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Behavioral effects of the highly selective serotonin releasing agent 5-methoxy-6-methyl-2-aminoindan

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Abstract

The behavioral effects of 5-methoxy-6-methyl-2-aminoindan (MMAI) were examined using the drug discrimination procedure and direct observation for classification of the characteristic syndrome induced by MMAI. The stimulus effects of MMAI were studied in 5 different groups of rats trained to discriminate MMAI (1.71 mg/kg; 8 μM/kg), MDMA (3,4-methylenedioxymethamphetamine; 1.75 mg/kg; 7.6 μM/kg), (+)-MBDB ((+)-*N*-methylamino-(1,3-benzodioxo-5-yl)-2-butanamine; 1.75 mg/kg; 7.18 μM/kg), (+)-amphetamine (1 mg/kg; 5.4 μM/kg), or LSD ((+)-lysergic acid diethylamide tartrate; 0.08 mg/kg; 186 nM/kg) from saline. In substitution tests in rats trained to discriminate MMAI from saline, all the compounds which fully mimicked MMAI were serotonin (5-hydroxytryptamine, 5-HT) releasing agents. This substitution is symmetrical for MDMA and (+)-MBDB. Nevertheless, the dose-response curve of MMAI is parallel to those of (+)-fenfluramine (*m*-trifluoromethyl-*N*-ethylamphetamine) and *p*-chloroamphetamine. The results also show that MMAI lacks amphetamine-like and LSD-like discriminative stimulus effects, suggesting that MMAI is neither a psychostimulant nor a hallucinogen. Tests of the discriminability of MMAI after 5-HT depletion with the selective serotonin synthesis inhibitor, *p*-chlorophenylalanine (2 × 200 mg/kg i.p., pretreatment 72 h before test), showed only saline appropriate responding. Prolonged block (ca. 1 week) of the MMAI cue by *p*-chlorophenylalanine further supports the conclusion that endogenous 5-HT is essential for MMAI discrimination. Fluoxetine (10 mg/kg) or paroxetine (2.5 mg/kg), both selective 5-HT uptake inhibitors, reduced the discriminability of MMAI to 40% and 50%, respectively. None of the antagonists (ketanserin, methiothepin, pindolol, yohimbine, haloperidol) used in antagonism tests inhibited the stimulus properties of MMAI. These results and data from radioligand binding studies support the conclusion that direct activation or inhibition of known neurotransmitter receptors did not play a significant role in the discriminative cue of MMAI. The administration of 5, 10, or 20 mg/kg of MMAI to rats induced a behavioral syndrome consisting of hypolocomotion with accompanying catalepsy-like posture, turning, Straub tail, flat body posture, and suppressed sleeping time. In general, this is qualitatively similar to what is seen after administration of 5-HT precursors or 5-HT receptor agonists. In conclusion, the data from the drug discrimination study and the behavioral syndrome induced by MMAI suggest that MMAI is a potential selective releaser of serotonin.

Key words: MMAI (5-methoxy-6-methyl-2-aminoindan); Drug discrimination; *p*-Chlorophenylalanine; Behavioral syndrome; 5-HT (5-hydroxytryptamine, serotonin) release; (Rat)

1. Introduction

Recent interest in our laboratory in the pharmacology of non-neurotoxic substituted phenethylamines has been focused on two areas: characterization of their behavioral effects and explanation of the mechanism(s) which lead to attenuation or loss of serotonergic neurotoxicity. We have developed novel, non-neurotoxic chemical structures that appear selectively to release endogenous stores of neuronal serotonin (Nichols et al., 1990; Johnson et al., 1991a,b; Huang et al., 1992). The effects of these molecules were initially identified using a two-lever drug discrimination procedure in rats trained to discriminate saline from 3,4-methylene-

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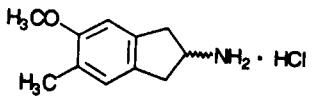

Fig. 1. Molecular structure of MMAI (5-methoxy-6-methyl-2-aminoindan).

dioxymethamphetamine (MDMA) or its α-ethyl homologue (MBDB). Many of these substances can potently mimic both MDMA and MBDB while at the same time showing significant pharmacological differences from hallucinogenic or stimulant training drugs. In vitro assays using rat brain synaptosomes showed some of these compounds to have extremely high selectivity for the serotonin carrier over that for dopamine or norepinephrine (Johnson et al., 1991b; Huang et al., 1992; Nichols et al., 1993). The most selective of the compounds developed to date is 5-methoxy-6-methyl-2-aminoindan (MMAI; Fig. 1).

It does not appear that compounds with similar pharmacology have been characterized in any other laboratories. Other structural modifications of amphetamine, such as *p*-chloroamphetamine, fenfluramine (*m*-trifluoromethyl-*N*-ethylamphetamine), and 3,4-methylenedioxymethamphetamine (MDMA) are the most well-known examples of compounds that release neuronal serotonin. However, these compounds are also central serotonergic neurotoxins (Schmidt et al., 1986; Battaglia et al., 1987; Johnson et al., 1990). In drug discrimination studies, the catecholaminergic effects of both *p*-chloroamphetamine and MDMA are pronounced (Schechter, 1986, 1988), and may be linked to their ability to cause degeneration of central serotonin neurons (Schmidt et al., 1986; Battaglia et al., 1987; Johnson et al., 1990).

By contrast, at near lethal or repetitive high dosages, MMAI produces no long-term changes in rat central monoamines or metabolites (Johnson et al., 1991b). This lack of toxicity may be related to its relative inactivity at dopamine or norepinephrine neurons. Indeed, the combined administration of MMAI with the catecholamine releasing agent (+)-amphetamine results in serotonin neuron destruction (Johnson and Nichols, 1991).

Rudnick and Wall (1992) have recently reported that the serotonin releasing action of MMAI is similar to MDMA, at least in membrane vesicles isolated from human platelets. Although MDMA inhibits binding of cocaine analogs to the dopamine transporter and releases dopamine accumulated by cells expressing the dopamine transporter, similar concentrations of MMAI lacked these actions.

Similarly, while both MDMA and MMAI are potent releasers of serotonin, the latter is almost completely inert at catecholamine uptake carriers, and completely lacks the serotonergic neurotoxic effect induced by the former. Receptor binding data also show that MMAI has relatively low affinity for a variety of other neuromodulator or neurotransmitter receptors, in each case IC_{50} values were higher than 10 micromolar (Nichols et al., 1993). Additionally, in this report we present data showing that MMAI also has low affinity for α_1-, α_2-, and β-adrenoceptors. These results have led us to conclude that MMAI is a potent and selective serotonin releasing agent.

Behavioral patterns evoked by the above mentioned derivatives of amphetamine are heterogeneous in nature and not well understood. Most of the experiments studying the behavioral effects of these agents employed only the drug discrimination paradigm, commonly used to study psychoactive drugs. While this procedure is sensitive and specific in classifying drug action, it is not useful in determining the overt behavioral profile or the mechanism of the serotonergic neurotoxicity associated with high doses of those drugs.

