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THE SIMULTANEOUS SEPARATION AND QUANTITATION OF THE ENANTIOMERS OF MDMA AND MDA USING GAS CHROMATOGRAPHY WITH NITROGEN-PHOSPHORUS DETECTION

K.M. Hegadoren, G.B. Baker and R.T. Coutts

Neurochemical Research Unit, Department of Psychiatry, and Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, CANADA, T6G 2B7.

ABSTRACT

A rapid and relatively inexpensive procedure is described for the simultaneous analysis of the enantiomers of MDMA and MDA. The method involves the use of extraction at basic pH followed by reaction with the unichiral reagent S-(-)-N-trifluoroacetylprolyl chloride and subsequent analysis on a gas chromatograph equipped with a nitrogen-phosphorus detector. The procedure has been applied to analysis in whole brain and individual brain regions in rats following administration of (±)-MDMA. Formation of MDA from MDMA was observed, and in the study in whole brain (at 1, 2, 4, and 8 h after MDMA injection) levels of the (-) enantiomer were higher than those of the (+) enantiomer in the case of MDMA while the reverse was true for the enantiomers of MDA; the same pattern was observed in the brain regions studied. MDMA was cleared from the brain more quickly than was MDA. Regional differences were observed in the levels of MDMA and MDA.

INTRODUCTION

There has been an increased interest and expressed concern in recent years in relation to "designer" amphetamines, so-called because they were designed in an attempt to circumvent federal government regulations regarding amphetamines. Two of these amphetamine congeners, 3,4-methylenedioxyamphetamine (MDA, "Love") and 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy"), have been reported to be neurotoxic to 5-hydroxytryptamine (5-HT) systems in rat brain in a similar manner to *p*-chloroamphetamine (Battaglia *et al.*, 1987).

Prior to 1985, when MDMA was placed on Schedule I by the Drug Enforcement Administration, this drug was used as an adjunct to psychotherapy. Given in dosages ranging from 75-150 mg, MDMA seemed to decrease negative emotional responses involved in perceived threatening situations and to facilitate communication regarding one's own feelings to one's family and friends (Greer and Tolbert, 1990). Although the therapeutic use of MDMA had diminished by the mid to late 80's, the recreational use, mostly by American college students, increased dramatically over the same time period. A survey of 369 college students in 1987 reported that 39% of the students had used MDMA at least once (Peroutka, 1990). Although it is reportedly used recreationally more for its ability to decrease anxiety and enhance self-awareness, at high doses MDA causes hallucinations, similar to those produced by LSD and mescaline (Nicholas and Oberlender, 1990). Peroutka (1990) expressed concern about the possibility of MDMA-induced serotoninergic neurotoxicity in humans in light of research demonstrating MDMA being neurotoxic to serotoninergic neurons in monkeys (Ricaurte *et al.,* 1988). Dowling (1990) in his review of postmortem reports from July 1985 to March 1986 found 16 cases where MDMA or the N-ethyl analog, N-ethyl-3,4-methylenedioxyamphetamine (MDE) were the underlying cause of death or were felt to have contributed to death.

Both MDMA and MDA exist as racemic mixtures, consisting of (+) and (-) enantiomers, all 4 of which are thought to have biological activity. The individual enantiomers of such drugs may have different individual pharmacokinetic and pharmacodynamic properties (Coutts and Baker, 1989) and the presence of one enantiomer may markedly affect the kinetic and dynamic properties and also the metabolic profile of the other enantiomer (Jamali *et al.,* 1989). For MDMA, the pharmacologic profile is complicated even further in that MDMA undergoes metabolic degradation to yield MDA, thus producing a complex interaction between 4

different enantiomeric compounds. Thus, the ability to separate the various enantiomers becomes of paramount importance when examining behavioural and pharmacological effects of MDMA.

Demethylation of MDMA to yield MDA has been demonstrated by a number of researchers (Yeh and Hsu, 1987; Fitzgerald *et al.*, 1989a,b). Fitzgerald and coworkers showed that MDA accumulated in blood of animals following acute administration of (±)-MDMA. The metabolic reaction was stereoselective in that more (+)-MDA was found than the (-) enantiomer. To examine the stereochemistry of (±)-MDMA metabolism, Fitzgerald *et al.* (1989a) utilized a gas chromatography-mass spectrometry (GC-MS) method which involved liquid-liquid extraction of (±)-MDMA and (±)-MDA from whole blood, followed by on-column derivatization using the unichiral reagent S-(-)-N-trifluoroacetylprolyl chloride. To our knowledge this methodology was not applied to other body fluids or tissues. On-column derivatization is useful when the derivatives are unstable. However, great care is needed when drawing up the different compounds into the syringe for injection into the gas chromatograph (GC) to ensure reproducibility of results.

