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Urinary excretion of deprenyl metabolites

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Abstract

(−)-Deprenyl metabolites in rat's urine, such as nordeprenyl, methamphetamine, amphetamine and *p*-hydroxy-methamphetamine were identified by HPLC–MS. After oral administration of 10 mg of pure (−)- and (+)-deprenyl to human volunteers, their urine was analyzed by gas chromatography. The concentration of methamphetamine was found to be overwhelming in the case of the (−)-isomer, while amphetamine and methamphetamine were excreted in equal amounts when (+)-deprenyl was administered. The metabolic processes of deprenyl resulted in metabolites possessing different lipophilicity, as it has been shown by planar displacement chromatography.

Keywords: Deprenyl metabolites; Amphetamines; Enzyme inhibitors

1. Introduction

(*R*)-(−)-N-Methyl-N-(1-phenyl-2-propyl)-N-propynylamine [(−)-deprenyl) is a selective inhibitor of monamine oxidase type-B (MAO-B) enzyme [1,2]. The inhibitor is presently used for the treatment of Parkinson's disease [3,4] and dementia of Alzheimer type [5,6]. Studies revealed that the beneficial effects of (−)-deprenyl in neurodegenerative disorders cannot be due solely to its irreversible MAO-B inhibitory action.

In addition, (−)-deprenyl increases the dopaminergic tone of the brain [7–9]. The neuronal rescue effect [10] and the reduction of cell apoptosis [10,11] seem to be MAO-independent consequences of (−)-deprenyl.

As early as 1978, Reynolds et al. [12] found amphetamines in human post-mortem parkinsonian brain after (−)-deprenyl treatment. Amphetamine was also identified in the urine of persons after they had taken (−)-deprenyl [13]. Heinonen et al. [15] reported that three major metabolites were eliminated in the human urine, and in plasma and cerebrospinal fluid after treatment with (−)-deprenyl. Both the serum level and the cerebrospinal fluid concentration of (−)-methamphetamine were about twice as high as that of (−)-amphetamine [14,15] and these metabolites were of levo form; no racemic transformation was found during metabolism of (−)-deprenyl [15–17]. Animals, such as rats [18–20], dogs [21], monkeys [22] and pigs [24] have also been widely used to study the metabolism of (−)-deprenyl. Methamphetamine and amphetamine were the major metabolites detected, with nordeprenyl [19–21,23] and unchanged deprenyl [19,22] being also sometimes found.

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Deprenyl metabolites were usually analyzed by gas chromatography. After derivatisation, HPLC has been used for determination of the three major deprenyl metabolites, nordeprenyl, methamphetamine and amphetamine [24]. Some recent works have employed GS–MS [25] and capillary electrophoresis for the metabolic studies on (−)-deprenyl [26,27]. The investigations confirmed that the chirality of (−)-deprenyl does not change during its metabolism.

The objectives of the present report are to identify metabolites of (−)-deprenyl in rat urine by HPLC–MS, and to compare human metabolism of (−)- and (+)-deprenyl.

2. Experimental

2.1. Chemicals

Standard compounds, such as (−)-deprenyl, (+)-deprenyl, nordeprenyl, methamphetamine, amphetamine, *p*-hydroxymethamphetamine and ephedrine were obtained from Chinoin Pharmaceutical Works (Budapest, Hungary). TLC 200×200 mm glass plates coated with a 0.2-mm layer of silica gel GF_{254} were obtained from E. Merck (Darmstadt, Germany). The highest purity solvents and other chemicals were purchased from commercial sources.

2.2. Apparatus

2.2.1. HPLC–MS

An HP 1090 Series II HPLC system (Hewlett-Packard, Palo Alto, CA, USA) was connected to an HP-59898 MS engine mass spectrometer equipped with an extended mass range (2000 U), a high energy dynode detector and an atmospheric pressure ionisation electrospray (API–ES) interface (HP 59987 A). A Harvard Type 22 syringe pump (Harvard Apparatus, South Natick, MA, USA) was employed to inject the samples into the electrospray ion source, and nitrogen gas was introduced into the solvent sample stream at a pressure of 50 p.s.i. (1 p.s.i.=6894.76 Pa). The fine spray formed was focused on to the spray shield (heated with nitrogen drying gas to 250°C) at a flow-rate of 8 l/min in order to facilitate solvent evaporation from the droplets in the spray chamber. The mass spectrometer, electrospray parameters and the capillary exit voltage (CapEx) were optimised with a standard solution to give molecular adduct ions ($[M+H]^+$) in the highest possible abundance and with minimum collision-induced dissociation (CID). To obtain maximum abundances within a scan for both the parent and fragment ions of deprenyl metabolites, the automatic changing facilities of CapEx were utilised by dynamic ramping. Thereby, the CapEx values were optimised for both the fragments and molecular ions of deprenyl metabolites. Data were acquired and analyzed by a Hewlett-Packard DOS ChemStation [24].