High doses of the serotonergic neurotoxins *p*-chloroamphetamine and MDMA produced stereotyped behaviors that included sniffing, lateral head-weaving, backpedaling, turning, and salivation (Hiramatsu et al., 1989; Gordon et al., 1991). Additionally, *p*-chloroamphetamine and fenfluramine evoke tremor, rigidity, forepaw treading, Straub tail, hind limb abduction, and lateral head-weaving, which are blocked by pretreatment with the 5-HT biosynthesis inhibitor *p*-chlorophenylalanine (Trulson and Jacobs, 1976). Blockade of behavioral effects of *p*-chloroamphetamine or fenfluramine by *p*-chlorophenylalanine pretreatment supports the hypothesis that those responses are mediated by the release of endogenous serotonin rather than a direct effect of *p*-chloroamphetamine or fenfluramine on postsynaptic serotonin receptors (Trulson and Jacobs, 1976).

Somewhat lower doses of *p*-chloroamphetamine, MDMA, fenfluramine and MMAI can also induce abnormal motor behavior. *p*-Chloroamphetamine and MDMA increase behavioral activity in animals; previous studies have suggested that this hyperactivity is partially mediated by release of endogenous serotonin (Callaway et al., 1990).

More recently, Callaway et al. (1993) have shown that fenfluramine (2.5 and 5 mg/kg) and MMAI (10 mg/kg) reduce motor activity. In that study, however, it was suggested that a mechanism other than 5-HT release contributed to the suppression of activity by fenfluramine or MMAI, since pretreatment with fluoxetine, a selective serotonin reuptake inhibitor, methiothepin, a non-selective 5-HT receptor antagonist, or *p*-chlorophenylalanine, did not antagonize MMAI-induced or fenfluramine-induced reductions of activity. However, methiothepin and fluoxetine cause inhibition of locomotor activity by themselves, and they may only

produce enhancement of any sedative action of MMAI and fenfluramine.

In view of the renewed interest in the role of serotonin in a number of mental disorders, including depression, panic and anxiety disorder, obsessive-compulsive disorder, and others, as well as the marked clinical success of the specific serotonin reuptake inhibitor fluoxetine (Prozac), a specific serotonin releasing agent might ultimately possess therapeutic significance. Such a compound would be expected to increase intrasynaptic serotonin concentrations, as would an uptake inhibitor. However, issues such as tolerance and turnover might differ for a releasing agent.

This report describes the further characterization of MMAI using the two-lever drug discrimination procedure. Cross-substitution tests, various pretreatments, and antagonists were used to define the behavioral effects of MMAI.

Additionally, the present study was designed to define further the site of action of MMAI induced behaviors such as sniffing, turning, head-weaving, Straub tail, flat body posture, immobility, hypolocomotion (sedation), sleeping, and salivation, in relation to serotonergic functions. For this purpose we investigated: (1) acute behavioral effects after MMAI administration, using higher doses (5, 10, and 20 mg/kg) than were employed in the drug discrimination studies, and (2) behavioral consequences after pretreatment with the serotonin synthesis inhibitor, *p*-chlorophenylalanine, on MMAI-induced behavior.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 200–220 g at the beginning of the drug discrimination study were divided into five groups ($n = 8$–15 per group), trained to discriminate MDMA · HCl, (+)-MBDB · HCl, (+)-amphetamine sulfate, LSD tartrate, or MMAI · HCl from saline. None of the rats had previously received drugs or behavioral training. Water was freely available in the individual home cages and a rationed amount of supplemental feed (Purina Lab Blox) was made available after experimental sessions so as to maintain approximately 80% of free-feeding weight. Lights were on from 07:00 to 19:00 h. The laboratory and animal facility temperature was 22–24°C and the relative humidity was 40–50%. Experiments were performed between 08:30 and 17:00 h each day, Monday–Friday.

For acute behavioral and biochemical studies male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighting 250 ± 50 g were used. The animals were kept in groups of 5 rats per cage, under the same conditions described above, but with free access to food and water.

2.2. Apparatus

Six standard operant chambers (model E10-10RF, Coulbourn Instruments, Lehigh Valley, PA) consisted of modular test cages enclosed within sound-attenuated cubicles with fans for ventilation and background white noise. A white house light was centered near the top of the front panel of the cage, which was also equipped with two response levers, separated by a food hopper (combination dipper pellet trough, Coulbourn model E14-06, module size 1/2), all positioned 2.5 cm above the floor. Solid state logic in an adjacent room, interfaced through a Med Associates interface to a Intel 486-based microcomputer, controlled reinforcement and data acquisition with a locally written program.

2.3. Discrimination training and testing

A fixed ratio (FR) 50 schedule of food reinforcement (Bioserv 45 mg dustless pellets) in a two-lever paradigm was used. The drug discrimination procedure details have been described elsewhere (Oberlender and Nichols, 1988, 1990). After habituation to the experimental conditions (one week after isolation in the individual home cages and at the beginning of the food deprivation) initial shaping was begun. During the first 2–3 sessions, rats were trained only to associate a characteristic noise (click) after lever pressing with a delivered food pellet (without drug injections). Initially, rats were shaped to lever press on an FR1 schedule so that one food pellet was dispensed for each press. Half of the rats were trained on drug-left, saline-right and the other half on drug-right, saline-left to avoid positional preference. Training sessions lasted 15 min and were conducted at the same time each day. Levers were cleaned between animals with 10% ethanol solution to avoid olfactory cues (Extance and Goudie, 1981). Only one appropriate lever was present during the first 10 sessions of initial learning (after beginning to administer saline or training drug i.p. 30 min before sessions). Afterwards both levers were present during all following phases of training, but reinforcements were delivered only after responses on the appropriate lever. Presses on the incorrect lever had no programmed consequences. As responding rates stabilized (during the next 15 sessions), the schedule of reinforcement was gradually increased to an FR50. Once at the FR50, training continued until an accuracy of at least 85% (number of correct presses × 100/number of total presses) was attained for eight of ten consecutive sessions (approx. 40–60 sessions). Once criterion performance was attained, test sessions were interspersed

between training sessions, either 1 or 2 times per week. At least one drug and one saline session separated each test session. Rats were required to maintain the 85% correct responding criterion on training days in order to be tested. In addition, test data were discarded when the accuracy criterion of 85% was not achieved on the two training sessions following a test session. Test drugs were administered i.p. 30 min prior to the sessions; test sessions were run under conditions of extinction, with rats removed from the operant chamber when 50 presses were emitted on one lever. If 50 presses on one lever were not completed within 5 min the session was ended and scored as a disruption. Treatments were randomized at the beginning of the study. For the time dependency test, the training dose of the training drug was administered 15, 30, 60, 90, 120, or 180 min prior to the test session.

2.4. Other behavioral studies

All behavioral experiments took place in a quiet room, at a temperature of 22–24°C between 09:00 and 16:00 h. Observation of animals was done in a plastic cage with dimensions 24 × 24 × 20 cm. The animals were habituated to the plastic cage by placing them individually in the cage for 30 min before the experiment.

Stereotyped behavior was recorded using a video camera, and rated using rating scales described by Nabeshima et al. (1984) with some modification. Briefly, stereotyped behavior was rated as follows: sniffing, flat body posture, Straub tail, and salivation (the intensity of these syndromes: 0 – absent, 1 – occasional, perceptible, 2 – weak, 3 – medium, 4 – constant). Head-weaving (the number of times the animals made a slow, side to side or lateral movement), turning (the number of times the animals laterally circled to the left or right over 360° within a relatively small area), and shaking behavior (head shaking plus a 'wet dog' shaking syndrome) were determined using the scale: 1 = 1–4 times, 2 = 5–10 times, 3 = 11–20 times, 4 = more than 20 times. Behavioral ratings were made for 12 periods of 10 min each. Locomotion, immobility (as the duration of catalepsy-like posture), and sleeping were recorded as the number of minutes of duration during each 10 min period of observation (the sum of minutes of locomotion, immobility, and sleeping is equal to the 10 min period of observation).

