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Disposition of Methylenedioxymethamphetamine and Three Metabolites in the Brains of Different Rat Strains and Their Possible Roles in Acute Serotonin Depletion

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ABSTRACT. 3,4-Methylenedioxymethamphetamine (MDMA) affects both dopamine and serotonin (5-HT) systems. One of its acute actions is to cause a reversible fall in steady-state brain 5-HT concentrations. To investigate the chemical basis of this acute effect, the brain levels of the parent compound and three major metabolites, 3,4-methylenedioxyamphetamine (MDA), 3,4-dihydroxymethamphetamine (DHMA) and 6-hydroxy-3,4-methylenedioxymethamphetamine (6-OHMDMA), were monitored, together with 5-HT levels, over a period of 6 hr in male Sprague–Dawley (SD) rats. The temporal relationships between drug concentrations of both stereoisomers and depletions were evaluated first. There was no correlation between the concentrations of the compounds measured and the extent of 5-HT depletion. Brain levels of MDMA and MDA were higher than plasma levels and exhibited a stereoselectivity in that (−)-MDMA and (+)-MDA levels were higher than those of their enantiomers. The relationship between the dose of (+)-MDMA and reduction in 5-HT levels was next investigated in SD male, SD female, and Dark Agouti (DA) female rats. These animals exhibit different capabilities of MDMA metabolism. There is a lower level of MDA, the N-demethylated metabolite of MDMA, in female SD rats than in males. Female DA rats are deficient in CYP2D isozymes, one of the enzymes responsible for demethylenation of MDMA to DHMA at pharmacological concentrations of substrate. There was a significant accumulation of MDMA in the brain and plasma of DA rats, but their 5-HT depletion was somewhat attenuated. The results indicated that MDMA was apparently not the single, causative agent for the acute 5-HT depletion, which may also involve a metabolite formed by CYP2D. BIOCHEM PHARMACOL 51;6:789–796, 1996.

KEY WORDS. MDMA; 5-HT depletion; MDMA and metabolites; MDMA disposition; Dark Agouti (DA) rats; CYP2D

MDMA‡ (Fig. 1) is a psychoactive and abused compound commonly known as "Ectasy," "E," or "Adam." In keeping with its structure as a ring-substituted amphetamine derivative, the compound affects both the dopamine and 5-HT systems. Its actions on the 5-HT system include reversible and irreversible depleting effects on steady-state levels of this neurotransmitter [1]. The reversible fall in 5-HT concentration is slow in onset, reaching a nadir about 3 hr after dosage, after which levels slowly return to control values [1] within 24 hr. The chronic, irreversible depletion, caused by selective damage of the 5-HT nerve terminals, is a neurotoxic effect of the drug. The reversible, acute depletion differs from the long-term effect of MDMA in its lack of stereoselectivity, i.e. the enantiomers of MDMA are comparable in their ability to cause this reversible effect. The mechanism of this action is not well understood, although inhibition of tryptophan hydroxylase [2] and enhancement of 5-HT efflux [3] have been proposed. This report summarizes results of a study investigating the role of MDMA and its metabolites in this reversible depletion.

A number of metabolites are formed from MDMA [4–7], and N-demethylation and demethylenation are important pathways involved in the formation of most of them. Demethylenation, which cleaves the methylenedioxy group to the catecholamine DHMA (Fig. 1), is catalyzed by CYP2D isozymes at low substrate concentration [8], and N-demethylation, which generates the primary amine MDA (Fig. 1), appears to be catalyzed, in part, by male specific cytochrome P450 isozymes.§ Another ring-hydroxylated metabolite, 6-OHM

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‡ *Abbreviations:* MDMA, 3,4-methylenedioxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; 6-OHMDMA, 6-hydroxy-3,4-methylenedioxymethamphetamine; DHMA, 3,4-dihydroxymethamphetamine; THMA, 3,4,6-trihydroxymethamphetamine; DA, Dark Agouti; SD, Sprague–Dawley; 5-HT, 5-hydroxytryptamine; HPLC-ECD, high performance liquid chromatography-electrochemical detection; TFAA, trifluoroacetic anhydride; and P450 or CYP, cytochrome P450.

