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Enantioselective metabolism of the designer drugs 3,4-methylenedioxymethamphetamine ('ecstasy') and 3,4-methylenedioxyethylamphetamine ('eve') isomers in rat brain and blood

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Abstract

The metabolism of MDA (3,4-methylenedioxyamphetamine), HMMA (3-hydroxy-4-methoxymethylamphetamine) and HME (3-hydroxy-4-methoxyethylamphetamin) of the popular designer drugs MDMA ('ecstasy', 3,4-methylenedioxymethamphetamine) and MDE ('eve', 3,4-methylenedioxyethylamphetamine) was determined in rat serum, whole blood and urine, as well as in whole brain structures (cortex and striatum) after subcutaneous administration of 20 mg/kg MDMA and MDE, respectively. MDMA and MDE were extracted from serum and homogenized brain structures using a solid-phase extraction procedure. The extracts were examined by a validated high-performance liquid chromatography procedure coupled with fluorimetric detection. Our results demonstrate that MDMA is metabolized to a higher degree than MDE, resulting in a higher concentration of neurotoxic dihydroxymetabolites and (S)-MDA. There was no difference between the metabolism of MDMA and MDE and its respective isomers. Different concentrations of the respective isomers of MDMA and MDE let us suggest an enantioselective metabolism for both MDMA and MDE.

Keywords: 3,4-Methylenedioxymethamphetamine; 3,4-Methylenedioxyethylamphetamine; Metabolism; Enantioselective; High-performance liquid chromatography with fluorimetric detection; Solid-phase extraction

In the last few decades, the synthetic drugs 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') and 3,4-methylenedioxyethylamphetamine (MDE, 'eve') have gained popularity as recreational drugs and are mainly used for their pleasurable stimulating effects, especially during so-called 'raves'. The popularity of these substances can be attributed to both their psychotropic effects and entactogenic effects [12]. These phenylethylamine derivatives induce feelings of euphoria, friendliness, closeness to others and empathy, and have been named 'entactogens' [6,12]. However, animal studies in rats and primates have shown that MDMA acts as a serotonergic neurotoxin [13]. Repeated administration of high oral doses of MDMA may produce long-term reductions in serotonergic neurons in humans. Chronic heavy use of ecstasy seems to be associated with persistent psychological deficits and cognitive impairment [11]. MDE produces a long-lasting, dose-related depletion of serotonin (5-HT) [14]. However, even at high dosage, MDE did not reduce the concentrations of either dopamine (DA) or norepinephrine (NE) on a long-term basis. When compared with MDMA, MDE was approximately only one-fourth as potent in producing a long-term depletion of 5-HT [14]. When dealing with amphetamine-like substances, it is important to remember that these compounds contain a chiral center. However, the methylenedioxyalkaneamines are virtually always used as racemic mixtures. Both isomers display different pharmacodynamic as well as different pharmacokinetic properties [9,12]. Chiral separation methods like chiral columns at HPLC have been found most useful in some pharmacokinetic investigations with respect to the compounds, and are

