In Vivo and in Vitro Metabolism of 3,4-(Methylenedioxy)methamphetamine in the Rat: Identification of Metabolites Using an Ion Trap Detector†

H. K. Lim and R. L. Foltz*

Center for Human Toxicology, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112

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Four biotransformation pathways of 3,4-(methylenedioxy)methamphetamine (MDMA) in the rat have been identified: N-demethylation, O-dealkylation, deamination, and conjugation (O-methylation, O-glucuronidation, and/or O-sulfation). The specific MDMA metabolites that have been identified are 3-hydroxy-4-methoxymethamphetamine, 4-hydroxy-3-methoxymethamphetamine, 3,4-dihydroxymethamphetamine, 4-hydroxy-3-methoxyamphetamine, 3,4-(methylenedioxy)amphetamine (MDA), (4-hydroxy-3-methoxyphenyl)acetone, [3,4-(methylenedioxy)phenyl]acetone, and (3,4-dihydroxyphenyl)acetone. All except 3,4-dihydroxymethamphetamine were present in the urine. The hydroxylated metabolites were excreted in the urine as the *O*-glucuronide and/or *O*-sulfate conjugates, but traces of free 4-hydroxy-3-methoxymethamphetamine and 4-hydroxy-3-methoxyamphetamine were also present in unhydrolyzed urine. *N*-Demethyl and 3-*O*-methyl phenolic amine metabolites of MDMA were consistently present in brain, liver, blood, and feces. MDMA was metabolized by the 10000*g* rat liver supernatant to 4-hydroxy-3-methoxymethamphetamine, 3,4-dihydroxymethamphetamine, MDA, and [3,4-(methylenedioxy)phenyl]acetone. Also, the 10000*g* rat brain supernatant metabolized MDMA to 4-hydroxy-3-methoxymethamphetamine, 3,4-dihydroxymethamphetamine, 4-hydroxy-3-methoxyamphetamine, and MDA.

Introduction

3,4-(Methylenedioxy)methamphetamine (MDMA, structure I in Figure 8) is a ring-substituted amphetamine derivative; hence its common reference in the literature as a "designer drug". It is related structurally to the psychomotor stimulant amphetamine and to the hallucinogen mescaline. Despite its structural resemblance to mescaline, MDMA has no hallucinogenic effects in animal models (*1–3*) or in humans (*4–6*); rather, MDMA has amphetamine-like subjective effects (*7–9*) which, together with its empathy-enhancing properties, have led to its widespread use as a recreational drug among college students (*6, 10*). Death from ingestion of MDMA is rare but has been documented in individuals with underlying cardiac disease (*11*). In view of its high potential for abuse and its structural similarity to MDA, a known neurotoxin (*12*), the U.S. Drug Enforcement Agency temporarily listed MDMA as a Schedule 1 drug in 1985. This created opposition from some psychiatrists who were using MDMA as an adjunct to psychotherapy to ameliorate anxiety or fear during the discussion of emotionally painful events (*13*). A successful legal challenge to the emergency scheduling of MDMA resulted in its removal from the temporary Schedule 1 list (*14*), but MDMA has now been placed on permanent Schedule 1 status (*15*).

In view of the controversy surrounding MDMA, research is under way to clarify the pharmacology and toxicology of the drug. The pharmacological activity of MDMA is apparently mediated through release and blockade of active reuptake of endogenous monoamine neurotransmitters like serotonin and norepinephrine (*16–18*). Stereoselectivity has been observed in these biochemical processes, the *S*-(+) enantiomer being more potent than the *R*-(−) antipode (*16–18*); in vivo the *S*-(+) enantiomer is mainly responsible for the pharmacodynamic effects of MDMA. Lyon et al. (*19*) recently demonstrated that MDMA binds with low affinity to 5-hydroxytryptamine (5-HT) receptor sites in the brain; the *R*-(−) enantiomer displays higher affinity toward the 5-HT receptor sites than the *S*-(+) antipode. This reverse in stereoselectivity, combined with observations that the release of neurotransmitter is calcium-independent (*17*) and that intact neurons are not required for release of neurotransmitter (*16*), argues against direct action of MDMA on postsynaptic receptors. Nevertheless, the possibility that MDMA acts directly on receptors cannot be totally excluded, since recent studies have demonstrated the presence of specific binding sites for MDMA in the rat brain (*20*).

Comparison of the acute lethality of five amphetamine-type hallucinogens among five animal species suggested that MDMA was the second most toxic compound of those tested (*21*). Recently, Commins et al. (*22*) provided neurochemical and histological evidence that MDMA is indeed toxic to the serotonergic and, to a lesser extent, catecholaminergic neurons in rat brain; histological examination of coronal brain sections revealed degeneration of axon terminals and cell bodies in the striatum and somatosensory cortex, respectively. Similar destruction of nerve terminals of serotonergic neurons has been observed in rat and monkey brains (*14, 23*). In addition to the histopathological changes, MDMA caused selective depletion of serotonin in the rat brain following both acute (*24, 25*) and

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* To whom correspondence should be addressed.

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multiple dosing (25) of MDMA. Furthermore, depletion of serotonin by MDMA was biphasic; the acute phase reached a maximum of 3–6 hours, and the later phase began 1 week after dosing (26) and continued for as long as 14 days (27). Only the second phase of serotonin depletion exhibited stereoselectivity for the S-(+) enantiomer (26). Although these authors speculated that the second phase of depletion was caused by metabolites of MDMA, until now only one MDMA metabolite (MDA) has been reported in rat (28) and human (29).

This report describes for the first time the structural elucidation and the distribution of metabolites of MDMA in the rat. We also provide evidence that rat brain enzymes can metabolize MDMA in vitro.

The initial identification of the MDMA metabolites was based on their electron ionization (EI) and chemical ionization mass spectra obtained on an ion trap detector. With this instrument we were able to obtain interpretable full-scan mass spectra on as little as a few picograms of MDMA (30–32) and MDA (33). This degree of sensitivity is particularly valuable in the investigation of brain metabolism of MDMA, since the drug-metabolizing enzymes are present in extremely low quantities in the brain (34, 35).

Experimental Procedures

Materials. MDMA, MDA, and 3,4-(methylenedioxy)ethylamphetamine (MDE) were purchased from Alltech Applied Science (Deerfield, IL). [3,4-(Methylenedioxy)phenyl]acetone was purchased from Fluka Chemical Co. (Hauppauge, NY). These standards were checked for purity by GC/MS analysis. Trifluoroacetic anhydride (99% pure) was obtained from Pierce Chemical Co. (Rockford, IL). All solvents used were glass distilled, HPLC grade, and were obtained from Burdick and Jackson (Muskegon, MI). The following reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI): sodium bisulfite, sodium hydroxide (98%), sodium acetate trihydrate (99+%), anhydrous sodium sulfate (99+%), potassium chloride (99+%), anhydrous dibasic potassium phosphate, anhydrous monobasic potassium phosphate (99+%), magnesium chloride hexahydrate (99%), 4-hydroxy-3-methoxybenzaldehyde (99%), 3-hydroxy-4-methoxybenzaldehyde (99%), 3,4-dimethoxybenzaldehyde (99%), anhydrous ammonium acetate, nitroethane (96%), powdered iron, 325 mesh (97%), lithium aluminium hydride (95%), potassium sodium tartrate tetrahydrate (99%), Celite 521, methylamine hydrochloride (98%), sodium cyanoborohydride (95%), and boron tribromide (1 M solution in dichloromethane). β-Glucuronidase (*Helix pomatia*, type H-1), D-glucose 6-phosphate (monopotassium salt), β-nicotinamide adenine dinucleotide phosphate (monosodium salt), and glucose-6-phosphate dehydrogenase (*Torula* yeast, in ammonium sulfate solution) were purchased from Sigma Chemical Co. (St. Louis, MO). Ammonium hydroxide and glacial acetic acid were obtained from VWR Scientific Co. (Salt Lake City, UT). Silica gel, 60–200 mesh, was purchased from American Scientific Products (Salt Lake City, UT).

