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STEREOCHEMICAL DIFFERENCES IN THE METABOLISM OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE *IN VIVO* AND *IN VITRO*: A PHARMACOKINETIC ANALYSIS

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ABSTRACT:

The *in vivo* *N*-demethylation of (+) and (−)3,4-methylenedioxymethamphetamine (MDMA) to 3,4-methylenedioxyamphetamine (MDA) was determined and the pharmacokinetic relationship between the two compounds calculated. The levels of MDA formed after iv administration of (+)MDMA to male rats were about 3 times greater than those for (−)MDMA, although the plasma levels of the parent drugs were comparable. Plasma MDA concentrations were lower in phenobarbital-pretreated rats, but SKF 525-A pretreatment, at the dose used, had minimal effects. In liver microsome experiments conducted with μM concentrations of (+)MDMA, 3,4-dihydroxymethamphetamine (*N*-methyl-α-methyldopamine) was shown to be the major metabolite. MDA was also formed *in vitro*, but the enantioselectivity was the opposite of that found *in vivo*, pointing out the difficulties in extrapolation of *in vitro* observations to *in vivo* disposition. The high levels of MDA observed after administration of (+)MDMA to intact animals suggest that this active metabolite could be important in the overall effects of (+)MDMA.

MDMA,[1] also called Ecstasy or Adam, is a recent entry into a group of phenylisopropylamines that are abused for their stimulatory and/or hallucinogenic activity (1). As the compound has also been used as an adjunct to group therapy in psychiatry, there is also interest in its legitimate medical use (2). The compound appears to be a mild hallucinogen and it has been shown to cause neurotoxicity in animals after large doses (3–6). The neurotoxicity is manifested as a reduction in 5-hydroxytryptamine levels and tryptophan hydroxylase activity (7, 8) and as lesions in the caudate nucleus (4, 9). One of the characteristics of the toxicity and depletion of 5-hydroxytryptamine is that the (+) isomer is more potent than the (−) (7). Similar enantiomeric differences were noted in the behavioral actions of the drug, and in this context the compound resembles methamphetamine, although the actual responses to the two drugs are different (10, 11). Like methamphetamine (12), however, MDMA undergoes *N*-demethylation to an active compound (13). The metabolite MDA, has similar actions to the parent drug (11) and the (+) isomer is more potent.

Knowledge of *in vivo* drug disposition is essential for the interpretation of experiments in intact animals, especially when an active metabolite is formed. MDMA presents a particularly important example of this need because its enantiomers form different amounts of an active metabolite and exhibit a difference in potency. This article describes the results of a study examining conversion of MDMA to MDA *in vivo* and *in vitro*. The *in vivo* disposition was monitored by determining plasma concentrations after iv administration to untreated animals and to others pretreated with agents that affect metabolic enzymes. Experiments with rat liver microsomes *in vitro* used drug concentrations in the same range as the plasma concentration.

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[1] Abbreviations used are: MDMA, 3,4-methylenedioxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; *N*-Me-α-MeDA, 3,4-dihydroxymethamphetamine (*N*-methyl-α-methyldopamine); $^2H_2$MDA, 1-(3′,4′-methylenedioxyphenyl)[1,2-$^2H_2$]isopropylamine; $^2H_2$MDMA, *N*-methyl-1-(3′,4′-methylenedioxyphenyl)[1,2-$^2H_2$]isopropylamine; α-MeDA, 3,4-dihydroxyamphetamine (α-methyldopamine); TFA, trifluoroacetic anhydride.

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Materials and Methods

Animals. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 280–330 g were used in all experiments. The rats were housed under a 12–12 hr light-dark cycle (lights on 7:00–19:00) and the room was maintained at 23–24°C. Rat chow and water were available *ad libitum*. All subjects were randomly assigned to treatment groups. In experiments *in vivo*, some rats were pretreated daily with phenobarbital-Na (80 mg/kg ip) for three days or with SKF 525-A (10 mg/kg, ip) 30 min before drug treatment. In the experiments *in vitro*, food was removed the night before microsome preparation.