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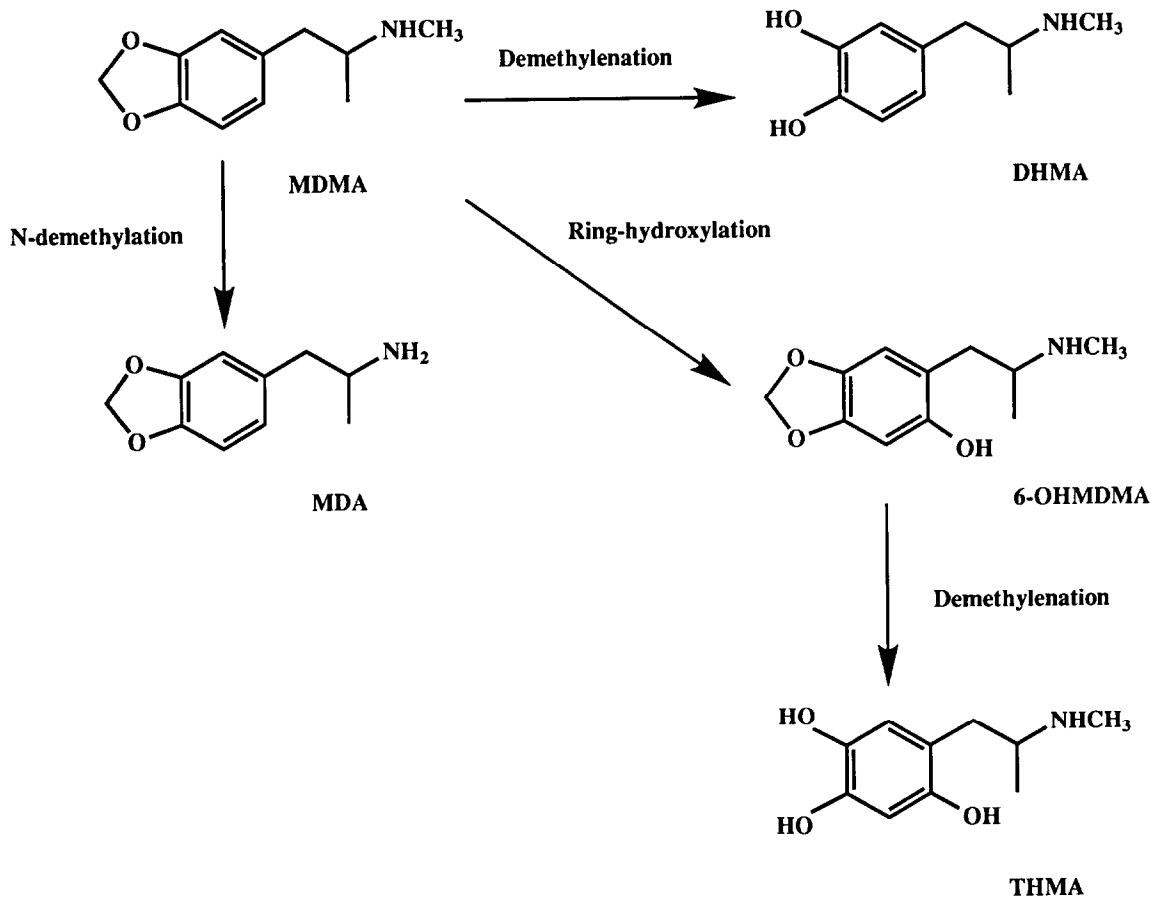

FIG. 1. 3,4-Methylenedioxymethamphetamine (MDMA) and its metabolites.

DMA (Fig. 1), has been shown to be demethylenated by CYP2D to THMA (Fig. 1) [7]. The latter is a potent but non-selective neurotoxin to both 5-HT and dopamine neurons after intraventricular injection [9]. Based on these observations, we investigated the *in vivo* disposition of MDMA and its possible relationship to 5-HT depletion. The temporal changes in drug concentrations and 5-HT depletion after each stereoisomer were followed in SD male rats. Then the effects of different doses of (+)-MDMA were examined in different rat strains. Male and female SD and female DA rats were utilized because of their differences in metabolic capabilities. The DA strain has been shown to be deficient [10] in CYP2D isozymes, and, as indicated above, the female SD rat has a decreased N-demethylation capability. By examining the nature of 5-HT depletion in these animals, we hoped to assess the participation of different metabolic pathways in the acute 5-HT-depleting actions of MDMA.

MATERIALS AND METHODS

Chemicals

(+)- and (−)-MDMA and MDA were obtained from the National Institute on Drug Abuse (Rockville, MD) as their hydrochloride salts. DHMA was prepared by the procedure described by Chavdarian *et al.* [11]. 6-OHMDMA [3-(6-hydroxy-3,4-methylenedioxyphenyl)-2-methylaminopropane] was synthesized according to the procedure described by Zhao *et al.* [12], and its deuterium substituted analog, D_2-6-OHMDMA [3-(6-hydroxy-3,4-methylenedioxyphenyl)-2-methylamino-(2,3-$^2H_2$) propane], was synthesized with the same protocol except that $LiAl^2H_4$ was substituted for $LiAlH_4$. Deuterium-substituted analogs of MDMA and MDA, 3-(3,4-methylenedioxyphenyl)-2-methylamino-(2,3-$^2H_2$) propane (D_2-MDMA) and 3-(3,4-methylenedioxyphenyl)-2-amino-(2,3-$^2H_2$) propane (D_2-MDA), were synthesized in this laboratory by procedures described earlier [13]. 5-HT creatinine sulfate and tyramine hydrochloride were purchased from the Sigma Chemical Co. (St. Louis, MO). TFAA was obtained from the Aldrich Chemical Co. (Milwaukee, WI). All other chemicals and solvents were obtained from common commercial sources.