Instrumentation. The gas chromatograph/mass spectrometer system used throughout this study consisted of a Hewlett-Packard Model 5890 gas chromatograph interfaced to a Finnigan MAT Model 800 ion trap detector. The data system for the ion trap detector consisted of an IBM AT microcomputer. The software (version 3.31), which contained both automatic gain control (AGC) and automatic reaction control (ARC) programs, was made available to us by Finnigan MAT. The ion trap manifold temperature was maintained at 220 °C during analysis.

The ion trap detector was autotuned according to the manufacturer's recommendations. Electron ionization (EI) mass spectra were acquired by repetitively scanning the mass range m/z 50–649 at a rate of 1 scan/s. Positive ion chemical ionization mass spectra were acquired by scanning the mass range m/z 100–649. Either methanol or acetone was used as the CI reagent gas. The reagent gas pressure within the ion trap was adjusted by opening the reagent gas metering valve to give an ion current intensity of approximately 2500 counts at the mass of the protonated reagent gas molecule. This adjustment was carried out with the automatic reaction control (ARC) function turned off, and with the B value (sensitivity) set to the minimum. For methanol, the ionization and reaction radio frequency (rf) levels were set at 3.5 and 26 amu, respectively. However, the ionization and reaction rf levels were set at 5 and 40 amu, respectively, when acetone was used as the reagent gas. Other operating parameters were the same as previously reported for acquisition of CI mass spectra using the automatic reaction control scan function (32).

MDMA and metabolites were separated chromatographically with a fused silica capillary column coated with dimethylsilicone (12.5 m × 0.2 mm i.d., 0.3-μm film thickness, Hewlett-Packard, Palo Alto, CA). The GC column was interfaced directly to the ion trap through the standard transfer line connector. Hydrogen served as both carrier gas (linear velocity = 60 cm/s) and buffer gas. The injector and transfer line temperatures were 260 and 220 °C, respectively. The oven temperature was held at 80 °C for 0.5 min after injection and then temperature-programmed to 300 °C at 10 °C/min. N-(Pentafluorobenzoyl)-MDMA (1 ng/μL) was injected to evaluate the performance of the GC/MS prior to analysis of samples.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded by using a 200-MHz IBM NR-200 spectrometer with tetramethylsilane as the internal standard. Chemical shifts are reported here in ppm downfield from tetramethylsilane.

In Vivo Metabolism. Six male Sprague-Dawley rats were used for the in vivo studies. The rats were administered either 5 or 20 mg/kg MDMA (calculated as free base) by gavage and were then placed in metabolic cages. Urine and feces were collected over 24 h. Feces homogenate was prepared in 3 volumes of 0.9% sodium chloride and then centrifuged at 10000*g* for 15 min. In this set of experiments, each rat served as its own control and was fed only the vehicle by gavage. In another set of experiments, rats were decapitated at either 6 or 24 h after drug administration. Blood was collected immediately and centrifuged at 3500 rpm for 15 min to separate the plasma. Also, brains and livers were removed; these tissues were homogenized in 3 volumes of cold 0.4% perchloric acid containing 0.05% sodium bisulfite. The supernatant, obtained from centrifugation of the homogenate at 10000*g* for 15 min, was stored at −20 °C until analysis. The blood, brains, and livers from saline-treated rats were used as control specimens and were processed in the same manner.

In Vitro Metabolism. Four male Sprague-Dawley rats were used for the in vitro experiments. The 10000*g* rat liver and brain supernatants were prepared by modification of previously reported procedures (36, 37). Rat brain (33% w/v) and liver (50% w/v) homogenates were prepared in 1.15% potassium chloride (KCl) solution. The 10000*g* supernatant was stored at −80 °C until required. The incubation procedure for in vitro brain and liver metabolic studies is a modification of that reported for LSD (38). All incubations, 4-mL total volume, contained 120 mM phosphate buffer, pH 7.4, 46 mM KCl, 2 mM magnesium chloride, 0.4 mM β-nicotinamide adenine dinucleotide phosphate ($NADP^+$), 4 mM glucose 6-phosphate (G-6-P), 0.4 unit of G-6-P dehydrogenase, 12 μM MDMA, and 0.8 mL of 10000*g* supernatant. After a 5-min preincubation, the reaction was started by addition of the NADPH-generating system and 10000*g* supernatant. The reaction mixture was further incubated for 2 hour at 37 °C. At the end of the incubation period, the reaction was stopped by immersing the test tubes in an ice bath prior to extraction. Control incubations were performed in the same manner, except for the omission of addition of MDMA.

Enzymatic Hydrolysis. The pH of the biological specimens (urine, plasma, feces, brain, and liver; 3 mL each) was adjusted to about 5 with 2 M sodium hydroxide, followed by addition of 1.5 mL of 1 M sodium acetate buffer (pH 4.8) containing β-glucuronidase (10 000 units/mL of sample). The samples were then incubated for 16 h at 37 °C.

Extraction and Derivatization. Each biological specimen in a 25-mL Teflon-lined, screw-capped test tube was spiked with 2 μg of MDE as the internal standard. The sample was basified to pH 9 with 2 M sodium hydroxide, saturated with sodium chloride, and extracted into 2 × 5 mL of dichloromethane/2-propanol (3:1 v/v) by gentle rocking for 15 min. The liquid phases were separated by centrifugation at 3500 rpm for 15 min. The pooled organic extract was washed with 5 mL of 0.1M ammonium

hydroxide solution and centrifuged as above. The organic extract was then dried over anhydrous sodium sulfate. Following centrifugation, the liquid phase was transferred to a 10-mL Teflon-lined, screw-capped test tube, and 50 μL of glacial acetic acid was added. The organic phase was evaporated to dryness under a gentle stream of air at 60 °C.

Ethyl acetate (100 μL) and trifluoroacetic anhydride (200 μL) were added to the residue, and the tube was tightly capped, hand vortexed (30 s), and then heated for 20 min at 80 °C. Just before analysis, the excess organic solvent and trifluoroacetic anhydride were removed under vacuum. The residue was reconstituted in 100 μL of ethyl acetate and hand vortexed for 30 s prior to injection of a 1-μL aliquot into the GC/MS.

Chemical Synthesis of Metabolites. The reference compounds were synthesized according to published synthetic routes. The purified reference compounds were all characterized by ^{1}H NMR and/or mass spectrometry.

(4-Hydroxy-3-methoxyphenyl)acetone (VII). Condensation of 4-hydroxy-3-methoxybenzaldehyde with nitroethane (*39*) gave 4-hydroxy-3-methoxy-β-nitrostyrene. Recrystallization of the nitrostyrene compound from methanol/water gave golden yellow crystals: ^{1}H NMR ($CDCl_3$) 2.47 (s, 3 H, CCH_3), 3.93 (s, 3 H, OCH_3), 6.17 (b s, 1 H, OH), 7.01 (m, 3 H, aromatic), 8.04 (s, 1 H, CH); CI-MS (TFA derivative), m/z 306 (MH^+, base peak).

The nitrostyrene compound was treated with ferric chloride, electrolytic iron powder, and concentrated hydrochloric acid by modification of the procedure of Morgan and Beckett (*40*) to give (4-hydroxy-3-methoxyphenyl)acetone as an oil. The oil was purified by elution from a silica gel column (60–200 mesh) with toluene/dichloromethane (9:1). Fractions containing the purified product were pooled. Evaporation of the organic solvent with a rotary evaporator yielded a golden yellow oil: ^{1}H NMR ($CDCl_3$), 2.12 (s, 3 H, CCH_3), 3.59 (s, 2 H, CH_2), 3.79 (s, 3 H, OCH_3), 6.31 (b s, 1 H, OH), 6.76 (m, 3 H, aromatic); CI-MS (TFA derivative), m/z 277 (MH^+, base peak).

