/mmol) and [^{3}H]myo-inositol (20–25 ci/mmol) were purchased from Dupont/NEN Corporation (Boston, MA) and Whatman GF/C glass fiber filters, from Brandel (Gaithersburg, MD). Penicillin, streptomycin, and Dulbecco's modified eagle medium (DMEM) were from GIBCO/BRL (Grand Island, NY), bovine serum, from Hyclone (Logan, UT), and tissue culture plates, from Falcon Corporation (Lincoln Park, NJ).

For in vivo studies, DOI, ketanserin, DOM, LSD, and DMT were dissolved in distilled water. MDL 100,907 was dissolved in 2% tartaric acid and diluted with distilled water. SB 206,553 was suspended in distilled water with a couple of drops of Tween 80. Doses were calculated as the salt, except for LSD, which was calculated as free base. All drugs were administered SC in a volume of 1 ml/kg.

Phosphoinositide Hydrolysis

Fibroblasts were grown in DMEM supplemented with penicillin (5 U/ml), streptomycin (5 mg/ml), and calf serum (9%). Transfected cells were plated into 24-well culture plates and allowed to adhere for 30 h in DMEM supplemented with 9% calf serum. Cells were subsequently washed once with 500 ml serum-free DMEM and then incubated for 18 h in 500 ml serum-free, inositol-free DMEM supplemented with 5 mCi/ml [^{3}H]-myo-inositol. Labeled cells were subsequently used for determination of [^{3}H]inositol phosphate (IP) formation (1) as described previously (7). Briefly, the reaction was terminated by aspiration of medium and addition of methanol. [^{3}H]Inositol phosphates were separated from phospholipids by solvent extraction followed by anion exchange chromatography to isolate [^{3}H]-IP. [^{3}H]-IP formation in intact choroid plexus isolated from the lateral and third ventricles of rats was analyzed by similar methodology as described previously (12). Dose–response curves were fit using Prism, a commercially available computer graphics/analysis program (GraphPad, San Diego, CA).

Radioligand Binding

Competition assays using 1 nM [^{3}H]mesulergine were performed in crude membrane preparations from transfected cells as described previously (21). Briefly, membranes were prepared by sonication and centrifugation. An aliquot of membrane (equivalent to 10 μg of protein) was incubated with ^{3}H-mesulergine and competing drug for 60 min. Bound radioligand was separated from free by rapid filtration. In saturation binding experiments, ^{3}H-mesulergine was varied from 0.25 to 8 nM, and nonspecific binding was determined by adding 10 μM methysergide.

Behavioral Subjects and Apparatus

Subjects were 72 male Sprague–Dawley rats (Harlen–Sprague–Dawley, Inc., Indianapolis, IN), individually housed and food deprived to 85% of their free-feeding body weight 1 week before the start of training. Following training and over the weekend, the animals were given enough food to maintain their weights at 85% of their expected nondeprived weights. All rats were maintained on a 12L:12D cycle with light onset at 0600 h.

Six commercially available operant chambers (BRS/LVE, Beltsville, MD, Model No. RTC-022), each housed in a sound attenuating chamber, were used. The operant chambers were equipped with two response levers located 5 cm above the floor and a liquid feeder centered between the two levers. White noise was used to mask extraneous auditory stimuli. The start of the session was signaled by the illumination of the house light (7.5 W bulb). All equipment was controlled by MS-DOS-compatible computers using the Operant Package for the Neuroscience software.

Behavioral Training

Rats were trained to lever press for milk reinforcement (Borden's condensed milk diluted 1:1 with water) on a continuous reinforcement schedule during daily 20-min training sessions. After shaping to lever press, the reinforcement contingency was changed to a VI 10-s schedule with a 15-s timeout and discrimination training began. The VI schedule was incremented by 10 s weekly until arriving at a VI 30-s schedule of reinforcement, which remained in effect for the duration of the experiment. The time-out contingency, a 15-s period after incorrect responses during which no reinforcement was available, served to facilitate discrimination learning. The training drugs were 0.125 mg/kg DOI dissolved in saline and 1.0 mg/kg ketanserin dissolved in distilled water, both administered SC. Training doses were selected based on the results of a pilot study that showed that, at these doses, when animals were tested on saline after discrimination acquisition, they responded about equally often on both levers.

