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The effect of selective serotonin reuptake inhibitors (SSRIs) on the pharmacokinetics and metabolism of perazine in the rat

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Abstract

The aim of this study was to investigate the effect of three selective serotonin reuptake inhibitors (SSRIs), fluoxetine, fluvoxamine and sertraline, on the pharmacokinetics and metabolism of perazine in a steady state in rats. Perazine (10 mg kg^{-1}, i.p.) was administered twice daily for two weeks, alone or jointly with one of the SSRIs. Concentrations of perazine and its two main metabolites (*N*-desmethylperazine and 5-sulfoxide) in the plasma and brain were measured 30 min and 6 and 12 h after the last dose of the drugs. Of the investigated SSRIs, fluoxetine and fluvoxamine significantly increased plasma and brain concentrations of perazine (up to 900 % and 760 % of the control value, respectively), their effect being most pronounced after 30 min and 6 h. Moreover, simultaneous increases in perazine metabolites concentrations and in the perazine/metabolite concentration ratios were observed. Sertraline elevated plasma and brain concentrations of perazine after 30 min. In-vitro studies with liver microsomes of rats treated chronically with perazine, SSRIs or their combinations showed decreased concentrations of cytochrome P-450 after perazine and a combination of perazine and fluvoxamine (vs control), and increased concentration after a combination of perazine and fluoxetine (vs perazine-treated group). Prolonged treatment with perazine did not significantly change the rate of its own metabolism. Chronic administration of fluoxetine or sertraline, alone or in a combination with perazine, accelerated perazine *N*-demethylation (vs control or perazine group, respectively). Fluvoxamine had a similar effect. The 5-sulfoxidation of perazine was accelerated by fluvoxamine and sertraline treatment, but the process was inhibited by administration of a combination of perazine and fluoxetine or fluvoxamine (vs control). Kinetic studies using control liver microsomes, in the absence or presence of SSRIs added in-vitro, demonstrated competitive inhibition of both *N*-demethylation and sulfoxidation by the investigated SSRIs. Sertraline was the most potent inhibitor of perazine *N*-demethylation but the weakest inhibitor of sulfoxidation. Results of in-vivo and in-vitro studies indicate that the observed interaction between perazine and SSRIs mainly involves competition for an active site of perazine *N*-demethylase and sulfoxidase. Moreover, increases in the concentrations of both perazine and metabolites measured, produced by the investigated drug combinations in-vivo, suggest simultaneous inhibition of another, yet to be investigated, metabolic pathway of perazine (e.g. aromatic hydroxylation).

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Introduction

The piperazine-type phenothiazine neuroleptics, perazine and perphenazine, are frequently combined with antidepressants in the therapy of psychotic and drug-

resistant depression, depression in schizophrenia and schizoaffective disorders, due to their clinical profile and spectrum of side-effects (Nelson 1993; Keck et al 1994; Sproule et al 1997). Moreover, selective serotonin reuptake inhibitors, (SSRIs) which are used as antidepressants (Lane et al 1995), attenuate negative symptoms of schizophrenia (Silver & Nassar 1992; Goff et al 1995; Avenoso et al 1997).

Although perazine belongs to the group of phenothiazines with piperazine structure in a side chain, it is not a potent antagonist of D_2 receptors. Furthermore, it is a weak antagonist of D_1, α_1, 5-HT_2 and M_1 receptors. Therefore perazine rarely produces side-effects in the central or autonomic nervous systems. In contrast to many other phenothiazine neuroleptics, perazine does not negatively influence mood, and some clinicians even attribute certain antidepressant properties to it. For these reasons, perazine is often used in geriatric patients and in combination therapy. However, in the presence of strong cytochrome P-450 inhibitors, such as SSRIs, a possible substantial increase in perazine concentration in-vivo may evoke certain side-effects (e.g. drowsiness, extrapyramidal symptoms, cardiovascular effects) which are concentration dependent (Baldessarini et al 1988; Midha et al 1994; Vergese et al 1991).

SSRIs are known to be substrates and inhibitors of cytochrome P-450. CYP2D6 is strongly inhibited by fluoxetine and paroxetine, and CYP1A2 by fluvoxamine (Crewe et al 1992; Brøsen et al 1993; Jeppesen et al 1996). The isoenzymes CYP3A4 and CYP2C19 are moderately inhibited by fluvoxamine and fluoxetine (Kobayashi et al 1995; von Moltke et al 1995; Jeppesen et al 1996). Like fluoxetine and paroxetine, sertraline is a substrate (Kobayashi et al 1999) and an inhibitor of CYP2D6 in-vitro (Crewe et al 1992), but does not exert inhibition in-vivo to any great extent in man (Preskorn 1997).

In both man and rats, perazine is metabolized by sulfoxidation in a thiazine ring, by *N*-demethylation in the piperazine side chain and by aromatic hydroxylation in position 3, by *N*-oxidation and degradation of a piperazine ring (Breyer 1969, 1972; Kanig & Breyer 1969); however, it not yet known which enzymes catalyse those metabolic pathways of the drug. Clinical studies with another phenothiazine neuroleptic of the piperazine-type, perphenazine, have shown that the pharmacokinetics of this neuroleptic is related to polymorphic CYP2D6 (Dahl-Puustinen et al 1989). However, it is still unknown which metabolic pathway of the neuroleptic is catalysed by this isoenzyme. Our unpublished results, obtained using specific cytochrome P-450 isoenzyme inhibitors, indicate that *N*-demethylation of perazine in the rat is catalysed by CYP2D1, CYP2B and CYP1A2, and its sulfoxidation by CYP2D1 and CYP2B. CYP2C and CYP3A do not seem to be involved in these metabolic pathways. Murine CYP2D1 is a counterpart of human CYP2D6, showing a high similarity of the amino-acid sequence and catalytic activity (Gonzalez 1990; Kobayashi et al 1995; Laurenzana et al 1995). In the case of tricyclic antidepressants, isoenzyme 2D6 has been found to be responsible for aromatic hydroxylation (Spina et al 1987; Steiner et al 1988; Brøsen et al 1991). This may also be true for perazine and other phenothiazine neuroleptics. Thus metabolic interactions between perazine and SSRIs seem quite possible.