The stationary phase was Superspher RP-Select B, 4 μm, 250×3 mm column (Merck, Darmstadt), and the mobile phase was a 7:3 (v/v) mixture of acetonitrile and 100 m*M* ammonium acetate with 0.45 ml/min flow-rate.

2.2.2. Gas chromatography

An HP-6890 gas chromatograph with nitrogen–phosphorus detection (NPD) was used. The stationary phase was a 0.33- μm layer of Ultra 2 (5% phenylmethylsilicone and 95% methylsilicone) (Supelco, Bellefonte, PA, USA) in a fused-silica capillary column (12 m×0.2 mm I.D.), and the flow-rate of the carrier gas was 0.6 ml/min. The injector temperature was 280°C, and the detector temperature was 300°C. Separations were achieved by temperature programming from 120 to 310°C at 15°C/min. The ion source temperature was 200°C, and 70 eV ionisation energy was applied.

2.2.3. Thin-layer chromatography

Lipophilicity of some phenylisopropylamines was determined by reversed-phase thin-layer chromatography on TLC silica plates GF_{254} impregnated with a solution of paraffin in hexane (10%, v/v). Developments were done in 40%, 50%, 60% aqueous methanol and acetone solutions, the spots identified under UV light at 254 nm, and TLC characteristics measured. R_M values were calculated in the usual way, and the $R_{M,0}$ values were approximated from the content of the organic modifier versus R_M lines by the use of a Quattro Pro program. To improve the

calculated lipophilicity data, displacement thin-layer chromatography on TLC silica plates GF_{254} was also performed. The mobile phase was 3% triethanolamine displacer in a chloroform carrier.

2.3. Treatments and clean-up of samples

Albino Wistar rats of both sexes (weighing 120–150 g) were injected subcutaneously with (−)-deprenyl in a dose of 10 mg/kg, and urine was collected for 24 h.

Healthy male volunteers (between 80 and 90 kg of body mass) took a single dose of either 10 mg (−)-deprenyl or 10 mg of (+)-deprenyl, and urine was collected for 6 h.

Urine samples were acidified with 10% (v/v) concentrated hydrochloric acid, extracted with chloroform–ethyl acetate (3:1, v/v) to remove the acidic components; the pH was adjusted to 10 with 2.5 *M* sodium hydroxide, and the basic components were extracted with chloroform–ethyl acetate (3:1, v/v); the organic layer was evaporated in a vacuum. Metabolites were identified directly without any derivatization.

3. Results

HPLC–MS was used to identify deprenyl metabolites in the rat urine. Fig. 1 shows the total ion current of a HPLC run. The highest peak at 4.5 min is methamphetamine, with the other peaks at 3.13, 3.77 and 6.82 min being *p*-hydroxymethamphetamine, amphetamine and deprenyl, respectively. Fig. 2 gives the electrospray mass spectrum of *p*-hydroxymethamphetamine, where the characteristic masses are 166 135 and 107, that is $[M+H]^+$; $[M+H]^+$ with the loss of $-NH-CH_3$, and $[M+H]^+$ with the loss of $-CH(-CH_3)-NH-CH_3$.

After administration of (−)- and (+)-deprenyl to human volunteers, their urine was analyzed. In Fig. 3 gas chromatograms of human urine extracts are presented. In the case of (+)-deprenyl (right side), the peak of amphetamine (at about 2.28 min) and that of methamphetamine (at 2.65 min) have similar areas, while the metabolism of (−)-deprenyl (left side) resulted in a large excess of methamphetamine in comparison with amphetamine. Peaks in Fig. 3 were identified by standard substances as well as by the use of GC–MS.

