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DETERMINATION OF PLASMA AND BRAIN CONCENTRATIONS OF TRAZODONE AND ITS METABOLITE, 1-*m*-CHLOROPHENYLPIPERAZINE, BY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

A sensitive and specific gas chromatographic procedure is described for the quantitation of trazodone and its active metabolite, 1-*m*-chlorophenylpiperazine (mCPP), in plasma and brain. After addition of internal standards, the samples were extracted with benzene and the extracts divided into two portions. One portion was evaporated to dryness, the residue dissolved in methanol and the solution injected into a gas chromatograph equipped with a nitrogen-selective detector, for trazodone quantitation. To the remaining half of the extracts, 100 μl of heptafluorobutyric anhydride solution were added and the metabolite was measured as the heptafluorobutyryl derivative by electron-capture detection. Gas chromatography–mass spectrometry was used to confirm the specificity of the analyses.

The kinetic profile of trazodone and its metabolite was investigated after oral administration of trazodone (25 mg/kg). The parent drug and its metabolite both accumulated in brain, reaching concentrations several times those in plasma. More mCPP than the parent compound entered the brain; the ratio of the area under the curve for trazodone to mCPP in plasma was about 4, whereas in brain it was only about 0.8.

INTRODUCTION

Trazodone,2-{3-[4-(*m*-chlorophenyl)-1-piperazinyl]propyl}-*s*-triazolo[4,3*a*]pyridin-3-(2*H*)-one hydrochloride, a non-tricyclic antidepressant, is extensively metabolized in man and animals, only a small percentage being excreted unchanged in the 24-h urine and faeces[1]. The main products of elimination are hydroxylated derivatives[2,3] and oxotriazolopyridinepropionic acid originating from oxidative cleavage of the parent compound[2,4,5]. There is no evidence that these metabolites are biologically active.

One metabolic pathway includes oxidation of the phenylpiperazine system of the trazodone molecule, with the formation of 1-*m*-chlorophenylpiperazine (mCPP), which has recently been isolated as the glucuronide from rat urine[6]. Pharmacological studies indicated that mCPP was more active than trazodone as an inhibitor of the

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membrane uptake mechanism for serotonin (5HT) in the brain[7], a property shared by some tricyclic antidepressants[8]. Subsequent studies showed that mCPP behaved like a 5HT agonist in the central nervous system[9–11]. Some workers[12] reported that trazodone acted as a central 5HT agonist at high doses but at lower doses it exerted anti-5HTergic activity, suggesting that the former effect might be induced by its metabolite. It is therefore of interest to follow the time courses of the production and elimination of mCPP, because in the studies mentioned[12] the metabolite was not directly identified in trazodone-treated animals. Moreover, in seeking a relationship between the plasma and tissue concentrations and pharmacological effects it is necessary to quantitate both the metabolite and trazodone.

Methods available for the quantitation of trazodone use spectrofluorimetry[1], ^{14}C-labelled compound[2,4], gas chromatography (GC) with flame-ionization detector or mass fragmentographic techniques[13], and are aimed only at the analysis of the parent compound. This paper reports a GC method for the quantitation of trazodone in biological samples, using a simpler and more sensitive procedure, *viz.*, rapid extraction and the use of a nitrogen-selective detector. The procedure includes quantitation of the metabolite by GC with electron-capture detection. GC–mass spectrometry (MS) was used to confirm the presence of the drug and its metabolite. Plasma and brain concentration curves were plotted for both compounds after oral administration of trazodone to rats.

EXPERIMENTAL

Trazodone hyydrochloride was supplied by Angelini (Rome, Italy), mCPP hydrochloride by Aldrich-Europe (Beerse, Belgium), benperidol by Janssen (Beerse, Belgium) and 4-amino-1-(6-chloro-2-pyridyl)piperidine hydrochloride by Clin-Midy (Montpellier, France). Heptafluorobutyric anhydride (HFBA), as a 25% (v/v) solution in ethyl acetate, was obtained from Pierce (Rockford, IL, U.S.A.). Formic acid, *n*-heptane, chloroform and benzene were obtained from Carlo Erba (Milan, Italy).

