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Letter to the Editor

Simultaneous determination of buspirone, gepirone, ipsapirone and their common metabolite 1-(2-pyrimidinyl)piperazine in rat plasma and brain by high-performance liquid chromatography

Sir,

We report some analytical data on the simultaneous determination of buspirone, gepirone and ipsapirone, three novel anxiolytic agents [1,2], and their common metabolite 1-(2-pyrimidinyl)piperazine (PmP), which arises from cleavage of the parent drug's side-chain (Fig. 1) [2,3]. Animal studies have indicated that this metabolite tends to concentrate in the brain and retains some of the pharmacological effects of its parent compounds [1–4]. These results, together with the fact that PmP interacts particularly with central α_2-adrenoceptors [4–6], suggest that its formation may be a pharmacologically important metabolic pathway.

A comprehensive investigation of the pharmacokinetics and pharmacodynamics of buspirone, gepirone or ipsapirone requires the determination of the parent drug and the active metabolite in body fluids and tissues, particularly the

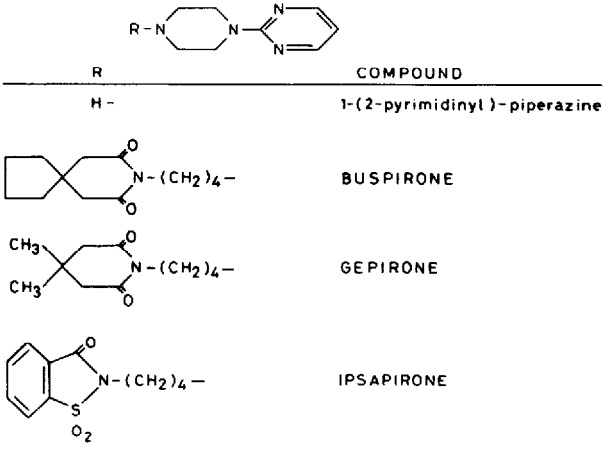

Fig. 1. Structures of buspirone, gepirone, ipsapirone and their common metabolite 1-(2-pyrimidinyl)piperazine.

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brain. Moreover, in seeking a relationship between brain concentrations and pharmacological effects, it is desirable to determine the metabolite and the parent drug simultaneously. Previous methods for the analysis of these structurally related compounds were focused only on one parent compound, (i.e., buspirone) by either radioimmunoassay [7] or capillary gas chromatography–mass spectrometry (GC–MS) [8], or the metabolite PmP by GC with electron-capture detection (ECD) [9] or GC–MS [10], or have been dependent for separation on high-performance liquid chromatographic (HPLC) systems, one for the parent compound (buspirone and gepirone) and another for PmP, because of differences in the retention behaviour of the parent drug and the metabolite [3].

Based on the extraction procedure for determination of buspirone and PmP in biological specimens [3,9,10], we have developed a gradient elution system for the simultaneous determination of ipsapirone, gepirone or buspirone and their common metabolite PmP in plasma and brain.

EXPERIMENTAL

Buspirone, gepirone, PmP and 1-(2-*p*-fluoropyrimidinyl)piperazine (*p*-FPmP) as the hydrochloride salts were kindly supplied by Mead Johnson Labs. (Evansville, IN, U.S.A.). Ipsapirone hydrochloride was kindly supplied by Troponwerke (Cologne, F.R.G.). Acetonitrile of HPLC grade was used. All other reagents and solvents were of analytical-reagent grade.

The analyses were conducted using an HPLC gradient system (Hewlett-Packard 10848) controlled by a computer terminal (HP 79850B LC), in conjunction with a variable-wavelength UV detector set at 235 nm. Separation was achieved on a μBondapak C_{18} reversed-phase column (30 cm × 3.9 mm I.D., particle size 10 μm) (Water Assoc., Milford, MA. U.S.A.). Solvent A was 0.01 *M* potassium dihydrogen phosphate (pH 2.85) and solvent B was 0.01 *M* potassium dihydrogen phosphate (pH 2.85)–acetonitrile (60:40). For elution during routine analysis a linear gradient from 10 to 90% of solvent B was applied in 25 min. All separations were performed at ambient temperature at a flow-rate of 1.5 ml/min.

