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STEREOCHEMISTRY OF THE METABOLISM OF MDMA TO MDA

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Summary

The chiral derivatizing reagent N-trifluoroacetyl-L-prolyl chloride (LTPC) was used to form diastereomers of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) which were resolved on an achiral gas chromatographic column using a mass spectrometer as a detector. Rats were subcutaneously dosed with 40 mg/kg of (+/-) MDMA·HCl and blood was obtained by decapitation four hours after dosing. Plasma was separated and extracted. The extract was derivatized on-column with LTPC. In addition to the two MDMA isomers, the demethylated metabolites, S(+) and R(-)-MDA were identified. In all experimental groups (male rats, food deprived male rats, female rats, post partum female rats, and mice) dosed with racemic MDMA, higher levels of the S(+) isomer of MDA relative to the R(-) MDA isomer were observed. This may be significant since it has been shown that the S(+) isomer of MDMA is the more neurotoxic isomer of the racemic drug of abuse MDMA.

The methylenedioxy analogs of amphetamine became popular when clandestine laboratories, attempting to bypass Drug Enforcement Agency (DEA) schedulings, sequentially altered the structure of 3,4-methylenedioxyamphetamine by adding alkyl groups to the nitrogen, producing drugs with a high potential for abuse. The Drug Enforcement Agency's attempts to regulate these drugs, as well as pharmacological and toxicological research, lag behind the emergence of these agents on the street.

MDA (see fig.1) has been abused since the late sixties and early seventies. By the late 1970's, the metabolism of MDA had been studied (1-4) and a considerable amount of data had accumulated on it's toxicity (5-8) including reports of a number of human fatalities (9-13). MDA became a schedule one drug under the Comprehensive Drug Abuse Prevention and Control Act of 1970. About the same time as MDA was beginning to be understood pharmacologically, its N-methyl analog, MDMA (see fig.1), was introduced on the street as "Ecstasy".

MDMA abuse appears to have become widespread and, in 1985, the DEA used it's emergency powers to classify MDMA as a schedule one drug. At that time, there was very little data on the pharmacological and toxicological effects and no data on the metabolism of MDMA. For a review of early research on MDMA see the proceedings of the conference on MDMA (14). Recently there has been intensive research into the pharmacological effects of MDMA (15-18), with an emphasis on separating the different effects of the S(+) and R(-) enantiomers (19-23). One important finding is that both isomers of MDA and the S(+) isomer of MDMA produce a long lasting serotonin (5-HT) neurotoxicity characterized by a reduction of 5-HT, 5-hydroxy indole acetic acid, and 5-HT uptake sites in the rat brain (24-30). Recently, two independent

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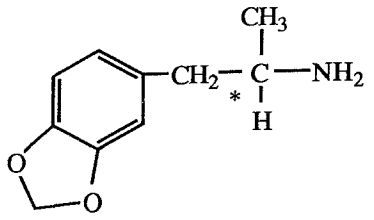
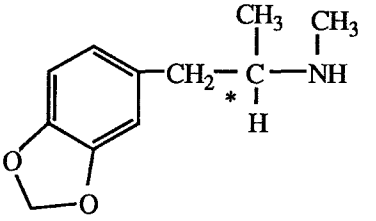
groups have demonstrated that MDMA is a serotonin neurotoxin in nonhuman primates (31,32).



MDA



MDMA

Fig. 1
Structure of 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA). The asterisk indicates the chiral center

We have previously identified MDA as a major metabolite in urine and plasma from rats dosed with racemic MDMA·HCl (33). Subsequently, MDA has been identified as a metabolite in humans (34). Much work was done administering separate enantiomers of MDA and MDMA to rats but no data were available on the stereochemistry of the demethylation of MDMA to MDA. The purpose of this work was to investigate the plasma concentrations of the enantiomers of MDMA and MDA after dosing with racemic MDMA·HCl.