2.5. Radioligand binding studies

The radioligand receptor binding assays to α- and β-adrenoceptors were performed by the Nova Pharmaceutical Corporation using their PROFILE Novascreen program, as generously provided by a contract with the National Institute of Mental Health. MMAI was screened at a concentration of 10 micromolar against the ligands: [^{3}H]prazosin, [^{3}H]RX781094, and [^{3}H]dihydroalprenolol.

After the Novascreen indicated some affinity of MMAI for α_2-adrenoceptors, binding to this site was assessed in our laboratory by the methods of Brown et al. (1990) with minor modifications. Male Sprague-Dawley rats (180–200 g) were killed and the brains dissected rapidly on an ice-cold surface. Cerebral cortex was used for the assay. Brain tissue was homogenized in 25 volumes of Tris buffer (50 mM Tris for [^{3}H]clonidine binding and 50 mM Tris plus 5 mM EDTA for [^{3}H]yohimbine binding) at 0°C before initial centrifugation (at 40 000 × g for 15 min at 4°C). The pellet was re-suspended in fresh buffer before second cycles of centrifugation. Incubations were carried out at 25°C using the following ligands, together with 10 μM of phentolamine to estimate non-specific binding: 1 nM [^{3}H]clonidine or 1 nM [^{3}H]yohimbine. Binding displacement at each concentration (50 nM–10 μM) of MMAI was determined in triplicate.

2.6. Drugs

The training drugs, dosages, and sources, used in this study were as follows: (+)-amphetamine sulfate (1 mg/kg, 5.4 μM/kg), Smith Kline and French Laboratories, Philadelphia, PA), 3,4-methylenedioxymethamphetamine hydrochloride (MDMA; 1.75 mg/kg, 7.62 μM/kg)) and S-(+)-*N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine hydrochloride ((+)-MBDB; 1.75 mg/kg, 7.18 μM/kg); both were synthesized in our laboratory (Nichols et al., 1986), (+)-lysergic acid diethylamide tartrate (LSD; 0.08 mg/kg, 186 nM/kg, NIDA), and 5-methoxy-6-methyl-2-aminoindan hydrochloride (MMAI; 1.71 mg/kg, 8.0 μM/kg) synthesized in our laboratory (Johnson et al., 1991b). All training drugs were dissolved in 0.9% saline and were injected intraperitoneally in a volume of 1 ml/kg, 30 min before the session.

Other drugs used during the drug discrimination study include: (+)-fenfluramine hydrochloride (synthesized in our laboratory; Nichols et al., 1973), fluoxetine hydrochloride (a generous gift of the Eli Lilly Laboratories, Indianapolis, IN), paroxetine hydrochloride (Beecham Pharmaceuticals, Surrey, UK), 2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI, synthesized in our laboratory), ketanserin (Research Biochemicals, Natick, MA), methiothepin (Hoffmann-La Roche, Nutley, NJ) and haloperidol (Mylan Pharmaceuticals, Morgantown, WV). Pindolol, *p*-chloroamphetamine hydrochloride, cocaine hydrochloride, clonidine hydrochloride, yohimbine hydrochloride and *d,l-p*-chlorophenylalanine methyl ester hydrochloride, all were purchased from Sigma Chemical Co. (St. Louis, MO). *p*-Chlorophenylalanine at a dose of 200 mg/kg

(total dose: 400 mg/kg) was dissolved in a volume of 4 ml/kg and injected i.p. 72 and 48 h before MMAI or saline. Paroxetine, fluoxetine, and ketanserin were administered 60 min before MMAI (90 min before the test session). Pretreatments with pindolol, methiothepin and yohimbine were 30 min before MMAI (60 min before the test session).

Drugs and dosages used in behavioral observations were as follows, MMAI: 5.0, 10.0, 20.0 mg/kg, fluoxetine: 10.0 and 20.0 mg/kg and *p*-chlorophenylalanine 200 mg/kg (total dose: 400 mg/kg). Dosages of MMAI were dissolved in a volume of 1 ml/kg and injected s.c. directly before recording. *p*-Chlorophenylalanine was administered dissolved in a volume of 4 ml/kg and injected 72 and 48 h before MMAI or saline.

Drugs used for radioligand binding studies were: [^{3}H]clonidine (spec. act. 57.8 Ci/mmol; New England Nuclear), [^{3}H]yohimbine (spec. act. 88 Ci/mmol; Amersham, Chicago, IL), and phentolamine hydrochloride (Sigma Chemical Co., St. Louis, MO).

2.7. Data analysis

Data from the drug discrimination study were scored in a quantal fashion, with the lever on which the rat first emitted 50 presses in a test session scored as the 'selected' lever. The percentage of rats selecting the drug lever (%SDL) for each dose of test compound was determined. The degree of substitution was determined by the maximum %SDL for all doses of the test drug. 'No substitution' is defined as 59% SDL or less, and 'partial' substitution is 60–79% SDL. If the drug was one which completely substituted for the training drug (at least one dose resulted in a %SDL = 80% or higher) the method of Litchfield and Wilcoxon (1949) was used to determine the ED_{50} and 95% confidence interval (95% C.I.). This method also allowed for tests of parallelism between dose-response curves of the drug and the training drug with 95% confidence limits (C.L.). If the percentage of rats disrupted (%D) was 50% or higher, an ED_{50} value was not determined, even if the %SDL of nondisrupted animals was higher than 80%. ANOVA with analysis of repeated measures was used for comparison of the mean response rate (defined as the number of lever presses per min) from the first 20 training sessions after saline and after drug with the corresponding response rate after one year of training.

The results from behavioral observations are expressed as the mean ± S.E. and were analyzed by one- or two-way analysis of variance with significant differences between groups determined by the Student Newman-Keuls test with homogenous 'N' (Hinkle et al., 1979).

Results of the binding displacement studies were analyzed using a 4-parameter non-linear least squares regression analysis program to estimate the IC_{50} (concentration of competitor displacing 50% of specifically bound radioligand).

3. Results

3.1. Shaping and discrimination training

All 15 rats learned the initial 1.71 mg/kg (8.0 μM/kg) MMAI vs. saline discrimination. The mean number of sessions to criterion (85% correct responding for eight of ten consecutive sessions) was 45 (range: 40–60). There was no difference in this parameter compared with (+)-MBDB vs. saline discrimination training to attain accuracy criterion (Oberlender and Nichols, 1990). MMAI discrimination was an easy task for the animals to learn, as judged by the length of time necessary to acquire the discrimination in comparison with racemic MDMA, for which as many as 65 sessions were necessary (Oberlender and Nichols, 1988). By contrast, the rats trained to discriminate (+)-amphetamine from saline more rapidly learn the discrimination task. The mean number of sessions to criterion for this group was only 31 ± 2 (Oberlender and Nichols, 1988).