Thirty minutes after injection with either of the training drugs, animals were placed in the operant chamber and reinforced for responding on the correct lever. For one-half the animals, responding on the right lever was DOI correct and responses on the left lever were ketanserin correct; for the remainder of the animals, the reverse was true. Throughout training, DOI and ketanserin were alternated every other day and training was given Monday through Friday. Discrimination learning was monitored twice weekly by calculating the percentage of correct lever responses (number of responses on the correct lever/total number of responses) during a 2.5-min extinction period at the beginning of the training session. These data provided a measure of discrimination learning un-

confounded by reinforcement. During the remaining 17.5 min of the session, responses were reinforced on a VI 30-s schedule of reinforcement. Training continued until choice behavior, for the whole group, during the 2.5-min extinction test periods averaged greater than 85% correct for both DOI and ketanserin. In the experiments described below a subset of the original 72 rats trained on the DOI–ketanserin discrimination was used for testing and most rats served as subjects in multiple experiments.

DOI–Ketanserin Dose–Response Curve

After acquisition of the DOI–ketanserin discrimination, a dose–response function was determined during 5-min extinction test sessions. For this experiment, a subset of the 72 trained rats were assigned to one of five groups (n = 8) matched for choice behavior during saline tests sessions.

Antagonist Studies

To determine if SB 206,553 or MDL 100,907 would block DOI discrimination, independent groups of rats (n = 12) were pretreated with SB 206,553 (8.0, 4.0 and 2.0 mg/kg or vehicle) or with MDL 100,907 (0.025, 0.013, 0.006, 0.003 mg/kg or vehicle) 15 min before 0.125 mg/kg DOI. Also included in the latter study was a vehicle–vehicle group. Animals were tested during 5-min extinction test sessions given 30-min after DOI administration.

In addition to studying the effects of increasing doses of MDL 100,907 on DOI discrimination, we also investigated whether a single dose of MDL 100,907 would shift the DOI dose–response curve to the right. In this experiment, 65 animals were assigned to one of five groups. All groups were pretreated with saline. Fifteen minutes later each group was injected with one of four doses of DOI (0.015, 0.03, 0.06, 0.125 mg/kg) or saline. Thirty minutes following injection with DOI or saline, the animals were placed in the operant chamber and tested during 5-min extinction test sessions. Following a week of retraining, the same five groups of rats were pretreated with 0.003 mg/kg MDL 100,907, 15 min prior to receiving injections of either 0.06, 0.125, 0.25, 0.5 mg/kg or saline. Thirty minutes following DOI or saline injections, the animals were tested during 5-min extinction test sessions.

Substitution Tests

Rats were tested for choice behavior during 5-min extinction test sessions after the administration of the 5-$HT_{2A/2C}$ receptor agonists LSD (0.016, 0.008, 0.004, and 0.002 mg/kg), DOM (0.25, 0.125, 0.06, and 0.03 mg/kg) and the 5-HT_{2A} receptor antagonists MDL 100,907 (0.2, 0.1, 0.05, and 0.025 mg/kg) and LY 53,857 (1.0, 0.5, 0.25, and 0.125 mg/kg). In addition, DMT (6.0, 3.0, and 1.5 mg/kg) was administered to determine whether its cue properties substituted for either the 5-$HT_{2A/2C}$ receptor agonist, DOI, or the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin. For each drug tested, a saline group was included. All tests compounds were administered SC. Rats were assigned to one of four or five groups (n = 12) matched on the basis of their choice behavior after saline administration. Test doses of drug were administered 30 min before placing the animals in the operant chamber. After the 5-min extinction tests, the animals were returned to their home cages. No more than one test was given each week with a minimum of 4 retraining days between test sessions.

RESULTS

Agonist Properties of DMT in Cell Lines

For receptors such as the 5-HT_{2A} and 5-HT_{2C} subtypes, which are linked to the phosphoinositide hydrolysis signaling cascade, the formation of [^{3}H]-inositol monophosphate (IP) is routinely used as an index of receptor activation. We therefore used this biochemical measure for an initial evaluation of the pharmacological properties of DMT. In fibroblasts expressing either the 5-HT_{2A} or 5-HT_{2C} receptors, DMT elicited a marked increase in [^{3}H]-IP formation (Fig. 1). DMT was a more potent agonist at 5-HT_{2C} than at 5-HT_{2A} receptors (EC_{50} = 49 and 983 nM, respectively). The maximum increases elicited by DMT were nearly equal to those produced by 5-HT (90 and 85% of the maximum 5-HT response at 5-HT_{2A} or 5-HT_{2C} receptors, respectively). These results suggested that DMT has the ability to function as an agonist at both 5-HT_{2A} and 5-HT_{2C} receptors. To confirm that the phosphoinositide hydrolysis signal reflects an interaction with the appropriate receptor, two antagonists were evaluated—ketanserin and mianserin. These drugs are nearly equipotent at competing for 5-HT_{2A} receptors, but mianserin is about 25 times more potent at 5-HT_{2C} receptors (9). The relative potency of mianserin and ketanserin at blocking the phosphoinositide hydrolysis response to DMT was 1:2 in 3T3/2A cells and 1:20 in 3T3/2C cells (data not shown), confirming that activation of intracellular signaling by DMT reflects agonist properties at 5-HT_{2A} and 5-HT_{2C} receptors, respectively.

