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# Analysis of the Enantiomers of 3,4-Methylenedioxy-N-Ethylamphetamine (MDE, "Eve") and Its Metabolite 3,4-Methylenedioxyamphetamine (MDA) in Rat Brain

Kathy M. Hegadoren, Glen B. Baker, and Ronald T. Coutts

*Neurochemical Research Unit, Department of Psychiatry and Faculty of Pharmacy of Pharmaceutical Sciences University of Alberta, Edmonton, Alberta, Canada*

The methylenedioxy analogues of amphetamine are used recreationally despite concerns raised regarding potential neurotoxicity of the parent compounds and a number of metabolites. Much has been written regarding 3,4-methylenedioxymethamphetamine (MDMA; "Ecstasy"), but there is less information available on 3,4-methylenedioxy-N-ethylamphetamine (MDE; "Eve"), despite recent reports indicating the abuse of this drug and its potentially serious side effects. An assay procedure was developed for the simultaneous quantitation of both enantiomers of MDE and its metabolite MDA; the method involves derivatization with an optically pure reagent and analysis on a gas chromatograph equipped with a capillary column and a nitrogen-phosphorus detector. Brain levels of the enantiomers of MDE and MDA were examined in the rat at different time periods after acute i.p. injections of racemic MDE and the results were compared with levels of MDMA and MDA obtained after i.p. injection of MDMA in a previous study from our laboratories. The levels of the enantiomers of MDE and MDA achieved at 1, 4, and 8 hr were lower than in the case of MDMA. Stereoselective differences in brain levels of enantiomers of the parent drug and metabolite were much less marked with MDE than with MDMA, but where these small differences did exist in the case of MDE, the (R)-(−) vs (S)-(+) relationship was opposite to that reported for MDMA.

**Key Words:** Chirality; Methylenedioxy analogues of amphetamine; Gas-liquid chromatography; Metabolic N-dealkylation

## Introduction

The methylenedioxy analogues of amphetamine (Figure 1) have been classified as "designer amphetamines," a term used to describe a variety of recreationally used amphetamine derivatives designed specifically to circumvent federal laws regarding controlled substances. Both 3,4-methylenedioxyamphetamine (MDA; "Love") and its N-methyl analogue 3,4-methylenedioxymethamphetamine (MDMA; "Ecstasy") are used recreationally, with increasing popularity, particularly by U.S. college students (Peroutka, 1990). There are increasing numbers of reports of psychiatric complications induced by MDMA use, including paranoid psychosis, anxiety, and depressive symptoms (McGuire and Fahy, 1991; Series et al., 1994). Another recreationally used methylenedioxy analogue, 3,4-methylenedioxy-N-ethylamphetamine (MDE; "Eve") has been the focus of recent media attention as a "rave" drug, used at all-night dance clubs (Tehan et al., 1993). Reports of sudden collapse and death have appeared in newspapers in England. Case reports include toxic psychosis and life-threatening hyperthermia after MDE ingestion (Battaglia et al., 1987; Tehan et al., 1993).

Many researchers have expressed concerns about the use of MDMA and MDA in light of evidence of neurotoxic changes to 5-hydroxytryptamine (5-HT; serotonin) neurons in rats and monkeys after acute or chronic administration (Battaglia et al., 1987; Schmidt et al., 1987; Kleven et al., 1989). The acute neurochemical effects of MDE, which include reductions in levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) and

Address reprint requests to Dr. Glen B. Baker, Neurochemical Research Unit, Department of Psychiatry, University of Alberta, Edmonton, Alberta, Canada, T6G 2B7.

