Pergamon Press Offices:

U.S.A. Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523, U.S.A.

U.K. Pergamon Press Ltd., Headington Hill Hall, Oxford OX3 0BW, England

CANADA Pergamon of Canada, Ltd., 75 The East Mall, Toronto, Ontario M8Z 5W3, Canada

AUSTRALIA Pergamon Press (Aust) Pty. Ltd., P O Box 544, Potts Point, NSW 2011, Australia

FRANCE Pergamon Press SARL, 24 rue des Ecoles, 75240 Paris, Cedex 05, France

FEDERAL REPUBLIC OF GERMANY Pergamon Press GmbH, 6242 Kronberg/Taunus, Pferdstrasse 1, Federal Republic of Germany

---

Library of Congress Cataloging in Publication Data

Main entry under title:

The Psychopharmacology of hallucinogens.

Papers of a conference held in Bethesda, Md., Dec. 21-22, 1976, and sponsored by the Division of Research of the National Institute on Drug Abuse.

1. Hallucinogenic drugs--Congresses. 2. Psychopharmacology--Congresses. 3. Pharmacology, Experimental--Congresses. I. Stillman, Richard C. II. Willette, Robert. III. National Institute on Drug Abuse. Division of Research.
[DNLM: 1. Hallucinogens--Pharmacodynamics--Congresses. 2. Behavior--Drug effects--Congresses. 3. Psychopharmacology--Congresses. QV77.7 P974 1976]
RM315.P754 615'.7883 78-14019
ISBN 0-08-021938-1

Psychopharmacology of Hallucinogens

LS

PHARMACOLOGICAL EFFECTS OF (±)-, (S)-, and (R)-MDA*†

by

Gerald M. Marquardt, Victor DiStefano, and Lydia L. Ling
Department of Pharmacology and Toxicology
University of Rochester

MDA is recognized as a frequently abused drug within our drug subculture (26). The ingestion of 1.0-1.5 mg/kg of MDA·HCl has been reported to result in a sense of well-being, increased taste sensations, heightened self-awareness, increased introspectiveness, and general mood elevation (1,26,49). Although MDA does not cause the colorful imagery and marked distortion of reality produced by other hallucinogens (e.g., LSD), it produces distinct visual and related sensory changes (peripheral visual field changes and auditory hyperacuity), creates a "three-dimensionality" to music, and sometimes causes visual imagery which disappears when the subject concentrates his attention on it (1,26,49). The only adverse reaction to moderate doses (1.0-1.5 mg/kg MDA·HCl) seems to be a marked physical exhaustion, lasting as long as two days after ingestion (26,42).

Larger doses of MDA (7.5 mg/kg) cause clonic convulsions which may develop into tonic convulsions and death (43). Several deaths resulting from the ingestion of MDA have been reported in the United States and Canada (48). Despite this mortality, the illicit use of MDA continues. Unfortunately, Louria (31) is substantially correct when he states that very little is known about MDA.

Thiessen and Cook (48) have reviewed the MDA literature. They stated that the study by Gunn et al. (18) was the most comprehensive examination of the pharmacologic effects of MDA. The latter report demonstrated that effects of MDA on motor activity, respiratory rate, blood pressure, and uterine and intestinal contractions were similar to those produced by amphetamine. Ogawa (40) and Daly et al. (10) have suggested that MDA produces sympathomimetic effects by an indirect, amphetamine-like action involving the release of norepinephrine (NE) from noradrenergic nerve terminals and inhibition of the reuptake of NE.

Several methylenedioxyphenyl (MDP) compounds are the hallucinogenic components of nutmeg (44). There is little evidence, however, that the potency of MDP hallucinogens is related to the methylenedioxy moiety (22). 3-Methoxy-4,5-methylenedioxyamphetamine (MMDA), MDA, and 3,4,5-trimethoxyamphetamine (TMA) are products of the metabolism of myristicin, safrole, and elemicin, respectively, by rat liver homogenates (3). MMDA, MDA, and TMA, produced by the in vivo metabolism of myristicin, safrole, and elemicin, respectively, are the metabolites believed to produce hallucinogenic effects observed after the ingestion of myristicin, safrole, and elemicin, respectively (46).

