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N-Dealkylation of Chlorimipramine and Chlorpromazine by Rat Liver Microsomal Cytochrome P450 Isoenzymes

MASSIMO VALOTI, MARIA FROSINI, MITRI PALMI, FRANCESCO DE MATTEIS* AND GIANPIETRO SGARAGLI

*Centro Interdipartimentale di Ricerca sul Metabolismo dei Farmaci Neuropsicotropi, Istituto di Scienze Farmacologiche, Università di Siena, Via Piccolomini 170, 53100 Siena and *Dipartimento di Anatomia, Farmacologia e Medicina Legale, Università di Torino, Italy*

Abstract

The role of different cytochrome P450 isozymes (CYP) in the *N*-demethylation of chlorimipramine and chlorpromazine has been investigated in liver microsomes from rats by studying the effects of multiple subchronic doses of chlorimipramine, chlorpromazine, phenobarbital and β-naphthoflavone on the *N*-demethylation of ethylmorphine, mono-*N*-demethyl-chlorimipramine and chlorpromazine and on the hydroxylation of aniline.

With control microsomes, CYP-dependent metabolism of chlorimipramine and chlorpromazine (100 nmol; 30 min incubation) resulted in the formation of predominantly chlorimipramine (46·5 ± 4·9 nmol) whereas chlorpromazine (14·1 ± 0·9 nmol) accounted for only part of the overall metabolism of chlorpromazine. Multiple doses of chlorimipramine increased the capacity of microsomes to *N*-demethylate ethylmorphine (9·8 ± 0·73 and 6·08 ± 0·06 nmol min^{-1} (mg protein)$^{-1}$ for chlorimipramine-treated and control rats, respectively) as well as itself (4·65 ± 0·25 and 3·10 ± 0·33 nmol min^{-1} (mg protein)$^{-1}$, respectively). Multiple doses of chlorpromazine induced aniline-hydroxylase activity (1·11 ± 0·16 and 0·94 ± 0·06 nmol min^{-1} (mg protein)$^{-1}$ for chlorimipramine and control microsomes, respectively) but the capacity to *N*-demethylate itself was unchanged. Phenobarbital treatment induced ethylmorphine *N*-demethylation activity, but did not affect *N*-demethylation activity, towards chlorimipramine and chlorpromazine. In control microsomes the *N*-demethylation capacity of chlorimipramine or chlorpromazine (0·160 ± 0·025 and 0·015 ± 0·003 nmol min^{-1} (mg protein)$^{-1}$, respectively) was one order of magnitude lower than that of chlorimipramine or chlorpromazine. The capacity to *N*-demethylate either chlorimipramine or chlorpromazine was increased by treatment with either phenobarbital or β-naphthoflavone. In control microsomes, sulphaphenazole markedly inhibited both chlorimipramine-*N*-mono- and di-*N*-demethylation, whereas quinidine markedly inhibited the rate of formation of chlorpromazine.

The CYP2C and CYP2D subfamilies seem to be involved in the mono *N*-demethylation of chlorimipramine and chlorpromazine, respectively. Moreover the CYP1A and CYP2B subfamilies might participate in the *N*-demethylation of either chlorimipramine or chlorpromazine. This could have important implications in the clinical use of chlorimipramine and chlorpromazine in view of the genetic polymorphism of CYP2C and CYP2D isozymes in man.

Chlorimipramine and chlorpromazine have a long clinical history as psychoactive agents and have been extensively studied in experimental psychopharmacology. Although chlorpromazine is an antipsychotic agent and chlorimipramine an antidepressant, the two drugs share several pharmacological features and their structures (Dahl & Strandjörd 1977), lipophilicity (Gigon et al 1983), plasma protein binding (Curry 1970) and metabolic pathways have many similarities.

The removal of one or both terminal methyl groups from the dimethylaminopropyl side chain

Correspondence: M. Valoti, Centro Interdipartimentale di Ricerca sul Metabolismo dei Farmaci Neuropsicotropi, Institute of Pharmacological Sciences, University of Siena, Via Piccolomini 170, 53100 Siena, Italy.

(*N*-demethylation), is the reaction pathway of major importance in the metabolism of chlorimipramine and chlorpromazine, producing metabolites which seem capable of crossing the blood–brain barrier and which have psychopharmacological properties (Bickel 1980; Breyer-Pfaff 1980).

In a recent study the plasma pharmacokinetics of chlorimipramine, chlorpromazine and their *N*-demethyl metabolites were investigated in healthy volunteers given a single oral dose of the parent compounds (Della Corte et al 1993). Although restricted to a single dose and to a healthy population the study indicated that the two drugs are processed in a remarkably different manner. At the same dosage, areas under the 24-h plasma concentration–time curve ($AUC_{0-24\,h}$) were fivefold those for chlorimipramine than for chlorpromazine, despite the slower apparent absorption rate of chlorimipramine. Plasma chlorimipramine levels reached a mean peak value three times that of chlorpromazine. The demethyl metabolite kinetics of chlorimipramine were elimination-rate limited whereas those of chlorpromazine were formation-rate limited.

A subsequent single-dose kinetic study of chlorimipramine and chlorpromazine in rat tissues also highlighted differences in the disposition of the drugs; chlorpromazine was metabolized more slowly and therefore had a longer persistence (Sgaragli et al 1995). In that study hepatic concentrations of parent compound and the *N*-demethylated metabolites were compared and the rate of *N*-demethylation was found to be much slower for chlorpromazine than for chlorimipramine.

