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Cerebral Pharmacokinetics of Ipsapirone in Rats After Different Routes of Administration

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Abstract—Ipsapirone, a putative non-benzodiazepine anxiolytic, was extensively metabolized in rats to 1-(2-pyrimidinyl)piperazine (1-PP) which accumulated in the brain. Neither the route of administration (i.p. or p.o.), nor prolonged administration of ipsapirone or 1-PP affected their accumulation in the rat brain. The cytochrome P450 level and ethylmorphine *N*-demethylase activity in rat liver microsomes were unchanged by chronic treatment with ipsapirone or 1-PP. The results indicate that 1-PP may contribute to the α_2-adrenoceptor antagonism of ipsapirone in rats and that chronic treatment with the drug does not affect its biotransformation to 1-PP.

Ipsapirone is a putative non-benzodiazepine anxiolytic drug which specifically interacts with the central 5-HT_{1A} binding sites (Glaser & Traber 1985), and does not display any of the side effects of diazepam (Traber et al 1984). We found earlier (Danek et al 1988) that 1-(2-pyrimidinyl)piperazine (1-PP) is one of the metabolites of ipsapirone in rats, and that it tends to accumulate in brain tissue.

Comprehensive pharmacological studies of the central actions of ipsapirone and its metabolite 1-PP were recently carried out in rats and mice (Giral et al 1987; Maj et al 1987; Tatarczynska et al 1989). The results of those investigations indicate that ipsapirone and 1-PP can exert different, and even opposing, central actions in rats and mice. Ipsapirone exhibits 5-HT_{1A} antagonism and at high doses it can inhibit 5-HT_2 and α_2-adrenoceptor function while 1-PP acts like a 5-HT_2 agonist and a potent α_2-antagonist. Since no information was available on the temporal distribution of ipsapirone and 1-PP, we undertook the study of the pharmacokinetics of both the parent compound and the metabolite in the rat brain after pharmacologically effective doses of ipsapirone and 1-PP (Maj et al 1987; Tatarczynska et al 1989) were given either intraperitoneally or orally.

We also investigated the effect of prolonged administration of ipsapirone or 1-PP on cytochrome P450 levels and on ethylmorphine *N*-demethylase activity in rat liver microsomes, as well as the effect of proadifen, (SKF525A) a non-specific blocker of enzymatic activity, on the levels of ipsapirone and 1-PP in brain.

Materials and Methods

Animals

Male Wistar rats, 200–230 g, kept under normal laboratory conditions with free access to food (Bacutil) and tap water, received ipsapirone hydrochloride (Troponwerke) as a single dose of 20 mg kg^{-1} i.p. or p.o. (0·049 mmol kg^{-1}), or 1-PP [1-(2-pyrimidinyl)piperazine dihydrochloride, Aldrich] in a single dose of 8 mg kg^{-1} i.p. or p.o. (0·048 mmol kg^{-1}). In one series of experiments rats received proadifen (SKF525A hydrochloride, Koch and Light Laboratory) (50 mg kg^{-1} i.p.) 2 h before ipsapirone (20 mg kg^{-1} i.p.). The rats used for the enzymatic study received the test substances in drinking water for two weeks, ipsapirone at 20 mg kg^{-1}/day or 1-PP at 5 mg kg^{-1}/day (since this dose of 1-PP was expected to produce a brain concentration of 1-PP similar to that found after the single dose of ipsapirone 20 mg kg^{-1} p.o.). Control animals received 0·9% NaCl (saline).

The animals were decapitated at different times after administration of ipsapirone or 1-PP (for the pharmacokinetic study), or 24 h after withdrawal of aqueous solution of ipsapirone or 1-PP (in the enzymatic study). The rats treated with proadifen before ipsapirone were killed 30 min after administration of ipsapirone. All brains and livers were dissected immediately after decapitation.