4-Hydroxy-3-methoxymethamphetamine (III) was prepared from the ketone VII by modification of the procedure of Morgan and Beckett (*40*). The free base of methylamine was liberated from the hydrochloride salt by stirring together 0.24 mol of methylamine hydrochloride, 0.22 mol of sodium carbonate, and 250 mL of methanol at room temperature for 30 min and then filtered into a 500-mL three-neck round-bottom flask containing 0.04 mol of (4-hydroxy-3-methoxyphenyl)acetone. After the mixture was refluxed for 2 h, 0.032 mol of sodium cyanoborohydride was added portionwise over 20 min at room temperature. Reflux was resumed for another 5 h, and during this time, the pH of the solution was maintained at neutrality by addition of 4 M methanolic hydrochloric acid as needed. The organic solvent was removed with a rotary evaporator, and the residue was taken up in 200 mL of water. The solution was acidified at pH 2–3 with 6 M hydrochloric acid, extracted with ethyl acetate, then basified to pH 9–10 with 6 M sodium hydroxide, and finally saturated with sodium chloride. The mixture was extracted with ethyl acetate; the pooled organic extract was then dried over anhydrous sodium sulfate, and the ethyl acetate was removed by evaporation to give an oil, which was purified by silica gel column chromatography (60–200 mesh) and eluted with a solvent mixture of toluene/dichloromethane/methanol (2:2:1). Fractions containing the 4-hydroxy-3-methoxymethamphetamine were pooled, and the organic solvent was evaporated to yield a dark brown oil: ^{1}H NMR ($CDCl_3$) 1.10 (d, J = 6 Hz, 3 H, CCH_3), 2.4 (s, 3 H, NCH_3), 2.71 (m, 3 H, CH_2CH), 3.79 (s, 3 H, OCH_3), 5.06 (b s, 2 H, NH and OH), 6.71 (m, 3 H, aromatic); CI-MS (TFA derivative), m/z 388 (MH^+, base peak).

4-Hydroxy-3-methoxyamphetamine (IV) was synthesized by reduction of 4-hydroxy-3-methoxy-β-nitrostyrene with lithium aluminium hydride ($LiAlH_4$) by slight modification of the procedure of Morgan and Beckett (*40*). The modifications were as follows: (1) The nitrostyrene was dissolved in a tetrahydrofuran/ether mixture (1:10), and the solution added to $LiAlH_4$. (2) The excess $LiAlH_4$ was decomposed with a saturated solution of potassium sodium tartrate. The pH of the resulting solution was adjusted to 9 with 4 M HCl and the solution filtered through Celite. The product was purified by silica gel column chromatography as described for compound III. Fractions containing the product were pooled, and the organic solvent was evaporated to yield a dark brown oil: ^{1}H NMR ($CDCl_3$) 1.14 (d, J = 6 Hz, 3 H, CCH_3), 2.55 (m, 2 H, CH_2), 3.14 (m, 1 H, CH), 3.71 (s, 3 H, OCH_3), 4.30 (b s 3 H, NH_2 and OH), 6.71 (m, 3 H, aromatic); CI-MS (TFA derivative), m/z 374 (MH^+, base peak).

(3,4-Dihydroxyphenyl)acetone (IX). Synthesis of (3,4-dimethoxyphenyl)acetone was carried out according to the procedure reported by Morgan and Beckett (*40*). The compound was purified by silica gel chromatography (60–200 mesh) and eluted with a mixture of toluene/dichloromethane (9:1). Removal of the organic solvent from the pooled fractions containing the product left an amber color oil: CI-MS, m/z 195 (MH^+, base peak).

The ketone was then demethylated by using boron tribromide in dichloromethane, according to the method of McOmie et al. (*41*). The reaction was quenched by addition of methanol (*42*); subsequent evaporation yielded a dark oil. Purification of (3,4-dihydroxyphenyl)acetone was by silica gel chromatography; the elution solvent was toluene/dichloromethane/ethyl acetate (2:5:1). Fractions containing the product were pooled and evaporated to give a light brown oil: CI-MS (TFA derivative), m/z 359 (MH^+, base peak). The EI mass spectrum was consistent with that reported for the trifluoroacetyl derivative of (3,4-dihydroxyphenyl)acetone (*43*).

3,4-Dihydroxymethamphetamine (V). The synthetic route for preparation of 3,4-dimethoxymethamphetamine was identical with that reported by Morgan and Beckett (*40*). This amine was purified by silica gel column chromatography (60–200 mesh) and eluted with a solvent mixture of toluene/dichloromethane (9:1). Evaporation of the organic solvent gave an amber color oil: CI-MS (TFA derivative), m/z 306 (MH^+, relative intensity = 4%). Demethylation of this amine was performed as previously described for compound IX. The dark oil obtained after evaporation was purified by flash chromatography, eluting with dichloromethane/methanol (3:1). The fractions containing the product were combined and evaporated to a light brown oil: ^{1}H NMR (DMSO-d_6) 1.09 (d, J = 6.4 Hz, 3 H, CCH_3), 2.53 (s, 3 H, NCH_3), 2.53 (m, 2 H, CH_2), 3.01 (m, 1 H, CH), 6.58 (m, 3 H, aromatic), 8.99 (b s, 3 H, NH and OH); CI-MS (TFA derivative), m/z 470 (MH^+, base peak).

3-Hydroxy-4-methoxymethamphetamine (II). The procedures used for synthesis and purification of this amine were the same as that for compound III except that the starting material was 3-hydroxy-4-methoxybenzaldehyde. The product was purified by silica gel chromatography as described for compound III. The solvent was evaporated to a dark brown oil: ^{1}H NMR (DMSO-d_6) 0.92 (d, J = 6 Hz, 3 H, CCH_3), 2.29 (s, 3 H, NCH_3), 2.48 (m, 2 H, CH_2), 2.66 (m, 1 H, CH), 3.73 (s, 3 H, OCH_3), 5.50 (b s, 2 H, NH and OH), 6.65 (m, 3 H, aromatic); CI-MS (TFA derivative), m/z 388 (MH^+, base peak).

Results and Discussion

Identification of in Vivo Metabolites. The trifluoroacetylated extracts of hydrolyzed urine from dosed and control rats were analyzed by capillary column GC/MS under both electron ionization (EI) and positive ion chemical ionization (CI) conditions. The CI total ion current chromatogram of hydrolyzed urine from one of the dosed rats showed eight peaks that were not present in the CI total ion current chromatogram from the hydrolyzed control urine (Figure 1). Full-scan CI mass spectra were obtained for all eight peaks. However, only compounds corresponding to peaks A and C–E were present in sufficient quantity to permit the acquisition of full-scan EI mass spectra. The minor chromatographic peaks B and F–H, which are barely detectable in Figure 1, were clearly evident in ion current chromatograms of selected ions (not shown). Tentative identification of the metabolites by interpretation of their mass spectra was confirmed by comparison of the mass spectra with the spectra obtained for synthetic reference compounds prepared as described under Experimental Procedures.

The EI and CI mass spectra of peak A are shown in Figure 2. The mass spectra of peak A and its retention time relative to MDE were identical with those of the

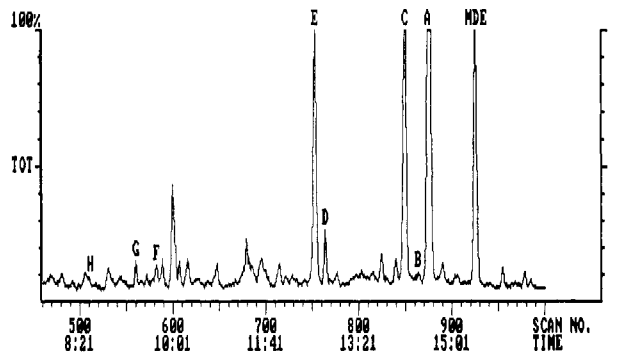

Figure 1. Total ion current chromatogram (positive ion chemical ionization) of a derivatized extract of urine collected from a rat dosed with 5 mg/kg MDMA.