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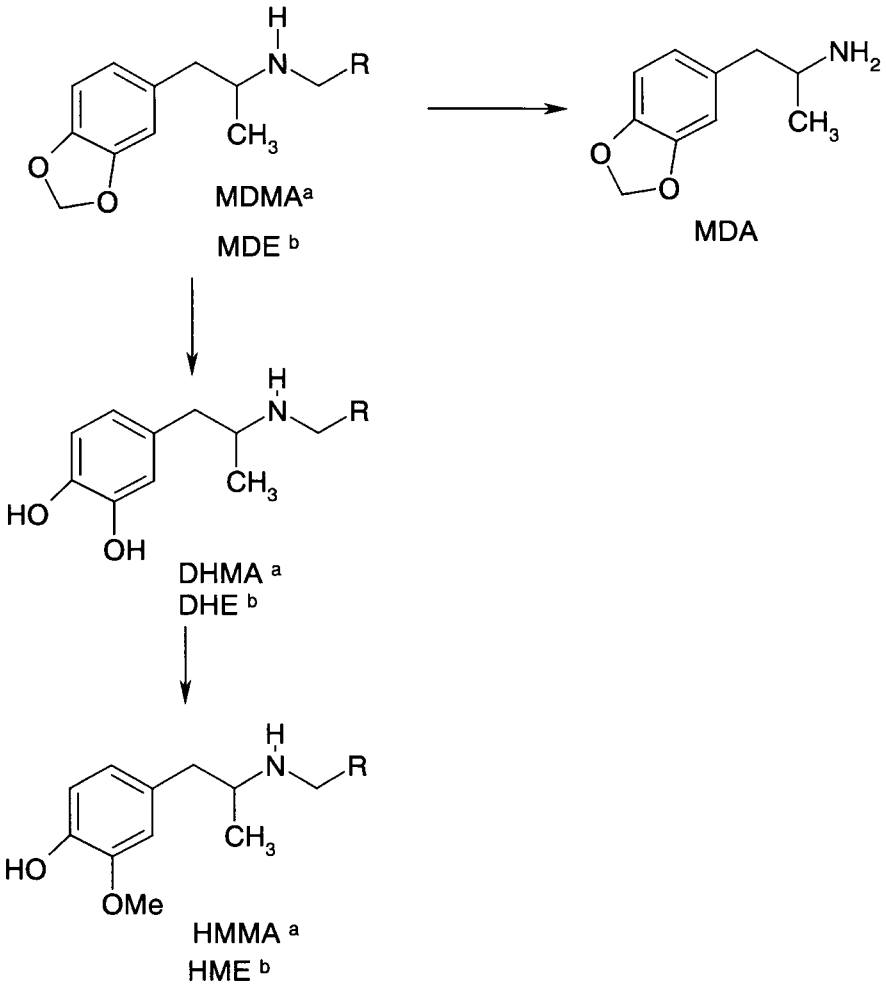


Fig. 1. Main metabolites of racemic 3,4-methylenedioxymethamphetamine (MDMA) and N-ethyl-3,4-methylenedioxymethamphetamine (MDE). (a) R = CH_3; (b) R = C_2H_5.

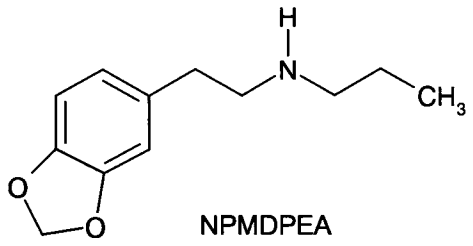
routinely performed to confirm the use of drugs of abuse in workplace drug testing.

MDMA, MDE and MDA (3,4-methylenedioxyamphetamine) are all methylenedioxyamphetamine derivatives (cf. Fig. 1). MDA is both a synthetic drug itself and an important metabolite of MDMA and MDE by N-dealkylation. Other important metabolites are the derivatives 3-hydroxy-4-methoxymethylamphetamine (HMMA) in rats [9] and in humans [5] and its analogue 3-hydroxy-4-methoxyethylamphetamin (HME) in healthy human volunteers [4]. The latest metabolites are formed via 3,4-dihydroxy derivatives (DHMA, DHE) by subsequent methylation at the hydroxyl group at position 3 of the aromatic ring. Kreth et al. [8] identified the cytochrome P 450 (CYP 450 2D6 and CYP 450 1A2) isozymes catalyzing the oxidative metabolism of the amphetamine derivatives MDMA, MDE and MDA. Several CYP 450 isozymes can contribute to microsomal oxidative metabolism of methylenedioxyamphetamines [8,10].

The aim of this study was to confirm the metabolism of MDMA and MDE in blood and brain of the rats and by this way to explain the different neurotoxicity of both amphetamines.