Methods

All rats were Sprague Dawley (250-350 g) from Dominion labs (Dublin, VA). Food deprived males were part of another experiment and were studied to see if this group had different levels of MDMA and MDA enantiomers than normal male rats. They were kept on a restricted diet consisting of 12-15 grams of rat chow and water ad libitum for 60 days. Postpartum females were studied because doses of MDMA that were not lethal to normal females, killed females that had recently given birth (J.A.R. unpublished results). Postpartum females were 5 days post parturition. ICR mice were also obtained from Dominion labs and weighed 35-40 g. To obtain sufficient quantities of mouse plasma for analysis blood from 7 mice was pooled. All animals were dosed with 40 mg/kg (+/-) MDMA·HCl in saline, subcutaneously, and were sacrificed 4 hours later. This dose and route of administration had been used in previous studies (33). The 4 hour time point was chosen because previous studies showed significant amounts of MDA at this time point after MDMA adminstration (33). Blood was collected into heparinized tubes after decapitation. Plasma was separated after centrifuging at 2000 rpm.

Quantitation of MDA and MDMA enantiomers in plasma was done by liquid-liquid extraction followed by on-column derivatization using the chiral derivatizing reagent LTPC, similar to a method described for whole blood (35). The internal standard, racemic methoxyphenamine (20 microliters of 20 ng/microliter in water), was added to 500 microliters of plasma. Samples were alkalinized with 500 microliters of 1 N NaOH and extracted with 1000 microliters of extraction solvent (10:10:1; ethyl acetate:hexanes:1-butanol). After vortexing and centrifuging the organic layer was pipetted and was back extracted into 250 microliters of 1 N HCl. Samples were vortexed, centrifuged, and the organic layer was discarded. The aqueous layer was alkalinized with 500 microliters of 1 N NaOH and extracted with 25 microliters of 1%

isopropanol in chloroform. Diastereomers were formed on-column by filling a 10 microliter syringe with 3 microliters of the chloroform layer, 0.2 microliters of air, 1 microliter of 0.1 M LTPC in chloroform, and rapidly injecting this onto the gas chromatograph/mass spectrometer (GC/MS). The GC was operated in the split mode with a split ratio of 15. Initial oven temperature was 220 °C, initial time 2 min, program rate 20 °C/min, and final temperature 290 °C. The injection port temperature was 275 °C. A Hewlett Packard methylsilicon capillary column (12.5 m x 0.2 mm x 0.33 um; length x i.d. x film thickness) was used to separate the diastereomers. The MS was operated in selected ion mode. MDA and MDMA were quantitated on the 162 m/z ion relative to the 148 m/z ion of the internal standard.

Students T test was the statistic used to compare amounts of MDA enantiomers formed. A p value of .005 was arbitrarily chosen as the cut off for determining if there was significant difference between the amounts of the two MDA isomers formed

Racemic, S(+) and R(-)-MDA and MDMA had been previously synthesized in our laboratories as their hydrochloride salts (35).

Results

Until the present study no data had been presented quantitating the amounts of MDA formed by rats dosed with MDMA. Figure 2 shows the amount of MDA and MDMA in each group four hours post injection.