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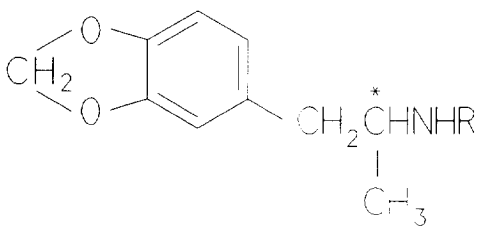

**Figure 1.** Chemical structures of MDE and MDMA and their N-dealkylated metabolite, MDA. MDE: R = $CH_2CH_3$; MDMA: R = $CH_3$; MDA: R = H. * denotes chiral center.

tryptophan hydroxylase activity in the frontal cortex and the hippocampus, are similar to those seen with MDMA and MDA. However, there is greater recovery of enzyme activity and of 5-HT and 5-HIAA levels after MDE (Johnson et al., 1987; Stone et al., 1987). This has led to the suggestion that MDE might be a less potent neurotoxin than MDMA or MDA. The basis for this difference in potency has not been established.

The mechanisms involved in the neurochemical changes seen after administration of these compounds remain unclear, but it has been suggested that one or more metabolites may be involved in the neurotoxic effects of the parent compounds (Molliver et al., 1986; Schmidt, 1987; Yeh and Hsu, 1987; Zhao et al., 1992). MDMA undergoes metabolic N-dealkylation to yield MDA, which could contribute to both the behavioral effects and the longer-term neurotoxic effects. Lim and Foltz (1988) reported that MDA appeared to be the major metabolite of MDMA formed in rat liver incubate. However, other metabolic paths have been identified and include O-demethylenation and aromatic hydroxylation to produce catechol-containing metabolites (Lim and Foltz, 1988; Lim and Foltz, 1991). In fact, Lim and Foltz (1991) found that the major metabolite of MDMA in both rat and mouse urine was 4-hydroxy-3-methoxymethamphetamine. The trihydroxy-methamphetamine metabolite of MDMA, but not the dihydroxy-metabolite, has been shown to produce decreases in 5-HT levels, suggestive of neurotoxic potential (Johnson et al., 1992; Zhao et al., 1992). The metabolism of MDA has also been studied (Kumagai et al., 1991; McCann and Ricuarte, 1991; Nakagawa et al., 1993). Many of the products of O-demethylenation seen after MDMA administration are also seen after MDA, the only difference being the presence of the N-methyl group in the case of MDMA. Enblin et al. (1990) examined the metabolites of MDE in human urine and identified three metabolic paths: N-deethylation, oxidative deamination followed by side-chain oxidation to yield benzoic acid derivatives, and O-demethylenation. The metabolic paths are similar to those seen in animal studies with MDMA and MDA. However, no quantitative nor stereoselectivity data were reported.

As with many of the amphetamine analogues, MDMA, MDA, and MDE contain chiral centers. Although it is the racemates of these compounds that are usually used recreationally, animal studies have been conducted on the comparative effects of the individual enantiomers. Several researchers have found stereoselective effects of MDMA and MDA on behavior and on the potency of the neurochemical effects on 5-HT systems (Nichols, 1986; Rosecrans and Glennon, 1987; Schmidt et al., 1987; Steele et al., 1987). Stereoselective differences in the levels of the enantiomers of both MDMA and MDA in whole blood and in brain and liver tissues of rats after acute administration of racemic MDMA (*rac*-MDMA) have been reported, with significantly higher levels of (R)-(−)-MDMA than (S)-(+)-MDMA, and the reverse with MDA—higher levels of (S)-(+)-MDA than (R)-(−)-MDA (Fitzgerald et al., 1989; Cho et al., 1990; Hegadoren et al., 1993). Tucker et al. (1994) examined the kinetics of the demethylenation of MDMA and also found stereoselectivity favoring formation of (S)-(+)-MDA over (R)-(−)-MDA. Nakagawa et al. (1993) demonstrated that the metabolism of MDA also contained stereoselective pathways. Metabolism and stereoselectivity could play a role in the observed differences in the potency and the duration of the neurochemical effects seen after administration of MDE compared with MDMA and MDA. In the investigation reported here, we developed an assay for simultaneous analysis of the enantiomers of MDE and MDA (a proposed metabolite) and applied it to rat brain at different time periods after the acute administration of racemic MDE (*rac*-MDE). The results were compared to those of a previous study in which we measured the enantiomers of MDMA and MDA in rat brain at the same time intervals after injection of racemic MDMA.