The determination of MDMA, MDE, MDA, HMMA and HME in biological samples has been based mainly on gas chromatography coupled with mass spectrometry (GC-MS) [4,9]. GC-MS provides excellent sensitivity and selectivity, but MDMA and its analogues require derivatization [4,9]. Also HPLC has been used for the determination of these structures. Unfortunately, the methods using ultraviolet or diode-array detection lack sensitivity [17]. The extraction procedures described use multiple liquid–liquid extraction or solid–liquid extraction steps [9]. The solid-phase extraction (SPE) from Brunnenberg and Kovar [3] and Spitzer et al. [16] was used in our experiments, and for detection we used HPLC coupled with fluorescence detection.

MDE, MDMA, HME, HMMA and MDA were synthesized according to Braun et al. [2]. Substances for analysis of the metabolism in biological subjects were obtained from Fluka (Buchs, Switzerland) and Merck (Darmstadt, Germany). Water was deionized and twice distilled. Rat plasma references of Sprague–Dawley rats were obtained



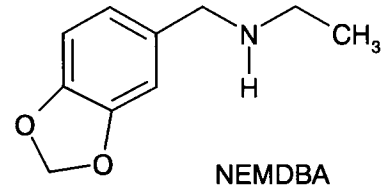


Fig. 2. Structures of the internal standards *N*-propyl-3,4-methylenedioxyphenethylamine (NPMDPEA) for MDMA samples and *N*-ethyl-3,4-methylenedioxybenzylamine (NEMDBA) for MDE samples.

from Charles River Laboratories (Sulzfeld, Germany). The plasma, cortex and striatum samples of the Sprague–Dawley rats were provided by two place preference studies in our working group. Plasma and brain structures were kept below −20 °C until analysis. For purifying the enzyme solution PD10 columns (Pharmacia, Freiburg, Germany) and SPE CBA columns (200 mg) (Separtis, Germany) were used. HPLC was carried out on a Chiral-CBH Guard column, 5 μm, 10 × 3.0 mm I.D., and a Chrial CBH column, 5 μm, 150 × 4 mm I.D. The HPLC station comprised a gradient pumping system L 6200, a LaChrom autosampler L 7200, and a LaChrom fluorescence detector L 7480 from Merck (Darmstadt, Germany).

For the hydrolysis of the conjugates in plasma and brain structures the β-glucuronidase/arylsulfatase solution was purified according to the manufacturer's instructions by means of size-exclusion chromatography (SEC). A 1 ml aliquot of plasma or homogenized cortex or striatum was treated with 125 μl purified enzyme and 875 μl 0.1 M sodium acetate buffer (pH 5.2), and then incubated at 37 °C for 24 h. Protein was precipitated by adding 1 ml of an aqueous solution of PEG 6000 (polyethylene glycol) under cooling in an ice bath for 5 min. As internal standard for the MDMA samples we used *N*-propyl-3,4-methylenedioxyphenethylamine (NPMDPEA) and for the MDE samples *N*-ethyl-3,4-methylenedioxybenzylamine (NEMDBA) (Fig. 2), synthesized in our working group. A total of 200 ng/ml internal standard was dissolved in the samples. The samples were centrifuged at 2875 × *g* for 5 min at 20 °C. The supernatant was subjected to SPE on cation-exchange columns (CBA). After conditioning with 2 ml acetonitrile, 1 ml 0.1 M HCl and 3 ml 0.1 M sodium acetate buffer (pH 6.5) the samples were applied. The elution was carried out with 2 ml acetonitrile/water/1 M HCl (8:1:1). The samples were evaporated to dryness under nitrogen at 45 °C and taken up in 250 μl water. A total of 100 μl of the extract was injected into the HPLC: the mobile phase consisted of 20 mmol potassium dihydrogenphosphate, 50 mM di-natriumethylenediaminetetraacetic acid (Na-EDTA) and 8% methanole for MDMA, and 7% isopropanole for MDE samples, respectively. The pH value of the mobile phase for MDMA samples was 6.70, and for MDE samples 6.44. The flow-rate was 0.7 ml/min. Detection was carried out with a fluorescence detector. The extinction wavelength was set to 286 nm. The emission wavelength was 322 nm for all substances. The analytic procedure was validated according to the ICH guidelines [1].