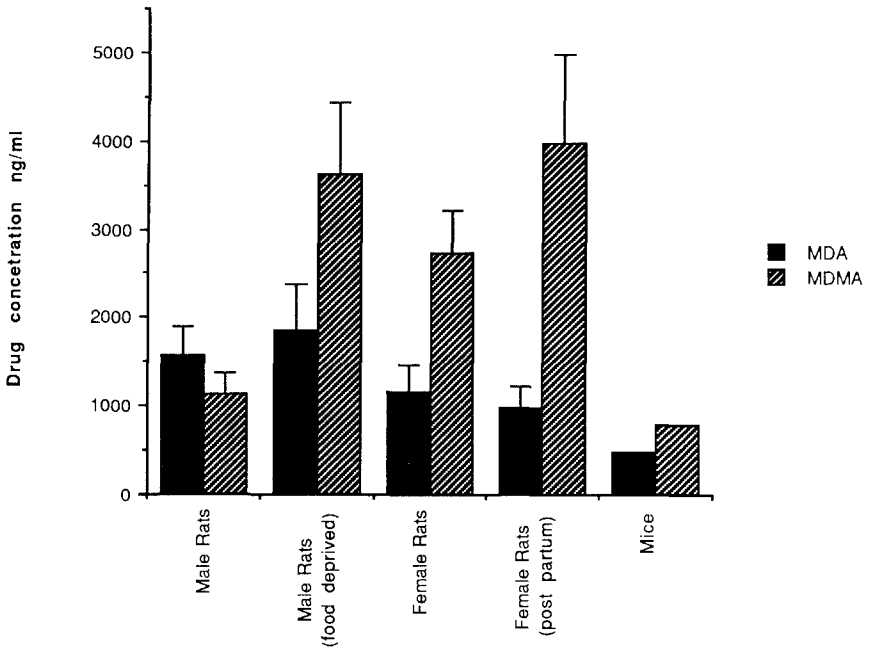


Fig. 2

The mean amount of MDA and MDMA in plasma four hours post injection of 40 mg/kg subcutaneous (+/-) MDMA·HCl (N=5 for rats). Error bars represent standard deviations. Mice represent pooled plasma (N=7).

It was found that the level of MDMA in male rats at the 4 hour time point was significantly lower than that of female rats. Post partum females had similar levels of parent drug and metabolite as normal females. Food deprived rats had higher levels of the parent drug than normal male rats.

Not surprisingly, MDA was identified as a metabolite of MDMA in mice as well as rats.

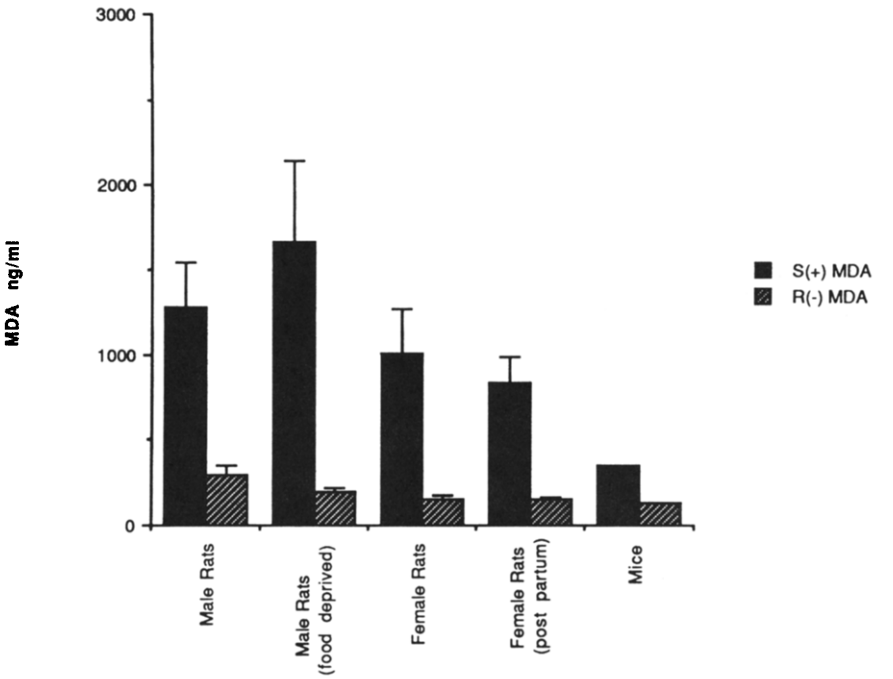


Fig. 3

The mean enantiomeric composition of MDA in plasma four hours post injection of 40 mg/kg subcutaneous (+/-) MDMA·HCl (N=5 for rats). Error bars represent standard deviations. Mice represent pooled plasma (N=7).

Figure 3 demonstrates the stereochemistry of the demethylation of MDMA to MDA for each experimental group. For rats, the hypothesis that equal amounts of the isomers are formed was rejected at p=.005, in favor of the alternative, that the S(+) isomer is preferentially formed. The ratio of S(+)-MDA to R(-)-MDA in rats dosed with racemic MDMA varies between 4.3 and 8.7 depending on the experimental group. Analysis of plasma from pooled samples of mouse blood showed that S(+)-MDA is preferentially formed in this species as well, with the ratio of S(+) to R(-) being 2.6.