There is little literature data on the effect of SSRIs on the pharmacokinetics and metabolism of phenothiazine neuroleptics after their co-administration. Such an interaction has recently been shown between perphenazine and paroxetine in man (Özdemir et al 1997), and has been thoroughly studied between promazine or thioridazine and the three SSRIs fluoxetine, fluvoxamine or sertraline in rats (Daniel et al 1999a, b). All of these SSRIs elevated the plasma and brain concentrations of promazine up to 300% of the control treated with promazine alone (Daniel et al 1999b). In the case of thioridazine, a very potent interaction was observed only with fluoxetine which increased the concentration of the neuroleptic up to 1200% of the control treated with thioridazine alone (Daniel et al 1999a). Fluvoxamine showed a similar tendency, while sertraline tended to decrease the level of thioridazine.

The aim of this study was to investigate a possible effect of the three SSRIs fluoxetine, fluvoxamine and sertraline on the pharmacokinetics and metabolism of perazine in a steady state in rats. It seemed very interesting to determine whether the piperazine-type phenothiazine neuroleptic perazine behaved pharmacokinetically like promazine (aliphatic type), or rather like thioridazine (piperidine type) when given jointly with SSRIs.

Materials and Methods

Drugs and chemicals

Perazine (dimaleate) was obtained from Labor (Wrocław, Poland). Fluoxetine hydrochloride was purchased from Eli Lilly (Indianapolis, USA), sertraline hydrochloride from Pfizer Corp. (Brussels, Belgium) and fluvoxamine maleate from Duphar (Weesp, Hol-

land). Perazine 5-sulfoxide and *N*-desmethylperazine were synthesized in our laboratory as described previously (Daniel et al 1998). NADP, glucose-6-phosphate and glucose-6-phosphate-dehydrogenase were purchased from Sigma (St Louis, MO). All the organic solvents of HPLC purity were supplied by Merck (Darmstadt, Germany).

Animal procedure

Male Wistar rats, 240–270 g, were kept under standard laboratory conditions. To avoid a possible drug interaction at the level of absorption from the gastrointestinal tract, and to achieve a better correlation of the drug concentrations with their metabolism, the investigated psychotropics were administered intraperitoneally. Perazine (dimaleate, 10 mg kg^{-1}, i.p.) was administered to rats twice daily for two weeks, alone or jointly with one of the investigated SSRIs (fluoxetine or sertraline hydrochlorides, 5 mg kg^{-1}, i.p.; fluvoxamine maleate, 10 mg kg^{-1}, i.p.). The doses of the investigated drugs were of pharmacological magnitude, since they are known to produce neuroleptic-like effects (catalepsy produced by perazine) (Maj et al 1978), antidepressant effects (SSRI activity in chronic mild stress) (Muscat et al 1992; Willner 1997) and certain behavioural or biochemical alterations in animal tests (Maj et al 1989; Maj & Moryl 1992, 1993; Fuller et al 1995). The doses used produced concentrations in rat plasma equivalent to those observed in blood plasma of psychiatric patients (Breyer-Pfaff et al 1983; Tremaine et al 1989; Caccia et al 1990; Goodnick et al 1994).

The animals' trunk blood was collected in tubes moistened with a 30% solution of sodium citrate, and their brains were rapidly removed and stored frozen in solid CO_2. Blood samples were centrifuged at 2000 *g* for 30 min. Concentrations of perazine and its main metabolites (5-sulfoxide and *N*-desmethylperazine) in the plasma and brain were measured at 30 min (C_{max}), 6 and 12 h (slow disposition phase) after the last dose of the drugs. The time intervals, as well as the two-week treatment schedule (steady state), was chosen on the basis of pharmacokinetic studies with perazine, carried out in our laboratory (data not published). Liver microsomes were prepared by differential centrifugation in 20 mM Tris/KCl buffer (pH 7.4) and by washing with 0.15 M KCl at 24 h after withdrawal of the drugs according to a conventional method. This procedure deprived microsomes of the presence of drugs administered in-vivo, which was confirmed in our experiment by the HPLC method described below.

Extraction of perazine and its metabolites from the plasma, brain and microsomal suspension

The brains were homogenized in distilled water (1:3 w/v). The homogenates (about 6 mL) were alkalinized with 3 M NaOH to achieve a pH of 12 (approx. 300 μL of NaOH). Perazine and its metabolites were extracted with dichloromethane and hexane (1:1, 6 mL) for 45 min. After cooling overnight at −20°C, the samples were centrifuged at 2000 *g* for 15 min. The organic phase containing the neuroleptic and its metabolites was transferred to clean tubes and evaporated under nitrogen at 40°C. After precipitation of proteins (1.5 mL of the plasma, or 1 mL of the microsomal suspension + 600 μL of methanol), plasma or microsomal suspension samples were mixed with 3 mL of distilled water. After alkalinization to a pH of 12 (80 μL of 3 M NaOH), extraction and evaporation of the organic phase were performed as above. During the whole procedure the samples were protected from light.

HPLC measurement of perazine and its metabolites

Concentrations of perazine and its main metabolites (5-sulfoxide and *N*-desmethylperazine) were assessed in the plasma, brain and microsomal suspension by the HPLC method previously developed by us (Daniel et al 1998). The residue obtained after evaporation of the plasma or brain extracts was dissolved in 100 μL of the mobile phase described below. A sample (20 μL) was injected into the HPLC system LaChrom (Merck-Hitachi), equipped with a UV detector, a L-7100 pump and a D-7000 System Manager. The analytical column (Econosphere C18 5 μm, 4.6 × 250 mm) was purchased from Alltech (Carnforth, UK).

The mobile phase consisted of an acetate buffer with a pH of 3.4 (containing 1 mL of triethylamine in 1 L) and acetonitrile in the proportion 30:70. Elution proceeded at an ambient temperature at flow rates of 0.6 mL min^{-1} (1–10 min), 1.2 mL min^{-1} (10.1–18 min) and 1.8 mL min^{-1} (18.1–24 min). Absorbance was measured at a wavelength of 254 nm.