For statistical analysis, the data were analyzed using completely randomized one-way analysis of variance (ANOVA) with post-hoc Newman–Keuls corrected *t*-test. Data are given as means ± SEM (standard error of the means) and a P value of <0.05 was accepted as significant.

Our findings suggest a similar metabolism in cortex and striatum of racemic MDMA and both its isomers after s.c. administration. This metabolism seems to differ from the metabolism found in plasma (Fig. 3). In plasma, the metabolites MDA and HMMA showed nearly the same concentration. In cortex and striatum, the concentration of the metabolite MDA was increased in comparison to HMMA. This might suggest an additional pathway of metabolism in brain structures after the passage of the blood–brain barrier. This could also explain the increased concentration of (S)-MDA versus (R)-MDA in cortex and striatum in comparison to plasma. Neither in brain structures nor in serum did we

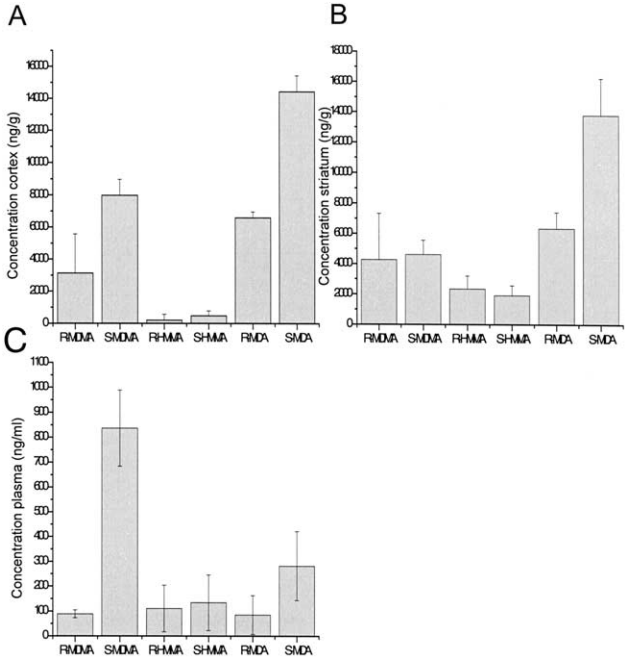


Fig. 3. Results of metabolism after s.c. administration of 20 mg/kg racemic MDMA in cortex (A), striatum (B) and plasma (C). Shown are mean values ± SEM of ten rats in each group.

Table 1
Concentrations of MDMA and its metabolites after s.c. administration of 20 mg/kg (R)- or (S)-MDMA[a]

	Cortex (ng/g)	Striatum (ng/g)	Plasma (ng/ml)
(R)-MDMA	10935 (±1650)	5249 (±1795)	102 (±1)
(R)-HMMA	100 (±41)	326 (±41)	8 (±4)
(R)-MDA	3671 (±1012)	7318 (±304)	6 (±3)
(S)-MDMA	2707 (±491)	1062 (±58)	431 (±96)
(S)-HMMA	2282 (±750)	2701 (±751)	664 (±39)
(S)-MDA	10532 (±1935)	17441 (±916)	12 (±5)

[a] Data shown are mean values ±SEM of ten rats in each group.

Table 2
Concentrations of MDE and its metabolites after s.c. administration of 20 mg/kg (R)- or (S)-MDE[a]

	Cortex (ng/g)	Striatum (ng/g)	Plasma (ng/ml)
(R)-MDE	11294 (±1164)	13548 (±803)	7411 (±2816)
(R)-HME	288 (±798)	415 (±115)	67 (±8)
(R)-MDA	399 (±131)	919 (±305)	64 (±12)
(S)-MDE	7702 (±1250)	8508 (±969)	5673 (±69)
(S)-HME	161 (±353)	15 (±2)	34 (±9)
(S)-MDA	83 (±14)	87 (±46)	19 (±5)