Figure 4 shows the enantiomeric content of MDMA in plasma 4 hours post injection. Adding the amounts of S(+)-MDA and S(+)-MDMA, and comparing this with the combination of R(-)-MDA and R(-)-MDMA, shows that at the 4 hour time point more of the S(+) isomer of MDMA is accounted for. This suggests that R(-)-MDMA is being metabolized to a compound that is undetected by the present analytical scheme.

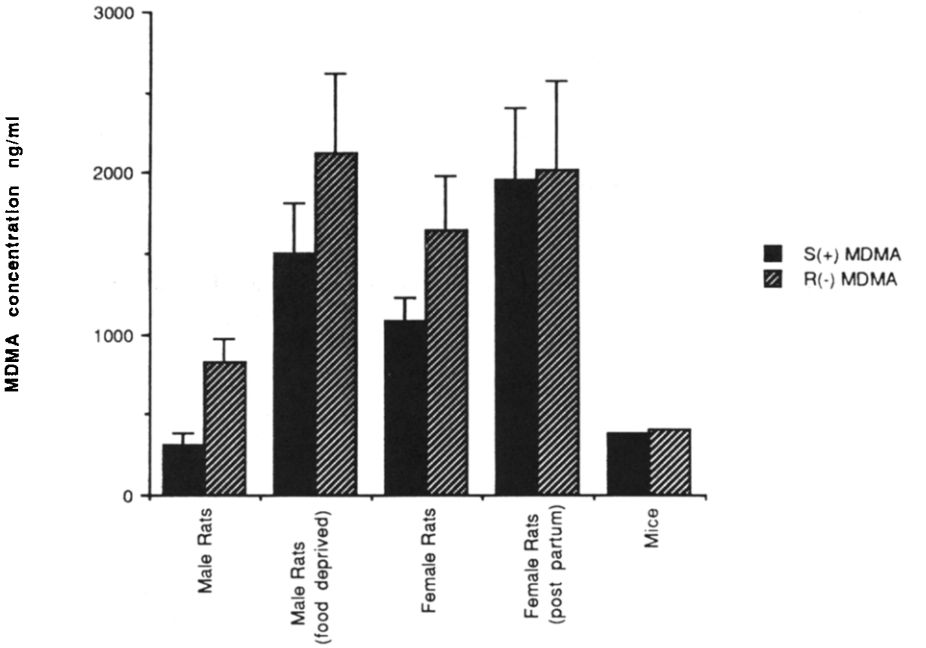


Fig. 4

The mean enantiomeric composition of MDMA in plasma four hours post injection of 40 mg/kg subcutaneous (+/-) MDMA·HCl (N=5 for rats). Error bars represent standard deviations. Mice represent pooled plasma (N=7).

Discussion

MDA is formed by N-demethylation of MDMA. N-Demethylation is thought to proceed through a carbinolamine intermediate produced by cytochrome P-450 oxidation of the carbon alpha to the N-methyl group, or through N-oxidation followed by rearrangement to the primary amine (36). The results presented here are consistent with the observation that the S(+) isomers of N-alkylamphetamines, are more extensively metabolized by dealkylation then their enantiomers (37). It has been suggested that N-dealkylation of R(-) enantiomers of amphetamines proceeds through a mechanism of N-oxidation, while S(+) enantiomers are N-dealkylated through alpha carbon oxidation (37).

This study showed a sex difference in the amount of parent drug in the plasma of rats 4 hours post dosing. Others have shown that sex related differences in drug metabolism first appear when animals begin to mature sexually, suggesting that some drug metabolizing enzymes are under hormonal control. For barbiturates, which have prolonged action in females compared with males, the sex related difference is related to the presence of testosterone. Administration of testosterone to ovariectomized female rats shortens the duration of anaesthesia by hexobarbitol. N-Demethylation, the metabolic step involved in the conversion of MDMA to MDA, of some narcotics such as morphine, meperidine, and methadone proceeds more rapidly in males than in females (38).

The group of post partum females were included in this study to determine if the metabolic profile of this group was significantly different than normal females. The increased lethality of this group (J.A.R. unpublished results) could not be explained by a difference in the levels of MDMA or MDA at the 4 hour time point. Additionally post partum females had similar levels of the enantiomers of both the parent and metabolite as normal females.