## Materials and Methods

### *Chemicals*

The hydrochloride salts of *rac*-MDE and -MDA and the individual enantiomers of MDE and MDA were supplied by the Health Protection Branch, Department of Health and Welfare, Canada. The optically pure derivatizing reagent, N-trifluoroacetyl-L-prolyl chloride (TFPC), was purchased from Aldrich (Milwaukee, WI). This reagent was chosen because it is commercially available and has been used by other researchers for the chromatographic analysis of the enantiomers of MDMA and a number of other compounds (Hermanssen and Von Bahr, 1980; Silber and Reigelman, 1980; Fitzgerald et al., 1989; Aspeslet et al., 1994). Another derivatizing reagent, heptafluorobutyryl-L-prolyl chloride (HFPC), was obtained from Drs. J. Hubbard and G. McKay, College of Pharmacy, University of Saskatchewan,

Saskatoon, Canada, and had been prepared according to the reported procedure of Lim et al. (1986).

### *Animals*

Male Sprague-Dawley rats were given acute intraperitoneal injections of *rac*-MDE (10 mg/kg, based on free base weight) and killed at 1, 2, 4, and 8-hr post-administration by guillotine decapitation. Brain tissues were removed, frozen immediately, and stored at −80° C until the time of analysis. All procedures involving the use of the rats were approved by the Health Sciences Animal Welfare Committee, University of Alberta.

### *Sample Preparation and Analysis*

Analysis of levels of the enantiomers of MDE and MDA were conducted using a modification of a gas chromatography (GC) procedure developed in our laboratories (Hegadoren et al., 1993). Tissue samples were homogenized in ice-cold 0.1N perchloric acid and centrifuged. The supernatant, to which the internal standard 2-(4-chlorophenyl)ethylamine had been added, was basified with 10% $Na_3PO_4$ and shaken with a solvent mixture (4 mL of toluene:ethyl acetate, 9:1) for 10 min. After a brief centrifugation, the organic layer was retained and was taken to dryness; the residue was reacted with TFPC in a heating block at 60° C for 30 min. The reaction mixture was then partitioned between toluene (100 μL) and saturated sodium borate buffer (1 mL). The organic layer was retained and an aliquot was injected on a gas chromatograph equipped with a fused silica capillary column (25 m × 0.32 mm i.d.) coated with a 0.52-μm film thickness of 5% phenyl methyl silicone (Hewlett Packard Co., Palo Alto, CA) and a nitrogen phosphorus detector. The carrier gas was pure helium (Linde, Union Carbide) at a flow rate of 2 mL/min. The detector was purged with pure hydrogen (Linde, Union Carbide) at 3.5 mL/min mixed with dry air (Linde, Union Carbide) at 80 mL/min. Temperatures at the injection port and detector were 250° C and 325° C, respectively. The initial oven temperature of 105° C was programmed to increase at a rate of 10° C/min to 270° C. Coupled GC-MS was used to confirm the structures of the derivative of MDE. The mass spectrometer (Hewlett Packard 5985A with HP 7920 data system) was operated in the electron-impact mode, and the GC conditions were as described above. Operating conditions for the mass spectrometer were as follows: ion source temperature, 200° C; interface temperature, 275° C; column pressure, 34.5 kPa; accelerating voltage, 2200 eV; ionization voltage, 70 eV; scan speed, 200 amu/sec; and dwell time, 200 msec.

### *Purity and Stability of Reagent*

The reagent TFPC has been reported to racemize to a small extent on standing (Adams et al., 1982). To monitor this, we stored the TFPC at 0–4° C and reacted standards of individual enantiomers of the drugs of interest with the reagent at various times throughout the experiment. As further confirmation of the MDE and MDA values that were obtained with TFPC, we repeated the experiment at the 1-hr time interval using HFPC, a reagent that has been reported to be less prone to racemization (Lim et al., 1986). The extraction and reaction conditions were as described above, except that the reaction with HPFC was allowed to proceed at room temperature instead of 60° C.

