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Note

Gas chromatographic–mass spectrometric study of urinary metabolism of melperone*

CLAUS KÖPPEL*

Reanimationszentrum, Klinikum Rudolf Virchow, Standort Charlottenburg, Freie Universität Berlin, Spandauer Damm 130, D-1000 Berlin 19 (F.R.G.)

JOACHIM TENCZER

Abteilung Toxikologie, Landesuntersuchungsinstitut für Lebensmittel, Arzneimittel und Tierseuchen Berlin, Invalidenstrasse 60, D-1000 Berlin 21 (F.R.G.)

and

KARLA IBE

Reanimationszentrum, Klinikum Rudolf Virchow, Standort Charlottenburg, Freie Universität Berlin, Spandauer Damm 130, D-1000 Berlin 19 (F.R.G.)

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Melperone, 1-(4-fluorophenyl)-4-(4-methyl-1-piperidinyl)butanone, is a butyrophenone derivative with a wide spectrum of neuroleptic properties [1–5]. Clinically, the drug has been found to be effective in the treatment of alcoholic predelirium, anxiety, agitation and confusion, especially in geriatric patients [6–9]. The frequency of side-effects is rather low [10,11]. In contrast to other neuroleptic drugs, it has a stabilizing effect on cardiac function [12–14]. The plasma elimination half-life of melperone is ca. 3–4 h after oral or intravenous administration [15,16]. After intramuscular injection, a half-life of 6 h has been determined. The bioavailability of an oral dose is in the range of 60%. After an oral dose of 100 mg of melperone, non-linear elimination kinetics were observed. This finding seems to indicate saturation of the hepatic metabolizing enzymes.

Several metabolites of melperone have been described [15]. The keto function of melperone is reduced to the corresponding alcohol II (Fig. 1). Two further metabolites are 4-fluorobenzoic acid (XVI) and 4-fluorohippuric acid (XVII). Additionally, a metabolite with the fluorine atom replaced by a hydroxyl group

*Dedicated to Professor Dr. Karl-Heinz Beyer on the occasion of his 60th birthday.

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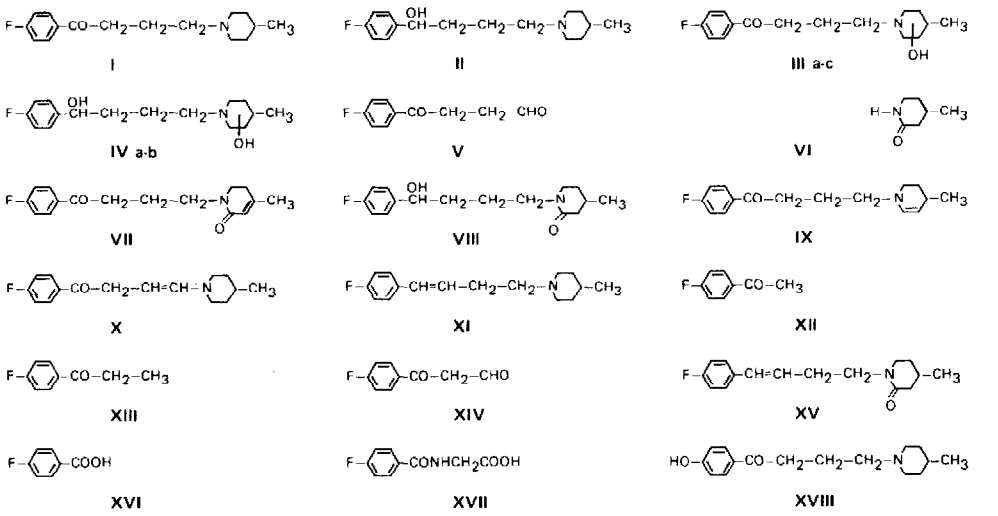

Fig. 1. Melperone, its metabolites, artifacts and derivatives.

(XVIII) has been postulated [17]. By hydrolysis of urine samples from patients with melperone overdose and acetylation of the extracts, Pfleger and co-workers [18,19] found 4-fluorophenyl-4-ketobutyric aldehyde (V), a melperone derivative with a double bond in the methylpiperidine ring (IX) and a further unidentified metabolite, but no XVIII.