Animals

Male and female SD rats (Charles River Breeding Laboratories, Wilmington, MA), weighing 200–250 g, and female DA rats (Bantin–Kingman Breeding Laboratories, Fremont, CA), weighing 175–200 g, were used. These weights reflect a common age of about 2 months. The animals were housed in a 12-hr light–12-hr dark cycle at 23–25°. Food and water were available *ad lib*.

The animals of each strain were randomly assigned to the

different treatment groups. MDMA hydrochloride in 0.9% NaCl was administered subcutaneously at a volume of 2 mL/kg at the indicated doses. Control animals received only saline. The animals were decapitated at the indicated times after dosage, and the brains were removed rapidly and bisected. The tissue was stored at −80° until assayed. One half of the brain was used for determination of 5-HT and DHMA by HPLC-ECD procedures, and the other half was used in GC-MS analyses of MDMA, MDA, and 6-OHMDMA. Blood was also collected into heparinized test tubes after decapitation. The blood samples were centrifuged at 13,500 *g* for 3 min, and the plasma (1 mL) was frozen and stored at −80° until assayed.

HPLC-ECD Determination of 5-HT and DHMA

The frozen brain was weighed and homogenized (Polytron homogenizer) for 30 sec in 0.5% perchloric acid containing 50 mM thiourea and 0.33 mM tyramine as the internal standard. The homogenate was then centrifuged at 12,000 *g* for 10 min, and 300 μL of the supernatant was filtered through a 0.45 μm syringe filter (Millipore, Type HA) to a microinjection vial. The samples (at 4°) were analyzed by an autosampler system (Gilson model No. 231).

The chromatography utilized a reversed-phase ODS column (Biophase ODS, 5 μm, 4.6 × 250 mm, Bioanalytical Systems Inc., Lafayette, IN) and an electrochemical detection system, i.e. a glassy carbon working electrode set at +0.7 V (vs Ag/AgCl) obtained from Bioanalytical Systems Inc. (Lafayette, IN). The mobile phase was 0.1 M citrate at pH 3.5 containing 1 mM octyl sodium sulfate, acetonitrile, and methanol in a 8:0.5:1 ratio with a flow rate of 0.7 mL/min. Peak heights were determined with a Hewlett Packard 3390 Integrator (Palo Alto, CA). External standards were prepared from authentic compounds in the ranges of 0.1 to 0.8 nmol/mL for 5-HT and 0.01 to 0.08 nmol/mL for DHMA. The retention times for 5-HT, tyramine, and DHMA were about 33, 24, and 29 min, respectively. The minimum quantifiable concentration (within 95% confidence limits) of DHMA under these conditions was between 0.030 and 0.060 nmol/g brain.

Chemical Demethylenation

The demethylenation of MDMA can occur chemically by the actions of hydroxyl radical, generated by reduction of superoxide by metal ions [14, 15]. In the course of assay development, artifactual DHMA formation, presumably mediated by chemical reaction, occurred during tissue extraction and storage of homogenate. This artifact was eliminated by the inclusion of thiourea in the homogenization buffer and low temperature manipulation of the samples. Substantial amounts of DHMA were formed in the absence of thiourea, and, for this reason, thiourea was added routinely to the perchloric acid prior to homogenization. This artifact has not been considered in earlier studies of MDMA metabolism by the brain (e.g. Ref. 16) so that the concentrations of metabolites reported here may be substantially lower than previously reported.

GC-MS Analysis of MDMA, MDA, and 6-OHMDMA

The tissues were homogenized in 3% perchloric acid containing 20 nmol each of D_2-MDA, D_2-MDMA and D_2-6-OHMDMA, added as internal standards. The plasma samples were treated with 2 mL of 3% perchloric acid containing the same internal standards as mentioned above. After centrifugation, 1.5 mL of the supernatant from brain or plasma was transferred to a 50-mL extraction tube containing 2 mL of a $NaHCO_3/Na_2/CO_3$ buffer (1.5 M, pH 9.5) and 10 mL of methylene chloride. The mixture was shaken, and the organic layer was collected and evaporated to a volume of 100 μL. To the concentrated extract were added 15 μL of acetonitrile and 100 μL of TFAA, and the mixture was heated at 60° for 15 min. The excess TFAA was allowed to evaporate in a fume hood, and the derivatized residue was reconstituted into 120 μL of acetonitrile. A volume of 1 μL of the acetonitrile solution was injected into the GC-MS system.