Chemicals and Drugs. (+) and (−)MDMA and (+) and (−)MDA were obtained from the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD). *N*-Me-α-MeDA was synthesized in our laboratory by procedures described by Chavdarian et al. (14). $^2H_2$MDA and $^2H_2$MDMA were synthesized by procedures described earlier (15). α-MeDA was a gift from Merck Sharp and Dohme Laboratories (West Point, PA). SKF 525-A was a gift from Smith Kline & French Laboratories (Philadelphia, PA). Phenobarbital-sodium was purchased from Amend Drug and Chemical Co. (Irvington, NJ). α-Chloralose, NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and HEPES were obtained from Sigma Chemical Co. (St. Louis, MO). TFA was purchased from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were reagent grade and obtained from common commercial sources.

Cannulae Implantation. Vascular-Access-Ports (Model SLA, Norfolk Medical Products, Skokie, IL) were implanted 24 hr before blood sampling. Prior to insertion, the port was filled with sterile heparinized saline (20 units/ml). After pretreatment with atropine methylbromide (1.0 mg/kg, sc), the rats were anesthetized with diethyl ether. The dorsal and ventral cervical areas of the rat were shaved and incisions were made dorsally and on the right side of the neck over the jugular vein. The

catheter attached to the port was tunneled under the skin from the back to the neck area and the port was sutured into the back. The right external jugular vein was isolated, the distal end tied, and a catheter was inserted into the vein and advanced into the atrium. The neck wound was sutured and the rats were injected im with 60,000 units of penicillin G procaine suspension.

Blood Sampling. On the morning of an experiment, the rats were removed from their cages, weighed, and the ports flushed with 0.6 ml of heparinized saline (20 units/ml) using a 1 ml syringe attached to a 24 gauge Huber point needle. Because of difficulties in handling rats after injection of (+)MDMA, the effect of anesthesia (α-chloralose, 55 mg/kg, ip 30 min before MDMA) on the metabolism of MDMA was tested. Experiments comparing untreated and α-chloralose-treated animals revealed no significant difference in pharmacokinetics and MDA formation, and in all subsequent experiments, animals were anesthetized with α-chloralose before MDMA administration. The effects of phenobarbital (80 mg/kg, ip daily for 3 days) and SKF 525-A (10 mg/kg, ip) 30 min before MDMA) on the metabolism of MDMA were also tested under anesthesia.

(+) or (−)MDMA (10 mg/kg) was dissolved in 0.9% saline and injected iv *via* the port in a volume of 1.0 ml/kg. The ports were flushed with 0.6 ml of heparinized saline after the MDMA was administered. Blood samples were taken 5, 10, 20, 30, 60, 120, 180, 300, 420, and 600 min after drug injection. The needle was inserted into the port and 0.6 ml of fluid removed and retained. Blood (0.5 ml) was withdrawn into a heparinized syringe, and the original 0.6 ml of fluid was re-injected, followed by 0.5 ml of heparinized saline. The dead space volume in the Vascular-Access-Port is 0.15 ml, and dye studies have demonstrated that the withdrawal of 0.6 ml totally flushes the system.[2] The blood samples were centrifuged at 13,500*g* for 3 min, and the plasma frozen and stored at −80°C until assayed.

Enzyme Preparation. Rats were decapitated and their livers removed, cleaned, and weighed. Portions of liver (10 g) were homogenized in 30 ml of 1.15% KCl using a Potter-Elvejhem homogenizer and Teflon pestle. The homogenates were centrifuged at 9000*g* for 20 min in a refrigerated centrifuge. The supernatants were recentrifuged at 100,000*g* for 60 min. The resulting pellets were washed with the same volume of fresh 1.15% KCl and recentrifuged at 100,000*g* for 30 min. The pellets were stored at −80°C until required.