### *Calibration*

Calibration curves were constructed with each assay run and were prepared by spiking drug-naive brain supernatant after homogenization and centrifugation with known quantities of *rac*-MDE and -MDA and the internal standard 2-(4-chlorophenyl)ethylamine. Peak height ratios of each of the four enantiomers to the internal standard were used to calculate linear regression lines, and the peak-height ratios from the brain extracts were compared to those on the calibration curves to calculate the quantities of the enantiomers in the brain sample. Mean absolute recovery rates were calculated using 300 ng and 25 ng of the individual enantiomers in spiked supernatant. The stabilities of the derivatives were also examined by storing derivatized samples at −80° C and reinjecting into the gas chromatograph 2, 6, 10, and 14 days later.

### *Statistics*

Analyses of variance, with enantiomer as the repeated factor and hour as the between factor, were conducted for both MDE and MDA. Multiple $F$ tests ($p < .05$) were conducted to calculate the critical difference required between each set of enantiomers to achieve statistical significance.

## Results

### *Quantitation*

The enantiomers of MDE and MDA were readily separated and quantitated. Over a range of 25–2000 ng for (R)-(−)- and (S)-(+)-MDE and 10–250 for (R)-(−)- and (S)-(+)-MDA, the standard curves were linear, with correlation coefficients > 0.99 obtained routinely. Brain concentrations calculated from the calibration curves were adjusted to take into account the small quantities of impurity found when each of the enantiomers was

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assayed separately. The mean recovery rates of 300 ng and 25 ng of MDE ($N$ = 4) were [(R)-(−) = 89.5% and 83.6%; (S)-(+) = 91.2% and 84.9%, respectively]. Recoveries of (R)-(−)- and (S)-(+)-MDA had been reported by us previously (Hegadoren et al., 1993). Typical chromatographs of derivatized brain extracts from rats treated with vehicle or *rac*-MDE are shown in Figure 2. A peak representing an endogenous unidentified substance is present immediately in front of derivitized R-(−)-MDA, but does not interfere with the determination of peak heights. The proposed mass fragmentation of the derivative of MDE is depicted in Figure 3. The mass fragmentation of the derivatives of MDA and p-chlorophenylethylamine have been previously described (Fitzgerald et al., 1989; Hegadoren et al., 1993). Intra- and inter-assay coefficients of variation for (R)-(−)- and (S)-(+)-MDE at concentrations of 25 to 500 ng ($N$ = 6) ranged from 4.1% to 10.2% and 9.5% to 11.6%, respectively. The derivatized samples, stored at −80° C and reinjected into the gas chromatograph after 2, 6, 10, and 14 days, provided almost identical data, indicating that solutions of analyte remained stable over the 2-week time period.

