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Determination of 4-(4-chlorophenyl)-4-hydroxypiperidine, a metabolite of haloperidol, by gas chromatography with electron-capture detection

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Abstract

An electron-capture gas chromatographic procedure was developed for the analysis of 4-(4-chlorophenyl)-4-hydroxypiperidine (CPHP), a metabolite of haloperidol. The assay involved basic extraction of this metabolite from the biological samples, followed by back-extraction with HCl. After basification of the acid phase, extractive derivatization with pentafluorobenzoyl chloride in toluene was conducted. The pentafluorobenzoyl derivative was quantified on a gas chromatograph equipped with a fused-silica capillary column, an electron-capture detector and a printer-integrator. N-(3-Trifluoromethylphenyl)piperazine was carried through the procedure as an internal standard and calibration curves were determined for each assay run. The procedure was demonstrated to be linear and reproducible and was utilized to detect and quantify CPHP in urine, plasma, brain and liver samples from rats treated with haloperidol. The structure of the derivatized metabolite was confirmed by gas chromatography–mass spectrometry.

Keywords: Haloperidol; 4-(4-Chlorophenyl)-4-hydroxypiperidine

1. Introduction

Although haloperidol has been one of the most frequently prescribed antipsychiotics for many years, much still remains unknown about its metabolic fate. In recent years, it has been reported to be metabolized to a number of biologically active metabolites that may be responsible for some of the pharmacological and toxicological effects of haloperidol [1–11]. The pyridinium metabolite (HP^+) of haloperidol, for example, is an analogue of the dopaminergic neurotoxin N-methyl-4-phenylpyridinium (MPP^+) and was therefore suggested to be involved in some of the motoneuron side effects of haloperidol [1–9,11]. This hypothesis was supported by the observations that HP^+ was neurotoxic both in vitro [7,8] and in vivo [9]. HP^+ and some other metabolites of haloperidol were also shown to inhibit monoamine oxidase [10] and dopamine and noradrenaline uptake [11].

Soudijn et al. [12], in a study utilizing radiolabelled haloperidol, demonstrated that *p*-fluorobenzoylpropionic acid is an N-dealkylation product of haloperidol but 4-(4-chlorophenyl)-4-hydroxypiperidine (CPHP) (Fig. 1), the other portion of the haloperidol molecule, was not detected because the

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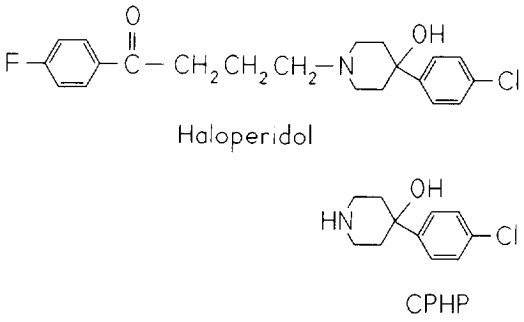

Fig. 1. Structures of haloperidol and its metabolite CPHP [4-(4-chlorophenyl)-4-hydroxypiperidine].

radioactive label was not on this part of the molecule. We have previously demonstrated that CPHP is produced by liver microsomal preparations from various animal species in the presence of NADPH [3,4,6]. Using a gas chromatographic (GC) procedure with nitrogen phosphorus detection, Ablordeppey et al. [13] detected CPHP in rat liver after intraperitoneal injection of haloperidol but not after oral administration. These workers provided no mass spectrum for the unequivocal identification of the peak; although the sensitivity of the method was not stated, it appears to be about 200 ng/g of liver. In the present study, a more sensitive procedure using electron-capture detection (ECD) was developed for the analysis of CPHP. The assay was utilized to detect and quantify this metabolite in different biological samples from rats treated with haloperidol. The structure of the derivatized CPHP was confirmed using combined gas chromatography–mass spectrometry (GC–MS).

2. Experimental

2.1. Chemicals

CPHP and pentafluorobenzoyl chloride (PFBC) were purchased from Aldrich (Milwaukee, WI, USA). Glass-distilled toluene and sodium carbonate were obtained from BDH (Toronto, Canada) and Fisher Scientific (Ottawa, Canada), respectively. The internal standard N-(3-trifluoromethylphenyl)-piperazine hydrochloride (TFMPP) was purchased from Research Biochemicals International (Natick, MA, USA). All chemicals were of the highest grade commercially available.