Analysis of response rate data for the first 20 drug or saline discrimination sessions (after attaining the accuracy criterion) and for the 20 last training sessions for MMAI or saline – after one year of training – revealed no significant difference between the initial response rate and the response rate after one year (87 ± 27 versus 96 ± 38 presses/min after MMAI, and 106 ± 38 versus 110 ± 31 presses/min after saline, respectively, $P > 0.05$). Differences between pressing rates in MMAI and saline sessions were significant; the response rate for MMAI sessions was lower than the response rate for saline, independent of the time when experiments were conducted ($P < 0.05$).

3.2. Time-response tests

In the time-response tests for the MMAI training dose (1.71 mg/kg; 8.0 μM/kg) more than 80% of the rats selected the drug appropriate lever between 15 and 60 min. A rapid onset after i.p. administration was evident as 10/12 rats tested 15 min after being injected selected the drug lever. The %SDL remained at or above 83% through 60 min, then rapidly decreased to 56% by 90 min. At 150 min post-injection only 3/10 rats continued to respond on the drug appropriate lever.

3.3. Cross-substitution with MDMA and (+)-MBDB

As seen in Table 1, both MDMA and (+)-MBDB fully substituted in MMAI-trained rats. We have previ-

ously shown (Johnson et al., 1991b) that MMAI substitutes in animals trained to discriminate MDMA or (+)-MBDB from saline. Thus, symmetrical substitution occurs between MMAI, MDMA and MBDB. The slopes of the dose-response curves for MDMA or (+)-MBDB were significantly different from the dose-response curve for the training drug (MDMA vs. MMAI, (+)-MBDB vs. MMAI). However, the dose-response curves of MDMA and (+)-MBDB were parallel to each other in MMAI-trained rats.

3.4. Substitution tests with other serotonergic releasers, psychostimulants and hallucinogens

The results of substitution tests with (+)-fenfluramine and *p*-chloroamphetamine are shown in Table 2. Both of these serotonergic agents produce full substitution. Furthermore, both dose-response curves are parallel to that of MMAI.

Table 2 also contains substitution test data for (+)-amphetamine and cocaine, prototypic psychostimulants. Neither compound substituted in MMAI-trained rats. However, test doses chosen for (+)-amphetamine and cocaine were clearly centrally active, as a large percentage of rats was disrupted by both drugs at the highest doses administered. In a previous study, we have shown that no substitution of MMAI occurs in rats trained to discriminate LSD or (+)-amphetamine from saline (Johnson et al., 1991b). (There is an error in this reference: the data in the %SDL column of Table IV should be all zeros). In MMAI-trained rats LSD produced partial substitution: 75% SDL with 47% disruption at a dose of 186 nmol/kg. The highest tested dose of LSD (372 nmol/kg) caused disruption of 12/13 tested animals. DOI, an hallucinogenic amphetamine derivative, did not mimic MMAI in rats trained to discriminate MMAI from saline but lowered the response rate. Thus, at the highest tested dose of DOI, 9/12 rats did not emit 50 responses during the 5 min test session.

Together with the data in Table 2, these results indicate that MMAI has neither psychostimulant-like nor hallucinogen-like effects. However, partial substitution of LSD in MMAI-trained rats did occur. Although the drug discrimination paradigm may produce false positives, to our knowledge there is no report of a false negative. That is, drug discrimination in rats has never indicated a compound to lack stimulus effects similar to a particular training drug, when the human psychopharmacology for the two was known to be similar.

Table 1
Data from substitution tests in MMAI-trained rats

Drug	Dose		*n*	%D	%SDL	ED_{50} (95% C.I.)
	μmol/kg	mg/kg				
Saline			14	0	0	
MMAI	2	0.43	12	0	33	2.64 μmol/kg
	3	0.64	13	0	54	(2.14–3.26)
	4	0.85	13	0	77	0.56 mg/kg
	6	1.28	13	0	100	(0.46–0.70)
	8	1.71	13	0	100	
MDMA	0.95	0.22	6	50	0	3.03 μmol/kg
	1.43	0.33	10	30	29	(1.98–4.62)
	1.90	0.44	11	36	29	0.70 mg/kg
	3.81	0.88	12	36	50	(0.46–1.10)
	5.70	1.31	11	36	71	
	7.62	1.75	11	27	100	
(+)-MBDB	0.90	0.22	11	36	29	1.44 μmol/kg
	1.79	0.44	12	42	67	(0.74–2.80)
	3.59	0.88	11	45	83	0.36 mg/kg
	5.38	1.31	11	55	80	(0.18–0.69)
	7.18	1.75	10	70	100	

Table 2
Data from substitution tests in rats trained to discriminate MMAI from saline

Drug	Dose		*n*	%D	%SDL	ED_{50} (95% C.I.)
	μmol/kg	mg/kg				
(+)-Fenfluramine	1.40	0.38	11	36	0	3.72 μmol/kg
	1.87	0.50	11	27	0	(3.13–4.43)
	2.80	0.75	13	15	18	0.997 mg/kg
	3.73	1.00	14	0	57	(0.84–1.19)
	5.60	1.50	11	18	89	
p-Chloroamphetamine	0.75	0.16	12	8	36	0.69 μmol/kg
	1.00	0.21	11	33	88	(0.44–1.08)
	1.50	0.31	13	23	90	0.14 mg/kg
	2.00	0.41	14	21	91	(0.09–0.22)
Fluoxetine	14.5	5.00	10	30	14	N.S.
	29.0	10.0	13	30	40	
(+)-Amphetamine	2.71	0.50	13	46	14	
	5.43	1.00	14	72	25	N.S. [a]
	8.14	1.50	5	80	0	
Cocaine	7.36	2.50	11	27	0	
	14.71	5.00	11	64	0	N.S.
	29.42	10.00	12	75	33	
DOI	0.14	0.05	11	18	11	
	0.28	0.10	11	18	22	N.S.
	0.56	0.20	11	45	33	
	1.12	0.40	12	75	67	
LSD	0.046	0.02	14	50	14	
	0.093	0.04	15	27	36	
	0.139	0.06	14	78	67	P.S. [b]
	0.186	0.08	15	47	75	
	0.372	0.16	13	92	100	
Yohimbine	0.39	1.0	8	25	17	
	0.59	1.5	6	0	17	N.S.
	0.78	2.0	10	10	44	
	1.56	4.0	8	100	–	
Clonidine	0.013	0.05	8	50	0	N.S.

[a] Although (+)-amphetamine did not produce substitution, it was potent in producing disruption, with an ED_{50} of 2.97 (1.59–5.54) μmol/kg, or 0.54 (0.29–1.02) mg/kg. [b] Although LSD produced partial substitution of 75% at a dose of 186 nmol/kg (0.08 mg/kg), it was also potent in producing disruption, with an ED_{50} of 99.2 (51.9–186) nmol/kg, or 0.037 (0.023–0.08) mg/kg.

Table 3
The data from antagonism tests in rats trained to discriminate MMAI from saline

Pretreatment drug	Dose (mg/kg)	Time of pretreatment	Test drug	n	%D	%SDL
Saline	–	30	MMAI	15	0	100
Fluoxetine	5.0	90	MMAI	12	25	78
	10.0	90	MMAI	13	62	40
Paroxetine	2.5	60	MMAI	13	38	50
Ketanserin	2.5	60	MMAI	10	10	78
Pindolol	1.0	60	MMAI	10	40	100
Methiothepin	0.05	30	MMAI	6	67	100
	0.10	30	MMAI	6	86	100
Yohimbine	1.5	30	MMAI	8	13	100
	2.0	30	MMAI	8	0	87
	3.0	30	MMAI	8	38	60
Haloperidol	0.1	60	MMAI	5	100	–
	0.5	60	MMAI	4	100	–

A variety of drugs were used as pretreatments, given 30, 60, or 90 min before MMAI. The dose of MMAI was 1.71 mg/kg, given 30 min prior to the test session.