Apparatus

GC determinations of trazodone were carried out on a Dani 3400 gas chromatograph equipped with a nitrogen–phosphorus detector (NPD). A glass column (1 m × 3 mm I.D.) packed with 80–100-mesh Gas-Chrom Q with 3% OV-1 as the stationary phase (Supelco, Bellefonte, PA, U.S.A.) was used. The temperatures of the column, detector and injector port were maintained at 270°C. The carrier gas was nitrogen at a flow-rate of 30 ml/min.

mCPP heptafluorobutyrate was analysed on a Carlo Erba Fractovap 2150 instrument equipped with a ^{63}Ni electron-capture detector. The chromatographic column was a glass tube (2 m × 3 mm I.D.) packed with 80–100-mesh Supelcoport with 3% OV-17 as the stationary phase (Supelco). The oven, injector port and detector temperatures were 205, 250 and 250°C, respectively. The carrier gas was nitrogen at a flow-rate of 35 ml/min.

GC–MS analysis was performed on an LKB 2091-051 instrument coupled with an LKB-2130 computer system for data acquisition and calculation. The gas chromatograph was operated under the conditions described above and mass spectra were collected in the electron-impact mode at 70 eV.

Animals

Male CD-COBS rats (Charles River, Como, Italy), average weight 250 g, were used.

Extraction from plasma

To 0.5–2 ml of heparin-treated plasma were added 50 μl of a methanolic solution of 4-amino-1-(6-chloro-2-pyridyl)piperidine (2 μg/ml) and water to a final volume of 2 ml, followed by 0.5 ml of 1 *N* sodium hydroxide solution and 10 ml of benzene. The samples were mechanically shaken and centrifuged. An 8.5-ml volume of the benzene phase was evaporated to dryness, the residue dissolved in 0.05 ml of a methanolic solution of benperidol (10 μg/ml)[13] and 1–2 μl were injected into the GC column described for trazodone analysis.

To the remaining benzene phase (1 ml), 100 μl of an ethyl acetate solution of HFBA (25%, v/v) were added and the samples were heated at 60°C for 30 min.

After the reaction the samples were washed with water (1 ml) and 5% aqueous ammonia solution (0.5 ml) and 1–2 μl of the benzene phase were injected into the GC column for mCPP analysis.

Extraction from brain

Brains were homogenized (9 ml/g) in cold acetone–1 *N* formic acid (85:15) and centrifuged. The supernatant was shaken twice with *n*-heptane–chloroform (4:1), the organic phase was discarded and the aqueous phase (0.5 ml) was used for drug extraction as described for plasma.

Internal standard calibration graphs

Drug-free plasma and brain samples containing known amounts of trazodone (0.05–0.5 μg) and mCPP (0.01–0.5 μg) were analysed concurrently with each set of unknown samples.

Calibration graphs were constructed by plotting the ratios of the peak areas of trazodone and mCPP to those of the respective internal standards and comparing the amounts of trazodone and mCPP added. Concentrations of trazodone and mCPP in the unknown samples were subsequently determined from the calibration graphs.

Recovery studies

Percentage recoveries were calculated by comparing the peak-area ratios of trazodone and mCPP heptafluorobutyrate after plasma and brain extraction with the peak-area ratios obtained by direct injection of standard solutions of trazodone or mCPP heptafluorobutyrate.