In-vitro studies of perazine metabolism

Perazine metabolism was studied in liver microsomes at a linear dependence of the product formation on time, and protein and substrate concentrations (Daniel et al 1998). To distinguish between the direct effect of SSRIs on perazine metabolism and changes produced by their

Table 1 Steady-state concentrations of perazine and its metabolites in the plasma and brain of rats at 30 min, 6 h and 12 h after perazine withdrawal; perazine was administered at a dose of 10 mg kg^{-1} (i.p.) twice daily for 2 weeks.

Time after perazine withdrawal	Perazine	*N*-desmethylperazine	Perazine 5-sulfoxide
Plasma (nmol ml^{-1})			
30 min	0.814 ± 0.089	0.133 ± 0.012	0.216 ± 0.033
6 h	0.287 ± 0.024	0.159 ± 0.012	0.158 ± 0.019
12 h	0.211 ± 0.024	0.106 ± 0.019	0.220 ± 0.059
Brain (nmol g^{-1})			
30 min	7.252 ± 1.319	1.361 ± 0.181	0.362 ± 0.037
6 h	0.901 ± 0.189	2.495 ± 0.479	0.386 ± 0.047
12 h	0.214 ± 0.030	0.629 ± 0.163	0.401 ± 0.097

Values are presented as means ± s.e.m., n = 5–6.

chronic co-administration, two experimental models were used. Model I involved pooled liver microsomes from three control rats. The rate of *N*-demethylation and sulfoxidation of perazine (perazine concentration: 10–50 nmol mL^{-1}) was assessed in the absence and presence of one of the investigated SSRIs added in-vitro (SSRI concentration: 50 nmol mL^{-1}). All the samples were prepared in duplicate. In Model II, liver microsomes from perazine- or SSRI-treated rats, or rats treated with a combination of the two, were used. Perazine was added to the incubation mixture in-vitro at a concentration of 50 nmol mL^{-1}. Incubations (Models I and II) were carried out in a system containing liver microsomes (0.5 mg of protein in 1 mL), a Tris/HCl buffer (20 mM, pH 7.4), $MgCl_2$ (2.5 mM), NADP (0.1 mM), glucose 6-phosphate (1.2 mM) and glucose-6-phosphate-dehydrogenase (0.3 U in 1 mL). The final incubation volume was 1 mL. After a 2-min preincubation, the reaction was initiated by introducing perazine. After a 10-min incubation, the reaction was stopped by adding 200 μL of methanol and cooling it down to 0°C. The metabolites formed during incubation were determined by HPLC analysis as described above.

Assessment of cytochrome P-450 and cytochrome b-5

Concentrations of cytochromes P-450 and b-5 in liver microsomes were determined according to the methods of Omura & Sato (1964) and Omura & Takesue (1970), respectively, using a Beckman DU-65 spectrophotometer. The protein content was assayed according to the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Calculations and statistics

For Lineweaver-Burk analysis, the lines were fitted to the obtained points using a linear regression, which allowed determination of K_m and V_{max} values. Inhibition constants were calculated from the equation $K_i = [I] \times [(K_p \times K_m^{-1}) - 1]^{-1}$ (competitive inhibition), where [I] = concentration of inhibitor, K_m = the apparent Michaelis constant and $K_p = K_m$ in the presence of inhibitor. In the case of mixed inhibition, the K_i value was determined from the secondary plot representing the K_m/V_{max} ratio as a function of inhibitor concentration. Statistical significance was estimated using analysis of variance followed by Dunnett's test.

Results

Table 1 presents absolute concentration values of perazine and its metabolites at steady state in rats. At all the time intervals, concentrations of the parent compound in the plasma were higher than those of its metabolites. Only after 12 h was the concentration of perazine in plasma lower than that of its sulfoxide, albeit still similar. After 30 min (C_{max}) the perazine concentration in the plasma was 6- and 4-fold higher than that of *N*-desmethylperazine and 5-sulfoxide, respectively. At longer time intervals (i.e. 6 and 12 h, a slow disposition phase), differences between plasma concentrations of the parent compound and its metabolites were not so big. In the brain, concentrations of the measured compounds, especially of *N*-desmethylperazine and perazine, were much higher than in the plasma. The brain concentration of the parent compound was 8-fold (30 min) and that of *N*-desmethylperazine 16-fold (6 h) higher than the respective plasma levels.