---

* MDA is β-3,4-methylenedioxyamphetamine. (S)- and (R)- denote the absolute stereochemical configuration about the α-carbon. (S)-MDA is dextrorotatory and (R)-MDA is levorotatory. (±)-MDA refers to the racemic drug.

†Portions of this work are described in: Marquardt, G. M. and DiStefano, V.: The hepatic microsomal metabolism of MDA. Life Sci. 15: 1603-1610, 1975.

Many MDP insecticide synergists are metabolized to the corresponding catechols by rat hepatic microsomes (6). Similar metabolism of MDA would result in the formation of α-methyldopamine (α-MeDA). This metabolite has been implicated in the actions of α-methyldopa (α-MeDopa), a drug which has central nervous system effects (e.g., sedation) (38,50) that are similar to the prolonged exhaustion after the ingestion of 1.0-1.5 mg/kg MDA·HCl (26).

Experimental results demonstrating the metabolism of MDA to α-MeDA suggest that (S)- and (R)-α-MeDA are at least partly responsible for some of the pharmacological and toxicological effects observed after the administration of (S)- and (R)-MDA, respectively. Evidence for the transformation of MDA to α-MeDA will be presented. Experiments suggesting that (S)- and (R)-α-MeDA may be at least partly responsible for the pharmacological-toxicological effects of (S)- and (R)-MDA, respectively, will be described.

METHODS

Rat Hepatic Microsomal Metabolism of MDA

Preparation of rat hepatic microsomes. Male Sprague-Dawley rats, weighing 150-200 g, were sacrificed by cervical dislocation; the livers were excised and immersed in ice cold 0.25 M sucrose. All subsequent procedures were carried out at 0-4°C. The livers were blotted dry, weighed, minced, and homogenized in 5 ml/g of 0.25 M sucrose by 3 strokes of a Teflon pestle in a Potter-Elvehjem homogenizer (size C; A. H. Thomas). Microsomes were prepared from the resulting 20% (w/v) homogenate according to the method of Leeling and Casida (28). The homogenate was centrifuged at 15,000 g for 30 min to sediment cellular debris. The supernatant was then centrifuged at 100,000 g for 60 min in a Beckman L3-50 preparative ultracentrifuge with a type 30 fixed-angle rotor to pellet the microsomes. The supernatant was discarded and the pellet was washed with 10 ml of 0.25 M sucrose. The pellet was resuspended in the original volume of 0.25 M sucrose to obtain the microsomal suspension used in the experiments. Protein content of the microsomal suspensions was determined by the method of Lowry et al. (32) using bovine serum albumin as a standard.

Incubation procedure. Incubations were carried out in a total volume of 2.0 ml containing 100 μmoles of TES buffer, 25 μmoles of $MgCl_2$, 21.2-228.0 nmoles of MDA, and the following components of an NADPH-generating system: 0.5 μmole of NADP, 15 μmoles of glucose-6-phosphate, and 0.5 unit of glucose-6-phosphate dehydrogenase. Incubations were started by the addition of 1.0 ml of the microsomal suspension (equivalent to 3 mg of protein) and were carried out aerobically, with shaking, at 37°C.

In some experiments the microsomal suspension was boiled before addition to the incubation medium, the NADPH-generating system was omitted, 0.5 to 2.0 μmoles of 2-diethylaminoethyl-2,2-diphenylvalerate (SKF 525-A) were added to the incubation medium before the addition of the microsomal fraction, or incubations were carried out in an atmosphere of $CO:O_2$ (9:1).

Duplicate samples and an incubation blank were removed at 10 min intervals for 40 min. Incubations were terminated by the addition of 0.8 ml of 20% $ZnSO_4$; 0.8 ml of saturated $Ba(OH)_2$ was added 5 min later. After an additional 5 min, the solution was cleared of particulate matter by centrifugation at 22,000 g for 10 min. Aliquots of the supernatant were diluted and analyzed for MDA and α-MeDA.

MDA and α-MeDA assays. MDA was determined spectrophotofluorometrically by the method of Gillespie (14). α-MeDA was assayed by the method of Laverty and Taylor (27).