A Hewlett Packard 5971 GC-MS system with a 12.5 m × 0.2 mm fused silica capillary column with cross-linked methylsilicone of 0.33 μm film thickness (Hewlett Packard, Palo Alto, CA) was employed in the selected ion monitoring mode. A temperature program, from 65° to 250° at a rate of 30°/min was used. Under these conditions, the retention times for the trifluoroacetyl derivatives of MDMA, MDA, and 6-OHMDMA were 5.62, 5.14, and 5.67 min, respectively. The ions chosen for the analytes (D_0) and internal standards (D_2) were D_0, 154 and D_2, 164 for MDMA; D_0, 162 and D_2, 164 for MDA; and D_0, 274 and D_2, 276 for 6-OHMDMA.

Standard curves were obtained by analysis of samples prepared from untreated brain by adding authentic compounds and internal standards to the brain homogenate over a range of 0.5 to 400 nmol. Minimal detectable quantities of these compounds under these conditions were: MDA, 0.058 pmol/g; MDMA, 0.247 pmol/g; and 6-OHMDMA, 0.311 pmol/g.

Statistics

Data are expressed as means ± SD, and statistical analyses were performed with Student's *t*-test.

RESULTS

Temporal Changes in MDMA and 5-HT Concentrations in SD Rats

The temporal disposition of MDMA and its relation to acute depletion were first studied in SD male rats. Brain concentrations of MDMA after 20 and 40 mg/kg are shown in Table 1. MDMA concentrations were dose dependent and peaked within the first 2 hr. The concentration of (−)-MDMA was significantly higher than (+)-MDMA at 3 and 6 hr for both dosages.

The depletion of 5-HT caused by 20 and 40 mg/kg doses of MDMA is shown in Fig. 2 (panels A and B, respectively). As reported before [1], the enantiomers were comparable in their abilities to deplete 5-HT, despite the fact that brain levels of (−)-MDMA were higher than those of (+)-MDMA at the later time points (3 and 6 hr). Maximum depletion was observed

TABLE 1. Brain concentrations of MDMA and MDA after 20 and 40 mg/kg MDMA

Time (hr)	Isomer	MDMA (nmol/g)		MDA (nmol/g)	
		20 mg/kg	40 mg/kg	20 mg/kg	40 mg/kg
0.5	(+)	57.33 ± 22.97	225.71 ± 47.33	2.81 ± 0.53	18.43 ± 7.11
	(−)	90.33 ± 22.29	ND*	2.40 ± 0.60	ND
1	(+)	ND	218.73 ± 40.49	ND	34.00 ± 13.32
	(−)	ND	247.14 ± 42.67	ND	10.01 ± 2.90†
1.5	(+)	81.07 ± 40.55	ND	15.71 ± 5.96	ND
	(−)	125.31 ± 3.55	ND	10.87 ± 1.16	ND
2	(+)	ND	174.85 ± 16.43	ND	59.32 ± 9.46
	(−)	ND	175.57 ± 35.40	ND	31.61 ± 5.39‡
3	(+)	43.66 ± 23.34	139.61 ± 15.14	35.74 ± 5.49	54.73 ± 0.77
	(−)	88.38 ± 9.91†	172.70 ± 5.11‡	19.76 ± 3.45‡	22.01 ± 4.20‡
6	(+)	6.16 ± 4.17	46.47 ± 8.76	23.21 ± 9.79	65.10 ± 11.22
	(−)	26.84 ± 5.09‡	73.77 ± 8.88‡	20.71 ± 4.50	23.00 ± 3.18‡

The concentrations of MDMA and MDA were determined in whole brain by the GC-MS procedures described in Materials and Methods. The rats received a single s.c. injection of drug (20 or 40 mg/kg) or vehicle (0.9% saline), and the brains were collected at the indicated times. Values are means ± SD of 3–4 animals.

* ND, not determined.

† Significantly different from the (+)-isomer, $P < 0.05$.

‡ Significantly different from the (+)-isomer, $P < 0.01$.

between 3 and 6 hr after dosage. In contrast to the continuous decline of brain and plasma MDMA after dosage (e.g. 20 mg/kg of the (+)-isomer in Fig. 3), 5-HT depletion increased gradually and reached a plateau between 3 and 6 hr (Fig. 2A). After peaking at about 1 hr, both brain and plasma levels of MDMA dropped to 10–20% of their highest concentrations at 6 hr. Thus, no correlation was evident between changes in brain MDMA concentration and the time course for depletion.