Lipophilicity of deprenyl and its tentative metabolites was investigated by reversed-phase thin-layer chromatography by determining the $R_{M,0}$ values (Table 1). Fig. 4 shows that planar displacement chromatography gives information similar to the determination of lipophilicity. The order of the potential metabolites in the displacement train is similar to that of $R_{M,0}$ values.

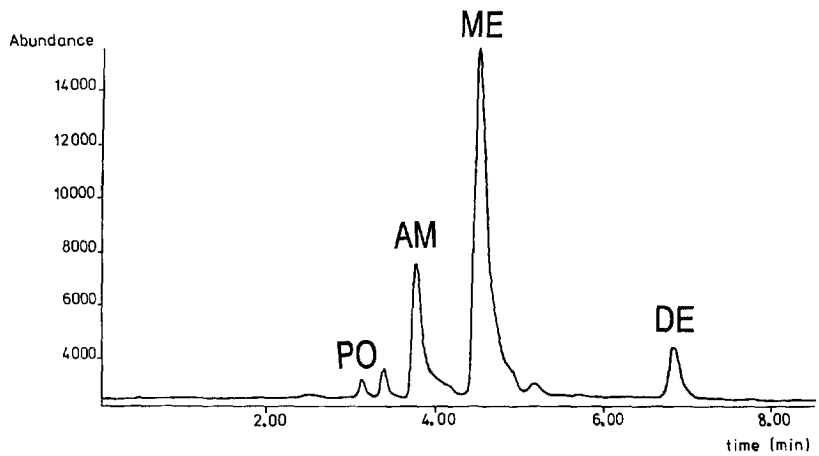

Fig. 1. HPLC identification of (−)-deprenyl metabolites extracted from rat urine. The highest peak (ME, 4.5 min) is methamphetamine, other peaks are *p*-hydroxymethamphetamine (PO, 3.13 min), amphetamine (AM, 3.77 min) and the unchanged (−)-deprenyl (DE, 6.82 min).

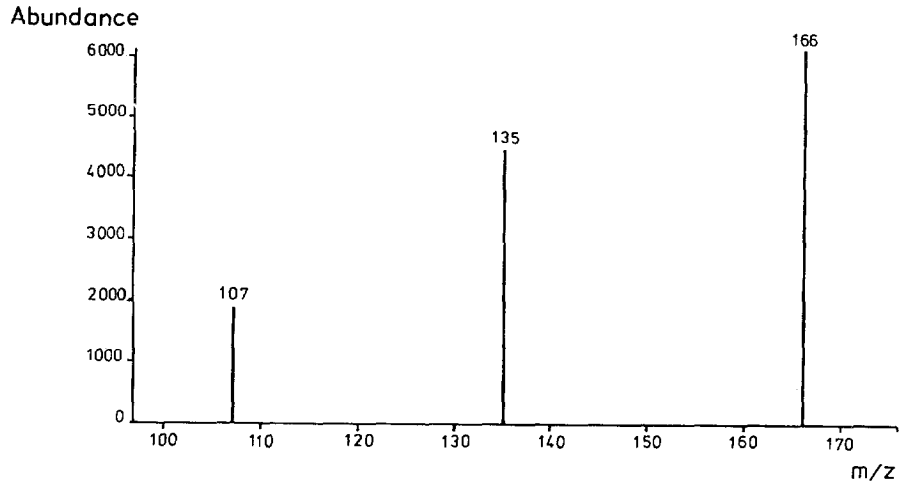

Fig. 2. Electrospray mass spectrum of *p*-hydroxymethamphetamine.

4. Discussion

Earlier findings pointed out the presence of *p*-hydroxyamphetamine and *p*-hydroxy-methamphetamine in rat urine treated with (−)-deprenyl [7]. Results of HPLC–MS confirmed that *p*-hydroxymethamphetamine takes part in the elimination of (−)-deprenyl from rats, and these investigations also proved the overwhelming role of methamphetamine in the metabolism.

Nordeprenyl, methamphetamine and amphetamine are the main deprenyl metabolites that were detected in human plasma, cerebrospinal fluid and urine after a single dose or continuous administration of the drug [7,24]. There was no significant change in the plasma level of metabolites if the single and multiple dosages were compared, that is, to say, the metabolites did not accumulate [12].