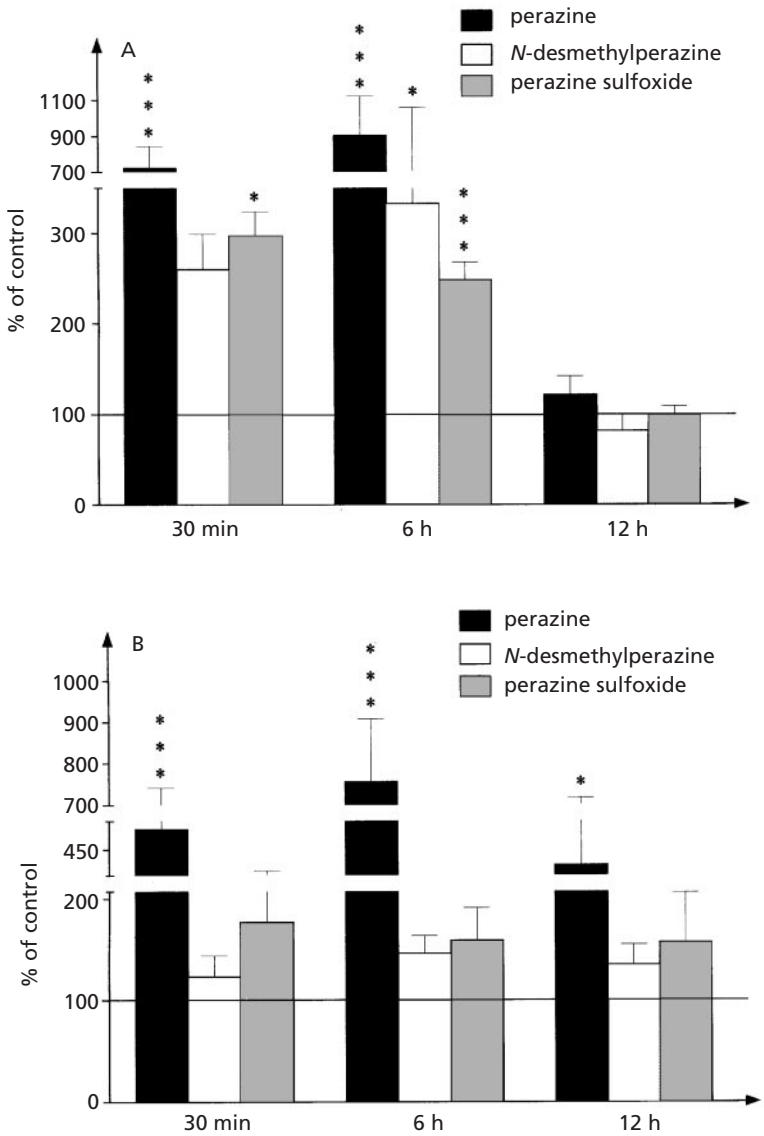

Figure 1 The influence of fluoxetine (hydrochloride, 5 mg kg^{-1}, i.p.) on the pharmacokinetics of perazine (dimaleate, 10 mg kg^{-1}, i.p.) after 2-week treatment of rats with a combination of the drugs. The plasma (A) and brain (B) levels of perazine and its metabolites at 30 min, 6 h and 12 h after withdrawal of the drugs are shown. $^{*}P < 0.05$, $^{***}P < 0.001$ (Dunnett's-test), compared with control (perazine-treated rats); n = 5–7. Absolute control values (animals treated with perazine alone) are presented in Table 1.

The investigated SSRIs increased the plasma and brain concentrations of perazine to a different extent. Fluoxetine (Figure 1) produced a marked elevation of the perazine level after 30 min and 6 h in the plasma: up to 700 and 900 % of the control, respectively (control = perazine-treated rats). A similar effect was observed at all the time intervals in the brain (up to 760 % of the control). These increases in neuroleptic concentration were accompanied by a significant rise in the metabolites levels in the plasma and a slight increase in the brain. Consequently, increases in the sum of perazine + metabolite concentration and in the perazine/metabolite concentration ratios in the plasma and brain were observed.

Fluvoxamine significantly raised the perazine level after 30 min: up to 760 and 430 % of the control in the plasma and brain, respectively (Figure 2). Simultaneously, an increase in *N*-desmethylperazine and 5-sulfoxide concentration was observed. At 6 h after perazine administration, the effect of fluvoxamine was less

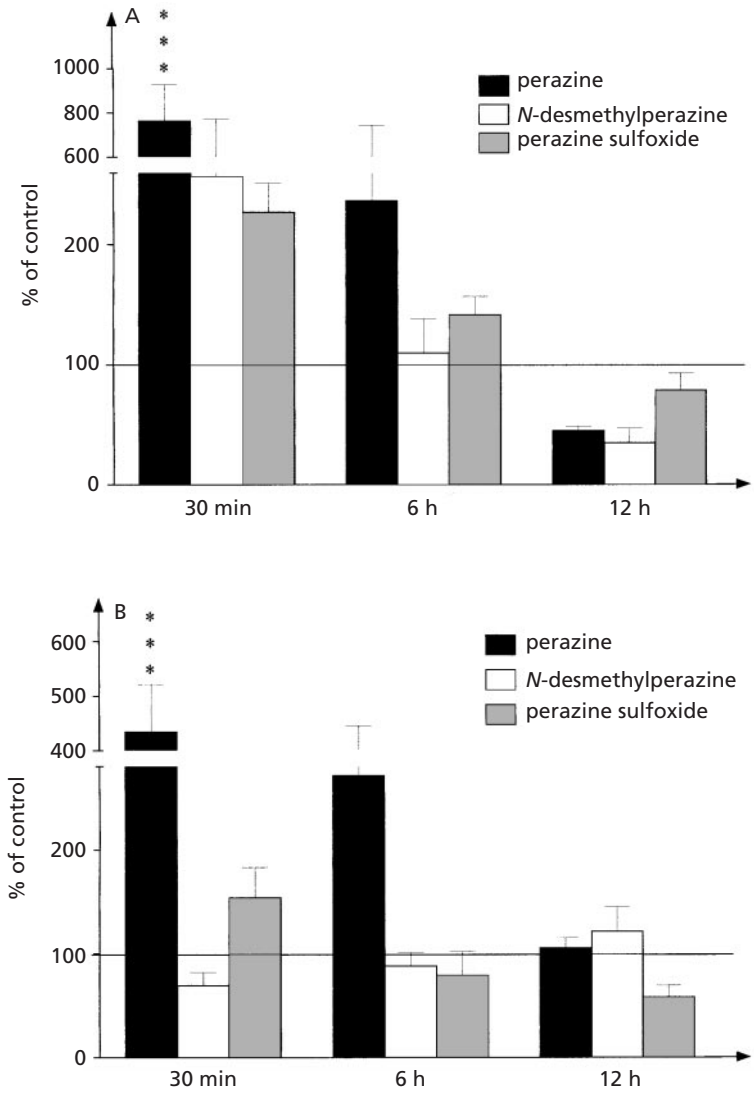

Figure 2 The influence of fluvoxamine (maleate, 10 mg kg^{-1}, i.p.) on the pharmacokinetics of perazine (dimaleate, 10 mg kg^{-1}, i.p.) after 2-week treatment of rats with a combination of the drugs. The plasma (A) and brain (B) levels of perazine and its metabolites at 30 min, 6 h and 12 h after withdrawal of the drugs are shown. ***$P < 0.001$ (Dunnett's-test), compared with control (perazine-treated rats); n = 5–7. Absolute control values (animals treated with perazine alone) are presented in Table 1.

pronounced, and after 12 h a decrease in neuroleptic concentration was seen. Like fluoxetine, fluvoxamine elevated the sum of concentrations of perazine + metabolites measured, and the perazine/metabolite concentration ratios after 30 min and 6 h in the plasma and brain.