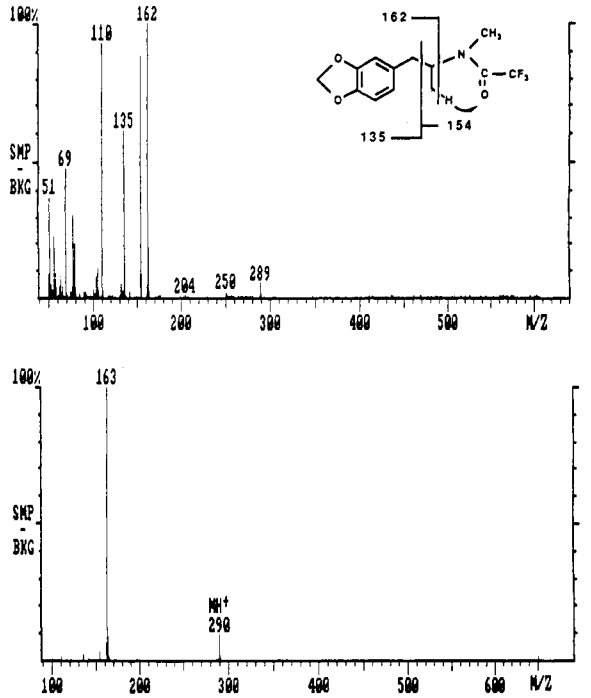

Figure 2. Electron ionization (top) and positive ion chemical ionization (bottom) mass spectra of the trifluoroacetyl derivative of MDMA (peak A in Figure 1).

N-trifluoroacetyl derivative of MDMA (*N*-TFA-MDMA). The EI mass spectrum contains diagnostic fragment ions resulting from a McLafferty rearrangement (m/z 162) and cleavage β to the nitrogen (m/z 135 and 154). The molecular ion is weak, but detectable, as is the protonated molecule in the CI mass spectrum. The most abundant ion in the CI mass spectrum (m/z 163) is formed by loss of $CH_3N{=}C(OH)CF_3$ from the protonated molecule.

Because the major fragmentation pathways observed in the mass spectrum of *N*-TFA-MDMA are evident in the mass spectrum of each of the nitrogen-containing metabolites of MDMA, a tentative structural identification is possible by interpretation of the mass spectra for each of these metabolites. For example, Figure 3 shows the EI and CI mass spectra of chromatographic peak C. The most abundant ion in the CI mass spectrum, at m/z 388 was presumed to be the protonated molecule. A molecular mass of 387 daltons indicated that the metabolite had an odd number of nitrogen atoms. Retention of the intact *N*-methyl-*N*-trifluoroacetamide group was further supported by the presence of an abundant ion at m/z 261 [$MH^+ - CH_3N{=}C(OH)CF_3$] in the metabolite's CI mass spectrum corresponding to the same major fragmentation

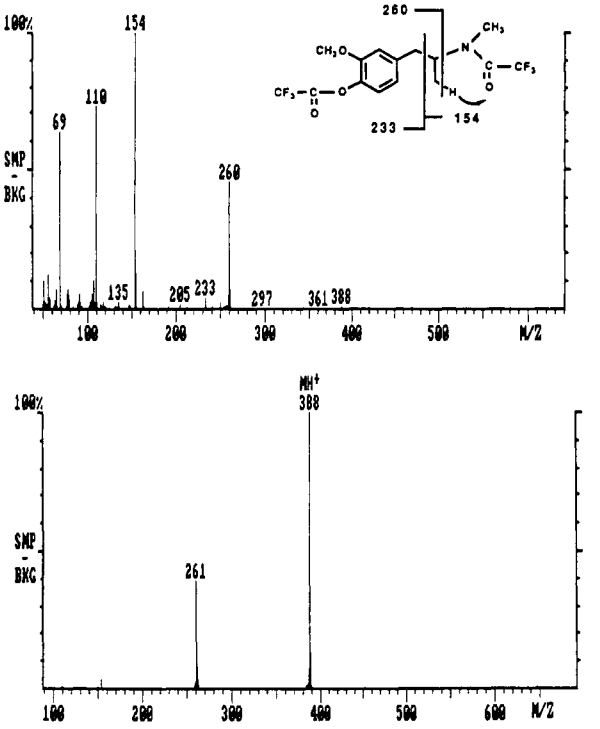

Figure 3. Electron ionization (top) and positive ion chemical ionization (bottom) mass spectra of the trifluoroacetyl derivative of MDMA metabolite III (peak C in Figure 1).

pathway observed in the CI mass spectrum of *N*-TFA-MDMA. The absence of structural change to the metabolite's side chain is further indicated by the abundant fragment ion at m/z 154 in the EI mass spectrum. Therefore, biotransformation must have occurred on the 3,4-methylenedioxy-substituted phenyl ring of the metabolite. The fragment ions at m/z 162 and 135 in the EI mass spectrum of *N*-TFA-MDMA are shifted to m/z 260 and 233 (98 daltons) in the EI mass spectrum of the metabolite. A mass shift of 98 daltons is consistent with the trifluoroacetylation of a phenolic functional group formed by O-dealkylation of the (3,4-methylenedioxy)phenyl group and subsequent O-methylation of one of the phenolic groups. The metabolite was further tentatively identified as 4-hydroxy-3-methoxymethamphetamine (III), on the basis of a report that the catechol *O*-methyltransferase that catalyzes the methylation reaction exhibits stereoselectivity for the *m*-hydroxyl group (*44*). This structural assignment was subsequently shown to be correct by comparison of the mass spectra (EI and CI) and relative retention time of this metabolite with those of the TFA derivative of synthesized 4-hydroxy-3-methoxymethamphetamine.

The EI mass spectrum of the TFA derivative of metabolite III shows no molecular ion, but a very weak protonated molecule (m/z 388) is present due to self-chemical ionization. Self-chemical ionization has been observed during acquisition of EI mass spectra with the ion trap detector when analyte concentrations are high (*45*).

The methanol CI mass spectra of the TFA derivatives of each of the MDMA metabolites exhibited prominent protonated molecules (Table I). All of the nitrogen-containing metabolites also showed a prominent CI fragment ion corresponding to loss of the *N*-trifluoroacetamide function. The protonated molecules were the only abundant ions in the methanol CI mass spectra of the MDMA metabolites that contain no nitrogen.

The EI mass spectrum of chromatographic peak D (Figure 4) shows the same m/z 260 and 233 fragment ions previously discussed in regard to the EI mass spectrum of

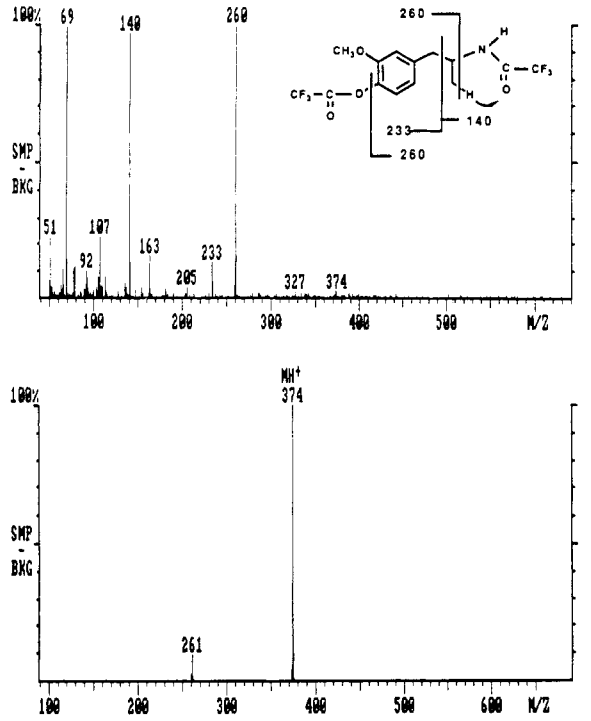

Figure 4. Electron ionization (top) and positive ion chemical ionization (bottom) mass spectra of the trifluoroacetyl derivative of MDMA metabolite IV (peak D in Figure 1).