Gas chromatography with NPD shows that (−)-deprenyl is preferentially metabolised to metham-

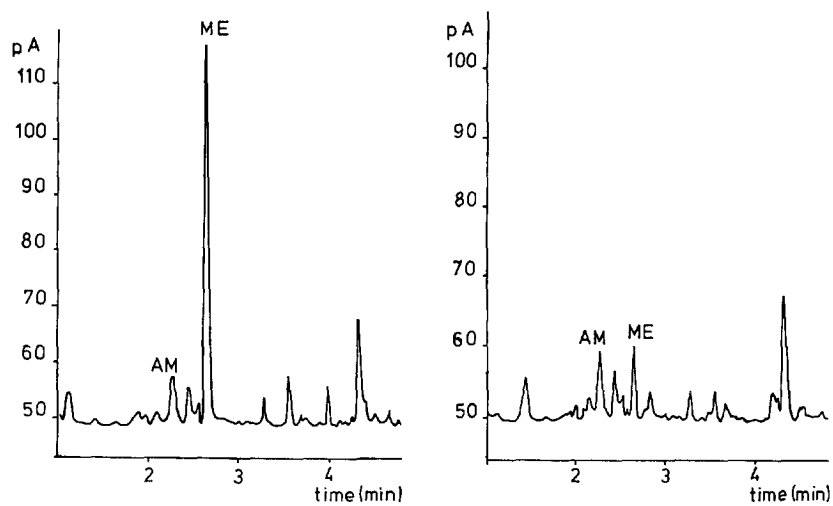

Fig. 3. Gas chromatograms of human urine extract after treatment of (−)deprenyl (left side) and (+)-deprenyl (right side). Peaks of methamphetamine (at 2.65 min) and amphetamine (at 2.28 min) are indicated with ME and AM.

Table 1
$R_{M,0}$ values for deprenyl (DE), and some of its potential metabolites such as nordeprenyl (ND), methamphetamine (ME), amphetamine (AM), ephedrine (EP) and *p*-hydroxymethamphetamine (PO)

Compound	$R_{M,0}$ value in methanol	$R_{M,0}$ value in acetone
DE	2.46	2.72
ND	1.47	1.85
ME	1.01	1.29
AM	0.87	1.07
EP	0.68	1.21
PO	0.66	1.13

phetamine, while oxidative dealkylation of (−)-deprenyl results in similar amounts of methamphetamine and amphetamine. The large excess of methamphetamine following (−)-deprenyl treatment may give a similarly enhanced catecholaminergic activity as that of (−)-deprenyl does [10]. Differences in the metabolic pattern of deprenyl enantiomers have been mentioned by Grace et al. [25]. Deprenyl was catabolized by recombinant cytochrome P 450 2D6. This enzyme also preferentially metabolised (−)-deprenyl compared to (+)-deprenyl. However, cytochrome P-450 2D6 favoured N-demethylation to N-depropynylation, which is rather unusual for an in vivo metabolism of any mammalian species.

$R_{M,0}$ and slope values obtained by reversed-phase thin-layer chromatography are proper characteristics of the lipophilicity as was discussed by Biagi et al. [28] in detail. The $R_{M,0}$ values (that is the R_M calculated for 0% of the organic modifier) are given in Table 1. It can be observed that an essential decrease of lipophilicity takes place during the loss of a propynyl group; that is through the metabolism of deprenyl to methamphetamine. Other potential metabolic alterations give either less change (such as demethylation to nordeprenyl) or need at least two steps to reach a similar alteration in lipophilicity (such as metabolism of deprenyl to amphetamine, ephedrine, *p*-hydroxymethamphetamine), which can be the reason that these metabolites could not be generally detected.

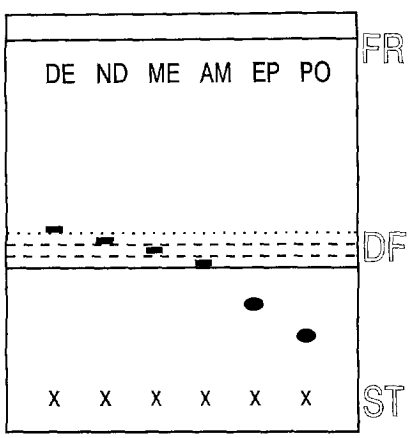

Fig. 4. Planar displacement chromatography gives a similar order of spots as measured by reversed-phase TLC. ST, DF and FR mean start, displacer fronts and carrier front, respectively. Other abbreviations as indicated in Table 1.

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