Of the investigated SSRIs, sertraline showed the weakest effect on the pharmacokinetics of perazine (Figure 3), with an increase in the neuroleptic concentration in the plasma and brain being noted after 30 min. Moreover, significant increases in *N*-desmethylperazine level (in the plasma and brain) and 5-sulfoxide level (in the brain) were reported. The increase in the sum of concentrations of perazine + metabolites was accompanied by alterations in the perazine/metabolite concentration ratios. The concentration ratio of perazine/*N*-desmethylperazine decreased in the plasma and increased in the brain, while that of perazine/5-sulfoxide increased in the plasma and remained unchanged in the brain.

Lineweaver-Burk analysis showed that SSRIs added to a liver microsomal suspension of control animals

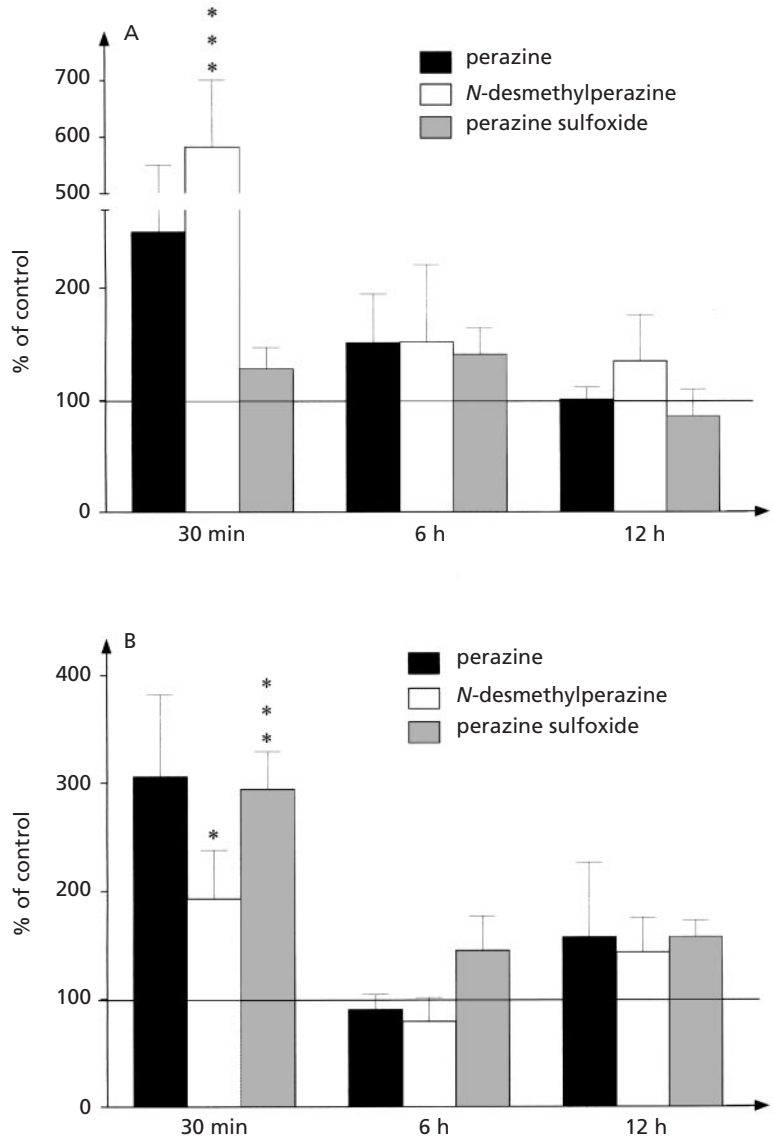

Figure 3 The influence of sertraline (hydrochloride, 5 mg kg^{-1}, i.p.) on the pharmacokinetics of perazine (dimaleate, 10 mg kg^{-1}, i.p.) after 2-week treatment of rats with a combination of the drugs. The plasma (A) and brain (B) levels of perazine and its metabolites at 30 min, 6 h and 12 h after withdrawal of the drugs are shown. $*P < 0.05$, $***P < 0.001$ (Dunnett's-test), compared with control (perazine-treated rats); n = 5–7. Absolute control values (animals treated with perazine alone) are presented in Table 1.

(Model I) competitively inhibited perazine *N*-demethylation and sulfoxidation (Figures 4 and 5). Sertraline was the most potent inhibitor of perazine *N*-demethylation (Figure 4) but the weakest inhibitor of sulfoxidation of all the SSRIs studied (Figure 5).

In-vitro studies with rat liver microsomes from rats treated chronically with perazine or one of the investigated SSRIs (the drugs administered in-vivo having been washed out from microsomes) showed that perazine decreased the total concentration of cytochrome P-450, while SSRIs had no influence on that parameter (Table 2). The investigated drugs did not change the concentration of cytochrome b-5. Chronic treatment with perazine alone did not significantly modify the cytochrome P-450 activity towards its own metabolism (Model II) compared with the control (control = saline-treated animals). In contrast, fluoxetine and sertraline administered alone significantly increased the specific