Compounds were extracted from plasma (1 ml) and brain homogenates (approximately 1 g) with benzene (6 ml), which represented the best compromise with respect to extraction efficiency and selectivity [3,9,10], using as internal standards *p*-FPmP (0.2 μg in 50 μl) for the metabolite and gepirone or ipsapirone (0.2 μg in 50 μl) for the parent compounds. The layers were separated by centrifugation at 600 *g* for 10 min and 50 μl of 6.5 m*M* hydrochloric acid were added to the organic extract to minimize volatilization of the compounds, particularly PmP. After brief mixing, the solvent was evaporated at 35–45°C under a gentle stream of nitrogen. The residue was dissolved in 100 μl of the mobile phase and 25–75 μl were injected into the HPLC instrument.

RESULTS AND DISCUSSION

Under the conditions described, buspirone, gepirone or ipsapirone, their common metabolite and internal standards were all resolved and all compounds of

interest eluted in a region of the chromatogram that was essentially devoid of endogenous components or other metabolites, allowing easy quantification in both plasma and brain. Approximate retention times were 5, 15, 18 and 20 min for PmP, gepirone, ipsapirone and buspirone, respectively (Fig. 2).

The selection of two internal standards (*p*-FPmP and gepirone or ipsapirone), in addition to improving the precision and accuracy of the determination of the respective structurally related compound, was useful during kinetic studies because of large differences in the plasma and/or brain concentrations of the metabolite and the parent drugs.

Calibration graphs for each compound based on the peak-height ratios to the respective internal standard were linear over the concentration range 0.05–10 nmol/ml. The limits of detection (0.05 nmol or better using 1 ml of plasma or approximately 1 g of brain tissue and intra- and inter-assay variability of standard replicates (generally less than 10%) were approximately as described previously for the isocratic elution of PmP and its parent compounds [3].

The kinetics of ipsapirone and gepirone were investigated in male CD-COBS rats (Charles River, Italy) by the procedure described. After an oral dose of 10 mg/kg both drugs formed significant amounts of PmP, the metabolite-to-parent drug area under the curve (AUC) ratios being approximately 1 and 14 in plasma and 5 and 60 in brain for ipsapirone and gepirone, respectively. Previous studies [4] have shown that in buspirone-treated rats (10 mg/kg, p.o) PmP achieves higher plasma and brain concentrations than its parent compound; together with the fact that the metabolite shows α_2-adrenoceptor affinity in in vitro binding studies and α_2 antagonist activity in vivo [2,5], this suggests that it is involved in the parent drug's ability to prevent central and peripheral effects of clonidine in the rat [6]. In man given an anxiolytic dose of buspirone, the metabolite was

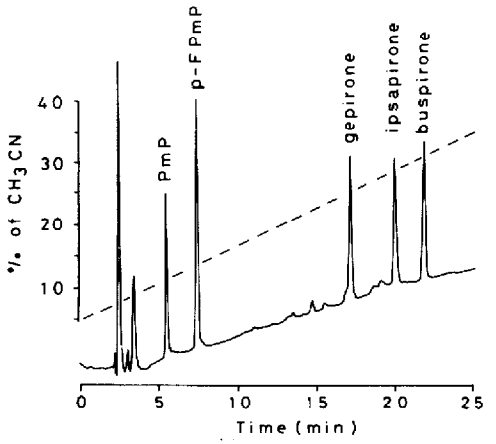

Fig. 2. Separation of buspirone, ipsapirone, gepirone, PmP and the internal standard *p*-FPmP on a μBondapak C_{18} (10 μm) column (30 × 3.9 mm I.D.). Mobile phase: (A) 0.0 1 *M* potassium dihydrogen phosphate (pH 2.85); (B) 0.01 *M* potassium dihydrogen phosphate (pH 2.85)-acetonitrile (60:40). Chromatogram of rat plasma spiked with PmP (0.1 μg/ml) and its parent drugs (0.2 μg/ml).

found at higher concentrations than its parent compound in plasma, as in the rat [4].

This method should facilitate the determination of buspirone, gepirone, ipsapirone and PmP in plasma and, perhaps more important, in brain tissue of animals given pharmacologically effective doses of these compounds, and should aid in establishing a quantitative relationship between brain concentrations of the parent drugs, their common metabolite and the central effects.

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