Temporal Changes in MDMA Metabolites

DHMA was only detected in male SD rats after the high 40 mg/kg dose used in toxicity studies [17], but the levels were highly variable, ranging from 0.081 to 0.275 nmol/g brain with no discernible time pattern. The levels of 6-OHMDMA were also variable, ranging from 0.087 to 0.233 nmol/g after 40 mg/kg and from 0.090 to 0.735 nmol/g after 20 mg/kg over the 6-hr experiment. Both hydroxylated metabolites showed no discernible pattern of time-dependent formation over the 6-hr experiment.

The major MDMA metabolite assayed in the brain was MDA. Brain concentrations of this compound rose gradually, in parallel with the plasma (data not shown) and reached a plateau between 1 and 3 hr (Table 1). For the most part, levels of the (+)-isomer were higher than those of the (−)-isomer in brain, and a similar tendency has been observed in plasma [18]. Six hours after dosing, concentrations of (+)-MDA were either

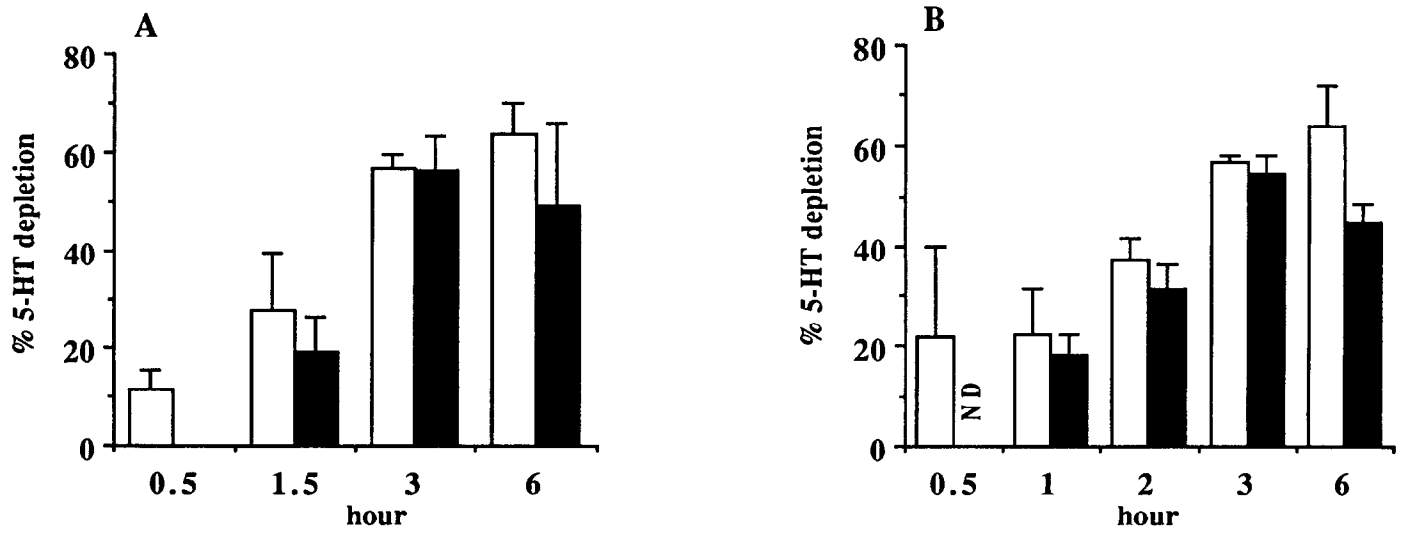

FIG. 2. Brain 5-HT depletion in male SD rats after subcutaneous MDMA doses of 20 mg/kg (panel A) and 40 mg/kg (panel B). The open bars refer to the (+)-isomer effects and the filled bars to the (−)-isomer effects. The vertical lines are the standard deviations resulting from at least 4 animals. ND = not determined. Control values of 5-HT = 2.48 ± 0.30 nmol/g for the 40 mg/kg experiment and 3.28 ± 0.43 for the 20 mg/kg experiment.