3.5. Antagonism tests

Table 3 presents the results of tests of MMAI discrimination following pretreatment with potential antagonists. The serotonin uptake blockers fluoxetine and paroxetine reduced the discriminability of MMAI to 40–50%. However, fluoxetine alone produces some drug lever selection in MMAI-trained rats (see Table 2, 40% at 10 mg/kg), so it is unlikely that doses of 5-HT uptake inhibitors could be selected that would completely block the cue. This result is similar to a previous study of fluoxetine in MDMA-trained rats (Nichols and Oberlender, 1990).

Also consistent with earlier studies of the behavioral effects of MMAI is the present finding that the 5-HT_2 receptor antagonist ketanserin did not block the MMAI cue. Although the discriminability of MMAI is attenuated somewhat (ca. 22%), this ketanserin dose will completely block the stimulus properties of compounds that act via 5-HT_2 receptor activation (Glennon et al., 1983; Glennon, 1985).

Pretreatment with behaviorally active doses of methiothepin or pindolol, non-selective 5-HT_1 receptor antagonists with high affinity for dopamine D_2 receptors and β-adrenoceptors, respectively, (Watson et al., 1992; Winter and Rabin, 1992; Callawey et al., 1993), also failed to antagonize the MMAI stimulus. However, methiothepin at a dose of 0.1 mg/kg produced strong sedation in MMAI-trained rats and caused disruption in 86% of the tested animals. This effect was probably due to enhancement by methiothepin of the sedative properties of MMAI. Yohimbine, an α_2-adrenoceptor antagonist with nanomolar affinity for 5-HT_{1A} and 5-HT_{1D} receptors (Hoyer, 1988), attenuated discriminability of MMAI to 60%. Haloperidol at both tested doses, 0.1 and 0.5 mg/kg, produced 100% disruption. Additionally, after pretreatment with the highest dose of haloperidol all rats showed catalepsy after the test (95 min after administration of haloperidol, unpublished observation). However, the haloperidol doses used in this study attenuated the stimulus effects of cocaine and amphetamine (at 0.5 mg/kg haloperidol attenuated the drug-lever response by 62% and 73%, respectively) in rats trained to discriminate saline from these latter two drugs (Callahan et al., 1991).

3.6. Effect of a 5-HT synthesis inhibitor on stimulus properties of MMAI

To test this hypothesis, animals trained to discriminate MMAI were pretreated with the 5-HT biosynthesis inhibitor *p*-chlorophenylalanine, at a dose regimen that has been shown to deplete central stores of 5-HT to about 15% of control values (Koe and Weissman, 1966). Table 4 presents the results of tests of the discriminability of MMAI after 5-HT depletion with *p*-chlorophenylalanine. All the rats showed only saline appropriate responding following the 1.71 mg/kg MMAI training dose. During training sessions for several subsequent days, rats fully responded appropriately after saline, but following MMAI administration they predominantly pressed the saline lever. During these drug-training sessions the rats were removed from the chambers after 5 min, until MMAI discriminability returned. Even at 8 days following the last *p*-chlorophenylalanine administration, only 10/14 rats tested responded on the drug lever. However, *p*-chlorophenylalanine pretreatment does not block discrimination in (+)-amphetamine trained rats (Schechter and Cook, 1975), and actually enhances the cue in

Table 4
Effect of the 5-HT biosynthesis inhibitor *p*-chlorophenylalanine on the discriminability of MMAI

Pretreatment drug	Dose (mg/kg)	Pretreatment time (h)	Test drug	n	%D	%SDL
None	–	–	Saline	14	0	0
None	–	–	MMAI	14	0	100
p-Chlorophenylalanine	200 + 200	72 and 48	Saline	5	0	0
p-Chlorophenylalanine	200 + 200	72 and 48	MMAI	10	10	0
p-Chlorophenylalanine	200 + 200	8 days	MMAI	14	23	82

The dose of MMAI was 1.71 mg/kg, given 30 min prior to the test session.

Table 5
Affinity of MMAI for adrenoceptor binding sites

Receptor selectivity	Radioligand	IC_{50} (μM)
α_1-Adrenoceptor	[^{3}H]Prazosin	> 10 [a]
α_2-Adrenoceptor	[^{3}H]RX781094	0.41 [a]
α_2-Adrenoceptor	[^{3}H]Yohimbine	1.58 ± 0.21 [b]
α_2-Adrenoceptor	[^{3}H]Clonidine	2.45 ± 0.35 [b]
β_1/β_2-Adrenoceptor	[^{3}H]Dihydroalprenolol	> 10 [a]

[a] Data obtained from the NIMH/NovaScreen Psychoactive Drug Discovery Program. [b] K_i values (in micromolar) obtained in our laboratory.

LSD-trained animals (Appel et al., 1982). Recently, Schechter has shown that *p*-chlorophenylalanine also blocks the discriminability of MDMA (Schechter, 1991). Thus, the prolonged block of the MMAI cue by *p*-chlorophenylalanine further supports the conclusion that endogenous 5-HT is essential for MMAI discrimination.

3.7. Radioligand binding studies

Table 5 contains IC_{50} data for MMAI from displacement experiments for α- and β-adrenoceptors. These data, in conjunction with previously reported results (Nichols et al., 1993) demonstrate the site selectivity for MMAI.

3.8. Behavioral pattern induced by different doses of MMAI

The scores of MMAI-induced behavioral syndromes, averaged for each 10 min interval and the time course of the effects of MMAI are shown in Figs. 2A–C. MMAI-induced stereotyped behaviors including sniffing (data not shown), turning (Fig. 2A), flat body posture (Fig. 2B), and Straub tail (Fig. 2C) were observed to occur in a dose-dependent manner. At the 10 and 20 mg/kg doses, MMAI-induced stereotyped behaviors reached a peak 20–30 min after injection, (second and third 10-min periods) and slowly decreased in the following time periods. The effects of 20 mg/kg MMAI continued longer than those of 10 mg/kg. However, all behavioral syndromes had returned to control levels by the last observation period. Additionally, head weaving, head shaking, and 'wet dog' shaking behavior was induced only at the highest dose of MMAI (20 mg/kg) but at weak intensity. Head weaving was observed in the first and second period (1.6 ± 0.6 and 1.2 ± 0.4, respectively; see Materials and methods for details of the rating scale) but then rapidly decreased to only 1–2 events/10 min in 2/5 rats. Shaking behavior, including head shakes and 'wet dog' shakes, was observed between 50 and 100 min at a frequency of 0.6 ± 0.4 per 10 min period.