Desensitization of 5-HT_{2C} Receptors by DMT

Time-course studies of the formation of [^{3}H]-IP in 3T3/2C cells revealed a profound desensitization that developed within 30 min of the addition of a supramaximal concentration of DMT (Fig. 2). When expressed as percent increase above basal, the DMT effect peaked at 30 min and then de-

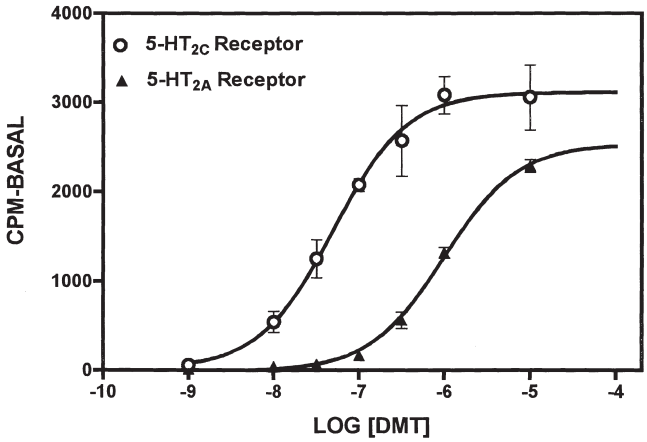

FIG. 1. DMT increases phosphoinositide hydrolysis in cell lines expressing the 5-HT_{2A} and 5-HT_{2C} receptors. Cells transfected with the 5-HT_{2A} (triangles) or the 5-HT_{2C} (circles) receptor were labeled overnight with [^{3}H]-inositol and then incubated in the presence of lithium and increasing concentrations of DMT for 15 min as described in the Method Section. The amount of [^{3}H]-IP formed was determined after purification by column chromatography and expressed as cpm in the presence of DMT minus basal values determined in the absence of DMT. The values plotted are mean ± standard error of the mean (SEM) of four separate experiments.

