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Note

Identification and quantitation of 1-(2-pyrimidinyl)piperazine, an active metabolite of the anxiolytic agent buspirone, in rat plasma and brain

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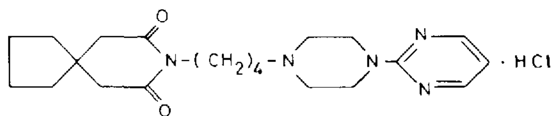
Buspirone, 8-[4-(2-pyrimidinyl)-1-piperazinyl]butyl-8-azaspiro[4,5]decane-7,9-dione hydrochloride, is a new type of anxiolytic agent[1] having a clinical efficacy comparable to diazepam[2]. Unlike diazepam, however, buspirone has no anticonvulsant activity and does not cause sedation or muscle relaxation[3–5]. The biochemical mechanism of its anxioselective action, however, is still not clear. The drug is unable to inhibit or stimulate binding of 3H-benzodiazepines and has no effect on the various neurotransmitter binding sites, interacting reasonably potently only with dopamine receptors[5–7]. Studies *in vitro*, however, may not be representative of the *in vivo* situation since in both man and animals buspirone is extensively metabolised[8] and an active metabolite may bind differently to the central nervous system. We have undertaken a determination of whether, after administration of buspirone, active metabolites are formed which may accumulate in the brain.

As a preliminary approach we investigated the possible hydrolysis of the pyrimidinylpiperazine side-chain of buspirone with the possible formation of 1-(2-pyrimidyl)piperazine (1-PP) (Fig. 1). That this reaction may occur was suggested by analogy with similar chemical structures. Thus, trazodone, 2-{3-[4-(*m*-chlorophenyl)-1-piperazine]propyl}-*s*-triazolo[4,3*a*]pyridin-3(2H)-one, and other psychotropic drugs containing a substituted phenylpiperazine in the side chain have been recently found to be metabolized by cleavage of the side chain to 1-*m*-chlorophenylpiperazine (*m*-CPP)[9,10]. This metabolite is biologically active[11–14] and accumulates in the brain



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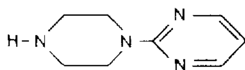

(II)

Fig. 1. Chemical structures of buspirone hydrochloride (I) and 1-(2-pyrimidinyl)piperazine (II).

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more specifically than its parent drugs[9,15,16] suggesting that the pharmacological effects of trazodone and its analogues are due, at least in part, to the formation of *m*-CPP.

We therefore developed an gas–liquid–electron-capture detection chromatographic procedure (GLC–ECD) which permits quantitation of 1-PP in biological samples. The procedure was used to confirm the presence of 1-PP in the plasma and brain of buspirone-treated rats and to follow the time course of the production and elimination of this metabolite.

MATERIALS AND METHODS

Buspirone hydrochloride and 1-PP hydrochloride were kindly supplied by Mead Johnson (Evansville, U.S.A.) and 1-*m*-trifluoromethylpiperazine by Clin–Midy (Montpellier, France). Heptafluorobutyric anhydride (HFBA) was obtained from Pierce (Rockford, IL, U.S.A.). Formic acid, *n*-heptane, chloroform and benzene (Pestanal grade) were obtained from Farmitalia–Carlo Erba (Milan, Italy).

Apparatus

1-PP heptafluorobutyrate was analysed on a Carlo Erba Fractovap 2150 chromatograph 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 180, 250 and 250°C, respectively. The carrier gas was nitrogen at a flow-rate of 40 ml/min.

Animals

Male CD-COBS rats (Charles River, Como, Italy), average weight 200 g, were used.

Extraction from plasma and brain

To 2 ml of heparin-treated plasma were added 50 µl of a methanolic solution of 1-*m*-trifluoromethylphenylpiperazine as internal standard followed by 1 ml of 0.5 *M* phosphate buffer (pH 10.5). The samples were mechanically shaken twice with 6 ml of benzene and the combined organic extracts concentrated to *ca.* 0.5 ml (cautiously because 1-PP is slightly volatile). Then 100 µl of an ethyl acetate solution of HFBA (25%, v/v) were added and the samples were heated at 60°C for 60 min. After the reaction the samples were washed with water (1 ml) and 5% aqueous ammonia solution (0.5 ml) and 1–3-µl aliquots of the benzene phase were injected into the GLC column.