Table I. Gas Chromatographic Relative Retention Times and CI-MS Data for Trifluoroacetyl Derivatives of MDMA and Its Metabolites

drug and metabolites	retention time rel to MDE	prominent CI ions, m/z (% of total ion current)
MDMA (I)	0.947	163 (100), 290 (MH^+, 9)
3-hydroxy-4-methoxymeth-amphetamine (II)	0.933	261 (25), 388 (MH^+, 100)
4-hydroxy-3-methoxymeth-amphetamine (III)	0.918	261 (13), 388 (MH^+, 100)
4-hydroxy-3-methoxy-amphetamine (IV)	0.825	261 (8), 374 (MH^+, 100)
3,4-dihydroxymethamphet-amine (V)	0.824	343 (? , 470 (MH^+, 100)
3,4-(methylenedioxy)-amphetamine (VI)	0.813	163 (100), 276 (MH^+, 90)
(4-hydroxy-3-methoxy-phenyl)acetone (VII)	0.629	277 (MH^+, 100)
[3,4-(methylenedioxy)-phenyl]acetone (VIII)	0.602	179 (MH^+, 100)
(3,4-dihydroxyphenyl)-acetone (IX)	0.552	359 (MH^+, 100)

chromatographic peak C, indicating that these two metabolites have the same phenyl ring substitution. However, the CI mass spectrum of chromatographic peak D indicates a molecular weight of 373, suggesting that this metabolite has one less methyl group than the metabolite corresponding to chromatographic peak C. Also, chromatographic peak D gave an EI fragment ion at m/z 140 [$CH_3CH{=}NHCOCF_3^+$] and a CI fragment ion at m/z 261 [$MH^+ - HN{=}C(OH)CF_3$], clearly indicating that it had lost the methyl group attached to the nitrogen in MDMA. Confirmation that chromatographic peak D corresponded to the TFA derivative of 4-hydroxy-3-methoxyamphetamine (IV) was based on the similarity of its mass spectra (EI and CI) and its relative retention time to those of the TFA derivative of synthesized 4-hydroxy-3-methoxyamphetamine.

Chromatographic peak E was readily identified as the TFA derivative of MDA (VI). The metabolite's EI and

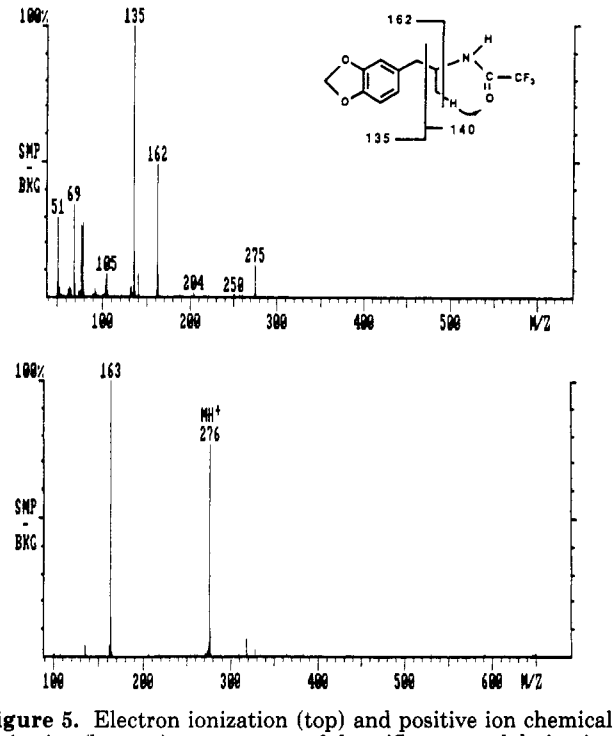

Figure 5. Electron ionization (top) and positive ion chemical ionization (bottom) mass spectra of the trifluoroacetyl derivative of MDMA metabolite VI (peak E in Figure 1).

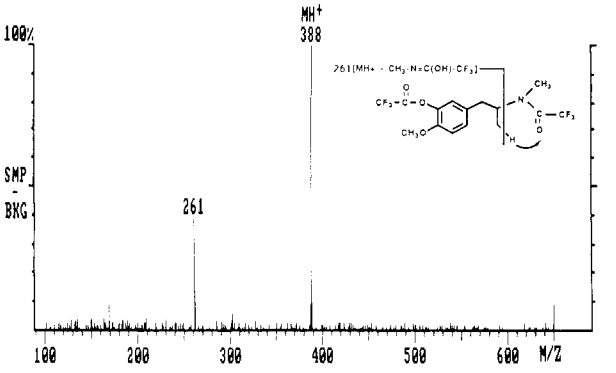

Figure 6. Positive ion chemical ionization of the trifluoroacetyl derivative of MDMA metabolite II (peak B in Figure 1).

CI mass spectra (Figure 5) show a molecular weight of 275 which suggests that the metabolite has one less methyl group than *N*-TFA-MDMA. The presence of an EI fragment ion at m/z 140 and of a CI fragment ion at m/z 261 [$MH^+ - HN{=}C(OH)CF_3$] indicates that the methyl group has been lost from the nitrogen of MDMA. The two most abundant EI fragment ions (m/z 162 and 135) indicate that the 3,4-(methylenedioxy)phenyl group remains unaltered in this metabolite. Conclusive identification of the metabolite as MDA was achieved by comparison of the EI and CI mass spectra of its TFA derivative and its relative retention time with those of a TFA derivative of authentic MDA.

The remaining MDMA metabolites identified in hydrolyzed rat urine were present in concentrations too low to yield interpretable EI mass spectra. However, good-quality CI mass spectra were obtained, and they provided the basis for tentative structural identifications. For example, the methanol CI mass spectrum of the very small chromatographic peak B is shown in Figure 6. This spectrum is nearly identical with the CI mass spectrum obtained for the TFA derivative of the metabolite identified as 4-hydroxy-3-methoxymethamphetamine (III, chromatographic peak C). We therefore concluded that

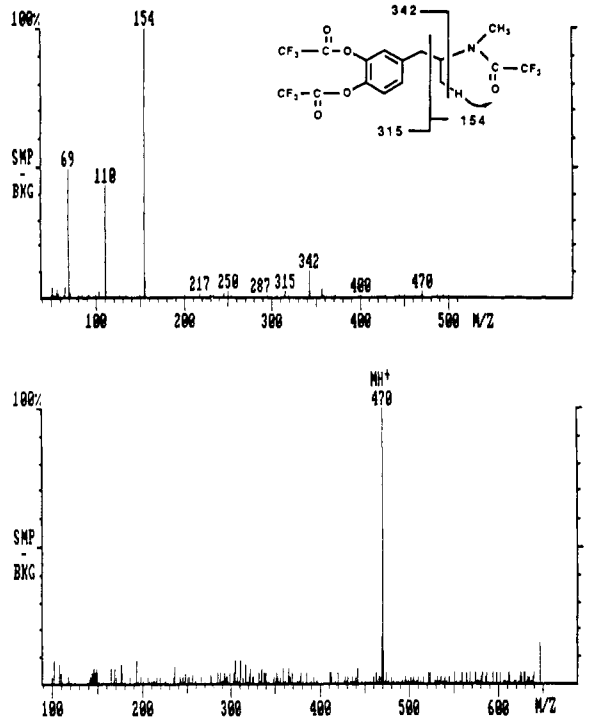

Figure 7. Electron ionization (top) and positive ion chemical ionization (bottom) mass spectra of the trifluoroacetyl derivative of MDMA metabolite V, identified as 3,4-dihydroxymethamphetamine.

it was isomeric with III and most likely corresponded to 3-hydroxy-4-methoxymethamphetamine (II). This conclusion was verified by comparison of the metabolite's relative retention time and its CI mass spectrum with those of synthesized 3-hydroxy-4-methoxymethamphetamine.