HPLC assessment of ipsapirone and 1-PP in the rat brain

The brains were homogenized in double-distilled water (1 : 4), and the homogenates were centrifuged for 25 min at 4500 *g*. To 2 mL of homogenate was added 100 μL of 0·1 M NaOH, 200 μL of ethanol and 4 mL of chloroform. The mixture was shaken mechanically for 30 min and centrifuged for 10 min at 3000 *g*. Three mL of the organic layer was transferred to another tube, and chloroform evaporated from the samples under reduced pressure. The residue was dissolved in 100 μL of methanol, and 10 μL of methanol solution was injected into the HPLC system (Kipp und Zonen LC 771) equipped with a stainless steel column (250 × 4 mm) packed with Li-Chrosorb RP-18 (Polskie Odczynniki Chemiczne, Lublin), a UV detector (235 nm) and an Orlita pump. The mobile phase was: 0·5 M phosphate buffer, acetonitrile, methanol, tetrahydrofuran (30:40:40:20) pH = 7·4. The flow rate was 1·5 mL min^{-1}. The retention times were: ipsapirone, 2·31 min; 1-PP, 3·04 min. For each series of measurements a separate calibration curve was prepared, and the brain concentration of ipsapirone or 1-PP was determined graphically from individual calibration curves. Sensitivity was 0·01 μg/sample of ipsapirone or 1-PP. Recovery was 61% for ipsapirone and 72% for 1-PP. Calibration curves were linear between 0·01–5 μg/sample ipsapirone and 0·01–7 μg/sample of 1-PP.

Enzymatic study

The livers were excised 24 h after the withdrawal of

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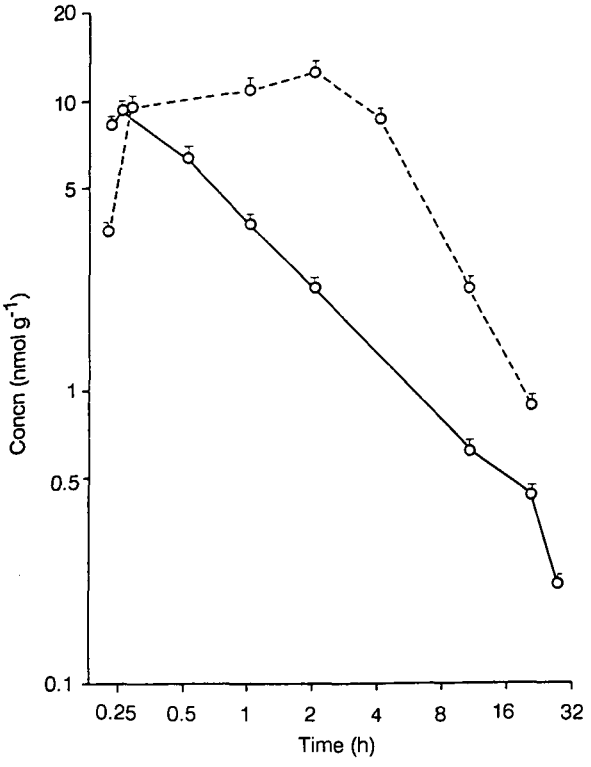

FIG. 1. The brain concentration–time curve of ipsapirone (unbroken line) and 1-PP (broken line) after i.p. administration of ipsapirone (20 mg kg^{-1}). Each point represents a mean ±s.e.m. of 5 rats.

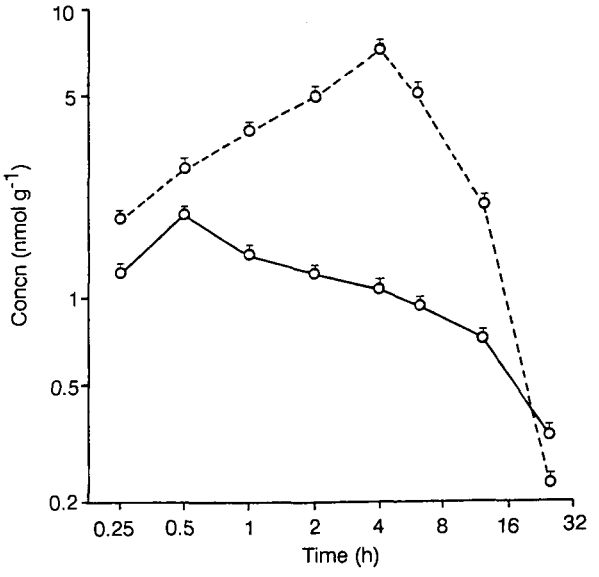

FIG. 2. The brain concentration–time curve of ipsapirone (unbroken line) and 1-PP (broken line) after oral administration of ipsapirone (20 mg kg^{-1}). Each point represents a mean ±s.e.m. of 5 rats.