***In Vitro* Incubation.** The standard incubation mixture consisted of 0.95 ml of a solution containing 150 μmol of HEPES buffer, pH 7.6, 1.0 μmol of NADP, 11.4 μmol of glucose-6-phosphate, 4.5 μmol of $MgCl_2$, 2.5 units of glucose-6-phosphate dehydrogenase, 0.05 ml of a solution of (+) or (−)MDMA (200 μM), 0.5 ml of hepatic microsomes (1–2 nmol/ml P-450) in a final volume of 1.5 ml. The final concentration of MDMA, 6.7 μM, was used because it is comparable to the range of plasma concentrations seen 1 hr after MDMA injection *in vivo*. All enzymatic reactions were carried out at 37°C and were started by adding the microsomal preparation. The reactions were stopped by adding 12% $HClO_4$ containing internal standards, [$^2H_2$]MDMA and [$^2H_2$]MDA, and immersing the incubation tubes in an ice bath. After centrifugation (13,500*g*, 5 min), the supernatant was divided into two samples: one aliquot was used for the assay of catechol metabolites using HPLC-EC, and the other for the assay of MDMA and MDA using GC/MS.

Catechol Analysis by HPLC-EC. The supernatants from $HClO_4$ precipitation were assayed for catechol metabolites by HPLC with electrochemical detection. These metabolites were separated by reverse phase chromatography using Biophase ODS 5 μm (4.6 × 250 mm, Bioanalytical Systems, Inc., West Lafayette, IN) and 0.1 M citrate buffer, pH 5.5, containing 7.5% methanol, 50 mg/liter EDTA and 15 mg/liter sodium octylsulfate at 0.9 ml/min flow rate. Electrochemical measurements were made using a glassy carbon working electrode set at +0.7V *vs.* Ag/AgCl reference electrode (LC-4, Bioanalytical Systems). In the analysis, the samples were injected directly onto the HPLC column without pretreatment by an autoinjector equipped with refrigeration. Samples were stable for at least 24 hr under these conditions. Signals were recorded with a Hewlett Packard 3390A recording integrator, and the peak height of each compound was compared with that of the standard sample. Retention times for α-MeDA and *N*-Me-α-MeDA were 13.0 min and 15.5 min, respectively.

MDMA and MDA Analysis by GC/MS. Volumes of 0.2 to 0.5 ml of plasma were treated with 2 ml of 3% $HClO_4$ containing internal standards [$^2H_2$]MDMA and [$^2H_2$]MDA, mixed and centrifuged for 20 min. The supernatant was transferred to a 50 ml extraction tube containing 2 ml of sodium carbonate buffer (1.5 M, pH 9.5) and 10 ml of dichloromethane. The resulting mixture was shaken and the organic layer collected, evaporated to 100 μl, and derivatized with TFA for injection into the GC/MS system. One ml of the supernatant obtained from *in vitro* experiments was transferred to a 50 ml extraction tube and processed in the same manner.

A Hewlett Packard 5971A GC/MS system was employed in the selected ion monitoring mode with [$^2H_2$]MDMA and [$^2H_2$]MDA as internal standards. Gas chromatography utilized a 12.5 m × 0.2 mm H-P fused silica capillary column with cross linked methylsilicone, .33 μ film thickness, operated with a temperature program from 70°C to 190°C at a rate of 40°C/min. Under these conditions, the retention times for MDMA-TFA and MDA-TFA were 4.3 and 3.7 min, respectively. The fragments monitored for quantitation are generated by β cleavage of the isopropylamine side chain (16). These fragments are major peaks in the electron impact mass spectra of phenylisopropylamines. d0-MDMA was monitored as the trifluoroacetylaminoethyl moiety with $M^+ = 154$, and the other three compounds were monitored at $m/z = 162$ (−MDA), or $m/z = 164$ ($^2H_2$ variants of MDA or MDMA). This latter fragment is the styrene generated by the elimination of trifluoroacetamide. Standard curves, made up and carried through the assay with each sample, had slopes that differed by less than 5%.