In view of the potential neurotoxicity of (±)-MDMA and (±)-MDA and the demonstrated differences in behavioural and neurochemical effects among the 4 enantiomers (Schmidt *et al.*, 1987; Steele *et al.*, 1987; Nichols and Oberlender, 1990; McKenna *et al.*, 1991), it is important that more work is done to examine the metabolic pathways and the stereochemistry involved. There is a need to be able to examine simultaneously levels of the 4 enantiomers in a number of tissues, especially brain.

Described here is a simple gas chromatography (GC) method utilizing nitrogen-phosphorus detection for the simultaneous measurement of (+)- and

(-)-MDMA and (+)- and (-)-MDA extracted from rat brain. The method involves extraction under basic conditions, followed by derivatization with S-(-)-N-trifluoroacetylprolyl chloride and separation of the derivatized enantiomers on a gas chromatograph equipped with a fused silica capillary column, a nitrogen-phosphorus detector and a printer/integrator.

MATERIALS AND METHODS

Sources of Drugs: Racemic MDA and MDMA were provided by the Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario. Individual enantiomers of these drugs were obtained from the National Institute on Drug Abuse (NIDA), Rockville, Maryland. S-(-)-N-trifluoroacetylprolyl chloride was purchased from Aldrich Chemicals (Milwaukee, Wisconsin).

Animals: Male Sprague-Dawley rats were randomly assigned to drug or vehicle treatment groups. The rats were injected intraperitoneally with either physiological saline or (±)-MDMA (10 mg/kg) dissolved in physiological saline. Animals were killed by cervical dislocation and decapitation 1, 2, 4 or 8 h post injection. Whole brain was removed immediately and frozen in solid carbon dioxide-cooled iso-pentane. All samples were stored at -80°C until analysis. Regional dissections were carried out on half brains of rats killed 2 h post injection. All procedures involving rats were conducted according to Canadian Council on Animal Care guidelines.

Derivatization: Tissue samples were partially thawed, weighed and homogenized in 5-10 vol of ice-cold 0.1 N perchloric acid. Homogenates were centrifuged at 12,000 g for 10 min at 0-4°C. Portions (1 ml) of the supernatants were used for analysis, and internal standard (1500 ng of *p*-chlorophenylethylamine) was added to all samples. Trisodium phosphate (200 μl of 10% solution) was added, followed by ethyl acetate (4 ml), and the tubes were shaken vigorously for 10 min and

centrifuged at 1,000 x g for 10 min. The organic phase was transferred to smaller test tubes and taken to dryness under a gentle stream of nitrogen in a heating block at 45°C. A derivatization solution, consisting of 2 μl of S-(-)-N-trifluoroacetylprolyl chloride per 100 μl toluene, was prepared and 100 μl were added to each of the tubes. The samples were vortexed and placed in a heating block at 60°C for 30 min, then removed and allowed to stand at room temperature for 10 min. A further 100 μl toluene and 1 ml saturated sodium borate solution were added. Each sample was vortexed briefly and centrifuged for 5 min. The toluene layer was retained and 1 μl portions were injected on the gas chromatograph.

Gas Chromatograph: A Hewlett Packard (HP) model 5890 gas chromatograph equipped with a nitrogen-phosphorus detector and linked to a HP 3392A integrator was used. A fused silica capillary column (25 m x 0.32 mm I.D.) coated with a 0.52 μm film thickness of 5% phenylmethyl silicone (HP Co., Palo Alto, CA, USA) was employed. 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.

Calibration: A standard curve ranging from 50 ng to 750 ng for each of the 4 enantiomers was run in parallel with each assay. These were prepared by adding 1500 ng of internal standard and varying amounts of (±)-MDMA and (±)-MDA to 1 ml of 0.1 N $HClO_4$. A comparison between using 0.1 N $HClO_4$ or brain supernatant from vehicle-treated rats in preparing the standard curves was carried out. No statistical difference was found, so for economic and ethical reasons, 0.1 N $HClO_4$ was used routinely. The ratio of the peak heights of the individual derivatized

enantiomers to that of the derivatized internal standard was calculated and plotted against the concentration of the individual enantiomers. Blank samples were also included in each assay.