The CI mass spectra of chromatographic peaks F–H are each characterized by a single prominent ion corresponding to the protonated molecule of the metabolite or its TFA derivative. Since the molecular weights of these metabolites were even numbers, they must have lost their amine functional groups. Since oxidative deamination is a well-established metabolic process, we anticipated that some of the MDMA metabolites might be ring-substituted phenylacetones. This was verified by initial identification of chromatographic peak F as (4-hydroxy-3-methoxyphenyl)acetone (VII), peak G as [3,4-(methylenedioxy)-phenyl]acetone (VIII), and peak H as (3,4-dihydroxyphenyl)acetone (IX) based upon their CI mass spectra. Confirmation of these identifications was based on comparison of their relative retention times and the CI mass spectra of each metabolite or its TFA derivative with those of the corresponding synthesized compounds (Table I).

The hydroxylated metabolites of MDMA were excreted in the urine primarily as *O*-glucuronide and/or *O*-sulfate conjugates. However, trace quantities of 4-hydroxy-3-methoxymethamphetamine and 4-hydroxy-3-methoxyamphetamine were detected in unhydrolyzed urine. Comparison of the relative peak sizes of MDMA metabolites in both EI and CI total ion chromatograms suggested that 4-hydroxy-3-methoxymethamphetamine was the major metabolite in the basic extract of rat urine.

In Vitro Metabolites. An additional metabolite of MDMA was detected in extracts of the 10000*g* rat brain and liver incubates. The CI mass spectrum of the metabolite's TFA derivative contained a single prominent ion (m/z 470) which was assumed to be the protonated molecule (Figure 7). The derivative's molecular weight of 469 and the EI fragment ion at m/z 154 [$CH_3CH{=}N(CH_3)$-$COCF_3^+$] indicate the the metabolite's side chain remains unaltered from that of MDMA. That biotransformation must have occurred on the 3,4-methylenedioxy-substituted phenyl ring of this metabolite is supported by the structurally diagnostic EI fragment ions at m/z 315 and 342, which are shifted 180 daltons from the fragment ions in the spectrum of *N*-TFA-MDMA at m/z 135 and 162, respectively (Figure 2). A mass shift of 180 daltons can be accounted for by trifluoroacetylation of two phenolic functionalities formed by O-dealkylation of the 3,4-(methylenedioxy)phenyl group. The structure of this metabolite was tentatively assigned as 3,4-dihydroxymethamphetamine (V). This was confirmed by agreement of the relative retention time and both EI and CI mass spectra of the TFA derivative of the metabolite with those of synthesized 3,4-dihydroxymethamphetamine.

Table II. Distribution of MDMA and Its Metabolites in the Rat

drug and metabolites	urine	feces	blood	liver	brain
MDMA (I)	+	+	+	+	+
3-hydroxy-4-methoxymethamphetamine (II)	+			+	
4-hydroxy-3-methoxymethamphetamine (III)	++	+	+	++	+
4-hydroxy-3-methoxyamphetamine (IV)	+	+	+	+	+
3,4-dihydroxymethamphetamine (V)					+
3,4-(methylenedioxy)amphetamine (VI)	+	++	++	+	++
(4-hydroxy-3-methoxyphenyl)-acetone (VII)	+		+	+	
[3,4-(methylenedioxy)phenyl]-acetone (VIII)	+		+	+	+
(3,4-dihydroxyphenyl)acetone (IX)	+				

In addition to 3,4-dihydroxymethamphetamine, we also identified 4-hydroxy-3-methoxymethamphetamine, MDA, and [3,4-(methylenedioxy)phenyl]acetone in the 10000*g* liver incubate. MDA appeared to be the major MDMA metabolite formed in rat liver incubate. With the exception of 3,4-dihydroxymethamphetamine, the identification of the other metabolites in extracts of rat brain incubate was based solely on comparison of the relative retention times of the metabolites with those of synthesized compounds. We were unable to acquire high-quality CI or EI mass spectra for these metabolites because of their very low concentrations and the presence of interferences from the biological matrices. We detected these metabolites by displaying profiles of ion currents at m/z values corresponding to the protonated molecules of the TFA derivatives of each of the metabolites. As a result, 4-hydroxy-3-methoxymethamphetamine, 4-hydroxy-3-methoxyamphetamine, and MDA were identified in the 10000*g* brain incubate. Better selectivity for ionization of MDMA and its metabolites in the trifluoroacetylated basic extract of rat brain was achieved when acetone was used as the reagent gas. This is because acetone has a higher proton affinity (194 kcal/mol) than methanol (180 kcal/mol) (*31*).

Distribution of Metabolites. The distribution of MDMA and its hydrolyzed metabolites in the rat is summarized in Table II. We have not yet determined quantitatively the relative amounts of each of the MDMA metabolites in the various fluids and tissues. However, some semiquantitative observations can be based on the relative sizes of the metabolite peaks in the ion current profiles. All of the identified metabolites were detected in the 24-h urine except for 3,4-dihydroxymethamphetamine. Our inability to find this metabolite in urine, even after increasing the MDMA dose from 5 to 20 mg/kg, can perhaps be explained by its total conversion to 4-