Brains were homogenized as previously described for *m*-CPP quantitation[15] and then processed as described for plasma.

Internal standard calibration graphs

Standard curves were determined for each experiment by adding known amounts of 1-PP and internal standard to drug-free plasma and brain homogenates and determining the ratio of the 1-PP to internal standard peak areas.

Recovery

Percentage recoveries were calculated by comparing the peak area ratios of 1-PP heptafluorobutyrate after plasma and brain extraction with the peak-area ratios obtained by direct injection of standard solutions of 1-PP heptafluorobutyrate.

RESULTS AND DISCUSSION

Derivatization of 1-PP with HFBA provided high sensitivity and specificity in the analysis of the metabolite. The HFBA derivatives of 1-PP and 1-*m*-trifluoromethylphenylpiperazine showed good chromatographic properties yielding symmetrical peaks with retention times of 8.0 and 7.3 min, respectively. Fig. 2 shows typical chromatograms of extracts from (A) a spiked brain sample, (B) a drug-free homogenized brain, and (C) the brain of a rat treated with buspirone (10 mg/kg). Benzene was ideally suited as extraction solvent because it extracted only a few impurities and no interferring substances from plasma or brain, as shown in Fig. 2, but yielding at the same time consistent recoveries of 1-PP. The recoveries during the preliminary kinetic study in the rat are summarized in Table I. In the 50–1000-ng range (the linearity range of HFBA–IPP) the metabolite was extracted reproducibly with a mean re-

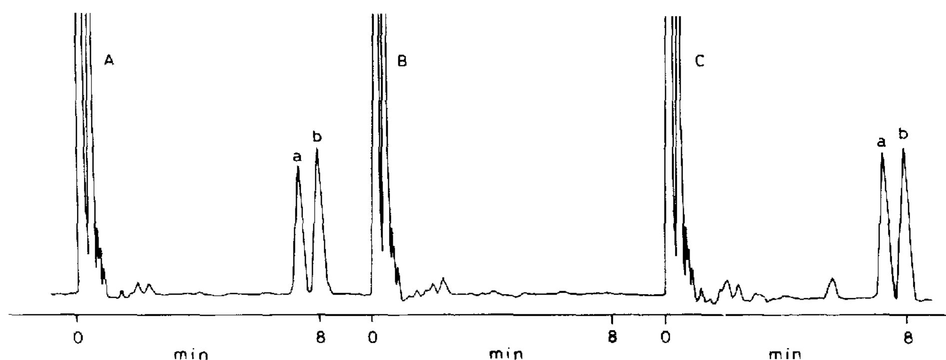

Fig. 2. Gas chromatograms of 1-(2 pyrimidinyl)piperazine heptafluorobutyrate (b) and the internal standard (a) from a spiked brain sample (A), from drug-free brain (B) and from brain of rats treated with buspirone (C).

TABLE I

RECOVERY OF 1-(2-PYRIMIDINYL)PIPERAZINE FROM PLASMA AND BRAIN

Each value is the mean of 4 determinations.

Sample	*Amount added (ng/ml or g)*	*Amount found ± S.D. (nl/mg or g)*	*Recovery ± S.D. (%)*
Plasma	50	45.5 ± 4.9	91 ± 9.9
	100	89.2 ± 6.9	89.2 ± 6.9
	250	220.5 ± 14.7	88.2 ± 5.9
	500	445.0 ± 30.8	89.0 ± 6.2
Brain	100	82.7 ± 8.4	82.7 ± 8.4
	250	206.2 ± 8.5	82.5 ± 3.4
	500	424.0 ± 21.5	84.8 ± 4.3
	1000	910.7 ± 61.8	91.0 ± 6.1