[a] Data shown are mean values ±SEM of ten rats in each group.

find any difference in the quality or quantity between the metabolism of the racemic MDMA or its isomers (S)-MDMA and (R)-MDMA (Fig. 3 and Table 1). Our findings for MDMA are partly in agreement with the results of Fallon et al. [5], who also detected a higher concentration of (S)-MDA versus (R)-MDA after administration of racemic MDMA in humans. This suggests an enantioselective metabolism of MDMA. For inexplicable reasons, however, we did not find a higher concentration of (R)-MDMA versus (S)-MDMA as seen with Fallon et al. [5] and as seems a logical consequence of the increased metabolism of (S)-MDMA to (S)-MDA.

Comparing the metabolism of MDMA and MDE, MDE is metabolized to a significantly lesser extent than MDMA (Fig. 4) which holds true for both the racemate and the isomers (S)-MDE and (R)-MDE (Table 2). Compared to MDMA, MDE shows a different relation of the MDE isomers in plasma and brain structures. In plasma we found more (R)-MDE than (S)-MDE, but the increased concentration of (S)-MDA as seen with MDMA could not be detected. However, judging from the different concentrations of (R)-MDE and (S)-MDE, an enantioselective metabolism for MDE could also be assumed which is in agreement with Spitzer et al. [16].

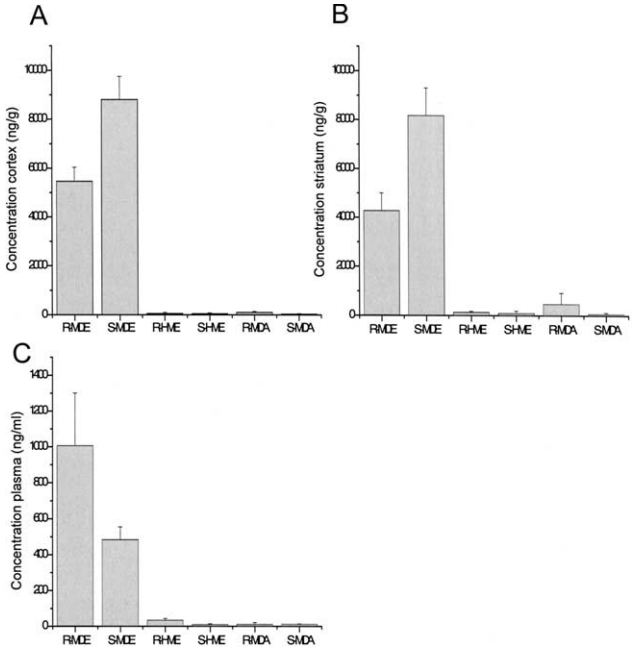


Fig. 4. Results of metabolism after s.c. administration of 20 mg/kg racemic MDE in cortex (A), striatum (B) and plasma (C). Shown are mean values ± SEM of ten rats in each group.

In brain structures, a reverse concentration of the MDE isomers was detected in comparison to plasma. We found a higher concentration of (S)-MDE versus (R)-MDE. This might suggest an enantioselective passage of MDE through the blood–brain barrier in that more (S)-MDE than (R)-MDE crosses the barrier.

MDMA is considered to be more toxic than MDE [14]. This is presently explained by high concentrations of the dihydroxymetabolites like DHMA and DHE [7] and by a high concentration of (S)-MDA [15] after metabolism of MDMA. Both theories are in agreement with our results, as we detected both an increased concentration of HMMA (being a metabolite of DHMA) and (S)-MDA after metabolism of MDMA in comparison to a significantly reduced concentration of HME (being a metabolite of DHE) and (S)-MDA after MDE metabolism.

In conclusion, our findings suggest an enantioselective metabolism for both MDMA and MDE. We propose that more (S)-MDE passes through the blood–brain barrier than its (R)-isomer. We also noticed increased concentrations of the toxic metabolites MDA and HMMA. This result is in agreement with other investigators regarding the increased toxicity of MDMA in comparison to MDE.

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