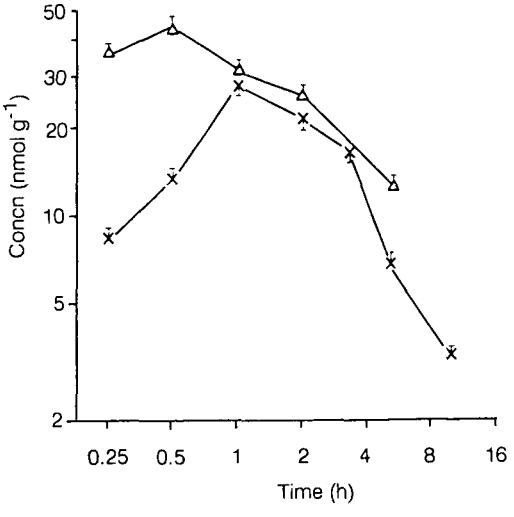

FIG. 3. The brain concentration–time curve of 1-PP after administration of 1-PP (8 mg kg^{-1} i.p. (Δ—Δ) or p.o. (×—×). Each point represents a mean ±s.e.m. of 5 rats.

ipsapirone or 1-PP. Microsomes were prepared conventionally by a differential centrifugation in Tris/KCl buffer (pH = 7·4). The cytochrome P450 level was assessed according to Omura & Sato (1964). Protein was assayed according to Lowry et al (1951). The demethylase activity in-vitro was determined by measuring formaldehyde formed from ethylmorphine (Daniel & Melzacka 1986). Formaldehyde was assayed according to Nash (1953).

Pharmacokinetic parameters were calculated graphically from response curves plotted on a linear time scale: biological half-life ($t_{\frac{1}{2}}$) from the terminal part of the time-concentration curve and area under the curve (AUC) with the trapezoidal rule and extrapolation to infinity. The highest mean concentration of ipsapirone or 1-PP obtained was defined as C_{max}.

The results were analysed statistically with Student's *t*-test.

Results

When ipsapirone was given to rats in a single dose (20 mg kg^{-1} i.p.) it penetrated the brain rapidly reaching a maximum concentration (9·59 nmol g^{-1}) about 15 min after administration. Its disappearance from the brain was slow; 24 h after administration ipsapirone was still present (Fig. 1). 1-PP was formed from ipsapirone in large amounts and penetrated the brain readily. Its maximum concentration (12·25 nmol g^{-1}, 27% greater than that of the parent drug) was reached 2 h after administration of ipsapirone. 1-PP was eliminated from the brain more rapidly than the parent compound (Table 1) and 24 h after drug administration, its level in the rat brain was below the limit of detection (Fig. 1).

After oral treatment with ipsapirone (20 mg kg^{-1}), the drug was again found in the CNS soon after its administration (t_{max} = 30 min) and it disappeared from the brain at a rate similar to that after i.p. administration. The maximum concentration of the metabolite was reached 4 h after oral ipsapirone administration and it was 3·5 times higher than that of the parent compound (7·12 and 1·94 nmol g^{-1}, respectively) (Table 1, Fig. 2). It is possible that, for the parent drug, the maximum concentrations have been underestimated because of rapid drug entry into the brain and the low time resolution of our study. However, this problem would not affect the other results or our conclusions.

Table 1. The maximum concentration (C_{max}), time to maximum concentration (t_{max}), half-life ($t_{\frac{1}{2}}$) and area under the curve (AUC) values for ipsapirone and 1-PP after administration of ipsapirone (20 mg kg^{-1} i.p. or p.o.) or after administration of 1-PP (8 mg kg^{-1} i.p or p.o.) to rats. C_{max} value is mean ± s.e.m. of 5 rats.