Data Analysis. Plasma MDMA and MDA levels and catechol levels were compared at each time point using Student's *t* test. Analysis of the error structure of the plasma concentrations showed that the standard deviation was linearly related to the mean value. With such an error structure, Zivin and Bartko (17) have pointed out that a log transformation allows a more balanced fitting to a model when the experimental values cover a wide range. Since the plasma concentration varied over three orders of magnitude, this transformation was employed.

The model used to emulate the data is depicted in fig. 1, in which all of the rate constants refer to first order processes, q_1 and q_2 designate the quantities of MDMA in the central and peripheral compartments, respectively, and q_3 is the quantity of MDA (assumed to distribute in only one compartment). The corresponding concentrations y_1 and y_3 are assumed to be proportional to q_1 and q_3 so that $q_1 = y_1V_1$ and $q_3 = y_3V_3$, where V_1 and V_3 are the apparent volumes of distribution. This com-

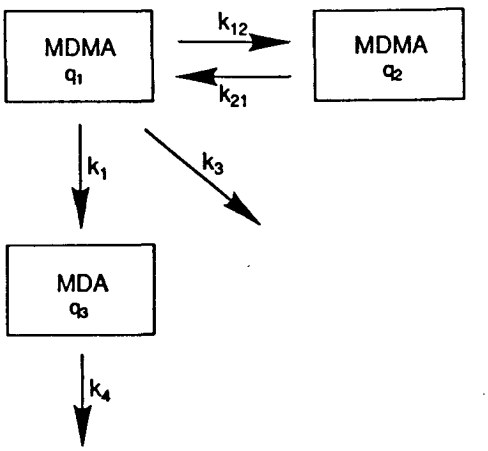

FIG. 1. *The model for two compartment decay of MDMA and first order formation and elimination of MDA.*

[2] Andrew Jacobson, personal communication.

partment system is described by the equations:

$$\frac{dq_1}{dt} = k_{21}q_2 - (k_{12} + k_3)q_1$$

$$\frac{dq_2}{dt} = k_{12}q_1 - k_{21}q_2$$

$$\frac{dq_3}{dt} = k_1q_1 - k_4q_3$$

$q_1(0) = q_0$; $q_2(0) = 0$; $q_3(0) = 0$ where q_0 is the dose. The solution is:

$$y_1 = \frac{q_0}{(\beta - \alpha)V_1}\left\{(k_{21} - \alpha)e^{-\alpha t}) - (k_2 - \beta)e^{-\beta t}\right\}$$

$$y_3 = \frac{k_1 q_0}{V_3}\left\{\frac{k_{21} - \alpha}{(k_4 - \alpha)(\beta - \alpha)}e^{-\alpha t} + \frac{k_{21} - \beta}{(k_4 - \beta)(\alpha - \beta)}e^{-\beta t} + \frac{k_{21} - k_4}{(\alpha - k_4)(\beta - k_4)}e^{-k_4 t}\right\} \quad (1)$$

where α and β are the roots of the quadratic equation

$$\lambda^2 - \lambda(k_1 + k_{12} + k_{21} + k_3) + k_{21}(k_1 + k_3) = 0$$

These functions were simultaneously fitted to experimental data representing measurements of y_1 and y_3 over the range $0 < t < 600$ min, using the BMDP iterative nonlinear regression program AR (18), and assuming equal variances for y_1 and y_3. Convergence problems were encountered when the model was fitted to data sets for individual animals, but disappeared when all the data were fitted simultaneously. This also allowed an evaluation to be made of the significance of differences in parameters between groups using the method of Motulsky and Ransnas (19), as described under *Results*.

Parameter estimates for AUC, t_1 and t_2 (the corresponding half-lives for α and β) and their standard deviations were estimated by reformulating equation (1) in terms of these parameters and fitting the transformed function to the same data. Values for AUC were calculated by integration of the appropriate equations from zero to infinite time.