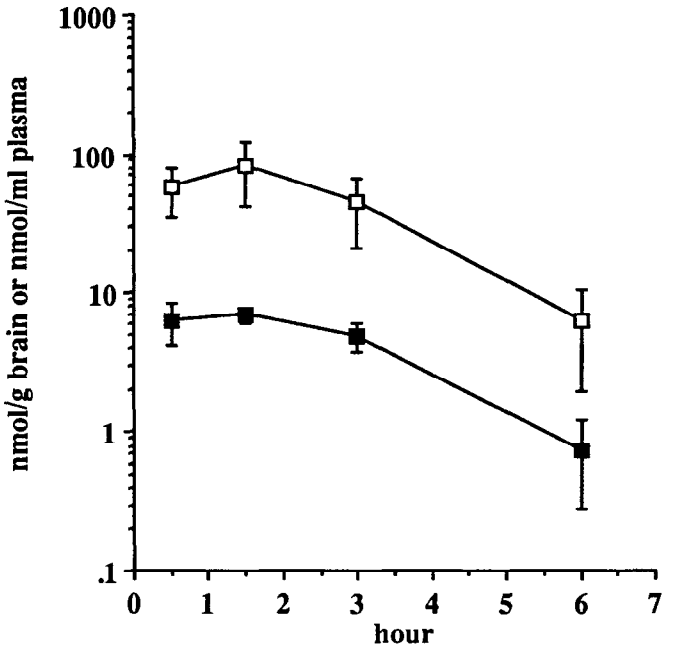

FIG. 3. Brain (□) and plasma (■) concentrations of (+)-MDMA after a subcutaneous dose of 20 mg/kg to male SD rats. The concentrations of MDMA were determined by procedures described in Materials and Methods. Values are means ± SD of 4 animals.

comparable to or greater than those of (+)-MDMA, but the levels of (−)-MDA were always lower than those of (−)-MDMA.

Sex and Strain Differences in MDMA Disposition

To assess the involvement of different metabolic pathways in 5-HT depletion, the actions of (+)-MDMA were examined in male and female SD and female DA rats. In studies with liver microsomes, N-demethylation activity was found to be much higher in male than female SD rats,* and the enzymes involved have very high K_m (mM) values. Demethylenation, the dominant metabolic pathway *in vitro* [18], was found to be catalyzed by CYP2D isozymes at low substrate concentrations (less than 10 μM) [8]. Female DA rats are deficient in CYP2D isozymes [10], and their liver microsomes exhibit only about 10% of the activity of SD rats [8]. The brain and plasma MDMA levels of this dose-depletion experiment with the three groups of rats are shown in Table 2.

Brain levels of (+)-MDMA increased non-linearly with dose (Table 2); that is, the increases in brain and plasma MDMA concentration were greater than the proportional increases in dose. The brain/plasma ratio was relatively fixed at about 7–10. (+)-MDMA levels in male and female SD rats were similar to each other but significantly lower than those of female DA rats. The differences were more drastic at lower doses, i.e. brain levels in the DA females were 15 times greater

* Landaw EM, Lin YP, Hiramatsu M, Kumagai Y, Schmitz DA, Zhu K and Cho Ak, manuscript in preparation.

TABLE 2. Brain and plasma concentrations of (+)-MDMA after different (s.c.) doses to rats

Dose (mg/kg)	SD male	SD female	DA female
	MDMA concentrations in brain (nmol/g)		
5	1.34 ± 0.13	0.74 ± 0.53	11.77 ± 2.46*†
10	9.29 ± 2.79	7.40 ± 1.78	19.99 ± 6.42*‡
20	43.66 ± 23.34	45.68 ± 10.62	63.79 ± 7.02§
40	121.79 ± 41.36	147.65 ± 44.06	114.24 ± 19.74
	MDMA concentration in plasma (nmol/mL)		
5	0.15 ± 0.02	0.00	1.45 ± 0.35*†
10	1.01 ± 0.18	0.87 ± 0.31	2.62 ± 1.03‡§
20	4.81 ± 1.12	6.17 ± 1.14	9.69 ± 1.87†§
40	ND‖	15.78 ± 3.92	16.69 ± 4.98

The concentrations of MDMA after (+)-MDMA were determined in rat brain as described in Table 1. The rats received a single s.c. injection of drug and were killed 3 hr later. Values are means ± SD of 4 animals.

* Significantly different from SD female rats, $P < 0.01$.
† Significantly different from SD male rats, $P < 0.01$.
‡ Significantly different from SD male rats, $P < 0.05$.
§ Significantly different from SD female rats, $P < 0.05$.
‖ ND, not determined.

than those of the SD females at 5 mg/kg but only about 2 times greater at 10 mg/kg, and no significant difference was observed at 40 mg/kg. A similar profile was also observed in plasma. The brain concentrations of MDA in the female SD rats were lower than the male (Table 3), presumably reflecting the differences in N-demethylation activity between the sexes. However, brain levels of MDA in the female DA rats were relatively proportional to dose and were higher than those of female SD rats.