Calculation of reaction rates. Reaction rates were calculated from the change in MDA or α-MeDA content of the medium during the interval 10-40 min after the beginning of incubation. Determination of rate based on the decline of the MDA concentration never differed from that based on the formation of α-MeDA by more than 5%. When this small difference was observed, the mean was chosen to represent the rate of metabolism. Reaction rate was routinely expressed as nanomoles of α-MeDA formed per milligram of protein per 30 min.

Identification of α-MeDA. In some experiments the incubation was terminated by the addition of 8.0 ml of 0.5 N $HClO_4$ containing 0.05% $Na_2S_2O_5$ to the incubation medium. The solution was cleared of precipitated protein by centrifugation at 30,000 g for 20 min. The α-MeDA was adsorbed onto alumina, washed four times with distilled water, and eluted with 0.3 N acetic acid according to the method of Crout (9). The eluates were lyophilized and KBr pellets were made from the resultant salts.

Behavioral and Toxic Effects in Mice

Behavioral and toxic effects of drugs were studied in male Swiss-Webster mice, weighing 18-22 g. All mice were housed individually and allowed free access to food and water. Drugs were administered in aqueous solution in a total volume of 5 µl/g. All drug dosages are expressed in terms of the free base.

Mice were sometimes pretreated with SKF 525-A, 10.0 mg/kg, i.p., 30 min before drug treatment. Mortality was recorded at 4 and 24 hours after drug treatment. LD_{50} values were estimated by the method of Litchfield and Wilcoxen (30).

Behavioral effects produced by (±)-MDA (40 mg/kg), (S)-MDA (20 mg/kg) and (R)-MDA (40 mg/kg, i.p.), were noted by two independent observers. The behavior of treated mice was compared with the behavior of control mice, which received normal saline (5 µl/g, i.p.), and with mice treated with d-amphetamine (d-Amph; 30 mg/kg), LSD (4 mg/kg), or mescaline (40 mg/kg) i.p. These dosages resulted in maximal behavioral effects with minimal toxicity. Neither observer knew the identity of the drug administered to the mice in a given experimental group. Each treatment group consisted of five mice.

Behavioral observations included recording the occurrence of ataxia, convulsions, and stereotyped behavior (characterized by repeated grooming activities) as well as salivation, changes in respiratory rate, motor activity, and the animal's reactivity to an external auditory stimulus(presented as a loud noise several feet from the animal). Any other unusual behavior was noted and described.

Cat Blood Pressure Experiments

Twenty-one mongrel cats, of either sex, weighing 2-4 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.p.); an endotracheal tube was inserted shortly before the surgical preparation of the animal. The right

carotid artery was cannulated and blood pressure was recorded on a Beckman Dynograph, model R-411, using a volumetric transducer (Beckman model 8503-4MB). The rate and depth of respirations were monitored with a standard bellows-type of respiratory cuff placed around the upper abdomen of the cat. Respirations were recorded from a volumetric transducer connected to the respiratory cuff. Heart rate was measured with a Beckman cardiotachometer coupler, type 9857, triggered by the EKG (lead II) which was recorded with needle electrodes. The tension of the nictitating membrane was recorded with a Grass force-displacement transducer, model FT03C. All drugs were administered intravenously, in a volume of 0.1-0.2 ml of normal saline per kg, via a cannula in the cephalic vein. Each drug injection was followed by a 1.0 ml wash with normal saline.

^{3}H-NE Uptake by Rat Hypothalamic Synaptosomes

Preparation of the synaptosomal fraction. Synaptosomes (P2 fraction) were prepared by a modification of the method of Gray and Whittaker (17). Male Sprague-Dawley rats, weighing 150-200 g, were sacrificed by decapitation and the hypothalamus of each animal was rapidly dissected from other brain tissue on dental wax by the method of Glowinsky and Iversen (15). After washing in 0.32 M sucrose, the tissue was blotted dry, weighed, and homogenized in 0.32 M sucrose (10 ml/g tissue) by 5-6 strokes of a Teflon pestle in a Potter-Elvehjem homogenizer. All procedures were carred out at 0-4°C.