Analyzing other behavioral syndromes which represent 'normal' patterns of behavior, in particular in the range between locomotion, motionless, and sleeping (Fig. 3), we observed that MMAI induced a unique behavioral effect. As shown in Fig. 3A, MMAI evoked 'sleeplessness' in rats, independent of dosage used (no significant difference between 5, 10, and 20 mg/kg). That is, although locomotion was reduced, and animals appeared sedated, they did not fall asleep, while approximately 90% of the control animals did. MMAI also produced suppression of locomotion (Fig. 3B) scored for each 10 min period as the time of duration of catalepsy-like posture. However, MMAI *did not* produce actual catalepsy, since after 10 or 20 mg/kg of

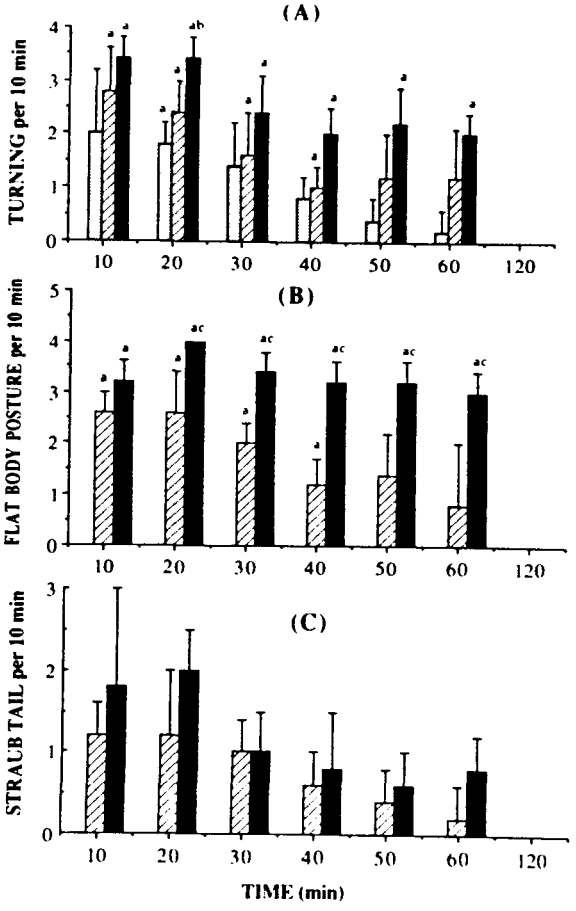

Fig. 2. MMAI-induced behavior in relation to the dose. Saline (open columns) or MMAI doses of 5.0 (stippled columns), 10 (hatched columns), or 20 mg/kg (black columns) were administered s.c. directly before recording. Flat body posture (B), and Straub tail (C, lower) were rated using the scale of intensity: 0 – absent, 1 – perceptible, 2 – weak, 3 – medium, 4 – constant. Turning behavior (A, upper) was determined for each period using the scale: 1 = 1–4 times, 2 = 5–10 times, 3 = 11–20 times, 4 = more than 20 times. Behavioral ratings were made for 12 periods of 10 min each. The mean ± S.E. is shown. $n = 5$ rats per group. A one- or two-way analysis of variance was used for statistical analysis of the data, with significant differences between groups determined by the Student Newman-Keuls test with homogenous 'N' (Hinkle et al., 1979), [a] $P < 0.05$ vs. saline, [b] $P < 0.05$ vs. 5 mg/kg MMAI, [c] $P < 0.05$ vs. 10 mg/kg MMAI.

MMAI rats did not maintain their limbs on a 10 cm high block for 20 s (data not shown). Times which the animals spent walking during 10-min periods of observation, after administration of 5, 10, and 20 mg/kg of MMAI, were not significantly different from saline administration (Fig. 3C).

3.9. Behavior induced by MMAI after 5-HT depletion by p-chlorophenylalanine

p-Chlorophenylalanine pretreatment did not significantly alter normal behavior, but had a tendency to increase non-stereotyped sniffing (data not shown). 5-HT depletion by *p*-chlorophenylalanine produced only partial (statistically nonsignificant) reduction of the sedation and 'sleeplessness' induced by 20 mg/kg MMAI alone (Fig. 4A and B). Tested rats spent the same time walking after MMAI administration either with or without *p*-chlorophenylalanine pretreatment (data not shown). As shown in Fig. 5A serotonin depletion inhibited completely the flat body posture induced by MMAI. The characteristic turning behavior produced by MMAI was also partially blocked (Fig. 5B). *p*-Chlorophenylalanine pretreatment before MMAI had no effect on Straub tail or shaking behavior in rats (data not shown).

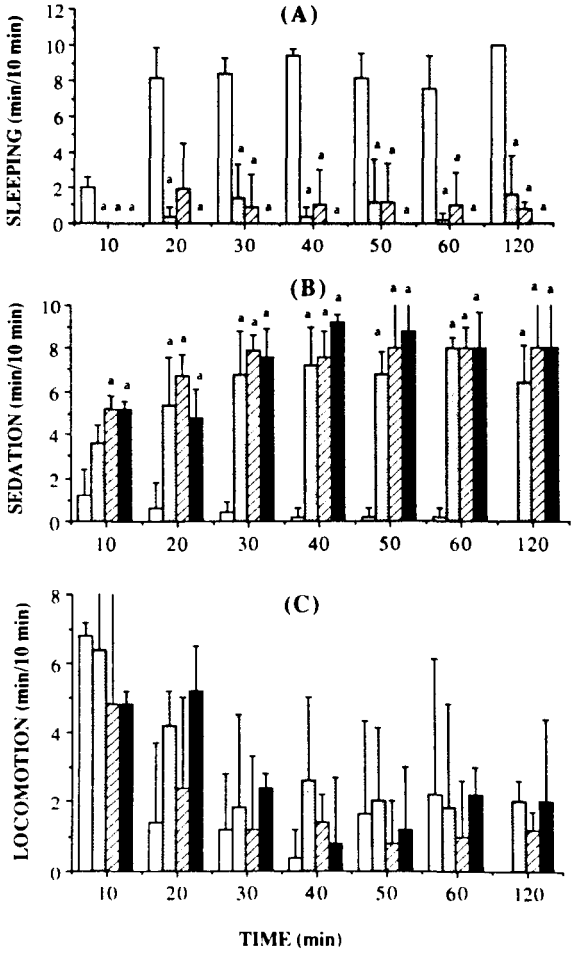

Fig. 3. MMAI-induced changes in 'normal' behavior. MMAI 5 (stippled columns), 10 (hatched columns), or 20 (black columns) mg/kg) or saline (open columns) was administered s.c. directly before observation. Sleeping (A), sedation (immobility defined as the duration of catalepsy-like posture, B), and locomotion (C, lower) were recorded as the number of minutes of duration during each 10 min observation period. The mean ± S.E. is shown. $n = 5$ rats per group. For statistical analysis of the data a one-way analysis of variance was used with significant differences between groups determined by the Student Newman-Keuls test with homogenous 'N' (Hinkle et al., 1979), [a] $P < 0.05$ vs. saline.