Substance	C_{max} (nmol g^{-1})	t_{max} (h)	$t_{\frac{1}{2}}$ (h)	AUC (nmol h g^{-1})
Ipsapirone (i.p.)	9·59 ± 0·72	0·25	11·88	25·24
1-PP	12·25 ± 0·84	2·00	4·08	94·92
Ipsapirone (p.o.)	1·94 ± 0·26	0·50	12·85	23·30
1-PP	7·12 ± 0·52	4·00	4·23	83·51
1-PP (i.p.)	43·29 ± 3·96	0·50	3·65	178·76
1-PP	28·96 ± 2·00	1·00	4·20	130·21

Table 2. The brain levels of ipsapirone and 1-PP in rats receiving proadifen (50 mg kg^{-1} i.p.) and ipsapirone (20 mg kg^{-1} i.p.) 2 h later. The animals were killed 30 min after ipsapirone administration. Each result is a mean of 5 animals ± s.e.m. *** $P < 0·001$, compared with rats receiving only ipsapirone (Student's t-test).

	Brain level (nmol g^{-1})	
Drug	Ipsapirone	1-PP
Control ipsapirone only	4·83 ± 0·39	10·91 ± 0·73
Proadifen + ipsapirone	19·35 ± 1·52***	2·65 ± 0·24***

Table 3. The effect of 14 days oral administration of ipsapirone (14 × 20 mg kg^{-1}/day) or 1-PP (14 × 5 mg kg^{-1}/day) on the liver microsomal cytochrome P450 level and formaldehyde formation from ethylmorphine. The rats were killed 24 h after substance withdrawal. Each result is a mean ± s.e.m. of 5 animals.

Drug	Cytochrome P450 level (nmol (mg protein)$^{-1}$)	Formaldehyde from ethylmorphine (nmol (mg protein)$^{-1}$) min^{-1}
Control (saline)	0·583 ± 0·050	1·396 ± 0·246
Ipsapirone	0·622 ± 0·049	1·484 ± 0·158
1-PP	0·567 ± 0·062	1·002 ± 0·227

Similarly the times of maximum concentrations are only approximate.

AUC values of ipsapirone after i.p. administration were similar to those after p.o. administration. The AUC values of 1-PP after i.p. or p.o. administration of ipsapirone significantly exceeded those of the parent drug and the ratios of the brain $AUC_{1\text{-PP}}/AUC_{ipsapirone}$ after each route of administration of the parent drug were similar (approx. 3·5) (Table 1).

When 1-PP (8 mg kg^{-1} i.p.) was given to rats, it readily penetrated the brain, reaching a maximum concentration 30 min after administration (43·29 nmol g^{-1}). Its elimination from the brain proceeded at a rate similar to that after ipsapirone administration; 5 h after administration its brain level was still relatively high (12·9 nmol g^{-1}) (Table 1, Fig. 3).

After oral administration of 1-PP (8 mg kg^{-1}) its brain concentration was lower than after i.p. administration; the maximum (28·96 nmol g^{-1}) was reached 1 h after oral administration of 1-PP (Table 1, Fig. 3).

Comparison of AUC values indicated that there was a greater accumulation of 1-PP in the rat brain after i.p. administration than after p.o. administration (Table 1).

Table 4. The levels of brain ipsapirone and 1-PP in rats receiving ipsapirone (14 × 20 mg kg^{-1}/day) or 1-PP (14 × 5 mg kg^{-1}/day) in drinking water at the time of withdrawal (A) and 24 h after withdrawal (B). Each result is a mean ± s.e.m. of 5–7 animals.

	Brain level (nmol g^{-1})			
	Ipsapirone		1-PP	
Drug	A	B	A	B
Ipsapirone	0·97 ± 0·27	0·47 ± 0·12	3·10 ± 0·60	0·37 ± 0·06
1-PP	—	—	2·60 ± 0·30	0·31 ± 0·06

Proadifen (50 mg kg^{-1}) blocked ipsapirone biotransformation when given to rats 2 h before the drug; the level of ipsapirone in the brain 30 min after administration was approx. 4 times higher in rats receiving proadifen (Table 2).