The group of food deprived male rats had higher levels of the parent drug than normal male rats. The effects of nutrition on drug metabolism is complex and has been reviewed (39). Drug levels were only determined at one time point in this study, therefore it is not appropriate to discuss mechanisms responsible for the observed difference.

The results of these experiments demonstrate that at the 4 hour time point higher levels of the S(+) enantiomer of MDA are observed relative to the R(-) isomer when rats are dosed with racemic MDMA. There are several possible explanations for this difference in isomers including: stereoselective absorption, distribution, elimination or metabolism. Since this study only looked at one time point in the pharmacokinetic profile of MDMA and MDA it is difficult to determine the mechanism responsible for the higher levels of the S(+) isomer of MDA. Currently we are studying the time course of these drugs using the intravenous and subcutaneous routes of administration. Preliminary data (unpublished) appear to rule out stereoselective absorption, distribution, elimination and saturation kinetics.

The higher levels of S(+) MDA relative to R(-) MDA are probably due to stereoselective metabolism. There are several possible mechanisms causing higher levels of the S(+) isomer. One mechanism is stereoselective demethylation preferentially forming the S(+) isomer. Another is that both isomers are demethylated and then the R(-) isomer of MDA is metabolised further. A third mechanism is that the R(-) isomer of the parent compound is metabolized to a compound different than MDA, depleting the substrate for demethylation.

The S(+) enantiomer of MDA being preferentially formed, regardless of the mechanism, is physiologically significant since the S(+) isomer of MDMA has been shown to cause the long term 5-HT neurotoxicity (27). One explanation of neurotoxicity of MDMA that has been suggested is that metabolism of MDMA to MDA is necessary for destruction of 5-HT neurons (29). The data presented here support this theory by providing a possible explanation of the stereoselectivity of the neurotoxicity of MDMA. When animals are dosed with S(+)-MDMA, sufficient quantities of S(+)-MDA may be formed to cause 5-HT neurotoxicity; but, when animals are dosed with R(-)-MDMA the quantities of R(-)-MDA formed may be insufficient to cause 5-HT neurotoxicity.

MDA given intracerebrally does not cause 5-HT neurotoxicity (40), suggesting that MDA is further metabolized in the periphery to the ultimate neurotoxic compound. The structure of the neurotoxic metabolite is currently unknown.

References

1. K.K. MIDHA, J. Chromatogr., 101 210-214 (1974).
2. K.K. MIDHA, J.W. HUBBARD, K. BAILEY and J.K. COOPER, Drug Metab. Disp., 6 623-630 (1978).
3. K.K. MIDHA, J. K. COOPER, A. BY, and J.-C. ETHIER, Drug Metab. Disp., 5 143-148 (1977).
4. G.M. MARQUARDT, V. DISTAFANO and L.L. LING, Biochem. Pharm., 27 1503-1505 (1978).
5. D.M. PATON, J.I. BELL, R. YEE and D.A. COOK, Proc. West Pharmacol. Soc., 18 229-231 (1975).