In a case of acute poisoning with melperone, several previously unknown metabolites and derivatives of melperone could be detected by a gas chromatography–mass spectrometry (GC–MS) screening procedure [20]. This finding prompted us to reinvestigate the urinary metabolism of melperone in patients after a therapeutic dose.

EXPERIMENTAL

Clinical studies

Urine was collected for 24 h after an oral dose of 3×25 mg of melperone (Eunerpan®) to five geriatric patients (three males, two females) with acute myocardial infarction, disorientation and psychomotoric agitation. Laboratory tests were normal with respect to liver and kidney function. Further medication included intravenous nitroglycerine at 3 mg/h. Urine was pooled and stored at $-20\,^{\circ}C$ prior to analysis.

Reagents and chemicals

Pure samples of melperone were generously provided by Nordmark-Werke (Uetersen, F.R.G.). Compound II was synthesized by reduction of I with sodium tetrahydroborate. Reaction of II with acetic acid yielded a 1:9 mixture of acetylated II (IIa) and XI. All reagents, analytical-reagent grade or better, were purchased from commercial sources and used without further purification.

Extraction

A 20-ml sample of urine was extracted twice at pH 3 and subsequently at pH 10 with diethyl ether (Nanograde®, Mallinckrodt, St. Louis, MO, U.S.A.). The organic solvent was removed with a dry stream of nitrogen. The residue was dissolved in 100 μl of methanol, and a 1–3 μl aliquot was used for GC–MS. Similarly, urine was extracted after incubation (37°C) with 0.5 ml of glucuronidase/sulphatase (Merck, Darmstadt, F.R.G.) at pH 5.5 for 30 min and after hydrochloric acid hydrolysis for 30 min at 100°C. Extracts were analysed directly, after acetylation with acetic anhydride and after methylation with diazomethane in diethyl ether.

Instrumentation

Mass spectra were run on a 4021 gas chromatograph–mass spectrometer with an Incos data system (Finnigan, San José, CA, U.S.A.). For GC a fused-silica capillary column (SE 54, 25 m × 0.32 mm I.D.; Macherey & Nagel, Düren, F.R.G.) was used with an injection port temperature of 280°C, and a column temperature programme of 75–300°C at 15°C/min. The column was directly coupled to the mass spectrometer. Kovats indices were determined by calibration of the column with a mixture of C_{12}–C_{22} *n*-alkanes. The ion source pressure was $4 \cdot 10^{-5}$ Pa in the electron-impact (EI) mode and $3 \cdot 10^{-3}$ Pa in the chemical ionization (CI) mode using methane. The multiplier voltage was 1200 V.

All samples were run in the EI (70 eV) and in the CI mode. Structural elucidation was based on reference mass spectra (MSDS library version 5.5E), determination of the molecular ion by chemical ionization, fragmentation pattern and formation of the corresponding derivatives after acetylation of the extracts.

RESULTS

EI and CI mass spectral data are summarized in Tables I and II. The basic mass spectral fragmentation pattern of melperone is depicted in Fig. 2. Analogous fragmentation steps were observed in the mass spectra of compounds II–IV, VII–XI and XV. CI with methane yielded the typical $[M+41]^+$, $[M+29]^+$ and $[M+1]^+$ ions and few fragment ions (loss of H_2, H_2O and HF).

In underivatized urine extracts (with and without glucuronide cleavage) I, II, IIIa–c, IVa and b, V, VI, VII, VIII and IX were identified. After acetylation, acetylated II (IIa), X and XI, and after hydrochloric acid hydrolysis, XII, XIII, XIV and XV could also be detected. The exact position of the hydroxyl and keto function in the methylpiperidine ring of IIIa–c, IVa and b, VI, VII, VIII and XV could not be determined from the mass spectra. Obviously, different isomers or stereoisomers of the metabolites III and IV were formed. The exact position of the double bond in the methylpiperidine ring of VII and IX could not be located from the MS data. Compounds XVI, XVII and XVIII could not be identified in either the underivatized extract or in the methylated extract using the extraction procedure described above.