2.2. Animals

Male Sprague–Dawley rats (Ellerslie Biosciences, Edmonton, Canada), 300–350 g, were housed in pairs in an environmentally controlled room using a 12 h light–12 h dark cycle. At the appropriate time animals were injected intraperitoneally with either vehicle or haloperidol. Urine samples were collected by placing the rats in metabolic cages. Animals were killed by decapitation 4 h after the last injection and the brains and livers were removed and immediately frozen on solid CO_2. Blood was collected from the neck region immediately after decapitation into a Vacutainer containing EDTA (Becton Dickinson Vacutainer Systems, NJ, USA) and centrifuged at 1000 *g* for 10 min in a Sorvall RT6000B Tabletop centrifuge (Sorvall Instruments, DE, USA).

2.3. Sample preparation

Rat tissues were weighed and homogenized in five volumes of ice-cold distilled water. An aliquot (1 ml) was removed from the homogenized sample and used in the analytical procedure. Appropriate calibration standards of CPHP were also prepared along with the samples by diluting standard solutions of authentic CPHP in control tissue homogenate prepared from drug-naive rats. The final calibration concentrations ranged from 6.25 to 800 ng per volume of tissue homogenate or body fluid for CPHP. In all studies, the volumes of tissue homogenate or biological fluids used in the calibration curves were the same as those of the samples taken from the haloperidol-treated rats; these calibration curves were included with each assay run.

2.4. Sample extraction

An aliquot (1 ml) of rat tissue homogenate or plasma was placed in an Eppendorf tube and the internal standard (TFMPP; 1 μg in 10 μl of distilled water) was added. The sample was basified by adding 0.5 ml saturated sodium carbonate and mixing briefly on a vortex-mixer. Glass-distilled toluene (6 ml) was added and the two phases were mixed for

10 min in an IkaVibrex VXR vortex-mixer (Janke and Kunkel, Staufen, Germany) and centrifuged for 5 min at 1000 *g* in a benchtop centrifuge (Sorvall GLC-2B general laboratory centrifuge, Du Pont, Wilmington, DE, USA). The toluene layer was transferred to a second 160×15 mm screw-cap culture tube to which was added 1 ml of 0.1 *M* HCl. The toluene–HCl mixture was vortex-mixed for 10 min and centrifuged for 5 min at 1000 *g*. The toluene layer was discarded and the remaining aqueous layer was basified with 1 ml saturated sodium carbonate. To this basic solution was added the derivatizing solution (10 μl PFBC in 4 ml toluene). The two phases were mixed for 10 min and centrifuged for 5 min at 1000 *g*. The toluene layer was retained and transferred to a 100×13 mm screw-cap culture tube. The organic solvent was evaporated in a Speed Vac SC110 (Savant Instruments, Farmingdale, NY, USA) and the residue was reconstituted in 0.3 ml of glass-distilled toluene. Of this solution, 1 μl was used for gas chromatographic (GC) analysis in all assays. Urine samples were diluted 100-fold with water and processed in the same way as other samples; the internal standard TFMPP (1 μg) was added to all samples and carried throughout the procedure described above. Calibration curves were conducted with each assay run.

2.5. Instrumental analysis

All samples were analysed using an HP 5790 gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with a ^{63}Ni electron-capture detector. The chromatographic column installed was a 25 m×0.32 mm I.D. HP-5 crosslinked 5% phenyl methyl silicone capillary column (1.05 μm film thickness; J & W Scientific, Palo Alto, CA, USA). The conditions for separation were as follows. An initial oven temperature of 105°C was maintained for 0.5 min, then increased to 295°C at a rate of 15 °C/min. The final oven temperature of 295°C was maintained for 7 min. The detector and injection port temperatures were held constant at 325 and 250°C, respectively. All injections of samples were carried out using the splitless mode of injection with a purge off time of 0.5 min. The ultra-pure helium (Union Carbide, Edmonton, Canada) carrier gas flow-rate and 10% argon-methane (Union Carbide) make-up gas flow-rate were 2.5 and 30 ml/min, respectively. The head pressure on the column was 45 psi.

Mass spectra (for confirmation of structures of the derivatives) were recorded in the electron-impact mode using a VG 7070E mass spectrometer (VG Instruments, Manchester, UK), linked to a Varian Vista gas chromatograph (Varian Instruments, Sunnyvale, CA, USA). The capillary column and the temperature program used for separation were the same as those used for electron capture GC analysis of these compounds.