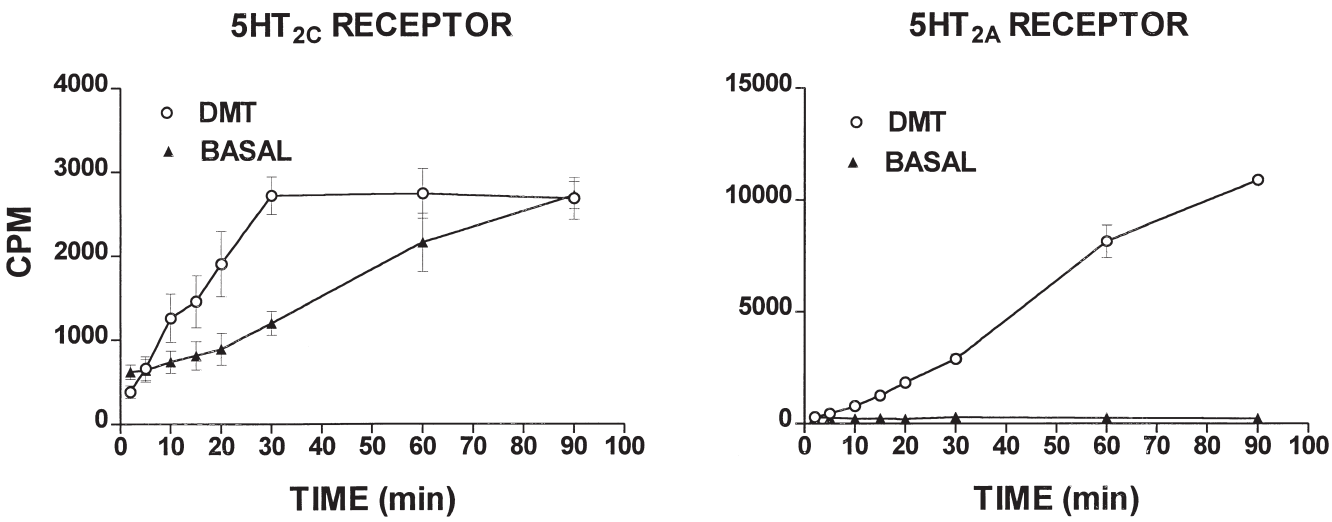

FIG. 2. Time-course of DMT-induced [^{3}H]-IP formation in cell lines expressing 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. Cells transfected with the 5-HT$_{2A}$ (right panel) or the 5-HT$_{2C}$ (left panel) receptor were labeled with [^{3}H]-inositol overnight and then incubated with a maximum concentration of DMT (100 μM or 10 μM, respectively) for the indicated times. The amount of [^{3}H]-IP formed was determined after purification by column chromatography as described in the Method Section. Basal (triangles) and DMT-basal (circles) values are plotted and represent the mean ± SEM of three separate experiments.

creased because the basal formation of [^{3}H]-IP continued to rise linearly. In contrast, in 3T3/2A cells, the 5-HT response was linear for at least 90 min (Fig. 2). The difference in basal increases in [^{3}H]-IP formation is consistent with evidence that only the 5-HT$_{2C}$ receptor shows detectable constitutive (agonist-independent) activity (unpublished results).

To determine if the reduced response to DMT in 3T3/2C cells was due to a K_D shift, a reduced maximum response, or both, cells were treated with 1 μM DMT for 60 min, then washed extensively to remove the drug and a dose response to DMT determined. Pretreatment with DMT blunted the phosphoinositide hydrolysis response, decreasing the maximum response to less than 1/3 that found in cells pretreated with vehicle (Table 1). The dose–response curve was shifted to the right in cells pretreated with DMT; however, the EC_{50} shift failed to reach statistical significance, probably because of the high variability in treated cells where the signal was small. Next, we determined if pretreatment with DMT reduced the density of 5-HT$_{2C}$ receptor sites labeled with [^{3}H]-mesulergine. Sixty minutes after treatment with DMT at a time when the maximum phosphoinositide hydrolysis signal was markedly reduced, the density of 5-HT$_{2C}$ receptors was not different from the density in untreated cells (Table 1). Thus, the changes in 5-HT$_{2C}$ receptor sensitivity were not mediated by changes in receptor density. The K_D value for ^{3}H-mesulergine was also unchanged by pretreatment with DMT.

Behavioral Studies of 5-HT$_{2A}$ Receptor Function

After 47 training sessions on the DOI vs. ketanserin discrimination, the animals averaged 85% or greater correct lever pressing during the 2.5-min test sessions. The data from the 2.5-min extinction test sessions for the final four extinction periods (two DOI and two ketanserin) showed correct lever choices averaged 90% for DOI and 90% for ketanserin. After acquisition, a dose–response curve was determined for several doses of DOI, ketanserin, and saline. Figure 3 shows that after training to discriminate 0.125 mg/kg DOI from 1.0 mg/kg ketanserin, animals detected changes in cue state in a drug-dependent manner.

To tease out the relative importance of 5-HT$_{2A}$ vs. 5-HT$_{2C}$ receptors in this discrimination, two new, selective antagonists were employed: MDL 100,907, a 5-HT$_{2A}$ receptor selective antagonist with low affinity for 5-HT$_{2C}$ receptors (16) and SB 206,553, which is more potent at 5-HT$_{2C}$ receptors than 5-HT$_{2A}$ receptors, although it also blocks 5-HT$_{2B}$ receptors (4). As shown in Fig. 4, MDL 100,907 potently and completely blocked the cue properties of DOI, while pretreatment with the 5-HT$_{2C/2B}$ antagonist SB 206,553 did not significantly alter discrimination of DOI, as evidenced by the fact that DOI

TABLE 1

DMT TREATMENT ELICITS DESENSITIZATION, BUT NOT DOWNREGULATION, OF THE 5-HT$_{2C}$ RECEPTOR

	Control	DMT Pretreatment
EC_{50} (nM)	312 ± 110	2631 ± 1703
E_{max} (% above basal)	136 ± 15	47 ± 6*
Basal (cpm)	2307 ± 288	2358 ± 223
K_D (nM)	2.1 ± 0.6	2.1 ± 0.8
B_{max} (fmol/mg protein)	10343 ± 2110	10379 ± 882

Fibroblasts expressing the 5-HT$_{2C}$ receptor were treated for 1 h with 1 μM DMT. After extensive washing, [^{3}H]-IP formation was determined in intact cells; 5-HT dose–response curves were analyzed using GraphPad Prism and the computer generated values (EC_{50} and E_{max}) were averaged for four separate determinations. Membranes were prepared for radioligand binding using ^{3}H-mesulergine as the radioligand as described in the Method sections. Saturation binding curves were analyzed by nonlinear regression using GraphPad Prism. Computer-derived values for K_D and B_{max} were averaged for four separate experiments. Values are mean ± SEM.