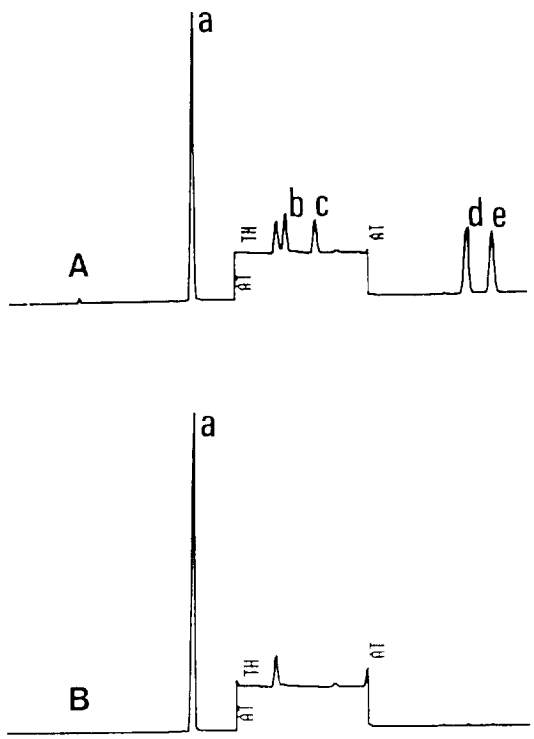


**Figure 2.** Typical chromatograms of derivatized brain extracts from rats treated with [A] rac-MDE or [B] vehicle. Retention times (in min) of the derivatives of (a) 2-(4-chlorophenyl)ethylamine (added internal standard), 19.08; (b) (R)-(−)-MDA, 20.63; (c) (S)-(+)-MDA, 21.12; (d) (R)-(−)-MDE, 23.64; and (e) (S)-(+)-MDE, 24.08. There were no interference peaks at the retention time corresponding to that of the internal standard. AT = points at which attenuation changes were programmed in.

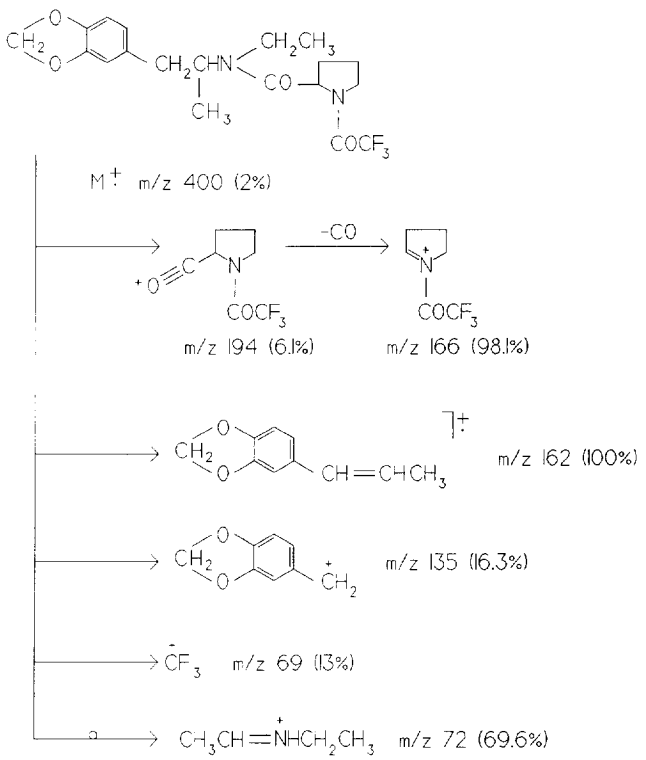


**Figure 3.** Proposed mass spectrometric fragmentation of the trifluoroacetylprolyl derivative of MDE. The percentages of relative abundances are indicated in parentheses.

In the confirmatory experiment at 1 hr in which analyses were done with both TFPC and HFPC, the levels of the enantiomers of MDE and MDA and the ratios to each other were very similar (R/S ratios for MDE of 0.97 and 0.98, respectively, and R/S ratios for MDA of 1.26 and 1.34, respectively).

## *Drug Levels*

Levels of the four enantiomers in whole brain are shown in Figure 4. By 8 hr, trace amounts of MDE and MDA were seen but were below our limits of detection. The levels of both MDE enantiomers at 1 hr and 4 hr were considerably lower than the previously reported levels of MDMA enantiomers after an equal dose (10 mg/kg) of *rac*-MDMA, while at 2 hr post-injection, the levels of both MDE enantiomers were lower than those of (R)-(−)-MDMA but not (S)-(+)-MDMA. There is significantly less MDA, especially the (S)-(+)-enantiomer, seen after MDE administration than is seen with MDMA.