Mass Spectrometry: Confirmation of structures of the derivatives was carried out using combined gas chromatography-mass spectrometry. 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: 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.

Stability of Derivatives: Stability of the derivatives of the drugs was examined by repeated GC analysis of the same set of standards and tissue samples six times over a one-week period.

Statistics: For the regional study at 2 h, statistical analyses were conducted using ANOVA followed by the multiple F test (α=0.05). In the time study, regression analysis was done on the differences between the (-) and the (+) enantiomers of MDMA and MDA over time.

RESULTS AND DISCUSSION

Under the conditions described above, the enantiomers of MDMA and MDA were readily separated and quantitated. The standard curve was linear over the range of 50-750 ng, with a correlation coefficient > 0.99 obtained routinely. Mean recovery of 200 ng of MDMA was 89.2% and 88.9% for the (-) and (+) enantiomers. Recovery of (+)- and (-)-MDA under the same conditions was 95.2% and 98.0%, respectively. Typical chromatograms of derivatized brain extracts from rats treated

with vehicle or (±)-MDMA are shown in Figure 1. The mass spectrometric data were consistent with the structure shown in Figure 2. The mass fragmentations of these derivatives have been described previously by Fitzgerald *et al.* (1989a), and we have shown in Figure 3 the proposed mass fragmentation of the derivative of the internal standard (*p*-chlorophenylethylamine). Intra- and inter-assay coefficients of variation for the 4 enantiomers ranged from 1.2 to 10.9%, and these values are shown in Table I.

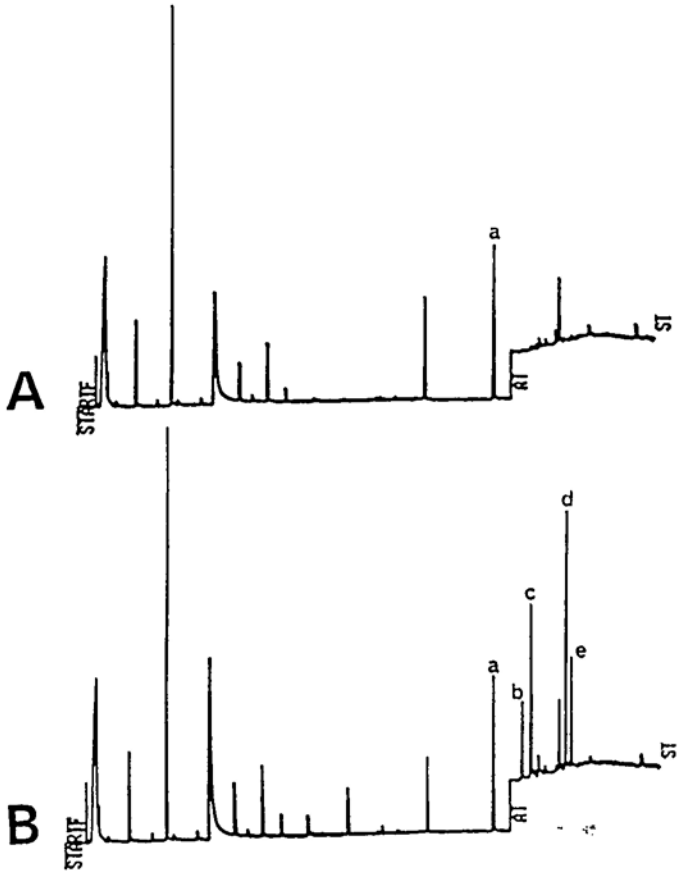

Figure 1: Typical chromatograms of derivatized brain extracts from rats treated with [A] vehicle and [B] (±)-MDMA. Retention times (in min) of the derivatives: [a] *p*-chlorophenylethylamine 14.39; [b] (-)-MDA 15.41; [c] (+)-MDA 15.69; [d] (-)-MDMA 16.88; and [e] (+)-MDMA 16.96.

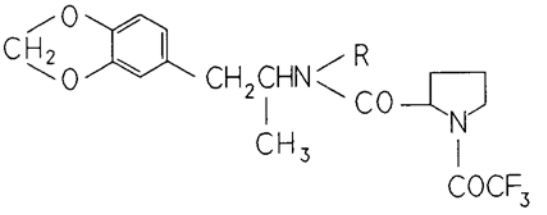

Figure 2: Structures of the derivatives of MDMA (R=CH_3) and MDA (R=H) formed in this assay procedure.