Results

The plasma concentrations of MDMA and MDA found in control, phenobarbital, and SKF 525-A-pretreated animals are shown in figs. 2, 3, and 4, respectively. The changes in MDMA and MDA concentrations were fitted to a model (fig. 1) that describes the loss of MDMA from an open, two compartment system and the first order formation and elimination of MDA. The pharmacokinetic parameters for the enantiomers and for the different drug treatments are summarized in table 1. At the early time points, the plasma concentrations of MDMA enantiomers were very similar, and as a result the volumes of distribution were also similar. However, plasma concentrations of MDA exhibited a marked enantioselectivity and were significantly higher after the (+) isomer (fig. 2). Using the AUC for MDA as a measure of the total quantity of this active metabolite available to the animal, AUC values for MDA were 3 times greater after (+) MDMA.

To assess differences in the microscopic parameters of the model in fig. 1, the volume of distribution of MDA was set at 1.077 liter/kg, based on estimates from separate experiments (data not shown). Using this value and a dose of 43.77 μmol/kg, values for the microparameters for each group were determined by nonlinear regression.

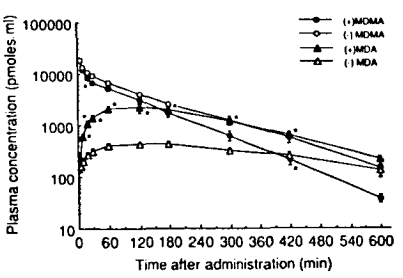

FIG. 2. *Time course of plasma MDMA and MDA concentration after iv injection of (+) or (−)MDMA.*

Each rat was administered (+) or (−)MDMA (10 mg/kg), iv. Values are the mean ± SE of 5–6 rats. * $p < 0.05$ *vs.* (−)MDMA.

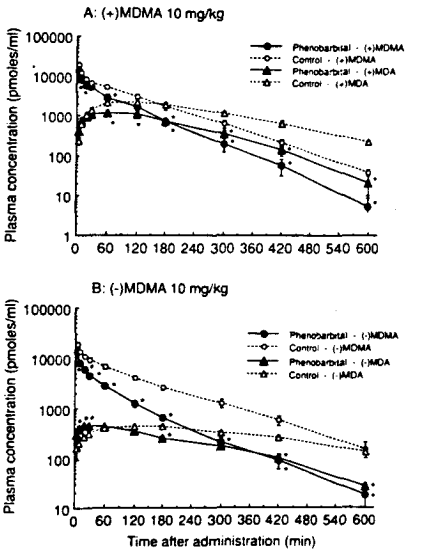

FIG. 3. *Effects of phenobarbital on the metabolism of (+) or (−)MDMA.*

Each rat was administered (+) or (−)MDMA (10 mg/kg iv). Phenobarbital (180 mg/kg, ip) was injected 3 days before experiments. Values are the mean ± SE of 5–6 rats. * $p < 0.05$ *vs.* control.

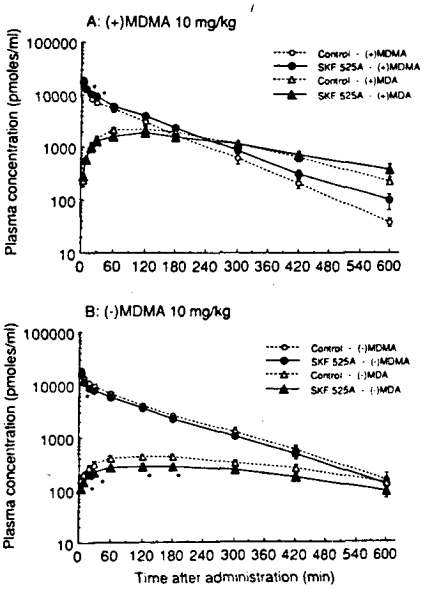

FIG. 4. *Effects of SKF 525-A on the metabolism of (+) or (−)MDMA.*

Each rat was administered (+) or (−)MDMA (10 mg/kg, ip). SKF 525-A (10 mg/kg, ip) was injected 30 min before MDMA injection. Values are the mean ± SE of 5–6 rats. * $p < 0.05$ *vs.* control.