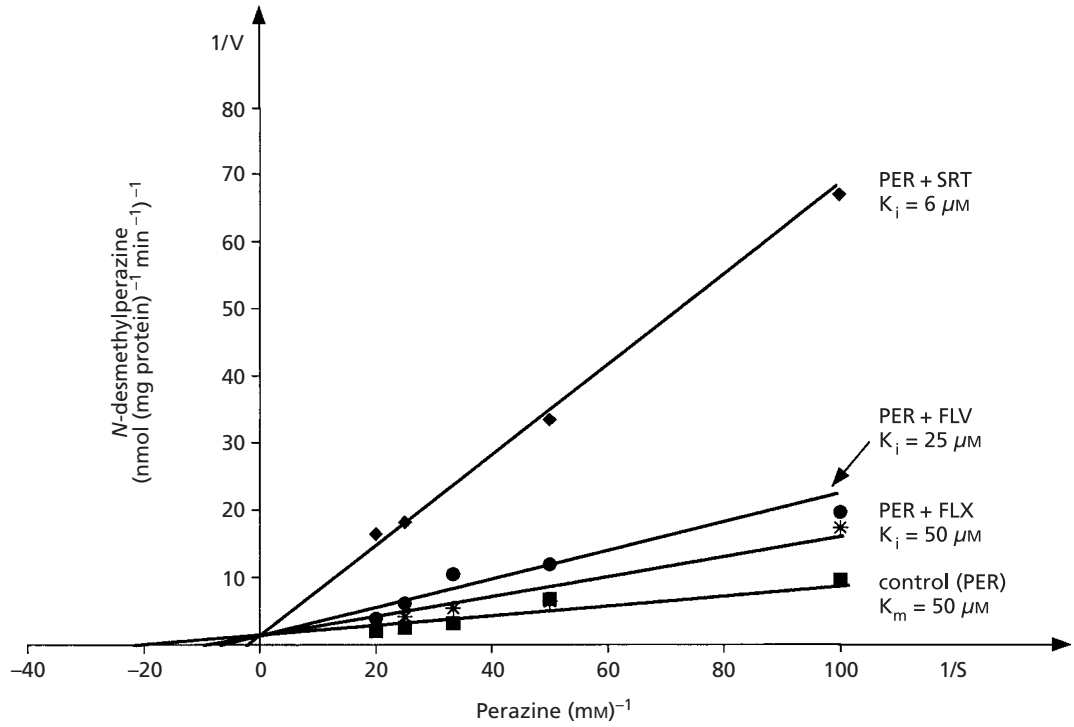

Figure 4 The kinetics of inhibition of perazine (PER) demethylation by fluoxetine (FLX), fluvoxamine (FLV) or sertraline (SRT) in-vitro. V = velocity of the reaction; S = concentration of perazine in the incubation mixture. The concentration of each inhibitor was 50 μM. The following values of kinetic constants were obtained: V_{max} = 0.32 nmol (mg of protein)$^{-1}$ min^{-1}; K_m (control, PER) = 50 μM, K_m(PER + FLX) = 100 μM, K_m (PER + FLV) = 150 μM, K_m (PER + SRT) = 454 μM.

(the amount of the product formed/mg protein) and the molecular (specific activity/amount of cytochrome P-450) activities of cytochrome P-450 towards perazine *N*-demethylation, while sertraline and fluvoxamine enhanced those activities towards perazine sulfoxidation. Consequently, chronic joint administration of perazine and fluoxetine increased the specific activity and tended to do so in the case of the molecular activity of cytochrome P-450 towards perazine *N*-demethylation compared to the perazine-treated group (Table 3). Instead, a combination of those drugs decreased the specific (compared with the control) and the molecular (compared with both the control and the perazine groups) activities of the cytochrome towards perazine sulfoxidation. Chronic treatment with a combination of perazine and fluvoxamine decreased the cytochrome P-450 concentration and specific activity of the cytochrome towards perazine sulfoxidation compared with the control (Table 3). Prolonged co-administration of perazine and sertraline raised the specific activity and tended to increase the molecular activity of cytochrome P-450 towards perazine *N*-demethylation compared with the perazine-treated animals (Table 3).

Discussion

These results show a differential effect of the investigated SSRIs on the pharmacokinetics and metabolism of perazine. Fluoxetine potently increased the plasma and brain concentrations of the neuroleptic after 30 min and 6 h, up to 900% of the control. A similar effect (i.e. an increase of perazine concentration up to 760% of the control) was observed with fluvoxamine after 30 min and persisted at 6 h. In comparison with fluoxetine and fluvoxamine, sertraline showed only a slight tendency to elevate the perazine concentration after 30 min. The observed increases in the concentration of *N*-desmethylperazine after co-administration of perazine with the investigated SSRIs may also have pharmacological significance, since the metabolite retains 50% of the parent compound activity (Rao 1989).

The observed increases in the concentration of perazine seem to be connected with direct inhibition of the neuroleptic's metabolism by SSRIs. Lineweaver-Burk analysis showed that the investigated antidepressants competitively inhibited the *N*-demethylation and sulfoxidation of perazine in-vitro, which expressed itself

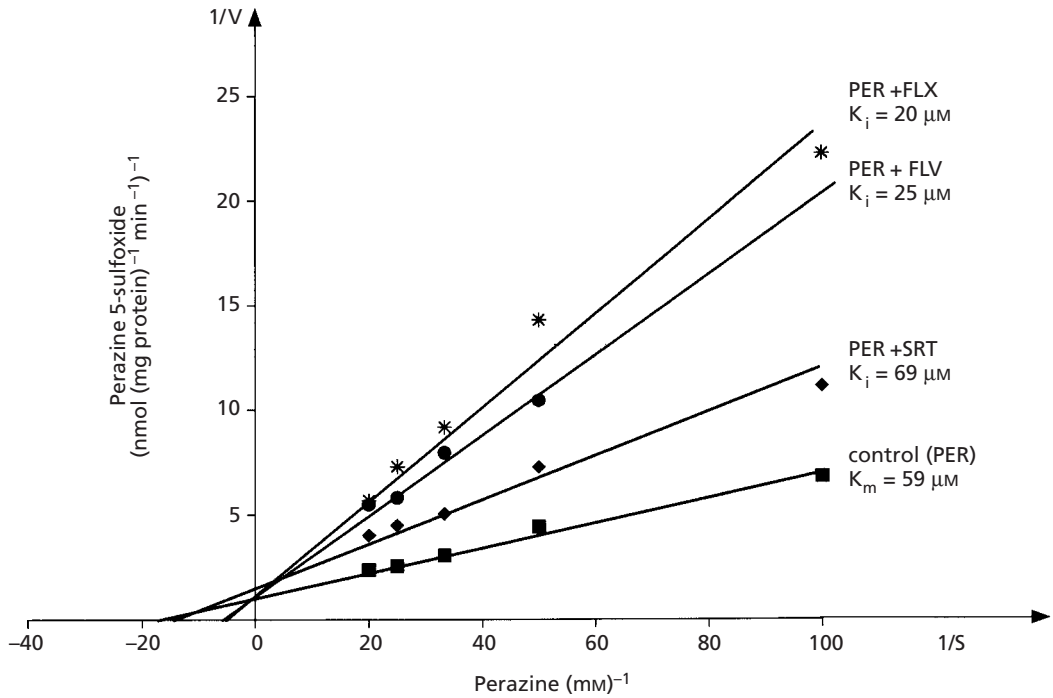

Figure 5 The kinetics of inhibition of perazine (PER) sulfoxidation by fluoxetine (FLX), fluvoxamine (FLV) or sertraline (SRT) in-vitro. V = velocity of the reaction; S = concentration of perazine in the incubation mixture. The concentration of each inhibitor was 50 μM. The following values of kinetic constants were obtained: V_{max} (control, PER) = 0.90 nmol (mg of protein)$^{-1}$ min^{-1}, K_m (control, PER) = 59 μM, K_m (PER + FLX) = 205 μM, K_m (PER + FLV) = 174 μM, V_{max} (PER + SRT) = 0.63 nmol (mg of protein)$^{-1}$ min^{-1}, K_m (PER + SRT) = 71 μM.