The homogenate was transferred to chilled polypropylene centrifuge tubes and centrifuged at 1000 g for 10 min to sediment cellular debris. The supernatant was decanted and centrifuged at 11,000 g for 20 min to pellet the synaptosomes. The pellet was resuspended in the same volume of 0.32 M sucrose and then centrifuged at 11,000 g for an additional 20 min to remove free norepinephrine (NE) and any other substances that might interfere with the transport of NE across the synaptosomal plasma membrane (29). This pellet was then resuspended in the same volume of 0.32 M sucrose to prepare the synaptosomal suspension used in the uptake experiments.

Incubation conditions. Fifty µl of normal saline (containing the appropriate concentration of drug) was added to 1.90 ml of incubation medium (containing 5.90 mM KCl, 1.20 mM $MgSO_4$, 1.28 mM $CaCl_2$, 112.20 mM NaCl, 31.10 mM Na·TES, 18.90 mM TES, 1.14 mM ascorbic acid, 0.06 mM Na_2EDTA and 12.50 µM nialamide) in 15 ml polypropylene beakers. One hundred µl of the synaptosomal suspension was added to each sample and preincubated at pH 7.40 for 5 min at 37°C. Incubations were started by the addition of 10 µl of ^{3}H-NE (l-noradrenaline, New England Nuclear Corp.; specific activity, 3.8 Ci/mmole; this solution was diluted to 20 µCi/ml ^{3}H-NE), resulting in a final NE concentration of 1.0 or 2.0 x 10^{-7}M. This was the concentration of NE used in the experiments unless otherwise stated.

Incubations were terminated 5 min later by filtration through 0.45 µm Millipore filters (type HAWP, Millipore Corp.). Filters were then washed with 5 ml of normal saline containing 1% BSA at 22°C. The filters were dried in glass scintillation vials heated to 100°C for 60 min. After the vials had cooled, 10 ml of Bray's solution (4) was added to each sample and the radioactivity was determined in a Packard Tri-Carb model 3385 liquid scintillation spectrometer. The counting efficiency was monitored by internal and external standards.

Samples of the ^{3}H-NE solution (10 µl) were counted in 10 ml of Bray's solution to determine the dpm/pmole NE. The protein concentrations of the synaptosomal suspensions were determined by the method of Lowry et al. (32).

Three hundred thirty µM α-MeNE or 150 µM metaraminol was included in some incubations to estimate the amount of ^{3}H-NE entering the synaptosomes by diffusion. The radioactivity of these diffusion blanks was routinely subtracted from that of samples not incubated with either α-MeNE or metaraminol to estimate the quantity of ^{3}H-NE actively taken up by synaptosomes.

In some experiments incubations were carried out in 0.32 M sucrose or in an incubation medium in which the sodium concentration was reduced from 143.3 mM to 31.1 mM in the latter solution (an equiosmolar concentration of sucrose or choline chloride was present to maintain the isotonicity of the incubation medium). In other experiments samples were incubated at 2°C or at 22°C.

Estimation of the percent of total ^{3}H-NE remaining as the unchanged catecholamine at the end of incubation. The percent of total radioactivity representing unchanged ^{3}H-NE was estimated by a modification of the method of Neff and Costa (30). Incubations were terminated by filtration and the total radioactivity present in the synaptosomes was determined in 10 ml of Bray's solution. To estimate radioactivity representing unchanged NE, filters were placed in 10 ml of 0.4 N $HClO_4$ containing 0.05% $Na_2S_2O_5$ and shaken overnight in a cold room (0-4°C). The extracted NE was adsorbed onto alumina and eluted with 2 ml of 0.3 N acetic acid (9) to determine recovery. One hundred ng of NE was added to the $HClO_4$ extract of some samples as an internal standard. The NE concentration of samples (500 µl) of the acetic acid eluates of samples (with or without the internal standard) was assayed by the method of Laverty and Taylor (27). In some of the samples radioactivity of the total acetic acid eluate was determined in 10 ml of Bray's solution. The fraction of total radioactivity representing unchanged NE was then calculated by dividing the radioactivity in the acetic acid eluate, corrected for recovery, by the total radioactivity in the synaptosomes on the Millipore filters.