*$p < 0.01$.

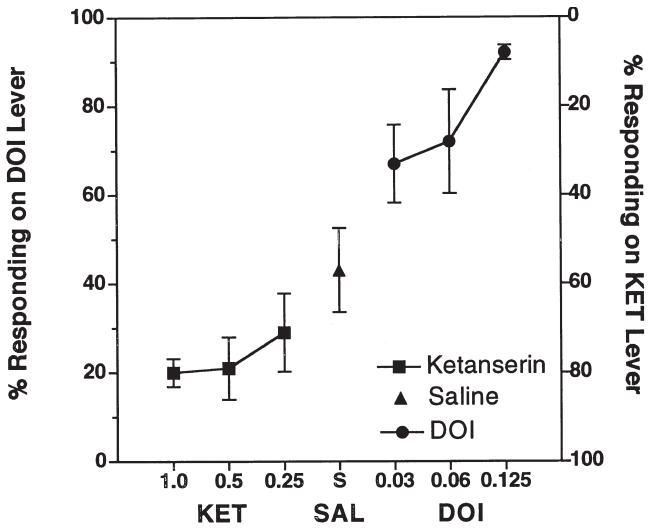

FIG. 3. DOI–ketanserin dose–response curve. The data shown are choice behavior as a function of varying doses of DOI (0.125, 0.0625, and 0.03 mg/kg), ketanserin (1.0, 0.5, and 0.25 mg/kg) and saline during 5-min extinction test sessions. Choice behavior is expressed as percent responding on the DOI lever on the left axis and percent responding on the ketanserin lever on the right axis. Data for antagonists are plotted from highest to lowest dose, contrary to convention, to illustrate the bidirectionality of this behavior. Mean response rates for the 5-min test sessions after DOI 0.125, 0.0625, and 0.03 mg/kg, were 72, 76, and 64, respectively. After ketanserin, 1.0, 0.5, and 0.25 mg/kg, mean response rates were 62, 89, and 87 responses per 5 min, respectively. For saline, mean responses during the 5-min test session were 87. The test doses are plotted as equal log units.

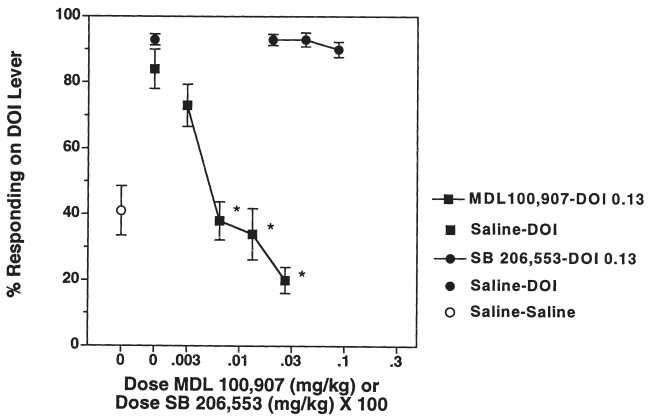

FIG. 4. Antagonist dose–response curves of MDL 100,907 and SB 206,553. The ability of different doses of MDL 100,907, SB 106,553, and saline to block DOI discrimination is shown. Five independent groups of rats were pretreated with MDL 100,907 15 min prior to injection with 0.125 mg/kg DOI. Thirty minutes following injection of DOI, rats were tested during 5-min extinction sessions. Following a week of retraining, the procedure was replicated with SB 206,553. Choice behavior is expressed as percent responding on the DOI lever. *Test dose of MDL 100,907 that significantly differed from saline controls injected with DOI ($p < 0.05$, Dunnett's t-test).

choice behavior in animals pretreated with SB 206,553 did not differ significantly from that of animals pretreated with vehicle ($p > 0.05$, Dunnett). After high doses of MDL 100,907 in combination with DOI, the animals responded on the ketanserin lever, suggesting that MDL 100,907's antagonist action now prevails, causing substitution for ketanserin. Additionally, MDL 100,907 shifts the DOI dose–response curve to the right with no reduction in maximum effect (Fig. 5), consistent with the interpretation that the blockade of DOI reflects a specific, surmountable action at 5-HT$_{2A}$ receptors.