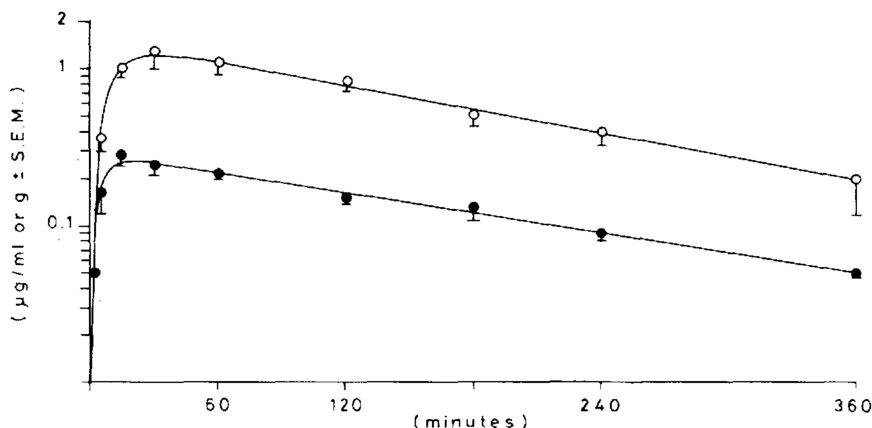

Fig. 3. Plasma (●) and brain (O) concentration–time curves for 1-(2 pyrimidinyl)piperazine after oral administration of buspirone hydrochloride (10 mg/kg) to rats.

covery of 89.3% and a coefficient of variation (C.V.) of between 6.7 and 10.9% from plasma and of 85.2% with a C.V. of between 4.1 and 10.1% from brain. Detection limits of 50 ng/ml or 100 ng/g were considered sufficient for our purposes.

Analysis of plasma and brain of rats treated orally with buspirone hydrochloride (10 mg/kg) showed that the biotransformation of buspirone in rats yielded measurable amounts of 1-PP in both plasma and brain. As can be seen from Fig. 3, at this oral dose of buspirone the plasma metabolite concentrations rose rapidly to a peak (0.28 ± 0.02 μg/ml) within 15 min after administration. After the peak, 1-PP disappeared in a mono-exponential manner with an apparent half-life ($T_{1/2}$) of 143 min. Of interest was the finding that 1-PP entered the brain with ease, this being similar to results reported for *m*-CPP[9,10]. Thus, even 5 min after oral administration of buspirone the brain metabolite concentrations were always higher than in plasma. Maximal concentrations were reached after 30 min with values (1.25 ± 0.23 μg/g) approximately five times higher than in plasma and declined thereafter with a $T_{1/2}$ comparable to the plasma $T_{1/2}$. The brain area under the curve (AUC) of 1-PP was approximately five times the plasma AUC (see Table II).

TABLE II

PEAK CONCENTRATIONS (C_{max}), HALF-LIVES ($T_{1/2}$) AND AREA UNDER THE CURVES (AUC) OF 1-(2-PYRIMIDINYL)PIPERAZINE IN PLASMA AND BRAIN OF RATS TREATED ORALLY WITH BUSPIRONE HYDROCHLORIDE (10 mg/kg)

Plasma and brain $T_{1/2}$ values were calculated assuming a one-compartment open model. AUC was calculated by the trapeizoidal rule and extrapolated to infinity.

Compartment	*C_{max} (μg/ml or g ± S.E.M.)**	*$T_{1/2}$ (min)*	*AUC (μg/ml or g × min)*
Plasma	0.28 ± 0.02	143	57
Brain	1.25 ± 0.23	124	250

* Observed values.

In conclusion these studies underline the similarity between the metabolic fate of buspirone and trazodone or its chemically related compounds. All these drugs undergo hydrolysis in the side-chain with formation of piperazine derivatives. These metabolites accumulate specifically in the brain, reaching concentrations several times greater than those in plasma. It has previously been shown that *m*-CPP may play a significant role in the pharmacological effects of its parent drugs[13–16]. Whether 1-PP also contributes to the pharmacological effect of the parent drug remains to be established. Animal studies now in progress indicate that 1-PP shares with buspirone the property of being active in the conflict test[8].

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