Neither ipsapirone (20 mg kg^{-1}/day) nor 1-PP (5 mg kg^{-1}/day) affected the cytochrome P450 level or the ethylmorphine N-demethylase activity in rat liver microsomes after prolonged (14 days) oral administration (Table 3).

Brain levels of ipsapirone and 1-PP, in rats receiving the substances in drinking water for 14 days, are presented in Table 4; these concentrations were in the same range as those found in the rat brain after a single oral dose of ipsapirone (20 mg kg^{-1}) or 1-PP (8 mg kg^{-1}), both at the time of withdrawal and 24 h later (Table 4).

Discussion

Following i.p. or p.o. administration of ipsapirone to rats, the fall in its brain concentration was biphasic. The drug was eliminated from the rat CNS at a moderate rate, and more slowly than its metabolite. Since the accumulation of ipsapirone in the rat brain, expressed in terms of AUC values, was similar after i.p. and p.o. administration of the same dose, it appears that the drug is completely absorbed from the gastrointestinal tract.

1-PP appeared rapidly in the brain after i.p. or p.o. administration of ipsapirone and its concentration exceeded that of ipsapirone. Similarly the AUC values of 1-PP after i.p. and p.o. administration of ipsapirone exceeded the corresponding AUC values of the parent drug. The ratios of AUC values (1-PP : ipsapirone) were similar after both routes of drug administration (3·7 and 3·4 after i.p. and p.o. administration, respectively). Therefore, it appears that biotransformation of ipsapirone into 1-PP and the elimination of both substances proceeded similarly after both i.p. and p.o. administration of the parent drug. These results suggest that the weak α_2-adrenolytic properties of ipsapirone (Maj et al 1987), may depend on rapid metabolism of ipsapirone to 1-PP and accumulation of 1-PP in rat CNS.

Intraperitoneal or oral adminstration of 1-PP to rats also led to accumulation of 1-PP in the rat brain. In contrast to ipsapirone administration, the brain AUC values after i.p. and p.o. administration of 1-PP were different (33% higher after i.p. treatment). This might suggest that 1-PP given to rats in a dose of 8 mg kg^{-1} orally was not completely absorbed from the gastrointestinal tract or that metabolism took place in the gut wall or in the intestine before its absorption.

The blockade of ipsapirone biotransformation by proadifen was seen as a significant increase in ipsapirone and a marked decrease in 1-PP concentration in the rat brain indicates that hepatic microsomal enzymes play an important role in biotransformation of ipsapirone into 1-PP.

It was found earlier by Caccia et al (1983, 1986) that buspirone, an anxiolytic with a chemical structure similar to that of ipsapirone, was metabolized to 1-PP in rats. However, in contrast to our results with ipsapirone, it was eliminated from the rat brain faster than the metabolite. Also Bianchi et al (1988) found that ipsapirone given to rats in a single oral dose of 10 mg kg^{-1} was eliminated from the blood faster than its metabolite 1-PP. These discrepancies may be due to differences in the rat strain used or to the different drug doses used.

Neither ipsapirone nor 1-PP affected the cytochrome P450 level or ethylmorphine *N*-demethylase activity in rat liver microsomes after prolonged administration of the substances. This finding suggests that at least the metabolic oxidation in the rat liver microsomes involving cytochrome P450 as the terminal oxidase are not disturbed by ipsapirone or its metabolite given chronically.

Since the brain levels of ipsapirone or 1-PP at the time of their withdrawal and 24 h after their prolonged oral administration in drinking water, were comparable with the levels of both parent and metabolites when they were administered to rats as a single dose orally, and since the oxidative metabolism in rat liver microsomes did not seem to be affected by prolonged administration of either substance, it appears that their accumulation in the rat brain after chronic administration of ipsapirone is predictable from single dose data.

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