Figure 6 shows the results of substitution tests with the 5-HT$_{2A/2C}$ receptor agonists LSD and DOM or with the 5-HT$_{2A}$ receptor selective antagonist MDL 100,907 or the 5-HT$_{2A/2C}$ antagonist, LY 53,857 (2). In addition, the effects of DMT are illustrated. Animals treated with either 0.004, 0.008, or 0.016 mg/kg of LSD made significantly greater percentage of their responses on the DOI lever (78, 83, and 89%, respectively) when compared to vehicle treated controls, $F(4, 56) = 5.013$, $p < 0.002$. Similarly, animals injected with DOM (0.03, 0.06, 0.125, and 0.25 mg/kg) made a significantly greater percentage of responses on the DOI lever (67, 77, 90, and 94%, respectively) than did vehicle treated controls; $F(4, 57) = 16.2$, $p < 0.0001$. Animals tested with the 5-HT$_{2A/2C}$ receptor antagonists MDL 100,907 or LY 53,857 both made significantly greater number of responses on the ketanserin lever relative to vehicle treated controls (Fig. 6). In animals treated with MDL 100,907, all four tests doses (0.025, 0.05, 0.1, and 0.2 mg/kg) of drug produced significantly greater responding (75, 82, 89, and 88%, respectively), $F(4, 57) = 8.9$, $p < 0.0001$, on the ketanserin lever relative to vehicle treated rats. Similarly rats administered 0.25, 0.5 and 1.0 mg/kg LY 53,857 all made a significantly greater, $F(4, 56) = 5.5$, $p < 0.0008$, percentage of their responses on the ketanserin lever relative to vehicle-treated animals. Based on the cumulative data of blockade and substitution tests, we conclude that the DOI–ketanserin drug–drug discrimination paradigm is a valid in vivo model to evaluate agonist vs. antagonist properties of DMT at 5-HT$_{2A}$ receptors. As illustrated in Fig. 6, animals tested following 1.5, 3.0, or 6.0 mg/kg DMT made significantly greater number of their responses, $F(4, 30) = 7.0$, $p < 0.0004$ on the DOI lever (80, 86, and 90%, respectively) than did vehicle treated controls (38%) suggesting that the discriminative stimulate effects of DMT bear a much stronger resemblance to those of the 5-HT$_{2A/2C}$ agonist DOI than the 5-HT$_{2A/2C}$ antagonist ketanserin. Based on the molar dose that elicits 80% responding on the DOI lever, DMT is approximately 1000-fold less potent than LSD.

Evidence That DMT Activates 5-HT$_{2C}$ Receptors in Choroid Plexus

The choroid plexus expresses a high density of 5-HT$_{2C}$ receptors and has in the past been used as a model system to evaluate drugs (8,13). Consistent with the results in recombinant cell lines, DMT produced a dramatic increase in phosphoinositide hydrolysis in choroid plexus with an EC$_{50}$ of 220 nM and a maximum response that was 25% lower than 5-HT (Fig. 7).

DISCUSSION

Renewed interest in the pharmacological characteristics of DMT in model systems has been generated by recent studies in humans of its hallucinogenic properties (19). These studies are one of the first controlled clinical evaluations of a hallucinogenic drug in nearly 2 decades. Theories of the mechanism of action of hallucinogenic drugs may now be tested in the rel-

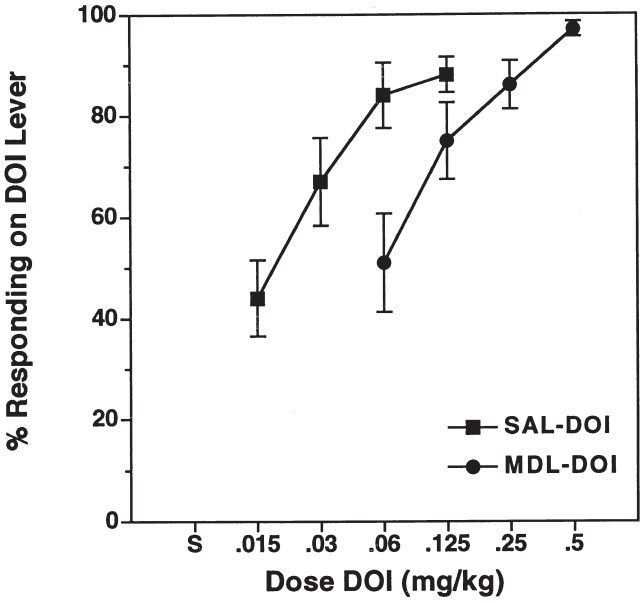

FIG. 5. Competitive antagonism of DOI by MDL 100,907. The ability of a single 0.003 mg/kg dose of MDL 100,907 to shift the DOI dose–response, curve to the right is shown. Five groups of animals were pretreated with saline; 15 min later they were injected with one of five doses of DOI (0.015, 0.03, 0.06, and 0.125) or saline. Thirty minutes following injection with DOI, the rats were tested during 5-min extinction sessions. Following a week of retraining, this procedure was replicated with the modification that the five groups of rats were pretreated with 0.003 mg/kg MDL 100,907. The mean per cent responding for animals tested on saline was 33 ± 8.7 (data not shown). Choice behavior is expressed as percent DOI lever selection.