6. R. YEE and J.I. BELL, Texas Rep. Biol. Med., 33 610 (1975).
7. W.M. DAVIS, I.W. WATERS, H.T. HATOUM, J.L. BUELKE and M.C. BRAUDE, Res. Comm. Chem. Path. Pharmacol., 17 575-582 (1977).
8. D.E. NICHOLS, M. ILHAN and J.P. LONG, Arch. Int. Pharmacodyn., 214 133-140 (1975).
9. A. POKLIS, M.A. MACKELL and W.K. DRAKE, J. Forensic Sci., 24 70-75 (1979).
10. T. LUKASZEWSKI, Clin. Tox., 15 405-409 (1979).
11. D. REED, R.H. CRAVEY and P.R. SEDGWICK, Clin. Tox., 5 3-6 (1972).
12. D.L. SIMPSON ADN B.H. RUMACK, Arch. Inter. Med., 141 1507-1509 (1981).
13. G. CIMBURA, J. Forensic Sci., 17 229-333 (1972).
14. J. Psychoactive Drugs, R.B. SEYMOUR, D. R. WESSON and D.E. SMITH Eds. 18 (1986)
15. R.P. CLIMCO, H. ROEHRICH, D.R. SWEENEY and J. AL-RAZI, Intl. J. Psychiat. Med., 16 359-371 (1986-1987).
16. D.M. STONE, D.C. STAHL, G.R. HANSON and J.W. GIBB, Eur. J. Pharmacol., 128 41-48 (1986).
17. D.R. GEHLERT, C.J. SCHMIDT, L. WU andW. LOVENBERG, Eur. J. Pharmacol., 119 135-136 (1986).
18. C.H. FRITH, L.W. CHANG, D.L. LATTIN, R.C. WALLS, J. HAMM and R. DOBLIN, Fund. Applied Tox., 9 110-119 (1987).
19. M.P. JOHNSON, A.J. HOFFMAN and D.E. NICHOLS, Eur. J. Pharmacol., 132 269-276 (1986).
20. T.D. STEELE, D.E. NICHOLS and G.K.W. YIM, Biochem. Pharamacol., 36 2297-2303 (1987).
21. R.A. LYON, R.A. GLENNON and M. TITELER, Psychopharmacology, 88 525-526 (1986).
22. M.D. SCHECHTER, Pharmacol. Biochem. Behav., 27 41-44 (1987).
23. R.A. GLENNON, M. YOUSIF, and G. PATRICK, Pharmacol. Biochem. Behav., 29 443-449 (1988).
24. G. RICAURTE, G. BRYAN, L. STRAUSS, L.SEIDEN and C. SCHUSTER, Science, 229 986-988 (1985).
25. C.J. SCHMIDT, L. WU and W. LOVENBURG, Eur. J. Pharmacol., 124 175-178 (1986).
26. D.M. STONE, D.C. STAHL, G.R. HANSON and J.W. GIBB, Eur. J. Pharmacol., 128 41-48 (1986).
27. C.J. SCHMIDT, J. Phamacol. Exp. Ther., 240 1-7 (1987).
28. D.L. COMMINS, G. VOSMER, R.M. VIRUS, W.L. WOOLVERTON, C.R. SCHUSTER and L.S. SEIDEN, J. Pharmacol. Exp. Ther., 241 338-345 (1987).
29. C.J. SCHMIDT, Eur. J. Pharmacol. 136 81-88 (1987).
30. G.A. RICAURTE, K.F. FINNEGAN, D.E. NICHOLS, L.E. DELANNEY, I. IRVIN and J.W. LANGSTON, Eur. J. Pharmacol., 137 265-268 (1987).
31. W. SLIKKER, S.F.ALI, A.C. SCALLET, C.H. FRITH, G.D. NEWPORT and J.R. BAILEY, Tox. Appl. Pharmacol., 94 448-457 (1988).
32. G.A. RICAURTE, L.S. FORNO, M.A.WILSON, L.E. DELANNEY I. IRWIN, M.E. MOLLIVER, and J.W. LANGSTON, J. Am. Med. Assoc, 260 51 (1988).
33. R. FITZGERALD, R. BLANKE, N. NARASIMHARCHARI, R. GLENNON and J. ROSECRANS, NIDA Reseach Monographs, 81 320 (1988).
34. K. VEREBY, J. ALRAZI and J.H. JAFFE, J. Am. Med. Assoc, 259 1649-1650 (1988).
35. R.L. FITZGERALD, R.V. BLANKE, R.A. GLENNON, M.Y. YOUSIF, J.A. ROSECRANS, and A. POKLIS, J. Chromatogr. 490, 59-69 (1989).
36. PETERSON and HOLTZMAN, Extrahepatic Metabolism of Drugs and Other Foreign Compounds, T.E. Gram Ed., p. 20, Spectrum Publication (1980).
37. J.CALDWELL, Amphetamines and Related Stimulants: Chemical, Biological, Clinical, and Sociological Aspects, J. CALDWELL Ed, p. 32, (1980).
38. R. KATO, Drug Metab. Review, 3 1-32 (1974).
39. R. KATO, Xenobiotica, 7 25-92 (1977).