Table 2 Hepatic metabolic parameters in rats after different pre-treatments; concentrations of cytochromes P-450 and b-5, and rates of perazine demethylation and sulfoxidation in liver microsomes of rats treated with perazine or antidepressants for 2 weeks.

Treatment	Cytochrome P450 (nmol (mg protein)$^{-1}$)	Cytochrome b-5 (nmol (mg protein)$^{-1}$)	Perazine *N*-demethylation		Perazine sulfoxidation	
			Specific activity DPER (nmol (mg protein)$^{-1}$ min^{-1})	Molecular activity DPER/cytochrome P450	Specific activity 5-SO-PER (nmol (mg protein)$^{-1}$ min^{-1})	Molecular activity 5-SO-PER/ cytochrome P450
Control	0.820 ± 0.030	0.658 ± 0.030	0.457 ± 0.046	0.570 ± 0.081	0.471 ± 0.031	0.576 ± 0.039
Perazine	0.634 ± 0.060*	0.609 ± 0.031	0.490 ± 0.040	0.781 ± 0.045	0.400 ± 0.021	0.647 ± 0.043
Fluoxetine	0.895 ± 0.027	0.587 ± 0.027	1.007 ± 0.203***	1.114 ± 0.206*	0.467 ± 0.075	0.516 ± 0.072
Fluvoxamine	0.794 ± 0.061	0.575 ± 0.020	0.686 ± 0.055	0.866 ± 0.031	0.853 ± 0.040***	1.109 ± 0.105***
Sertraline	0.739 ± 0.039	0.645 ± 0.011	1.175 ± 0.128***	1.590 ± 0.150***	0.833 ± 0.060***	1.137 ± 0.089***

Values are means ± s.e.m., n = 6; *$P < 0.05$, ***$P < 0.001$, compared with control (Dunnett's test). DPER, desmethylperazine; 5-SO-PER, perazine sulfoxide.

in-vivo in significant increases in the perazine concentration and the perazine/metabolite concentration ratio, evoked by fluoxetine or fluvoxamine. At 12 h after administration of a combination of perazine and fluoxetine or fluvoxamine, no interactions were observed, since concentrations of the drugs were probably too low to compete for active centres of enzymes.

Surprisingly, sertraline was the most potent inhibitor of *N*-demethylation in-vitro, though it had only a weak effect on the pharmacokinetics of perazine in-vivo.

Table 3 Hepatic metabolic parameters in rats after different pre-treatments; concentrations of cytochromes P-450 and b-5, and rates of perazine demethylation and sulfoxidation in liver microsomes of rats treated with perazine and/or antidepressants for 2 weeks.

Treatment	Cytochrome P450 (nmol) (mg protein)$^{-1}$)	Cytochrome b-5 (nmol (mg protein)$^{-1}$)	Perazine *N*-demethylation		Perazine sulfoxidation	
			Specific activity DPER (nmol (mg protein)$^{-1}$ min^{-1})	Molecular activity DPER/ cytochrome P450	Specific activity 5-SO-PER (nmol (mg protein)$^{-1}$ min^{-1})	Molecular activity 5-SO-PER/ cytochrome P450
Control	0.820 ± 0.030	0.658 ± 0.030	0.607 ± 0.065	0.755 ± 0.107	0.473 ± 0.043	0.579 ± 0.055
Perazine	0.630 ± 0.060*	0.609 ± 0.031	0.501 ± 0.068	0.779 ± 0.055	0.370 ± 0.020	0.596 ± 0.031
Perazine + Fluoxetine	0.879 ± 0.051 #	0.710 ± 0.014	0.931 ± 0.052 # # #	1.076 ± 0.094	0.316 ± 0.010*	0.361 ± 0.013* # # #
Perazine + Fluvoxamine	0.621 ± 0.053*	0.583 ± 0.036	0.486 ± 0.067	0.781 ± 0.079	0.305 ± 0.028*	0.488 ± 0.011
Perazine + Sertraline	0.818 ± 0.055	0.668 ± 0.058	0.855 ± 0.133 #	1.017 ± 0.115	0.435 ± 0.061	0.527 ± 0.058

Values are means ± s.e.m., n = 6; *$P < 0.05$, compared with control (Dunnett's test); # $P < 0.05$, # # # $P < 0.001$, compared with perazine-treated rats (Dunnett's test). DPER, desmethylperazine; 5-SO-PER, perazine sulfoxide.

However, of the investigated SSRIs, sertraline had the weakest inhibitory effect (via a mixed mechanism) on perazine sulfoxidation. This finding may imply that in-vivo sulfoxidation is a more important metabolic pathway of perazine elimination than is *N*-demethylation, or that sertraline does not reach a sufficiently high concentration in the vicinity of cytochrome P-450 to exert its inhibitory effect on perazine metabolism.

Adaptive changes in cytochrome P-450, produced by chronic administration of the drugs studied, seem to play a less important role in the observed pharmacokinetic interactions. Prolonged treatment with SSRIs accelerated *N*-demethylation or sulfoxidation of perazine. Joint administration of perazine with fluoxetine or sertraline to rats accelerated the *N*-demethylation of perazine compared with administration of perazine alone. The influence of the combination of the investigated drugs on sulfoxidation was of relatively little importance to the interactions. The decrease in molecular activity of cytochrome P-450 towards perazine sulfoxidation (compared with the perazine group), observed after chronic treatment with perazine + fluoxetine, results exclusively from enhancement of the total activity of the cytochrome in the liver, which is most probably linked to acceleration of *N*-demethylation.