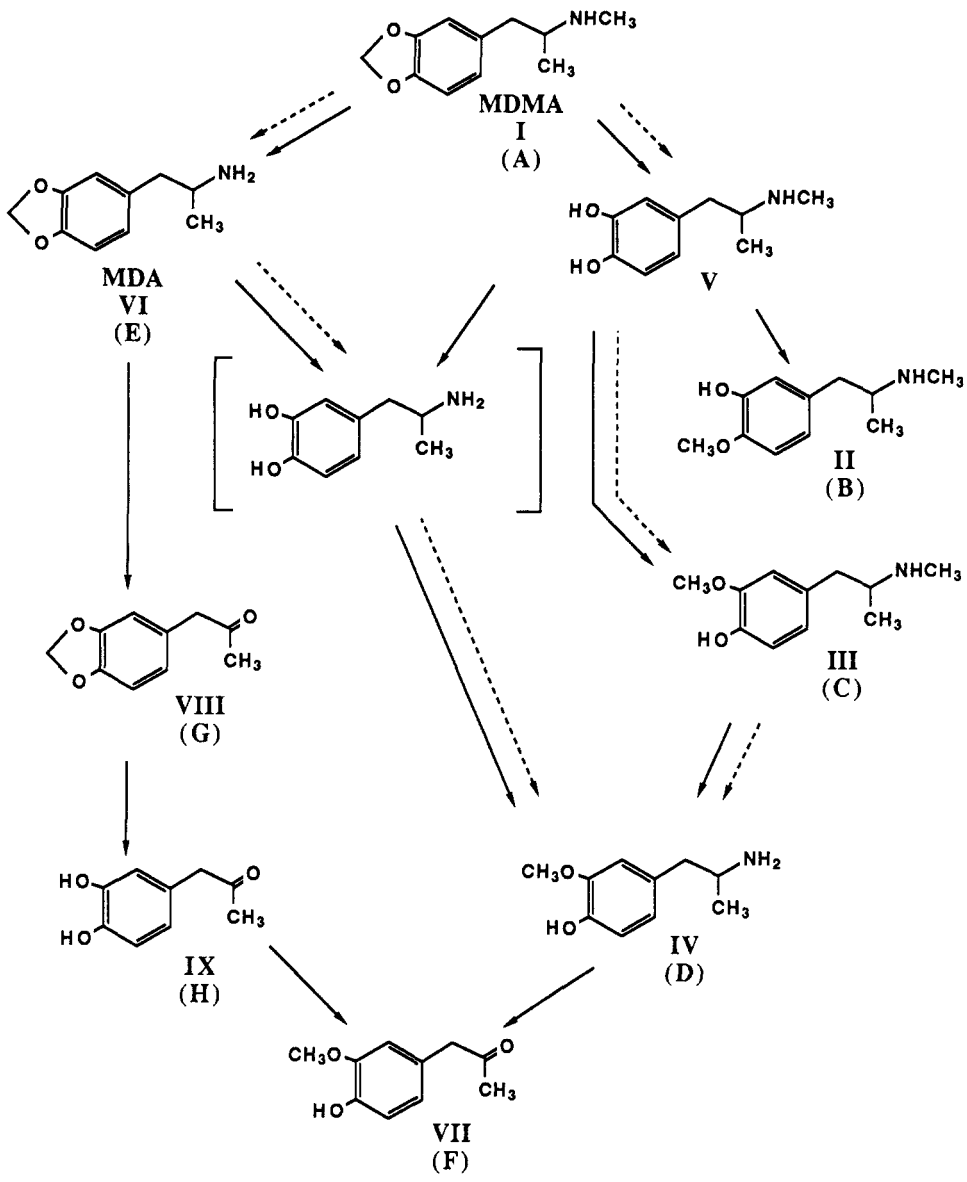

Figure 8. Metabolism of MDMA in the rat. Dotted arrows indicate metabolism occurring in the rat brain. The letters in parentheses correspond to the chromatographic peak labels shown in Figure 1.

hydroxy-3-methoxymethamphetamine via 3-*O*-methylation. Of the identified metabolites, only 4-hydroxy-3-methoxymethamphetamine, 4-hydroxy-3-methoxyamphetamine, and MDA were detected in the feces. The major metabolite in the feces was MDA, judging from the relative peak sizes of the metabolites. The metabolic profile of MDMA in the plasma is similar to that of the 24-h feces, except for two additional deaminated metabolites, (4-hydroxy-3-methoxyphenyl)acetone and [3,4-(methylenedioxy)phenyl]acetone. With the exception of [3,4-(methylenedioxy)phenyl]acetone, all of the metabolites were detected in the plasma 24 h after administration of 20 mg/kg MDMA. The metabolic profile of MDMA in rat liver is similar to that in the urine, except for the absence of 3,4-dihydroxymethamphetamine and (3,4-dihydroxyphenyl)acetone. However, only the *N*-demethyl and the 3-*O*-methyl phenolic amine metabolites of MDMA were detected in the liver 24 h after administration of 20 mg/kg MDMA.

Since MDMA is known to cause neurotoxicity for up to 14 days after a single dose (*27*), it is interesting to determine the metabolic profile of MDMA in the brain and also to examine the capacity of the brain to biotransform MDMA. All of the metabolites listed in Table II, except [3,4-(methylenedioxy)phenyl]acetone, were detected in the brain even 24 h after dosing with 20 mg/kg MDMA. The similarity in the profiles of the MDMA metabolites in the brain and the plasma suggests the possibility that these metabolites can penetrate the blood–brain barrier in the unconjugated form. However, brain metabolism could also contribute significantly to the brain concentration of these metabolites, since brain tissue in vitro has been shown to metabolize MDMA to each of the identified metabolites except for [3,4-(methylenedioxy)phenyl]acetone. This suggestion is consistent with a published report that the brain is able to cleave the methylenedioxy bridge of the prodrug of the potent dopamine agonist 10,11-(methylenedioxy)-*N*-propylnorapomorphine via O-dealkylation (*45*). Our demonstration of the ability of the rat brain to metabolize MDMA suggests the potential involvement of the metabolite(s) in MDMA-induced neurotoxicity, since one of the metabolites (MDA) is a known neurotoxin (*12*).

p-Hydroxyamphetamine has been reported to account for some of the pharmacological effects associated with amphetamine (*46*), so it would not be surprising if the phenolic amine metabolites of MDMA contribute to the psychopharmacology of MDMA, in view of the resemblance and/or potential conversion of the phenolic amine

metabolites of MDMA to endogenous catecholamine and indoleamine neurotransmitters, respectively.

Conclusions

The in vivo biotransformation pathways that have been identified for MDMA in rat are summarized in Figure 8. The metabolite in the brackets has not been detected but is a postulated intermediate in the formation of 4-hydroxy-3-methoxyamphetamine. MDMA was metabolized in the rat via O-dealkylation, N-demethylation, deamination, and conjugation (O-methylation, O-glucuronidation, and/or O-sulfation). Furthermore, the rat brain was shown to possess some of these biotransformation pathways (O-dealkylation, N-demethylation, and O-methylation). Hence, the metabolite(s) may have a significant role in the neurotoxicity and psychopharmacology of MDMA.

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Registry No. II, 117652-27-4; III, 117652-28-5; IV, 13026-44-3; V, 15398-87-5; VI, 4764-17-4; VII, 2503-46-0; VIII, 4676-39-5; IX, 2503-44-8; 4-hydroxy-3-methoxybenzaldehyde, 121-33-5; 4-hydroxy-3-methoxy-β-nitrostyrene, 6178-42-3; nitroethane, 79-24-3; methylamine, 74-89-5; (3,4-dimethoxyphenyl)acetone, 776-99-8; 3,4-dimethoxymethamphetamine, 33236-61-2.