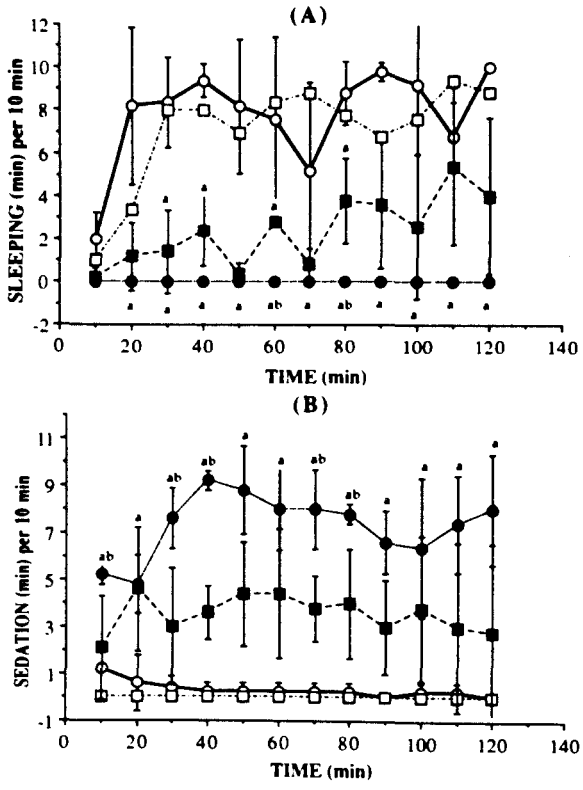

Fig. 4. Effect of inhibition of serotonin synthesis on the behavior induced by MMAI. Rats ($n = 5$ per group) received either *p*-chlorophenylalanine (200 mg/kg i.p.) or saline 72 and 48 h prior to MMAI (20 mg/kg s.c.) or saline injection (SAL-SAL (○), *p*-chlorophenylalanine-SAL (□), SAL-MMAI (●), *p*-chlorophenylalanine-MMAI (■)). The behavior induced by MMAI was observed for 2 h and determined for each 10 min period. Sleeping (A, upper graph), and sedation (B, lower graph), were recorded as the number of minutes of duration during each 10-min period of observation. The mean ± S.E. is shown. For statistical analysis of the data, a two-way analysis of variance was used with significant differences between groups determined by the Student Newman-Keuls test with homogenous 'N' (Hinkle et al., 1979), [a] $P < 0.05$ vs. SAL-SAL, [b] $P < 0.05$ vs. *p*-chlorophenylalanine-MMAI.

4. Discussion

The present results further confirm the serotonergic nature of the pharmacology of MMAI. In the substitu-

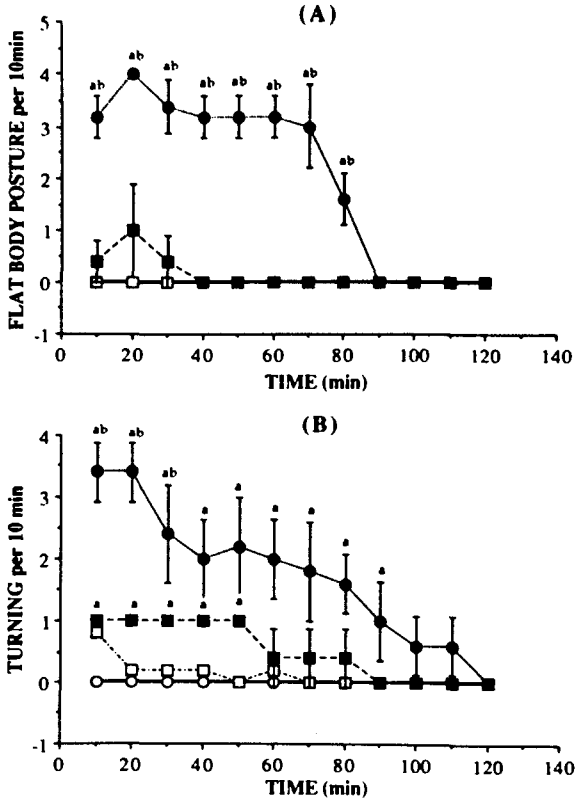

Fig. 5. Effect of inhibition of serotonin synthesis on the behavioral syndromes induced by MMAI. Male rats ($n = 5$ per group) received either *p*-chlorophenylalanine (200 mg/kg i.p.) or saline twice (72 and 48 h) before MMAI (20 mg/kg s.c.) or saline injection (SAL-SAL (○), *p*-chlorophenylalanine-SAL (□), SAL-MMAI (●), *p*-chlorophenylalanine-MMAI (■)). The behavioral syndromes were observed for 2 h and scored for each 10 min period. The intensity of flat body posture (A, the upper graph) was rated as: 0 – absent, 1 – occasional, 2 – weak, 3 – medium, 4 – constant. Turning behavior (B, lower graph) was rated as follows: 0 = absent, 1 = 1–4 times, 2 = 5–10 times, 3 = 11–20 times, and 4 = more than 20 times. The mean ± S.E. is shown. For statistical analysis of the data a two-way analysis of variance was used with significant differences between groups determined by the Student Newman-Keuls test with homogenous 'N' (Hinkle et al., 1979), [a] $P < 0.05$ vs. SAL-SAL, [b] $P < 0.05$ vs. *p*-chlorophenylalanine-MMAI.

tion tests all the compounds that substitute for MMAI are serotonin releasing agents. This substitution is symmetrical for MDMA and (+)-MBDB. Nevertheless, the dose-response curve of MMAI is not parallel to those of MDMA and (+)-MBDB. All the compounds which fully substituted for MMAI have multiple pharmacological effects: inhibition of serotonin uptake, release of serotonin, inhibition of MAO and additionally, for *p*-chloroamphetamine, inhibition of serotonin synthesis as well as effects on other monoamine systems. The lack of parallelism of dose-response curves of these compounds to the MMAI dose-response curve may indicate that the predominant pharmacological effect of MMAI can control discriminative responding in the rat, but may represent only part of a pharmacological complex of activity for compounds that fully substituted. Since both MDMA and (+)-MBDB have similar psychopharmacology in man, this could be interpreted to indicate that MMAI might possess similar clinical effects. However, fenfluramine produces symmetrical substitution in MDMA- or MBDB-trained rats, and also substitutes in MMAI-trained rats (Table 2). Since fenfluramine does not produce MDMA-like psychopharmacology in man, the likelihood must also be considered that MMAI may have clinical effects more like fenfluramine.

The results also show that MMAI lacks amphetamine-like discriminative stimulus effects, indicating that MMAI is not a psychostimulant. Partial substitution of LSD for MMAI but lack of substitution for MMAI in LSD-trained rats (Johnson et al., 1991b) and the fact that DOI, a 5-HT_2/5-HT_{1C} receptor agonist did not mimic MMAI, indicate that MMAI is not a hallucinogen.

Moreover, MMAI-trained rats completely lacked drug appropriate responding after pretreatment with the serotonin synthesis inhibitor, *p*-chlorophenylalanine, yet they maintained full responding after saline administration, in agreement with our hypothesis that MMAI acts by a release of endogenous stores of neuronal serotonin. This also corresponds to the previous report by Johnson et al. (1991a) that 5-HT modulation is more important than dopamine or norepinephrine for the discriminative cue of MDMA and MBDB. Additionally, the data from substitution tests and from cross-substitution studies between MMAI and MDMA or (+)-MBDB are consistent with the results of Rudnick and Wall (1993). Those workers found that MMAI displayed all of the in vitro characteristics of serotonin releasing amphetamines such as MDMA and *p*-chloroamphetamine. The ability of MMAI to inhibit serotonin accumulation and imipramine binding by platelet plasma membranes indicated that it interacted directly with the serotonin transporter. Although MMAI effects on the serotonin transporter were similar to those of MDMA, the two compounds had different effects on the dopamine transporter. MDMA inhibited binding of a cocaine analog to the dopamine transporter and released dopamine accumulated by cells expressing dopamine transporters, but similar concentrations of MMAI were inactive. The last finding demonstrated by Rudnick and Wall (1993) is consistent with the results presented here on the lack of substitution of (+)-amphetamine in MMAI-trained rats and the data published earlier (Johnson et al., 1991b) that MMAI did not mimic (+)-amphetamine in rats trained to discriminate amphetamine from saline.