RESULTS AND DISCUSSION

Flame-ionization detection is not sensitive enough for kinetic studies of trazodone and the biological extracts have to be cleaned up in order to eliminate interfering constituents[13]. The selective detector significantly improves the sensitivity and reduces interference from endogenous constituents. However, electron-capture detection is not very sensitive to high-molecular-weight compounds such as trazodone, which contain only one chlorine atom. We chose the alternative of using an NPD,

achieving high sensitivity and specificity with the nitrogen-containing compound involved. Under the experimental conditions used the ratio of the peak area of trazodone to that of the internal standard was linear in the range 1–10 ng per injection. An additional sample dilution was necessary at concentrations higher than 10 ng per injection; 1 ng per injection (2 μl) was the detection limit, corresponding to 0.05 μg per millilitre of plasma or per gram of brain. The greater response of mCPP heptafluorobutyrate to the ECD provided high sensitivity and specificity in the analysis of the metabolite. Concentrations down to 10 ng per millilitre or per gram of tissue could still be measured. Thus, evaporation of the biological extracts, which resulted in unpredictable losses of mCPP, was avoided. The ratio of the peak area of mCPP heptafluorobutyrate to that of the internal standard was linear in the range from 0.01 ng (2 μl) to 0.5 ng per injection. Extraction from plasma and brain homogenates using *n*-hexane, diethyl ether or benzene was investigated. Benzene was found to be the most suitable as it gave consistent recoveries of both trazodone and mCPP after a single extraction. A summary of the recoveries during the kinetic studies in rats is reported in Table I. Trazodone was extracted reproducibly with a mean recovery of 81 ± 1.3% and a coefficient of variation (C.V.) of 3.6–10%. The recoveries of mCPP from plasma samples were 82–94% with a C.V. of 3.3–9.7%; recoveries from brain homogenates were 83–86% with a C.V. of 4.6–7.2%.

Figs. 1 and 2 are typical chromatograms of extracts from (A) a spiked brain sample, (B) the brain of a rat treated with trazodone (25 mg/kg, p.o.) and (C) a drug-free homogenized brain. The extracts from drug-free plasma or brain show no peaks that could interfere with the analysis of trazodone or its metabolite. Retention times were 4.2 min for trazodone and 5.3 min for the internal standard. The retention times of heptafluorobutyrate derivatives of mCPP and 4-amino-1-(6-chloro-2-pyridyl)-1-piperidine were 4.2 min and 3.3 min, respectively. Specificity of the analysis was confirmed when unknown plasma and brain samples of rats given trazodone were analysed by GC–MS. The mass spectra were identical with those obtained after

TABLE I

RECOVERY OF TRAZODONE AND 1-*m*-CHLOROPHENYLPIPERAZINE FROM PLASMA AND BRAIN

Each value is the mean of five determinations.

Sample	*Amount added (μg)*	*Trazodone*		*1-m-Chlorophenylpiperazine*	
		Amount found (μg ± S.D.)	*Recovery (% ± S.D.)*	*Amount found (μg ± S.D.)*	*Recovery (% ± S.D.)*
Plasma	0.01	–	–	0.008 ± 0.001	82 ± 8
	0.05	0.040 ± 0.003	80 ± 6	0.045 ± 0.004	91 ± 8
	0.10	0.080 ± 0.004	80 ± 4	0.090 ± 0.003	90 ± 3
	0.25	0.205 ± 0.080	82 ± 3	0.230 ± 0.007	92 ± 3
	0.50	0.404 ± 0.038	81 ± 8	0.469 ± 0.029	94 ± 6
Brain	0.05	0.040 ± 0.003	81 ± 5	0.041 ± 0.003	83 ± 6
	0.10	0.080 ± 0.008	80 ± 8	0.085 ± 0.006	85 ± 6
	0.25	0.198 ± 0.010	79 ± 4	0.215 ± 0.009	86 ± 4
	0.50	0.417 ± 0.037	83 ± 7	0.430 ± 0.020	85 ± 4