The ratios in brain of the levels of the (R)-(−)- and (S)-(+)-enantiomers of MDE and MDA after *rac*-MDE

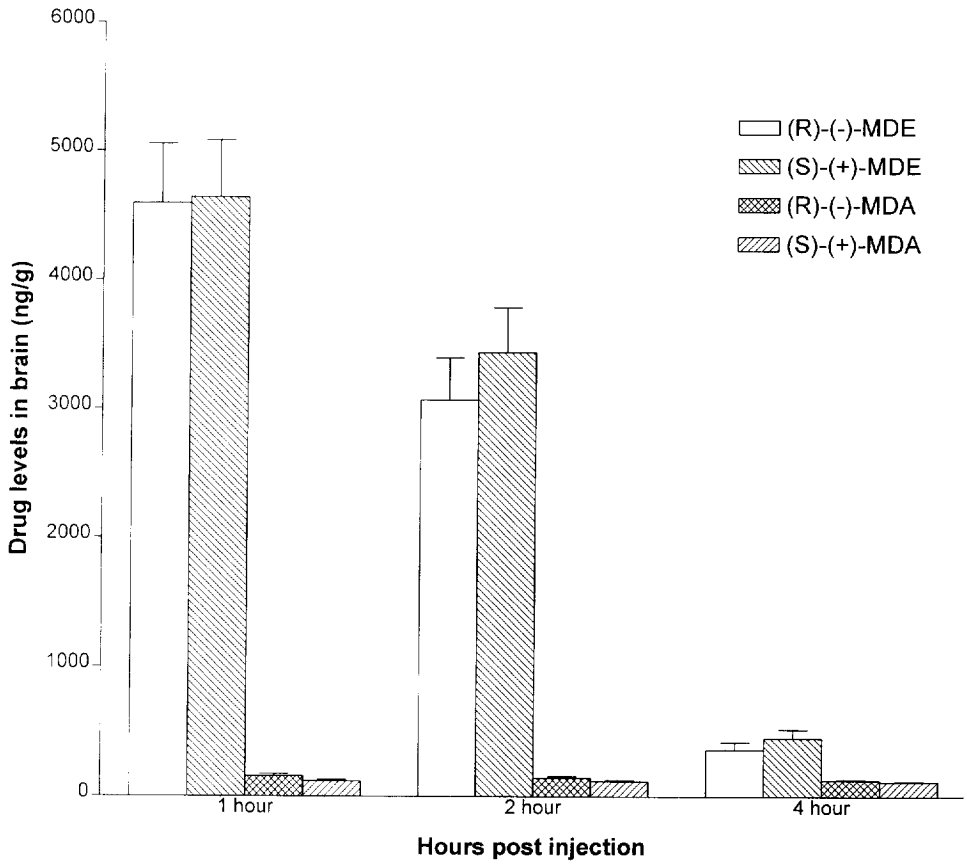


**Figure 4.** Whole brain levels of the enantiomers of MDE and MDA in rats after acute injections of *rac*-MDE (10 mg/kg). Values represent means ± SEM ($N$ = 7).

administration are markedly different than what has been previously reported after MDMA administration (Fitzgerald et al., 1989; Hegadoren et al., 1993) (Table 1). At 1 hr, there is no difference between the levels of (R)-(−)- and (S)-(+)-MDE and small but significant differences between the levels of (R)-(−)- and (S)-(+)-MDA, with (R)-(−) greater than (S)-(+) [MDE: $F$ (1, 18) = 87.5; MDA: $F$ (1, 18) = 17.7; $p < .05$]. At 2 hr there are small but significant stereoselective differences in the levels of both MDE and MDA, with more (S)-(+)-MDE and (R)-(−)-MDA than their antipodes. At 4 hr the difference between the levels of (R)-(−)- and (S)-(+)-MDA is no longer significant. Not only are the enantioselective differences much smaller in the case of MDE than MDMA, with regard to both the parent drugs and the MDA formed from them, the pattern of differences is reversed, as shown in Table 1.