Furthermore, several non-selective 5-HT receptor antagonists (Table 3) failed to inhibit the stimulus properties of MMAI. Many of them also have high affinity (nanomolar range) for sites other than sero-

tonin receptors. Ketanserin binds to 5-HT_2/5-HT_{1C} receptors but also to α_1-adrenoceptors, dopamine and histamine receptors (Leysen et al., 1981). Pindolol binds to β-adrenoceptors and to 5-HT_{1A}/5-HT_{1B} sites (Hoyer, 1988). In addition to its affinity for 5-HT_1 and 5-HT_2 receptors, methiothepin also binds to dopamine D_2 receptors, and the α_2-adrenoceptor blocker yohimbine also has affinity for 5-HT_{1D} and 5HT_{1A} receptors (Hoyer, 1988). Results from inhibition tests are consistent with radioligand binding data (Table 5 and Nichols et al., 1993). Enhancement of MMAI-induced sedation by pretreatment with methiothepin was noticeable in drug discrimination experiments and produced a relatively high percentage of disruption (Table 3). This may be related to inhibition of dopamine D_2 receptors by methiothepin. Two pieces of additional data suggest this as the explanation. First, pretreatment with the D_2 receptor antagonist haloperidol before MMAI produced 100% disruption. Second, administration of MMAI to rats trained to discriminate (+)-amphetamine resulted in long term (ca. 1 week) disruption (unpublished results).

The above facts do not fully explain why some compounds intensified the sedative effects of MMAI and dramatically lowered response rate in the drug discrimination paradigm. However, this may be a property that separates MMAI from other serotonin releasers. For example, MDMA had affinity for dopamine D_2 receptors (Lyon et al., 1986) similar to MMAI, but did not produce long term disruption after administration to amphetamine-trained animals (unpublished studies). Further, in drug discrimination studies with MDMA-trained rats pretreatment with haloperidol or methiothepin did not lead to a high percentage of disruption.

However, the results from the tests in which fluoxetine and paroxetine, both selective 5-HT uptake inhibitors, could only partially block the MMAI cue (60% and 50% of inhibition, respectively), and the partial substitution of fluoxetine indicate that release of neuronal serotonin from endogenous stores is more important for the discriminative cue of MMAI than the synaptic level of 5-HT. One additional fact compatible with this theory is the loss of MMAI discriminability for as long as central stores of serotonin were depleted by pretreatment with *p*-chlorophenylalanine.

Taken together, the results from substitution and from antagonism tests in rats trained to discriminate MMAI from saline lead to the conclusion that activation or inhibition of various other CNS receptors probably does not play a significant role in the discriminative stimulus properties of MMAI.

The data from our behavioral observations using high doses of MMAI support the hypothesis that a major effect of the administration of MMAI is the rapid release of stored serotonin. The short latency to the onset of the syndrome, less than 10 min following s.c. injection, indicates that this early effect of MMAI is primarily a reflection of a serotonin-releasing action rather than reuptake blockade (which takes 30 min, based on results from an unpublished comparison study with fluoxetine). The subcutaneous administration of high doses of MMAI (5, 10, and 20 mg/kg) to rats induced a behavioral syndrome consisting of hypolocomotion with accompanying catalepsy-like posture, flat body posture, the Straub tail, and suppressed sleeping-time. In general, the syndrome is qualitatively similar to that seen following administration of precursors or releasers of 5-HT or directly acting 5-HT receptor agonists (Jacobs, 1976; Trulson and Jacobs, 1976; Hillegaart et al., 1989), although there are also certain differences. For instance, MMAI did not induce tremor and reciprocal forepaw treading identified as a component of the 5-HT syndrome (Jacobs, 1976). Additionally, head, or head and body shakes were only weakly evoked by 20 mg/kg MMAI within the second hour after drug administration. This could be a result of the accumulation of serotonin released by MMAI then also activating 5-HT_2 receptors.

All the behavioral syndromes induced by MMAI are more similar to effects induced by 5-HT_1 receptor agonists rather than by 5-HT_2 receptor agonists. According to the latest proposed new nomenclature, central 5-HT receptors have been classified into four general populations, designated 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 based on data from functional, radioligand binding studies, and from molecular biology (Humphrey et al., 1993). The MMAI evoked outflow of neuronal serotonin from endogenous stores would increase the level of free 5-HT which then could presumably bind to serotonin receptors of the 5-HT_1 subtypes. Additionally, changes in the pattern of spontaneous locomotor activity of normal rats after administration of high doses of MMAI (i.e. the rat appears to be moving slowly, close to the wall and the floor of the Plexiglas cage) seem to be similar to those reported by Ahlenius et al. (1993) as characteristic for 5-HT_{1A} agonists. Callaway et al. (1993) suggested that the decrease in activity induced by MMAI or fenfluramine measured in open field using a Behavioral Pattern Monitor is not related to 5-HT release. However, the significant sedative properties of fenfluramine or MMAI observed in that laboratory occurred mostly during the first 30 min, i.e. a time at which rats present investigatory behavior, and at relatively high doses of both compounds which induced the serotonin behavioral syndrome described above. The changes of locomotor activity in rats seem to be a more complex animal behavior, which is under the control of more than one neurotransmitter system. The fact that the selective 5-HT uptake inhibitor fluoxetine, or the 5-HT synthesis inhibitor *p*-chlorophenylalanine, did not reverse or even partly antagonize the

fenfluramine-induced reduction of motor activity of rats in the study by Callaway et al. (1993), leaves no explanation for a mechanism responsible for the sedative properties of MMAI or fenfluramine. The potential antagonists used in that study as tools have sedative properties by themselves and could potentiate the reduction of locomotor parameters induced by MMAI or fenfluramine. During our studies with higher doses of MMAI we also observed some reduction of locomotor activity. This decrease appeared to be a consequence of development of the serotonin syndrome in treated rats, since no significant difference was noted between rats treated with MMAI or saline, in the time which animals spent in motion.

Blockade of the behavioral effect of MMAI by *p*-chlorophenylalanine pretreatment supports the hypothesis that this response is mediated by the release of endogenous serotonin rather than a direct effect of MMAI on serotonin receptors.

In conclusion, the behavioral and drug discrimination data suggest that MMAI is a selective releaser of stored neuronal serotonin. It is our working hypothesis that the human psychopharmacology of MDMA and MBDB is the result of a combined action in both serotonergic and catecholaminergic pathways (Schechter, 1986, 1988). The lack of any in vitro effect of MMAI on either dopamine or norepinephrine might thus be an indicator that its human psychopharmacology may be more similar to fenfluramine than to MDMA.

The widespread use of the nonselective agent, *p*-chloroamphetamine, to characterize the behavioral effects of 'serotonin release' may have led to erroneous conclusions. Any future studies should employ more specific drugs such as MMAI or its congeners.

Acknowledgments

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