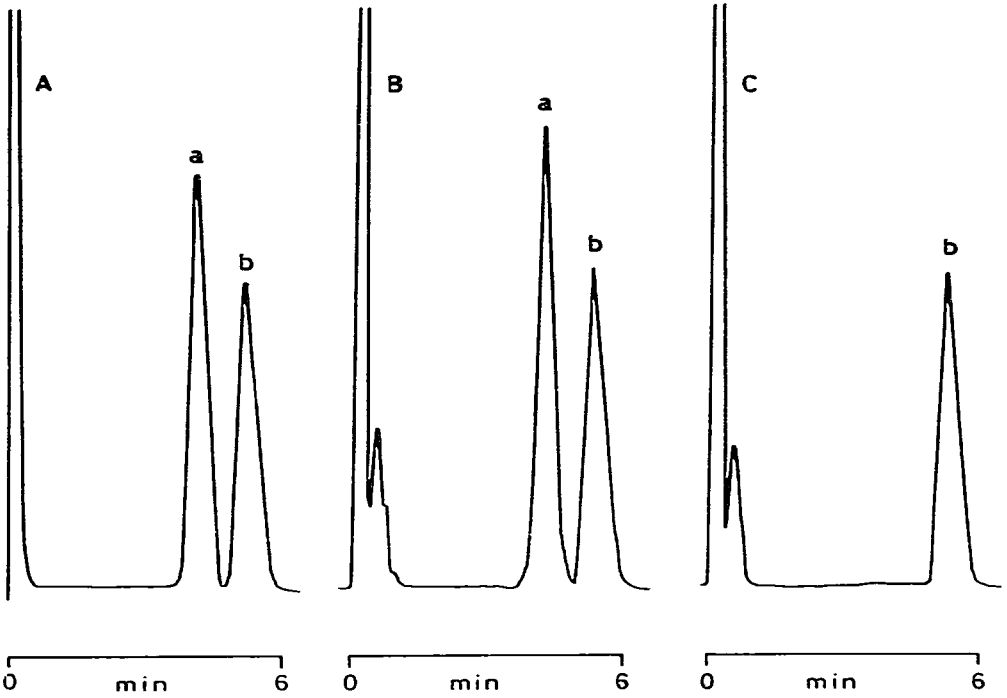

Fig. 1. Gas chromatograms of extracts from spiked brain sample (A), from brain of rats treated with trazodone (B) and from drug-free brain (C). Peaks: (a) trazodone; (b) internal standard.

injection of trazodone or mCPP heptafluorobutyrate. Fig. 3 shows the mass spectrum of mCPP heptafluorobutyrate. There is a base peak at m/e = 195 corresponding to the chlorophenylpiperazine moiety, which is further fragmented to give intense ions at m/e = 166, 139 and 111. In the high mass range the mass spectrum shows characteristic ions at m/e 373 and 357, which correspond to the loss of fluorine and chlorine, respectively, from the molecular ion. The mass spectrum of trazodone has been reported by other workers[13,14].

In order to collect information on the kinetic profile of trazodone and its active metabolite, male rats were treated orally with the parent compound (25 mg/kg) and the plasma and brain were analysed as described. Fig. 4 shows graphs of plasma and brain concentrations *versus* time. Trazodone was rapidly adsorbed, rising to peak plasma concentrations of 1.40 ± 0.40 μg/ml after 5 min. The plasma concentrations showed a biphasic decline thereafter, with an initial phase lasting about 30 min, followed by a second, slower phase with a half-life ($T_{\frac{1}{2}}$) of 50 min. Brain peak concentrations were reached after 15–30 min, declining thereafter in a monoexponential manner with $T_{\frac{1}{2}}$ = 77 min. Similar results have been obtained for the rat by other workers[1,2,13].

mCPP was detected in both plasma and brain 5 min after oral administration of the parent compound, reaching peak concentrations after 1–2 h. The metabolite disappeared from plasma ($T_{\frac{1}{2}}$ 97 min) more slowly than the parent compound, but its brain $T_{\frac{1}{2}}$ (83 min) was comparable to the brain $T_{\frac{1}{2}}$ of trazodone. The metabolite