TABLE I

KOVATS INDICES AND EI-MS DATA OF MELPERONE, ITS METABOLITES, ARTIFACTS AND DERIVATIVES

Compound*	Kovats index	*m/z* (intensity %)**
I	1990	M^+ 263 (3), 220 (1), 165 (8), 138 (3), 125 (7), 124 (6), 123 (29), *112* (100), 110 (10), 95 (19), 69 (12)
II	2070	M^+ 265 (10), 248 (3), 232 (1), 199 (1), 165 (3), 125 (6), 123 (5), 113 (10), *112* (100), 99 (16), 84 (4), 69 (11), 55 (12)
IIa acetate	2310	M^+ 307 (6), 290 (0.5), 265 (4), 264 (32), 248 (4), 246 (2), 165 (6), 125 (2), 123 (3), 113 (10), *112* (100), 110 (9), 69 (11), 55 (10)
IIIa	2180	M^+ 279 (3), 165 (8), 141 (62), *128* (100), 125 (6), 123 (28), 110 (27), 95 (18), 83 (10), 69 (10)
IIIb	2220	M^+ 279 (2), 206 (2), 195 (2), 165 (12), 141 (72), *128* (100), 125 (6), 123 (33), 110 (4), 95 (17)
IIIc	2230	M^+ 279 (<0.5), 278 (1), 261 (4), 250 (2), 165 (7), 150 (3), 141 (67), *128* (100), 125 (6), 123 (31), 95 (18), 85 (7)
IVa	2245	M^+ 281 (12), 264 (2), 255 (1), 137 (3), *128* (100), 110 (12), 97 (12)
IVb	2260	M^+ 281 (10), 264 (4), 250 (10), 135 (8), *128* (100), 115 (12)
V	1580	M^+ 180 (23), 136 (12), 135 (19), 133 (6), 125 (41), 123 (21), 109 (17), 95 (22), *56* (100)
VI	1160	M^+ *133* (100), 112 (31), 96 (4), 84 (11), 57 (58), 55 (40)
VII	2275	M^+ 275 (29), 274 (22), 258 (3), 165 (52), 150 (16), 138 (35), 137 (81), 125 (11), 124 (18), *123* (100), 112 (20), 110 (18), 95 (57), 55 (19)
VIII	2420	M^+ 279 (23), 260 (4), 236 (6), 210 (3), 155 (63), 154 (48), 148 (53), 141 (31), 133 (7), 127 (28), *126* (100), 114 (17), 113 (15), 109 (16), 98 (42), 69 (27)
IX	2060	M^+ 261 (79), 260 (41), 244 (59), 232 (18), 218 (22), 165 (8), *138* (100), 123 (97), 111 (72), 96 (38), 95 (58), 69 (22)
X	2065	M^+ 261 (17), 244 (18), 232 (7), 218 (8), 138 (24), 123 (23), *112* (100), 95 (4), 69 (8)
XI	1920	M^+ 247 (<0.5), 246 (1), 228 (1), 199 (1), 149 (4), 133 (4), *112* (100), 83 (4), 55 (8)
XII	1280	M^+ *138* (100), 123 (80), 95 (31), 85 (12), 55 (23)
XIII	1310	M^+ 152 (2), 124 (10), *123* (100), 96 (4), 95 (43), 75 (14), 69 (6)
XIV	1340	M^+ 166 (62), 165 (69), 147 (5), 140 (26), 125 (31), 124 (22), *123* (100), 95 (30), 75 (12)
XV	2280	M^+ 261 (23), 149 (15), *148* (100), 135 (11), 134 (10), 133 (18), 127 (11), 126 (83), 115 (11), 109 (14), 98 (48), 69 (16), 56 (22)

*See Fig. 1 for structures.
**M^+ = molecular ion; base peak (100%) italicized.