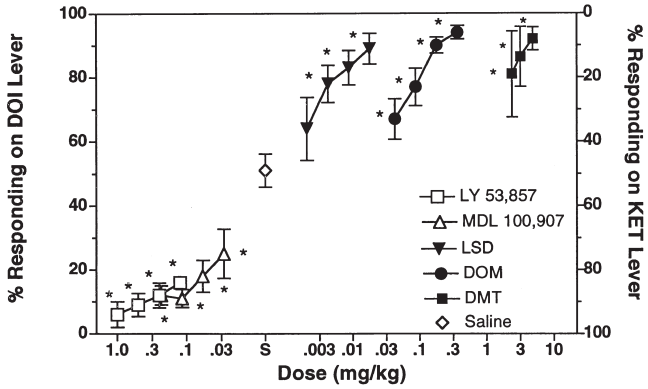

FIG. 6. Substitution tests with DMT compared to known 5-HT_2 receptor agonists and antagonists. Two 5-HT_2 receptor agonists (LSD and DOM), two antagonists (MDL 100,907 and LY 53,857) or DMT were tested to determine if they would substitute for either DOI or ketanserin. Four or five independent groups were administered one of three or four doses of drug or saline and tested for choice behavior during 5-min extinction test sessions. Choice behavior is expressed as percent DOI lever responding on the left axis and percent ketanserin lever responding on the right axis. The 5-HT_2 antagonists are plotted from high to low dose, contrary to convention. The saline point represents the mean of the four saline test groups. The doses are plotted as equal log units. DMT progressively decreased response rate from 76 ± 8 bar presses in saline-treated rats to 65 ± 8 after 1.5 mg/kg of DMT, 47 ± 10 after 3 mg/kg, and 46 ± 21 after 6 mg/kg. *Test doses that differed significantly from corresponding saline control ($p < 0.05$, Dunnett's *t*-test).

evant species, humans. It was, therefore, critical to evaluate the actions of DMT vis-à-vis current theories of hallucinogenic drug action. 5-HT_{2A} receptors (5) and, more recently, 5-HT_{2C} receptors (13) have been implicated in the actions of hallucinogens based on biochemical and behavioral assays in rats. DMT binds to both 5-HT_{2A} and 5-HT_{2C} receptors; however, only one report has appeared dealing with the second-messenger responses to DMT binding (3). We, therefore, undertook a detailed analysis of the pharmacological action of DMT at 5-HT_{2A} and 5-HT_{2C} receptors in cell lines expressing the cloned receptors. The properties of DMT were also explored in a behavioral model and a neurochemical model where 5-HT_{2A} and 5-HT_{2C} receptors are naturally expressed.

In a recombinant cell line expressing the cloned 5-HT_{2A} receptor, DMT functioned as an agonist, producing a maximum response in phosphoinositide hydrolysis assays that was nearly equal to 5-HT. This result does not agree with an earlier report (3), in which DMT was found to function as an antagonist at 5-HT_{2A} receptors in cerebral cortex slices. One possible explanation for this discrepancy is that cell lines expressing the cloned receptor do not reflect the properties in the in vivo drug–receptor interaction. We, therefore, examined the actions of DMT in a 5-HT_{2A} receptor agonist–antagonist drug discrimination paradigm, which provides a highly specific behavior in which to evaluate the properties of DMT in vivo (17). In rats trained to discriminate DOM from saline (6), DMT has been shown to generalize to DOM, an hallucinogenic drug with 5-$HT_{2A/2C}$ agonist properties. In the present work, rats were trained to discriminate the 5-$HT_{2A/2C}$ agonist