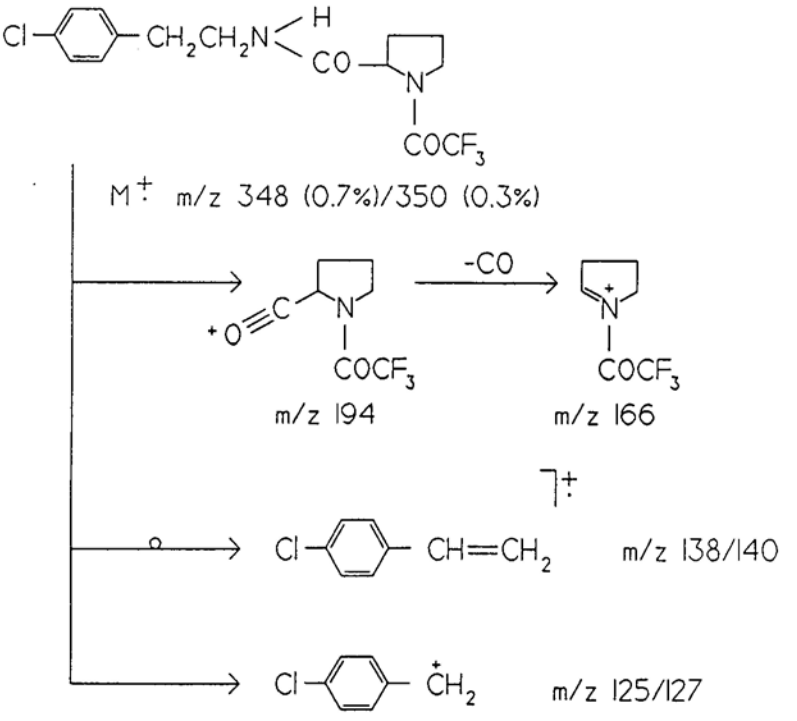

Figure 3: Proposed mass spectrometric fragmentation of the trifluoroacetyl-prolyl derivative of *p*-chlorophenylethylamine.

Table I: Coefficients of variation for the assay procedure.

Intra-assay c.v.s (n=6)

Enantiomer	50 ng	100 ng	250 ng	500 ng
(-)-MDMA	10.9	5.9	5.0	3.1
(+)-MDMA	9.7	4.9	4.9	3.0
(-)-MDA	4.8	3.8	2.0	1.2
(+)-MDA	4.6	3.0	2.7	2.0

Inter-assay c.v.s (n=11)

Enantiomer	50 ng	100 ng	250 ng	500 ng
(-)-MDMA	9.7	7.8	9.8	9.3
(+)-MDMA	5.6	7.8	8.8	8.9
(-)-MDA	5.8	6.9	3.9	6.7
(+)-MDA	6.2	7.6	3.7	6.0

Levels of the 4 enantiomers are shown in Figure 4. There is a significant linear relationship (MDMA:$F(1,20)=39.4$, MDA:$F(1,20)=42.3$, $p<0.0001$) between the (-) and (+) enantiomers of each of the drugs over time. At all time periods analyzed in this study, the levels of (-)-MDMA are significantly greater than those of (+)-MDMA. The reverse is seen with MDA, in that there is more (+)-MDA than (-)-MDA at the studied periods.

The pattern of decline in the levels of (+)-MDMA and (+)-MDA compared to the pattern seen with (-)-MDMA and (-)-MDA is different. At 1 and 2 h, there is significantly more (+)-MDMA than (+)-MDA. By 4 h, the difference is no longer significant and by 8 h the pattern has reversed, with significantly more (+)-MDA than (+)-MDMA. In the case of (-)-MDMA and (-)-MDA, (-)-MDMA remains higher

than (-)-MDA levels until the 8 h period. These data underscore the complexity of interactions among the different enantiomers of MDMA and MDA after administration of racemic MDMA.

Nichol and Oberlender (1990) indicated that the hallucinogenic effects of (±)-MDA last 10-12 h, while the effects of (±)-MDMA are of a shorter duration, 3-5 h. Fitzgerald *et al.* (1990) calculated the half-lives of both (+)- and (-)-MDMA in whole blood, after intravenous administration of 10 mg/kg of (±)-MDMA, to be approximately 1.7 h. To our knowledge no information is available on the half-lives of the enantiomers of MDMA in brain tissue.