TABLE 1

Pharmacokinetic parameters of MDMA and availability of MDA

Data from each group of animals were fitted to a two compartment model to obtain estimates of the parameters (± asymptotic standard deviation values). *Vd.* = dose/co; Clearance = dose/AUC.

Treatment	*Vd*	AUC	Clearance	Half-life	Ratio[a]
	liter/kg	*μmol/min/liter*	*liter/kg⁻¹ min⁻¹*	*min*	+/−
Control					
(+)MDMA	1.24	1136.7 ± 59.1	.038	73.8 ± 2.5	
(+)MDA		661.4 ± 38.0			
(−)MDMA	1.33	1431.5 ± 68.3	.030	100.7 ± 8.7	
(−)MDA		213.7 ± 12.6			3.09
Phenobarbital (80 mg/kg, 3 days)					
(+)MDMA	1.34	627.4 ± 37.6	.069	63.5 ± 2.5	
(+)MDA		272.4 ± 18.50			
(−)MDMA	1.27	572.2 ± 38.1	.076	66.2 ± 2.8	
(−)MDA		135.7 ± 10.1			2.01
SKF 525-A (10 mg/kg, 30 min before MDMA)					
(+)MDMA	1.42	1382.4 ± 55	.033	84.6 ± 2.5	
(+)MDA		772.0 ± 32.7			
(−)MDMA	1.48	1245.0 ± 54.7	.034	99.4 ± 3.9	
(−)MDA		146.5 ± 8.0			5.27

[a] Ratio of AUC values for (+) and (−) MDA.

The significance of the differences between the parameter values was determined in pooled data calculations (19) in which the value of one parameter is shared between two groups and the residual sum of squares for shared and unshared calculations is used to determine F value. Since the individual calculations indicated that V_1, the volume of distribution of MDMA, and the values of k_{12} and k_{21} did not differ significantly among the groups, mean values were estimated from the pooled data. Using these values, the differences between the microparameters, k_1, k_3, and k_4 were assessed. The results of these calculations are shown in table 2. The values for k_1, the rate constant for MDA formation, showed a differential sensitivity to phenobarbital and SKF 525-A pretreatment. Although k_1 was larger for the (+) than the (−) isomer, only k_1 for the (−) isomer was increased in phenobarbital-pretreated animals and decreased in SKF 525-A-pretreated animals. This difference in k_1 sensitivity to induction and inhibition could reflect a difference in the enzymology of the reaction for the two enantiomers. The values for k_3, the major elimination rate constant, was also greater for the (+) than for the (−) isomer, reflecting the longer half-life and higher plasma levels of the (−) isomer at the long time points (fig. 2). Phenobarbital pretreatment increased k_3 for both isomers, but SKF 525-A affected only that for the (+) isomer. The phenobarbital effect was reflected in the bioavailability of MDMA since AUC values for both isomers were reduced substantially (table 1). Values of k_4 were also greater in the phenobarbital-pretreated groups.

The *N*-demethylation and demethylenation reactions were examined *in vitro* with microsomal preparations using the range of concentrations found in plasma. MDA formation exhibited a two-phase time course (fig. 5), with a fast phase between 0–5 min and a subsequent slower, but continuous phase similar to the *N*-demethylation of methamphetamine (12). The enantiomeric selectivity was the opposite of that observed *in vitro*. Thus, after a 60 min incubation, MDA formation from (−) MDMA was greater by a factor of about 1.7 (table 3). Catechol formation,. however, favored the (+) enantiomer, and at 3 min, the ratio of (+) to (−) *N*-Me-α-MeDA was 1.3 (table 3). At this time point, the catechol was the dominant metabolite and accounted for

TABLE 2

Microscopic rate constants

Data from all animals were fitted for the two-compartment open model and the first order formation and elimination of MDA, as described in *Methods*. The values for volume of distribution of MDMA(V_1), k_{12}, and k_{21} were obtained from pooled data. V_1: 1.6517 ± 0.1518; k_{12}: 0.0617 ± 0.0108; k_{21}: 0.0370 ± 0.003.