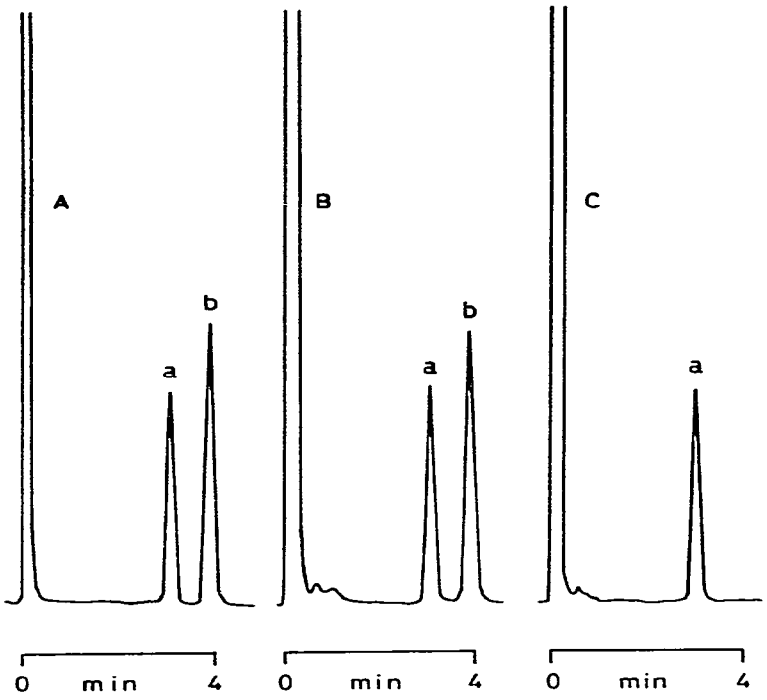

Fig. 2. Gas chromatograms of 1-*m*-chlorophenylpiperazine heptafluorobutyrate (b) and internal standard (a) from spiked brain sample (A), from brain of rats treated with trazodone (B) and from drug-free brain (C).

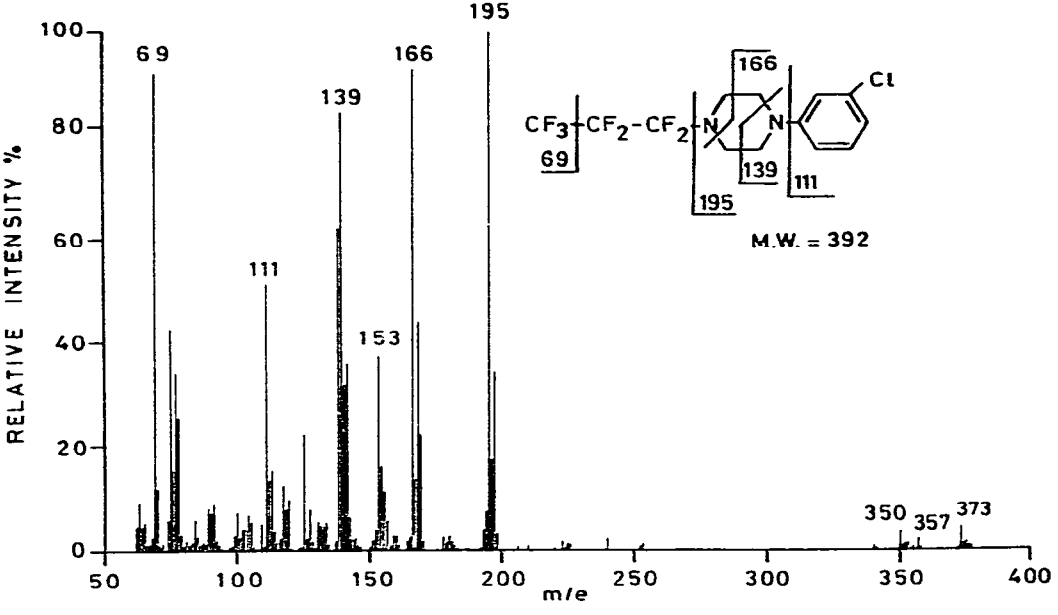

Fig. 3. Mass spectrum of 1-*m*-chlorophenylpiperazine heptafluorobutyrate.