Subcellular distribution of the ^{3}H-NE. Several incubations containing 1.0 ml of the synaptosomal suspension in a total volume of 20.6 ml were terminated by pouring the contents of each beaker into chilled polypropylene centrifuge tubes and centrifuging at 11,000 g for 20 min (at 0-4°). The supernatant was discarded; the pellet was washed with 1.0 ml of 0.32 M sucrose and then resuspended in 1.0 ml of 0.32 M sucrose. Two hundred µl of this suspension was then layered on top of 3.6 ml of a linear sucrose density gradient (freshly prepared by linearly diluting 1.60 M sucrose with 0.32 M sucrose using a Buchler Monostaltic Pump and a Buchler Autodensiflow) and centrifuged at 100,000 g for 2 hr, at 0°C, in a Beckman L3-50 preparative ultracentrifuge with an SW65L Ti rotor.

Four-drop fractions were then removed with the monostaltic pump and Autodensiflow and collected in glass scintillation vials. The radioactivity of each fraction was determined after the addition of 10 ml of Bray's solution.

Expression of results. The rate of active uptake of ^{3}H-NE by synaptosomes is expressed as pmoles of ^{3}H-NE per mg of synaptosomal protein per 5 min. The K_i of NE uptake inhibitors was determined by the method of Dixon (12).

RESULTS AND DISCUSSION

The effects of intraperitoneal doses of (±)-MDA, (S)-MDA, (R)-MDA, d-Amph, LSD, and mescaline on the behavior of mice are summarized in Table 1. The magnitude of an animal's behavioral response was estimated by comparing the behavior of treated mice with that of control mice treated with normal saline and with that of mice treated with either d-Amph, LSD, or mescaline. The magnitude of certain responses to the latter drug treatments was maximal, and the magnitude of similar responses to other drugs was estimated by comparison with these maximal responses. Maximal hallucinogenic behavior was produced by LSD, maximal ataxia resulted from treatment with mescaline, and all other maximal responses were produced by d-Amph.

TABLE 1. Behavioral Effects of d-Amph, LSD, Mescaline, (S)-MDA, (R)-MDA, and (±)-MDA in Mice[a]

Behavioral response[b]	Drug treatment (mg/kg, i.p.)					
	d-Amph (30)	LSD (4)	Mescaline (40)	(S)-MDA (20)	(R)-MDA (40)	(±)-MDA (40)
Salivation	↑ ↑ ↑	↑ ↑	↑	↑ ↑	↑	↑ ↑ ↑
Respiratory rate	↑ ↑ ↑	↑ ↑	↑	↑ ↑ ↑	↑	↑ ↑
Motor activity	↑ ↑ ↑	↑	↓ ↓	↑ ↑ ↑	↑	↑ ↑
Reactivity	↑ ↑ ↑	↑ ↑ ↑	↑	↑ ↑ ↑	↑	↑ ↑ ↑
Convulsions	+ + +	+	+	+ + +	+	+ + +
Stereotypy	+ + +	+	-	+ + +	+	+ +
Ataxia	+	+	+ + +	+	+ +	+ +
Apparent hallucinations	-	+ + +	+ +	-	+ + +	+ +

[a]Behavioral effects produced by these drug treatments were observed in six double-blind experiments, as described in the text.

[b]The magnitude of behavioral responses to the different drug treatments was estimated by comparing the behavior of treated mice with that of control mice, treated with 5 µl/g normal saline, i.p., and that of mice treated with either 30 mg/kg d-Amph, 4 mg/kg LSD, or 40 mg/kg mescaline, i.p.

Hallucinations were considered to be present when the animal looked at something with such intensity that it did not respond to external stimuli except touch, when it reared up and appeared to be fighting an imaginary animal, or when it seemed to be hiding from something by partially concealing itself under the bedding in a corner of the cage. This type of behavior was easily distinguished from other types of behavior, such as stereotypy, in all experiments, and was observed after the administration of LSD, mescaline, (R)-MDA, and (±)-MDA. Thus, the hallucinogenic activity of MDA could be separated from other behavioral effects and appears to result from the action of the (R)-isomer exclusively. (S)-MDA did not produce these apparent hallucinations. This observation is consistent with the report of Shulgin (45) that the hallucinogenic activity of 2,5-dimethoxy-4-methylamphetamine (DOM), another amphetamine structural analog, is due to an action of the (R)-isomer only.