TABLE II

CI-MS DATA OF MELPERONE, ITS METABOLITES, ARTIFACTS AND DERIVATIVES

Compound*	*m/z* (intensity %)**
I	304 (5), 292 (15), $[M+1]^+$: *264* (100), 262 (15), 244 (30)
II	306 (3), 294 (10), $[M+1]^+$: *266* (100), 264 (22), 248 (20)
IIa	348 (2), 336 (21), $[M+1]^+$: *308* (100), 288 (20)
IIIa	320 (3), 308 (16), $[M+1]^+$: *280* (100), 262 (35), 260 (10)
IIIb	320 (5), 308 (25), $[M+1]^+$: *280* (100), 262 (25), 260 (5)
IIIc	320 (6), 308 (17), $[M+1]^+$: *280* (100), 262 (20), 260 (10)
IVa	322 (10), 310 (25), $[M+1]^+$: *282* (100), 264 (30), 262 (20)
IVb	322 (5), 310 (26), $[M+1]^+$: *282* (100), 264 (30), 262 (10)
V	221 (5), 209 (27), $[M+1]^+$: *181* (100)
VI	154 (3), 142 (10), $[M+1]^+$: *114* (100)
VII	316 (2), 304 (10), $[M+1]^+$: *276* (100), 256 (15)
VIII	320 (6), 308 (10), $[M+1]^+$: *280* (100), 262 (30), 260 (10)
IX	302 (3), 290 (11), $[M+1]^+$: *262* (100), 260 (22), 242 (18)
X	302 (4), 290 (25), $[M+1]^+$: *262* (100), 260 (18), 242 (18)
XI	288 (6), 276 (30), $[M+1]^+$: *248* (100), 246 (30), 228 (13)
XII	179 (3), 167 (21), $[M+1]^+$: *139* (100), 119 (21)
XIII	193 (2), 181 (11), $[M+1]^+$: *153* (100), 133 (11)
XIV	207 (4), 195 (12), $[M+1]^+$: *167* (100)
XV	302 (7), 290 (12), $[M+1]^+$: *262* (100), 260 (10), 242 (18)

*See Fig 1 for structures.
**$[M+1]^+$ = protonated molecular ion; base peak (100%) italicized.

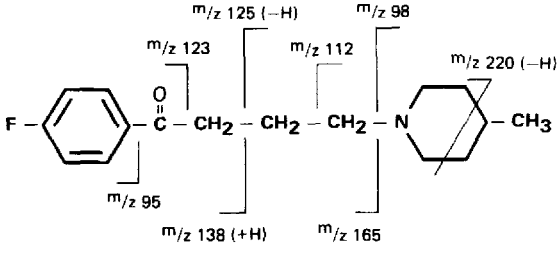

Fig. 2. Fragmentation pattern of melperone (molecular ion *m/z* 263).

DISCUSSION

Besides unchanged melperone, a variety of metabolites could be identified: II, IIIa–c, IVa–c, V, VI, VII and VIII. Compounds IX, X, XI and XV were probably artifacts generated by dehydration of the corresponding alcohols. Decarboxylation of 4-fluorophenyl-3-ketopropionic acid and 4-fluorophenyl-4-ketobutyric acid probably led to the artifacts XII and XIII. The mechanism of formation of the artifact XIV is not clear. Compounds XVI, XVII and XVIII could not be detected under the extraction conditions described. The mass spectrum of the unidentified acetylated metabolite described by Pfleger et al. [18] is identical with that of acetylated II (IIa). However, the mass spectrum that was related to the structure of IX by Pfleger et al. fits the structure of X much better, according to its fragmentation pattern.

The main metabolic pathways of melperone were: (1) reduction of the keto function; (2) hydroxylation and oxidation of the methylpiperidine ring at different positions; (3) hydroxylation and further oxidation of the alipathic C_4 chain; and (4) N-desalkylation. Many of the metabolites were products of a combination of these metabolic steps. Human metabolism of melperone is rather similar to that of other butyrophenones, such as haloperidol [21,22]. It remains to be established whether melperone metabolites are pharmacologically active. In patients not responding to neuroleptic therapy with haloperidol, the plasma concentration of reduced haloperidol (a structural analogue of II) seemed to be higher than in responders to therapy, although the haloperidol plasma levels in the two groups did not differ significantly [23].

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