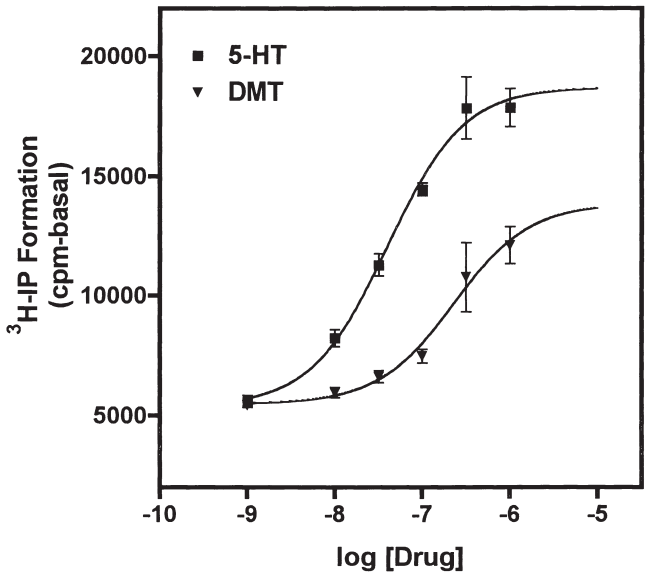

FIG. 7. DMT activates 5-HT_{2C} receptors in choroid plexus. Choroid plexi from individual rats were labeled with [^{3}H]-inositol for 3 h then incubated with increasing concentrations of serotonin (5-HT, squares) or DMT (triangles) for 30 min. The values presented are means ± SEM of triplicate determinations and are representative of two separate experiments. The EC_{50} values of DMT for these two experiments were 231 and 207 nM.

DOI from the 5-HT_{2A} antagonist ketanserin. In this paradigm, the DOI discrimination is exclusively mediated by activation of the 5-HT_{2A} receptor, because it is potently and completely blocked by the highly specific 5-HT_{2A} antagonist MDL 100,907 (16), but not by the 5-$HT_{2B/2C}$ receptor selective antagonist SB 206,553 (4). Previous studies of DOM (10) and DOI (15), in a drug–saline paradigm, led to a similar conclusion. In addition, data from our laboratory suggest that the discriminative stimulus effects of ketanserin in this paradigm reflect predominantly 5-HT_{2A} receptor antagonism. Support for this assertion comes from two findings. First, the 5-HT_{2A} selective antagonist MDL 100,907 potently substitutes for ketanserin at doses that do not compete for 5-HT_{2C} receptor sites in vivo (17). Second, the training dose of ketanserin was shown to selectively occupy 5-HT_{2A} but not 5-HT_{2C} receptors in rat brain based on ex vivo receptor autoradiography (17). Thus, we propose that the DOI–ketanserin paradigm gives a direct in vivo measure of agonist vs. antagonist activity at the 5-HT_{2A} receptor. The finding that DMT substitutes for DOI rather than ketanserin in ketanserin–DOI-trained rats provides strong evidence that, in vivo, DMT functions as a 5-HT_{2A} receptor agonist. The discrepancy between our biochemical results in a cell line and an earlier study in brain (3) may be related to the increased signal to noise in transfected cells, which enhances the ability to detect significant agonist effects.

DMT also behaved as an agonist (nearly comparable to 5-HT) in cell lines expressing the 5-HT_{2C} receptor. To evaluate the properties of DMT in a native system, we utilized the choroid plexus, a tissue that expresses the highest density of 5-HT_{2C} receptors in brain. Here, too, DMT was a robust agonist at activating phosphoinositide hydrolysis, although it gave a maximum phosphoinositide hydrolysis signal somewhat lower than 5-HT. The response to DMT in the transfected cell line showed a rapid and profound desensitization. Within 30-min after addition of DMT, the formation of [^{3}H]-IP leveled off and remained essentially unchanged for another 60 min. Desensitization of 5-HT_{2C} receptors is also evident with 5-HT or LSD as the agonist (unpublished results). This was in sharp contrast to the response of 5-HT_{2A} receptors, which was linear for at least 90-min with no evidence of desensitization. The markedly different regulatory properties of DMT could reflect differences in density in the two cell lines or different cellular microenvironments; however, these explanations seem unlikely, because the receptor cell lines were derived from the same parental cell line (3T3 fibroblasts) and receptor densities were equal. These results, therefore, suggest that the 5-HT_{2A} and 5-HT_{2C} receptors are regulated by distinct intracellular mechanisms. The present finding that 5-HT_{2C}, but not 5-HT_{2A}, receptors rapidly desensitize combined with the recent report (20) that the hallucinogenic effect of DMT in humans does not desensitize when the drug is given repeatedly provides additional evidence that, relative to the 5-HT_{2A} receptor, 5-HT_{2C} receptors play a less significant role in the action of DMT, and perhaps other hallucinogenic drugs.

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