3. Results and discussion

Some representative chromatograms of derivatized extracts of urine and plasma samples and brain and liver homogenates obtained from haloperidol-treated and control (vehicle-treated) rats are shown in Fig. 2. Extracts of brain and liver homogenate obtained from drug-free rats and plasma or urine samples showed no chromatographic peaks that interfered with the analysis of CPHP. The identities of the chromatographic peaks representing the derivatized CPHP and TFMPP were confirmed by comparing their GC retention times and mass spectra to those of authentic standards of CPHP and TFMPP, similarly derivatized. The mass spectra and proposed fragmentation patterns of the derivatized CPHP and TFMPP are depicted in Fig. 3 and Fig. 4. Haloperidol was run through the entire assay procedure and gave no interference peak in the analysis of CPHP.

Calibration graphs were obtained by analysing standards prepared in parallel with the samples for each assay run. The lower limit of quantitation was 5 ng/ml. Six- or eight-point calibration graphs were generated over the concentration range of 6.25 to 800 ng (0.03 to 3.78 nmol of CPHP) per volume of tissue homogenate, plasma or urine extracted. Regression analysis of the relationship between standard concentrations and the chromatographic peak-height ratio of the compound/internal standard yielded a linear relationship over the concentration range analysed, with a typical r^2 value >0.99. Calibration curves generated on different days were reproducible. Slopes and *y*-intercepts of the curves for the derivatives of CPHP were similar for all bio-matrices

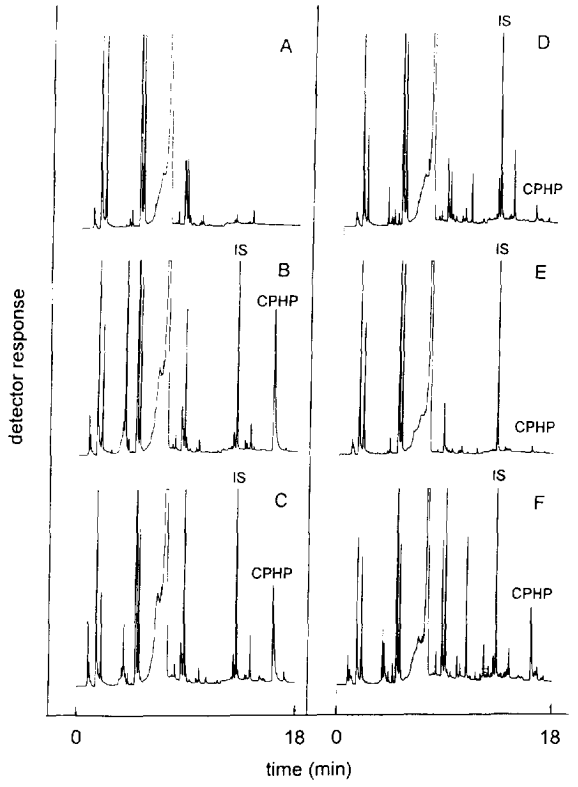

Fig. 2. Representative chromatograms of derivatized extracts of biological samples from haloperidol- or vehicle-treated rats. (A) Urine from drug-naive rats injected with vehicle; (B) urine from drug-naive rats spiked with authentic CPHP; (C–F) chromatograms of derivatized extracts of urine, plasma, brain and liver, respectively, from rats treated with haloperidol (5 mg/kg daily for three days); urine samples were collected for 24 h from day 2 to 3, and tissue samples were collected 4 h after the last dose of haloperidol on day 3. The GC retention times of the derivatives of the internal standard (IS) and CPHP were 13.2 and 16.2 min, respectively.

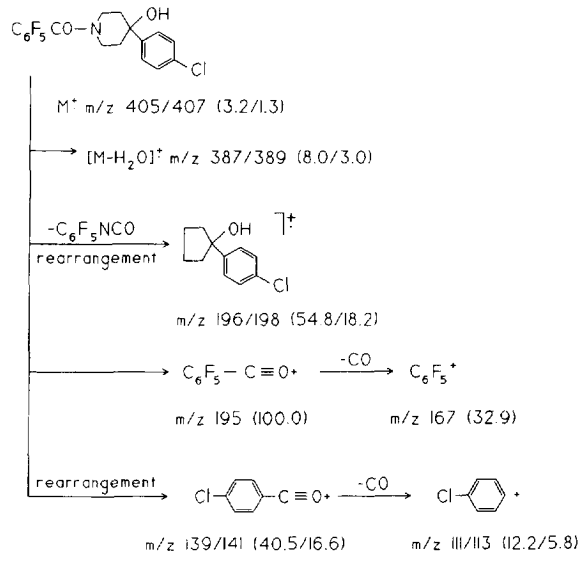

Fig. 3. Electron-impact mass fragmentation of the pentafluorobenzoyl derivative of CPHP. Values in parentheses represent % relative abundance.

and the mean values, based on μg of CPHP plotted on the X-axis, were 1.54 and 0.014, respectively. Recovery of the extraction/back extraction for blank urine samples spiked with 200 ng/ml CPHP was determined to be 77%. Intra- and inter-day coefficients of variation were 6.2 and 8.4% respectively for 50 ng/ml CPHP and 2.4 and 11.2% respectively for 400 ng/ml CPHP. These results indicate that the developed analytical procedure is both sensitive and reproducible.