**Table 1.** Enantiomeric Ratios [(R)/(S)] of Both Parent Drug and Metabolite in Rat Brain after Administration of *rac*-MDE or *rac*-MDMA (10 mg/kg, i.p.)

| | 1 Hr | 2 Hr | 4 Hr |
|---|---|---|---|
| *rac*-MDE | | | |
| (R)/(S)-MDE | 1.01 ± 0.009 | 0.89 ± 0.005* | 0.79 ± 0.013* |
| (R)/(S)-MDA | 1.31 ± 0.076* | 1.21 ±0.086* | 1.11 ± 0.074 |
| *rac*-MDMA | | | |
| (R)/(S)-MDMA | 1.20 ± 0.03* | 1.40 ± 0.048* | 2.16 ± 0.27* |
| (R)/(S)-MDA | 0.36 ± 0.015* | 0.41 ± 0.018* | 0.44 ± 0.013* |

Enantiomeric ratios for MDMA and its metabolite were calculated from data previously published by ourselves (Hegadoren et al., 1993). Results are expressed as means ± SEM ($n$ = 6 or 7). * Indicates significant difference between levels of (R)-(−) and (S)-(+) enantiomers ($p < .05$).

## Discussion

Our data indicate that TFPC is a suitable derivatizing reagent for the chiral separation of the compounds of interest, producing stable diastereoisomers that were readily separated and quantitated. However, TFPC has been reported to undergo some racemization and, as indicated by recent reports in the literature, it is important to monitor optical purity of reagents used (Allenmark, 1984; Wright and Jamali, 1993; Srinivas, 1994). Our monitoring and the added confirmation of the results obtained using HFPC give us confidence in the results reported here.

Although there is controversy regarding MDA's role in MDMA neurotoxicity (Molliver et al., 1978; Molliver et al., 1986), MDA could be an important intermediate

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preceding O-demethylenation to produce potentially neurotoxic catechol derivatives. No reports were found comparing the dihydroxy and trihydroxy metabolites of MDMA prior to N-demethylation to those following the loss of the N-methyl group in terms of neurotoxic potential and potency. The lower ratio of MDA to parent drug levels in the case of MDE compared to that with MDMA may be involved in the decreased potency to produce long-term effects on 5-HT in brain. The decreased potency of MDE relative to MDMA might also be related to the lower levels of the parent compound seen in brain tissues. The levels in brain of MDE are only approximately 50% of the levels of MDMA at 1 hr and 4 hr post-administration. Detailed pharmacokinetic studies are required in the future to address these matters adequately.

The differences in both the degree and pattern of stereoselectivity between the parent compounds MDMA and MDE and their N-dealkylated metabolite MDA are very interesting and somewhat surprising. Perhaps because N-deethylation becomes a minor metabolic path, the levels of MDA enantiomers reflect retention of stereoselectivity, but other less stereoselective paths overwhelm any impact N-deethylation has on the levels of the MDE enantiomers. Thus, initially levels of MDE do not demonstrate any stereoselectivity. Over time, as the levels of MDE enantiomers decline, small but significant differences are revealed. With both MDE and MDA, the differences between the sets of enantiomers at all time periods studied, even when significant, are small.

It has been suggested that the metabolic N-dealkylation of MDMA is catalyzed by the CYP450 family of enzymes (Hiramatsu et al., 1990). It is not presently known if a specific CYP450 isozyme is involved or whether a number of different isozymes can catalyze the reaction. Until the specific isozyme that catalyzes the N-demethylation of MDMA is known, we do not know whether it is the same isozyme involved in the N-deethylation of MDE and hampered by the ethyl group or whether it is a different isozyme with different kinetics. Cho et al. (1990) were unsuccessful in blocking MDMA N-dealkylation with the non-selective metabolic inhibitor SKF 525-A. Other metabolic pathways involving O-demethylenation and ring hydroxylation that are seen with MDMA and MDA are also likely to occur with MDE. Further metabolic studies are required to confirm this.

Our results show that marked differences exist between the levels in brain of enantiomers and of the parent drug and the metabolite MDA after MDE administration compared with MDMA. Further comparative studies between these two structurally similar compounds and an examination of the mechanisms involved in their long-term effects and the role that metabolism and chirality play in those effects, are needed. The continued recreational use of these drugs, despite the potential for toxic effects, makes further research critical.

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