Treatment	k_1 ($min^{-1} \times 10^3$)	k_3 ($min^{-1} \times 10^3$)	k_4 ($min^{-1} \times 10^3$)	Data Points
Control				
(+)MDMA	2.47 ± 0.24[a]	24.36 ± 2.12[a]	5.85 ± 0.51	98
(−)MDMA	0.63 ± 0.06	19.05 ± 1.70	6.19 ± 0.63	98
Phenobarbital 80 mg/kg for 3 days				
(+)MDMA	2.60 ± 0.27	35.41 ± 3.03[b]	10.32 ± 0.66[b]	85
(−)MDMA	1.13 ± 0.11[a]	37.80 ± 3.14[a]	8.32 ± 4.3[c]	114
SKF 525-A 10 mg/kg, ip 30 min before MDMA				
(+)MDMA	2.21 ± 0.22	20.23 ± 1.80[b]	5.78 ± 0.54	96
(−)MDMA	0.45 ± 0.04[a]	20.70 ± 1.82	5.92 ± 0.55	108

[a] $p < 0.01$ *vs.* (−)MDMA control.
[b] $p < 0.01$ *vs.* (+)MDMA control.
[c] $p < 0.05$.

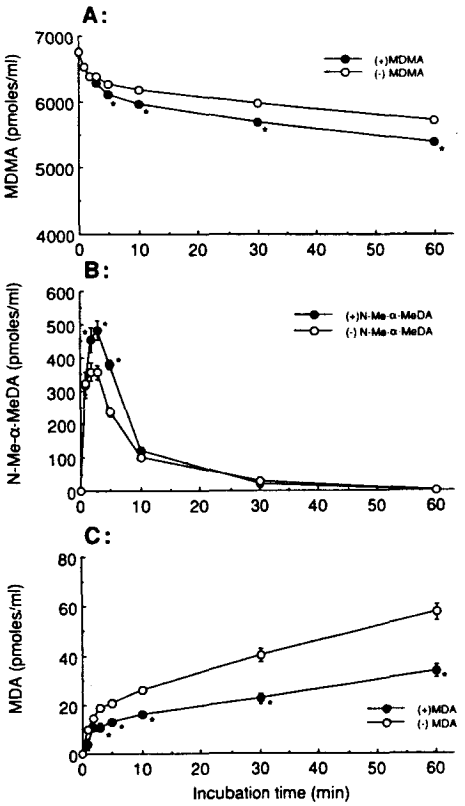

FIG. 5. *Time course of MDMA metabolism by rat liver microsomes.*

(+) or (−)MDMA (6.8 μM) were incubated with rat liver microsomes for 0–60 min. Final P-450 concentration was 1–2 μM. Values are the mean ± SE of 4 experiments. * $p < 0.05$ *vs.* (−)MDMA.

TABLE 3

Distribution of metabolite after microsomal metabolism of MDMA

MDMA (6.8 μM) was incubated for the indicated times with rat liver microsomes according to procedures described in *Methods*. Levels of MDMA and MDA were determined by GC/MS and those of *N*-Me-α-MeDA by HPLC-EC. The results are the mean ± SE of four experiments.