Levels of CPHP in urine, plasma, brain and liver in rats treated with haloperidol were quantified and the results are presented in Table 1. Urine contained by far the highest levels of CPHP, indicating that CPHP was rapidly cleared from the tissues and excreted.

CPHP has been reported to induce a delayed and persistent freezing action in *Rana pipiens* frogs at high doses [13]. It has also been reported to bind to sigma receptors (K_i=326 nM) but not the dopamine D2 receptor [14]. The present study demonstrated that CPHP levels in the brain at the dose regimen used are similar to the K_i value noted above for binding to sigma receptors.

This study provided unequivocal evidence that CPHP is an in vivo metabolite of haloperidol. The analytical procedure described here is a rapid and sensitive method for the analysis of CPHP in biological fluids and tissues and should be useful for future studies on the pharmacokinetic profile of CPHP after administration of haloperidol, alone or in combination with other drugs. Such studies will be very important since haloperidol is often given concomitantly with other drugs that compete for

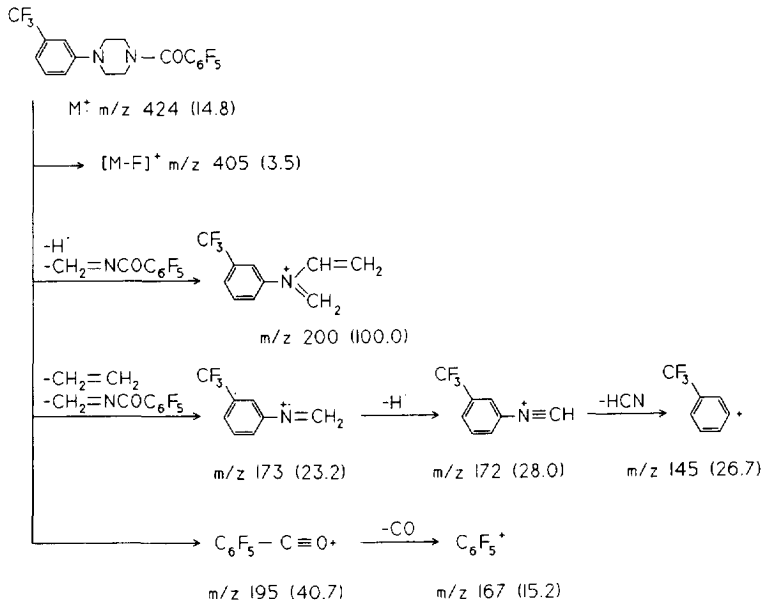

Fig. 4. Electron-impact mass fragmentation of the pentafluorobenzoyl derivative of the internal standard TFMPP. Values in parentheses represent % relative abundance.

cytochrome P-450 isoenzymes, although little is currently known about the role of individual P-450 isoenzymes in the formation of specific metabolites of haloperidol. It has been reported that co-administration of haloperidol with other drugs such as fluoxetine and fluvoxamine that act on such isoenzymes can result in elevated plasma levels of haloperidol in humans [15–17] as well as increased side effects [18]. An assay such as that described here will be very useful for studying the formation and disposition of CPHP under such conditions.

Table 1
Levels of the haloperidol metabolite CPHP in urine, plasma, brain and liver of rats after administration of haloperidol

Day	Urine[a] (μg/24 h)	Plasma (ng/ml)	Brain (ng/g tissue)	Liver (ng/g tissue)
1	110.0±16.0	13.1±2.2	88.7±13.1	492.0±63.5
3	205.9±33.9	21.6±3.2	127.9±19.1	1015.6±187.9

Rats (five per group) were either injected with 5 mg/kg haloperidol and sacrificed 4 h later or injected with 5 mg/kg haloperidol daily for three days and killed 4 h after last injection. Values are presented as mean±S.E.M.

[a]Urine samples were collected for 24 h on days 1–2 or on day 2–3 before the last injection. Representative GC traces related to determination of these values are shown in Fig. 2.

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