The acceleration of perazine demethylation in-vitro, observed after chronic treatment with perazine and fluoxetine or sertraline, should express itself in-vivo in an elevation of the *N*-desmethylperazine level at a decreased perazine level and perazine/*N*-desmethylperazine concentration ratio. Indeed, with combination of perazine and fluoxetine, an increase in the *N*-desmethylperazine concentration was observed; however, in contrast to the changes expected after induction, the concentration of the parent compound and the parent compound/metabolite concentration ratio rose, which indicates that the competitive inhibition of perazine *N*-demethylation by fluoxetine plays a more important role than does the adaptive alteration of cytochrome P-450 in relation to this metabolic process. Similar changes in the concentrations of perazine and *N*-desmethylperazine were observed after joint administration of perazine and fluvoxamine, though chronic treatment with this combination did not enhance the rate of perazine *N*-demethylation. However, apart from an increase in the concentration of *N*-desmethylperazine after concurrent administration of perazine and sertraline, a decrease in the perazine/*N*-desmethylperazine concentration ratio was observed after 30 min. It seems, therefore, that in such a case the increased activity of cytochrome P-450 towards perazine *N*-demethylation is of some, though not crucial, importance for the final result of the interaction, since the concentration of perazine showed a tendency to increase. Although sertraline was the most potent competitive inhibitor of perazine *N*-demethylation in-vitro, its direct effect on cytochrome P-450 in-vivo did not significantly surpass the enzyme induction. As already mentioned, sertraline probably did not reach a sufficiently high concentration in the vicinity of cytochrome P-450 in-vivo to overcome the adaptative acceleration of *N*-demethylation.

After prolonged administration of a drug combination, the final outcome of an interaction is a result of a direct effect on an enzyme and of adaptive changes produced by the drug combination. The results obtained in-vivo and in-vitro indicate that for perazine–SSRIs interactions a direct effect of the investigated antidepressants on cytochrome P-450 is of crucial importance. As previously mentioned, our unpublished data show that in the rat *N*-demethylation is catalysed by CYP2D1, CYP2B and CYP1A2, and *S*-oxidation by CYP2D1 and CYP2B. Thus competitive inhibition by the investigated antidepressants, of one or more enzymes catalysing the *N*-demethylation and sulfoxidation of perazine, is a key mechanism of their influence on the pharmacokinetics of the neuroleptic. Moreover, the observed in-vivo increase in the concentrations of both perazine and metabolites measured suggests simultaneous inhibition by SSRIs of another metabolic pathway of perazine, not yet investigated by us.

With inhibition of drug metabolism, an increase in the parent compound and a decrease in its metabolite concentration in plasma may be expected. In this study, increases in the concentrations of both perazine (characteristic of inhibition) and the metabolites measured (inconsistent with inhibition) were observed. This inconsistency may possibly arise from a metabolic pathway of perazine, not investigated in our experiment (e.g. aromatic hydroxylation), being inhibited by the antidepressants, which might have increased the concentration of substrate perazine and thus, indirectly, the amount of the metabolites formed. SSRIs are known substrates and inhibitors of CYP2D6, an enzyme which can catalyse aromatic hydroxylation (Crewe et al 1992; Jeppesen et al 1996; Preskorn 1997; Kobayashi et al 1999). However, the possibility exists that successive metabolism of the perazine metabolites measured may also be inhibited by the antidepressants.

Our earlier and present results show that the investigated SSRIs differently affect the pharmacokinetics of phenothiazines with different chemical structures (i.e. promazine (Daniel et al 1999a), thioridazine (Daniel et al 1999b) and perazine (studied here)). The most effective inhibitor of neuroleptic metabolism is fluoxetine, a well-known potent inhibitor of CYP2D6 (Crewe et al 1992; Jeppesen et al 1996); this finding confirms the importance of the CYP2D isoenzyme subfamily to the metabolism of phenothiazine neuroleptics. Fluoxetine elevated the plasma and brain concentrations of all three of the phenothiazines, its effect being most pronounced with thioridazine (a 1200 % increase), than perazine (a 900 % increase) or promazine (a 300 % increase). Fluvoxamine significantly affected the concentrations of perazine (a 760 % increase) and promazine (a 300 % increase), the effect only being slight in the case of thioridazine. Sertraline significantly increased the concentration of promazine (a 300 % increase), showing a similar effect with perazine and an opposite one with thioridazine. It seems that the effects of the investigated SSRIs on the metabolism of phenothiazine neuroleptics depend not only on the potency of antidepressants to inhibit cytochrome P-450 isoenzymes which catalyse the metabolism of phenothiazines, but also on the extent of contribution of the isoenzymes inhibited by SSRIs to the metabolism of neuroleptics and to the affinity of the latter for active centres of these isoenzymes.

It is quite likely that the interactions occurring in the rat also occur in man since similar metabolic pathways, amino acid sequences and catalytic activities of the cytochrome P-450 isoenzymes (especially CYP2D) are involved in perazine metabolism in the rat and man. Moreover, similar K_i values are obtained for fluoxetine and fluvoxamine, which reflect both the order of magnitude of the respective K_m values and the presumed concentrations of the antidepressants in the vicinity of cytochrome P-450 in-vivo (i.e. in the endoplasmic reticulum), in both the rat and man (Bergstrom et al 1992; Daniel & Wójcikowski 1999; Preskorn 1997). There are case reports of patients on neuroleptics who experienced increased CNS adverse effects when SSRIs were added to their treatment regimens (Sproule et al 1997).

In conclusion, fluoxetine or fluvoxamine co-administered jointly with perazine to rats substantially increases the concentrations of the neuroleptic and its *N*-demethylated and sulfoxidated metabolites via competitive enzyme inhibition. Such a tendency is also seen when sertraline is co-administered with perazine. Similar interactions may be expected in man, leading possibly to undesired enhancement of the perazine inhibition of the neurotransmitter receptors in the central and autonomic nervous systems.

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