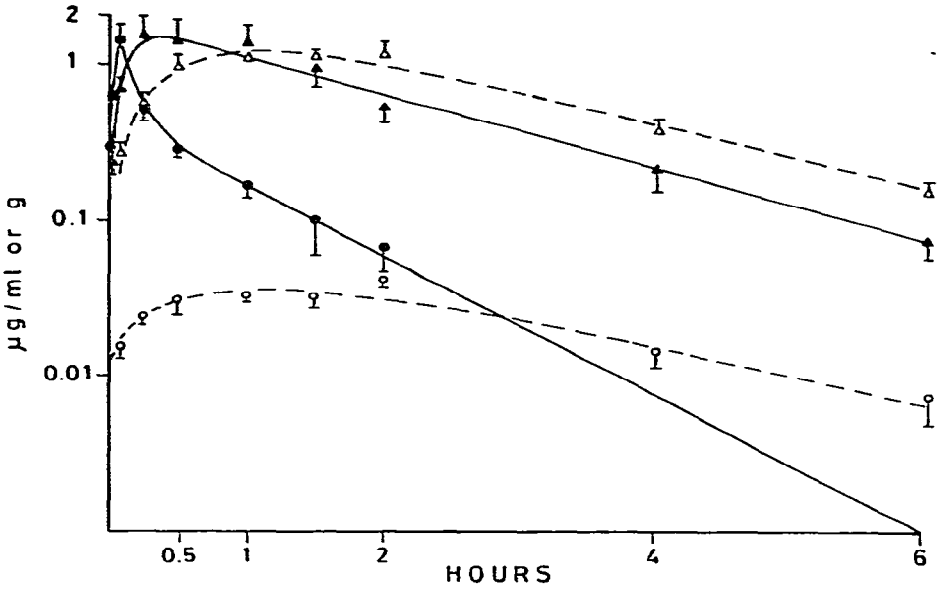

Fig. 4. Plasma (circles) and brain (triangles) concentration–time curves for trazodone (●, ▲) and 1-*m*-chlorophenylpiperazine (○, △) after oral administration of trazodone (25 mg/kg) to rats. The values are means ± S.D. for 4–6 rats.

accumulated more specifically than the parent compound in brain. These findings were reflected in the area under the curves (AUC) (Table II). At the oral dose of trazodone tested, the brain AUC was about 5 times greater than the plasma AUC, whereas the brain AUC of the metabolite was about 26 times greater than the plasma AUC. The AUC ratio of trazodone to mCPP was approximately 4 in plasma and 0.8 in brain. These results suggest that mCPP is a quantitatively significant metabolite of trazodone in the rat brain, confirming the suggestion[12] that it may play an important role as a 5HT agonist after relatively high doses of trazodone in the rat. Data not reported in detail indicate that the brain concentrations of mCPP after oral administration of trazodone to rat are comparable to those reached after pharmacologically and biochemically effective doses of mCPP[15]. Further studies are now in progress to elucidate the role of the metabolite in the pharmacological effects of the parent compound.

TABLE II

PLASMA AND BRAIN AREAS UNDER THE CURVES (AUC) OF TRAZODONE AND 1-*m*-CHLOROPHENYLPIPERAZINE

AUC values were calculated by the trapezoidal rule and extrapolated to infinity.

Compound	*Plasma AUC (μg/ml · min)*	*Brain AUC (μg/g · min)*
Trazodone	39.81	202.87
1-*m*-Chlorophenylpiperazine	10.01	262.12

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