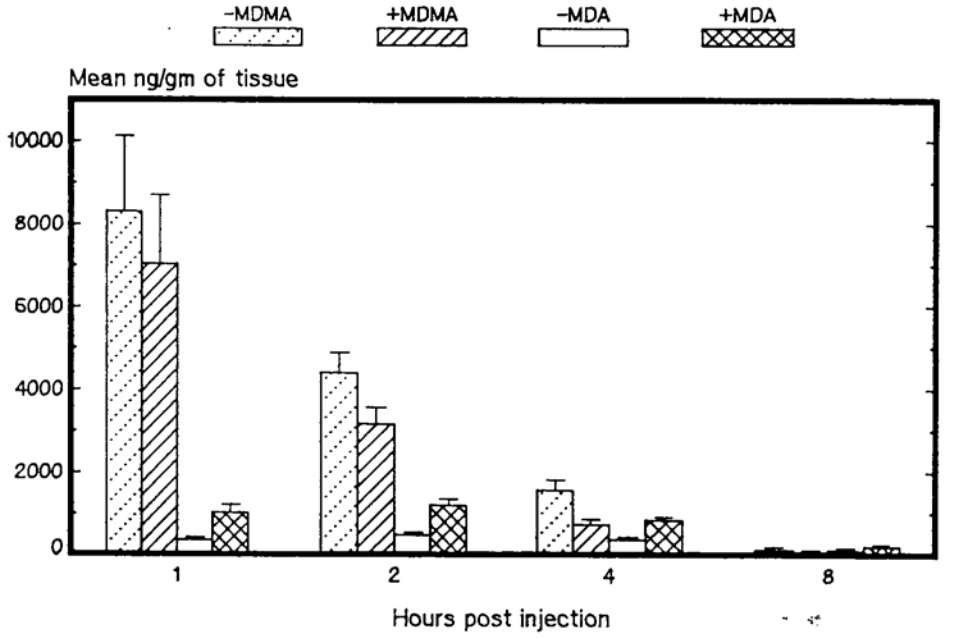

Figure 4: Whole brain levels of the enantiomers of MDMA and MDA after acute injections of (±)-MDMA (10 mg/kg). Values represent means ± S.E.M. (n=6).

Regional analyses of drug levels 2 h post injection are shown in Table II. The same pattern of ratio of the enantiomers is observed in the regions as in whole brain, i.e. levels of (-)-MDMA are higher than those of (+)-MDMA, while the reverse if true for the enantiomers of MDA.

Table II: Enantiomers of MDMA and MDA in whole brain and brain regions 2 h post injection of racemic MDMA (10 mg/kg i.p.).

	(-)-MDMA	(+)-MDMA	(-)-MDA	(+)-MDA
Whole brain	4412 ± 502	3172 ± 414	489 ± 54	1210 ± 155
Cortex	5994 ± 1318	4232 ± 922	510 ± 107	1289 ± 244
Hypothalamus	3804 ± 354	3010 ± 92	1730 ± 894	2300 ± 1084
Striatum	5529 ± 1108	2584 ± 392	897 ± 50	1862 ± 440
Hippocampus	5114 ± 758	3414 ± 549	986 ± 90	1547 ± 149
Pons	2311 ± 292	1492 ± 153	277 ± 39	658 ± 79
Cerebellum	2887 ± 264	1608 ± 320	272 ± 19	663 ± 32
Rest of brain	4470 ± 2378	3183 ± 1634	459 ± 153	1153 ± 23

Results represent ng/g and are expressed as means ± S.E.M. (n=4).

In summary, a rapid and relatively inexpensive gas chromatographic procedure has been developed for the simultaneous analysis of the enantiomers of MDMA and MDA. This method is readily applicable to brain tissue from rats and should prove helpful in furthering our knowledge of the pharmacokinetics of various amphetamine derivatives that are presently being used recreationally. There has been considerable discussion about the possible contribution of metabolites of MDMA and MDA to the neurotoxic actions of these drugs (Yeh and Hsu, 1987; Lim and Foltz, 1988; Hiramatsu *et al.*, 1990; McCann and Ricaurte, 1991; Zhao and Castagnoli, 1992), and presumably the method could be modified to permit analysis of these metabolites as well.

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