As mentioned before, DHMA was only detected in male SD rats after 40 mg/kg. It was not detected in females of either the SD or the DA strain after any of the doses used here. 6-OHMDMA was not detected in the brains of SD female rats at doses below 40 mg/kg. Female SD rats exhibited a level of 0.18 ± 0.38 nmol/g after doses of 40 mg/kg, whereas female DA rats appeared to have significant levels of the compound after doses of 10 (0.06 ± 0.02 nmol/g), 20 (0.15 ± 0.04 nmol/g), and 40 (0.15 nmol/g) mg/kg.

TABLE 3. Brain concentrations of MDA in different rats after different (s.c.) doses of (+)-MDMA

Dose (mg/kg)	MDA concentrations in brain (nmol/g)		
	SD male	SD female	DA female
5	2.88 ± 0.37	0.75 ± 0.34*	4.73 ± 0.73*†
10	14.51 ± 3.80	3.82 ± 1.78*	7.59 ± 1.52‡§
20	35.74 ± 5.49	15.85 ± 3.83*	18.05 ± 2.56*
40	75.72 ± 14.3	28.47 ± 4.40*	30.03 ± 8.42*

The concentrations of MDA in the animals of Table 2 were determined by procedures described in Materials and Methods. Values are means ± SD of 4 animals.

* Significantly different from SD male, $P < 0.01$.
† Significantly different from SD female, $P < 0.01$.
‡ Significantly different from SD female, $P < 0.05$.
§ Significantly different from SD male, $P < 0.05$.

5-HT Depletion After Different MDMA Doses

There was no sex difference in the 5-HT depletion of SD rats (Table 4). On the other hand, the female DA rat had a distinctively different dose–response pattern. In this strain, the depletion appeared to be independent of the increase in dose and remained in the 20–30% range even after the highest dose.

DISCUSSION

The parallel concentration–time curves of plasma and brain MDMA (Fig. 3) indicate that the two compartments are at a steady state within 30 min after a subcutaneous dose. Pharmacological effects, such as the stereotypy response [19] and the dopamine-releasing action of MDMA, appear to occur in parallel with its appearance in the plasma [20] and, by inference, in the brain. The 5-HT-depleting actions of the drugs seem to be much more complex. The extent of depletion is clearly not related to the concentration in brain and plasma, i.e. during the times of maximum effect (i.e. 3 and 6 hr), 5-HT depletion was the same for both stereoisomers although the levels of (−)-MDMA were significantly higher than the (+)-isomer at both 20 and 40 mg/kg. This stereoselective accumulation was also observed at 10 mg/kg by Hegadoren *et al.* [21].

Schmidt and Taylor [22] have proposed that the acute depletion of 5-HT is due to a rapid inactivation of tryptophan hydroxylase coupled with the carrier-mediated efflux of 5-HT from the nerve terminal. The ability of MDMA to release 5-HT has been characterized before by Rudnick and Wall [3], using plasma membrane vesicles of human platelets that have 5-HT transporters. These workers reported that MDMA can cause the release of preloaded 5-HT from these vesicles at concentrations (10 μM) lower than those found here for brain. On the other hand, the possibility of a direct effect of MDMA on tryptophan hydroxylase at the concentrations found is less likely. It was shown that neither 100 μM MDMA (the brain concentration of MDMA found here) nor 100 μM MDA affects tryptophan hydroxylase *in vitro* [2]. In addition, direct injection of MDMA into the brain has no effect on the enzyme activity [23]. Although a constant i.c.v. infusion of 600 μg of MDMA did decrease tryptophan hydroxylase activity, the concentrations of MDMA achieved after that protocol would be considerably higher than those found in this study. This evidence indicates that MDMA may not be the only causative agent for the acute depletion. Indeed, the reduced depletion observed in the CYP2D-deficient DA strain in the presence of higher MDMA concentrations also suggests that the parent compound is not entirely responsible for 5-HT depletion. Therefore, some of the metabolites of MDMA were considered for their role in causing acute depletion.

The possibility that the active metabolite(s) of MDMA mediates 5-HT depletion has been suggested before. Gollamudi *et al.* [17] reported that phenobarbital and SKF-525A pretreatment enhances and attenuates, respectively, the acute depletion at 3 hr after MDMA. Phenobarbital and SKF-525A alter MDMA pharmacokinetics presumably through their actions on metabolism since phenobarbital pretreatment increases the rate constant for MDMA elimination and SKF-525A pretreatment decreases it [18]. MDA, the most prominent metabolite assayed, has been shown to cause 5-HT depletion with a potency comparable to that of MDMA [24–27], so that the concentrations of MDA attained after MDMA were too low to be effective. Furthermore, the differences in brain levels between male and female SD rats would suggest that a sex difference in depletion should be seen if MDA is directly causing 5-HT depletion.