References

(1) Glennon, R. A., and Young, R. (1984) "Further investigation of the discriminative stimuli properties of MDA". *Pharmacol., Biochem. Behav.* **20**, 501–505.
(2) Glennon, R. A., Young, R., Rosecrans, J. A., and Anderson, G. M. (1982) "Discriminative stimulus properties of MDA and related agents". *Biol. Psychiatry 17*, 807–814.
(3) Nichols, D. E., Hoffman, A. J., Oberlander, R. A., Jacob, P., III, and Shulgin, A. T. (1986) "Derivatives of 1-(1,3-benzodioxol-5-yl)-2-butanamine. Representative of a novel therapeutic class". *J. Med. Chem.* **29**, 2009–2015.
(4) Anderson, G. M., Braun, G., Braun, U., Nichols, D. E., and Shulgin, A. T. (1978) "Absolute configuration and psychotomimetic activity". In *Absolute Configuration and Psychotomimetic Activity in QUASAR: Quantitative Structure Activity Relationships of Analgesics, Narcotic Antagonists and Hallucinogens* (Barnett, G., Trsic, M., and Willette, R., Eds.) NIDA Research Monograph 22, pp 8–15, National Institute on Drug Abuse, Rockville, Maryland.
(5) Downing, J. (1986) "The psychological and physiological effects of MDMA on normal volunteers". *J. Psychoact. Drugs* **18**, 335–340.
(6) Peroutka, S. J., Neuman, H., and Harris, H. (1988) "Subjective effects of 3,4-methylenedioxymethamphetamine in recreational users". *Am. J. Psychiatry* (in press).
(7) Kamien, J. B., Johanson, C. E., Schuster, C. R., and Woolverton, W. L. (1986) "The effects of (±)-methylenedioxymethamphetamine and (±)-methylenedioxyamphetamine in monkeys trained to discriminate (+)-amphetamine from saline". *Drug Alcohol Depend.* **18**, 139–147.
(8) Shulgin, A. T. (1986) "The background and chemistry of MDMA". *J. Psychoact. Drugs* **18**, 291–304.
(9) Snyder, S. H. (1986) "Enlightment in a pill". In *Drugs and the Brain*, pp 179–205, W. H. Freeman, New York.
(10) Peroutka, S. J. (1987) "Incidence of recreational use of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') on an undergraduate campus". *N. Engl. J. Med.* **317**, 1542–1543.
(11) Dowling, G. P., McDonough, E. T., and Bost, R. O. (1987) "Eve and Ecstasy: a report of five deaths associated with the use of MDEA and MDMA". *JAMA, J. Am. Med. Assoc.* **257**, 1615–1617.
(12) Ricaurte, G., Bryan, G., Strauss, L., Seiden, L., and Schuster, C. (1985) "Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals". *Science (Washington, D.C.)* **229**, 986–988.
(13) Greer, G. (1983) *MDMA: A New Psychotropic Compound and Its Effects in Humans*. Copyright 1983, 333 Rosario Hill, Santa Fe, NM 87501.
(14) Barnes, D. M. (1988) "New data intensify the agony over Ecstasy". *Science (Washington, D.C.)* **239**, 864–866.
(15) Drug Enforcement Agency (1988) "Schedules of controlled substances: scheduling of 3,4-methylenedioxymethamphetamine (MDMA) into Schedule 1 of the Controlled Substances Act: remand". *Fed. Regist.* **53**, 5156–5157.
(16) Nichols, D. E., Lloyd, D. H., Hoffman, A. J., Nichols, M. B., and Yim, G. K. W. (1982) "Effects of certain hallucinogenic amphetamine analogues on the release of [^{3}H]serotonin from rat brain synaptosomes". *J. Med. Chem.* **25**, 530–534.
(17) Johnson, M. P., Hoffman, A. J., and Nichols, D. E. (1986) "Effects of the enantiomers of MDA, MDMA, and related analogues on [^{3}H]serotonin and [^{3}H]dopamine release from superfused rat brain slices". *Eur. J. Pharmacol.* **132**, 269–276.
(18) Nichols, D. E. (1986) "Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class: entactogens". *J. Psychoact. Drugs* **18**, 305–313.
(19) Lyon, R. A. Glennon, R. A., and Titeler, M. (1986) "3,4-Methylenedioxymethamphetamine (MDMA): stereoselective interactions at brain 5-HT$_1$ and 5-HT$_2$ receptors". *Psychopharmacology (Berlin)* **88**, 525–526.
(20) Gehlert, D. R., Schmidt, C. J., Wu, L., and Lovenberg, W. (1985) "Evidence for specific methylenedioxymethamphetamine (Ecstasy) binding sites in the rat brain". *Eur. J. Pharmacol.* **119**, 135–136.
(21) Davis, W. M., Hatoum, H. T., and Waters, I. W. (1987) "Toxicity of MDA (3,4-Methylenedioxyamphetamine) considered for relevance to hazards of MDMA (Ecstasy) abuse". *Alcohol Drug Res.* **7**, 123–134.
(22) Commins, D. L., Vosmer, G., Virus, R. M., Woolverton, W. L., Schuster, C. R., and Seiden, L. S. (1987) "Biochemical and histological evidence that methylenedioxymethamphetamine (MDMA) is toxic to neurons in the rat brain". *J. Pharmacol. Exp. Ther.* **241**, 338–345.
(23) Ricaurte, G., DeLanney, L., Irwin, I., and Langston, W. (1988) "Toxic effects of MDMA on central serotonergic neurons in the primate: importance of route and frequency of drug administration". *Brain Res.* **446**, 165–168.
(24) Schmidt, C. J., Levin, J. A., and Lovenberg, W. (1987) "In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain". *Biochem. Pharmacol.* **36**, 747–755.
(25) Stone, D. M., Stahl, D. C., Hanson, G. R., and Gibb, J. W. (1986) "The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain". *Eur. J. Pharmacol.* **128**, 41–48.
(26) Schmidt, C. J., (1987) "Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine". *J. Pharmacol. Exp. Ther.* **240**, 1–7.
(27) Stone, D. M., Merchant, K. M., Hanson, G. R., and Gibb, J. W. (1987) "Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in brain of rat". *Neuropharmacology* **26**, 1677–1683.
(28) Gollamudi, R., Lopez, M., Leaky, J., Webb, P., and Slikker, W., Jr. (1988) "Metabolism of 3,4-methylenedioxymethamphetamine (MDMA) by rat liver microsomes". *Toxicologist* 8, 200 (Abstract 797).
(29) Verebey, K., Alrazi, J., and Jaffe, J. H. (1988) "The complications of 'Ecstasy' (MDMA)". *JAMA, J. Am. Med. Assoc.* **259**, 1649–1650.
(30) Lim, H. K., Sakashita, C. O., and Foltz, R. L. (1987) "Toxicological applications of the ion trap detector". Proceedings of the 35th ASMS Conference on Mass Spectrometry and Allied Topics, Denver, CO, pp 696–697.
(31) Lim, H. K., Sakashita, C. O., and Foltz, R. L. (1988) "The application of chemical ionization to drug analysis". *Spectra* **11**, 10–14.
(32) Lim, H. K., Sakashita, C. O., and Foltz, R. L. (1988) "Chemical ionization with the ion trap detector: application to a drug assay requiring a wide dynamic range". *Rapid Commun. Mass Spectrom.* **2**, 129–131.
(33) Lim, H. K., and Foltz, R. L., unpublished data.

(34) Sasame, H. A., Ames, M. M., and Nelson, S. D. (1977) "Cytochrome P-450 and NADPH cytochrome *c* reductase in rat brain: formation of catechols and reactive catechol metabolites". *Biochem. Biophys. Res. Commun.* **78**, 919–926.
(35) Naslund, B. M. A., Glaumann, H., Warner, M., Gustafsson, J. A., and Hansson, T. (1988) "Cytochrome P-450 *b* and *c* in the rat brain and pituitary gland". *Mol. Pharmacol.* **33**, 31–37.
(36) Coutts, R. T. , Prelusky, D. B., and Baker, G. B. (1984) "Determination of amphetamine, norephedrine, and their phenolic metabolites in rat brain by gas chromatography". *J. Pharm. Sci.* **73**, 808–812.
(37) Coutts, R. T., and Kovach, S. H. (1977) "Metabolism in vitro of *N*-methylamphetamine with rat liver homogenates". *Biochem. Pharmacol.* **26**, 1043–1049.
(38) Lim, H. K., Andrenyak, D., Francom, P., Foltz, R. L., and Jones, R. T. (1988) "Quantification of LSD and *N*-demethyl-LSD in urine by gas chromatography/resonance electron capture ionization mass spectrometry". *Anal. Chem.* **60**, 1420–1425.
(39) Gairaud, C. B., and Lappin, G. R. (1953) "The synthesis of ω-nitrostyrenes". *J. Org. Chem.* **18**, 1–3.
(40) Morgan, P. H., and Beckett, A. H. (1975) "Synthesis of some N-oxygenated products of 3,4-dimethoxyamphetamine and its *N*-alkyl derivatives". *Tetrahedron* **31**, 2595–2601.
(41) McOmie, J. F. W., Watts, M. L., and West, D. E. (1968) "Demethylation of aryl methyl esters by boron tribromide". *Tetrahedron* **24**, 2289–2292.
(42) Musson, D. G., Halldin, M. M., Karashima, D., and Castagnoli, N., Jr. (1986) "Unexpected adduct ion formation under chemical ionization conditions". *Biomed. Environ. Mass Spectrom.* **13**, 287–291.
(43) Midha, K. K., Hubbard, J. W., Bailey, K., and Cooper, J. K. (1978) "α-Methyldopamine, a key intermediate in the metabolic disposition of 3,4-methylenedioxyamphetamine in vivo in dog and monkey". *Drug Metab. Dispos.* **6**, 623–630.
(44) Mesnil, M., Testa, B., and Jenner, P. (1984) "Xenobiotic metabolism by brain monooxygenases and other cerebral enzymes". In *Advances in Drug Research* (Testa, B., Ed.) Vol. 13, pp 96–207, Academic, London.
(45) Eichelberger, J. W., Budde, W. L., and Slivon, L. E. (1987) "Existence of self chemical ionization in the ion trap detector". *Anal. Chem.* **59**, 2730–2732.
(46) Dougan D., Wade, D., and Duffield, P. (1987) "How metabolites may augment some psychostimulant actions of amphetamine". *Trends Pharmacol. Sci.* 8, 277–280.