Isomer	Time	Substrate Consumed	MDA	*N*-Me-α-MeDA
	min	*pmol/ml/nmol P-450*		
(+)MDMA	3	476.0 ± 41.2	10.8 ± 1.7[a]	481.6 ± 28.9[a]
(−)MDMA	3	380.9 ± 54.2	18.7 ± 1.7	354.7 ± 21.2
(+)MDMA	10	790.7 ± 35.8[a]	16.1 ± 1.1[a]	119.8 ± 13.2
(−)MDMA	10	575.7 ± 30.0	26.1 ± 1.5	100.0 ± 7.7

[a] $p < 0.05$ *vs.* (−)MDMA.

essentially all of the consumed substrate. The subsequent decline of the catechol appears to be due to its oxidation by a superoxide-mediated reaction.[3] The catechol metabolite of MDA, α-MeDA, was not detected in these studies, although it was present in separate incubations of MDA (data not shown).

Discussion

The study of psychoactive drugs requires *in vivo* experiments, and much of the pharmacology and toxicology of MDMA has been based on whole animal experiments. However, little is known of the pharmacokinetics of this compound in the animal in which it has been extensively studied. Although MDMA conversion to the active compound MDA is known (13, 20), these experiments demonstrate that MDA levels are subject to considerable enantiomeric differences. Since (+) MDA is more potent than (+) MDMA in some effects (11), and since its plasma concentration exceeds that of MDMA after 5 hrs, this compound could contribute significantly to MDMA pharmacology *in vivo*. Thus, in addition to enantiomeric differences in the pharmacology of MDMA (11, 21), differences in metabolism may participate in the overall pharmacology of this compound.

The bioavailability of MDMA and MDA was decreased after phenobarbital pretreatment. This observation is relevant to the work of Schmidt and Taylor (22), who have reported that phenobarbital pretreatment had no effect on MDMA-induced changes in tryptophan hydroxylase activity of serotonin concentrations. Because of the changes in bioavailability of both parent and active metabolites after pretreatment, the role of metabolism in these actions of MDMA cannot be assessed. The effects of SKF 525-A were minimal, but the dose employed was also minimal compared to those used in other studies (23). The choice of the low dose was based on earlier observations that demethylenation was extremely sensitive to this compound.[4] However, Battaglia and co-workers (24) have reported neither potentiation nor attenuation of neurodegeneration following MDMA administration to SKF 525-A-pretreated animals, so overall MDMA metabolism may not be sensitive to this agent.

The *in vitro* metabolic experiments were conducted at low concentrations (6.8 μM) to be consistent with the plasma concentrations, and the stereoselectivity for *N*-demethylation noted was opposite of that found *in vivo*. A possible explanation for this discrepancy is the involvement of the flavin-dependent amine oxidase in the reaction. Prior studies (13) had demonstrated the participation of this oxidase in MDMA conversion to MDA, and the related methamphetamine has also been shown to undergo *N*-demethylation by this enzyme system (12). The inability of phenobarbital pretreatment to increase k_i for the (+) isomer is also consistent with this possibility. This enzyme is very unstable, however (25), and its activity could be decreased substantially in microsomes. Attempts were made to assess this possibility by comparing activity in microsomes that had been stored at −80°C with a fresh preparation, but the change in activity was very small and did not reverse the enantioselectivity.[5] These observations point out the hazards of extrapolation of *in vitro* observations to *in vivo* metabolism and disposition. Although the substrate concentrations may be comparable between tissue and *in vitro* incubation mixtures, the activities of enzymes examined in subcellular preparations could be different from those in the intact tissue where cofactor levels are not fixed.

In summary, these studies have shown that the availability of MDA from MDMA *in vivo* exhibits a substantial stereoselectivity that is not reflected in metabolic studies *in vitro*. The effects of pharmacological manipulation on the pharmacokinetics are consistent with the involvement of cytochrome P-450 in the overall metabolism of MDMA and the availability of MDA from the (−) isomer. The higher *in vivo* availability of MDA from (+) MDMA may be the result of metabolic differences, but separate studies on MDA disposition will be required to establish this possibility.

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[3] M. Hiramatsu, *et al.* Manuscript in preparation.

[4] Unpublished data.

[5] Unpublished data.

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