The concentrations of the hydroxylated metabolites, DHMA and 6-OHMDMA, were so variable that direct assessment of their involvement is difficult. However, some conclusions regarding these metabolites may be drawn from the studies with the different rat strains. DHMA and the potentially neurotoxic THMA are formed by demethylenation of MDMA and 6-OHMDMA, respectively. Demethylenation of MDMA to DHMA had been studied in our laboratory with liver microsomes and exhibited two-site enzyme kinetics [8] with CYP2D involved in the low K_m system (less than 10 μM). Consistent with the deficiency of CYP2D in female DA rats, the brain levels of MDMA in this strain were much higher than those of SD rats after the low doses but were comparable to those of SD rats at high doses. Presumably at the high doses, alternative enzymes (i.e. enzymes with high K_m values) are available for metabolism in both strains. A similar phenomenon was observed for 6-OHMDMA, the precursor of THMA. 6-OHMDMA was detectable in the brains of female DA rats at 10 mg/kg, whereas SD females did not show its presence until the dose of 40 mg/kg had been administered. The higher levels of MDMA and 6-OHMDMA may be the result of reduced formation of DHMA and THMA in the CYP2D-deficient DA rats. Animals of this strain may accumulate their respective precursors after the lower doses, but at the higher doses alternative enzymes may metabolically remove MDMA and 6-OHMDMA in DA as well as SD rats.

Despite the extensive accumulation of unmetabolized MDMA, the DA females showed an attenuated 5-HT depletion relative to both male and female SD rats. This attenuated depletion profile suggests that a CYP2D-derived metabolite(s), such as DHMA or THMA, may play an important role in 5-HT depletion. In fact, Johnson *et al.* [9] have shown that

TABLE 4. Depletion profile of 5-HT in different rats after different (s.c.) doses of (+)-MDMA

Dosage (mg/kg)	% Depletion of 5-HT		
	SD male	SD female	DA female
5	44.69 ± 6.49	31.25 ± 7.22	28.20 ± 17.05
10	60.89 ± 9.29	63.15 ± 11.45	36.51 ± 4.66*†
20	56.34 ± 6.63	56.71 ± 12.38	23.76 ± 12.26*†
40	56.85 ± 1.50	53.90 ± 13.78	26.03 ± 16.83†

The concentrations of 5-HT in the animals of Table 2 were determined by procedures described in Materials and Methods. The concentrations of 5-HT in control animals were 2.295 ± 0.276 nmol/g for SD male, 2.147 ± 0.119 nmol/g for SD female, and 2.528 ± 0.358 for DA female.

* Significantly different from SD female, $P < 0.01$.

† Significantly different from SD male, $P < 0.01$.

direct intraventricular injection of DHMA has little chronic effect on 5-HT systems but THMA can cause lesions to both 5-HT and dopamine neurons 5 days after intraventricular injection. The acute effects of these CYP2D metabolites after direct CNS injection have not yet been reported. Schmidt and Taylor [2, 22] have shown that uptake blockers, such as fluoxetine and citalopram, can block or reverse the acute depletion of MDMA, indicating the involvement of the 5-HT transporter in such depletion. A similar observation was made by Hashimoto and Goromaru [28], who coadministered paroxetine with MDMA and subsequently blocked the acute depletion effect of the latter. However, fluoxetine, citalopram, and paroxetine are also inhibitors of CYP2D isozymes [29, 30]; therefore, the previous observations could also be interpreted as a response similar to that of the DA rats. Hashimoto and Goromaru [28] have also shown that the methylenedioxy compound, 1-piperonyl-piperazine, can attenuate the acute depletion when coadministered with MDMA. The same compound was also shown to alter the distribution of radioactivity in the brain of mice dosed with [^{3}H]MDMA [31]. 1-Piperonyl-piperazine was a fairly weak 5-HT uptake inhibitor and had no effects on release from rat brain synaptosomes. The presence of a methylenedioxy group on this drug raises the possibility that its action could be due to inhibition of metabolism either as a competitive substrate or as a mechanism-based inhibitor [32]. Taken together, the data suggest that acute 5-HT depletion caused by MDMA could also be due to an active metabolite, possibly formed by CYP2D.

In summary, while these studies have not identified the mechanism and the chemical component(s) responsible for the acute depleting action of MDMA on 5-HT stores, the observations, taken in concert with other reported data, suggest that MDMA itself may not be the only causative agent. Furthermore, the results of the DA rats suggest that metabolic pathways catalyzed by CYP2D are involved in the depletion.

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