To clarify the different kinetic behaviour in the *N*-demethylation pathway of the drugs, experiments have now been performed using rat-liver microsomal preparations. We treated rats with phenobarbital and β-naphthoflavone, two well known inducers of different CYP isozymes, and with chlorimipramine and chlorpromazine. It was hoped in this way to determine whether the higher *N*-demethyl metabolite levels observed in the rat in-vivo for chlorimipramine (compared with those for chlorpromazine) could be correlated with a higher rate of *N*-dealkylation by liver microsomal CYP in-vitro. There is evidence in man and other species that chronic treatment with chlorimipramine (but not with chlorpromazine) increases the rate of accumulation of the corresponding *N*-demethylated metabolites, but there is no evidence indicating whether these effects are a result of the different rate of elimination of the two metabolites or the different rate at which they are produced under these conditions. We therefore treated rats subchronically with chlorimipramine or chlorpromazine with the aim of establishing whether chronic treatment with chlorimipramine or chlorpromazine affects their own metabolism by *N*-dealkylation.

Materials and Methods

Chemicals

Chlorimipramine.HCl was from Ciba-Geigy (Milan, Italy) and chlorpromazine.HCl was from the Santa Maria Nuova Hospital (Florence, Italy). Mono- and di-*N*-demethylchlorimipramine and the *N*-dimethylated forms of chlorpromazine.HCl were from the National Institute of Mental Health, Rockville, MD, USA. NADPH, NADP, NADH and glucose-6-phosphate dehydrogenase were from Boehringer and Söehne, (Mannheim, Germany). All other reagents were obtained from E. Merck (Darmstadt, Germany).

Carbon monoxide was produced in a closed glass jar by adding sulphuric acid dropwise to formic acid at room temperature. It was withdrawn by use of a plastic 50-mL syringe connected to the reaction chamber by plastic tubing.

Drug treatment and preparation of liver microsomes

Male Sprague-Dawley rats, 180–200 g, from Charles River were housed in standard cages in a temperature- and light-controlled facility with free access to food (M.I.L.; Morini, San Polo d'Enza, Italy) and water. All experiments were performed in compliance with the Animal Care and Ethics Committee of the University of Siena, Italy.

After an acclimatization period of 10 days, a group (n = 5) of control rats was maintained on the same diet without any further treatment. The other animals were divided in four groups (n = 5 each) which were subjected to one of four treatments: $80\,mg\,kg^{-1}$ intraperitoneal phenobarbital then $1\,mg\,mL^{-1}$ in the drinking water for 1 week; $80\,mg\,kg^{-1}$ intraperitoneal β-naphthoflavone in olive oil for 3 days; $20\,mg\,kg^{-1}$ oral chlorimipramine or chlorpromazine (0·06 mmol) for 2 weeks. All rats were then fasted overnight before being killed.

Under diethyl ether anaesthesia the abdominal cavity was opened and the liver perfused in-situ through the vena cava with ice-cold normal saline. The liver was excised, chopped, suspended in 3 vols 0·25 M sucrose and homogenized on a Potter-Elvejhem blender fitted with a Teflon pestle. The homogenate was centrifuged at 10 000 *g* for 20 min and the resulting supernatant ultracentrifuged at 105 000 *g* for 1 h. The microsomal pellets were

suspended in 0·01 M Tris buffer, pH 7·60, containing 151 mM KCl, 1 mM EDTA and 20% glycerol, and stored in liquid nitrogen until use.

Essay of enzyme activity

Cytochrome P450 content was determined by means of the CO-difference spectrum of microsomes as described by Omura & Sato (1964). Cytochrome C (P450) reductase activity was calculated as described by Phillips & Langdon (1962). Aniline hydroxylase activity was measured by recording the formation of 4-aminophenol for 20 min according to the procedure described by Lake (1987). Ethylmorphine *N*-demethylase activity towards ethylmorphine was assayed by measuring the formaldehyde formed (Lake 1987).

N-demethylase activity towards chlorimipramine, chlorpromazine, mono-N-demethyl-chlorimipramine and mono-N-demethyl-chlorpromazine

Chlorpromazine, chlorimipramine or their NOR_1 derivatives (100 μM) were incubated at 37°C for 30 min in phosphate buffer (70 mM, pH 7·4) containing microsomal protein (0·5 mg mL^{-1}), in presence of NADPH-generating system (NADPH-GS; 1 mM NADPH, 4 mM glucose-6-phosphate and 1 unit glucose-6-phosphate dehydrogenase). The reaction was stopped by cooling the test tube in ice and adding NaOH (1 M; 0·3 mL) and internal standard (chlorpromazine for measurement of chlorimipramine and mono-*N*-demethyl-chlorimipramine *N*-demethylase activity, chlorimipramine otherwise; 100 nmol). In some experiments CYP reductase activity towards chlorpromazine was assayed. Chlorpromazine (100 μM) was added to microsomes previously bubbled with N_2 for 10 min in the presence of NADPH (1 mM) as cofactor and phosphate buffer (70 mM, pH 7·4). This reaction mixture was sealed in capped tubes.

The reaction mixtures were processed by one-step organic solvent extraction (hexane–isoamyl alcohol, 98 : 2, v/v). The metabolites and the parent drugs were subsequently determined by gas–liquid chromatography (GLC) as described elsewhere (Ninci et al 1986) with slight modifications. After derivatization with trifluoroacetic acid anhydride the samples were dried under N_2, resuspended in methanol and injected into a Perkin-Elmer 3B series gas chromatograph equipped with a 15 m × 0·53 mm i.d. DB-17 column and nitrogen-phosphorus detector. GLC analysis was performed by means of a temperature-programmed separation procedure, starting from an initial temperature of 150°C and increasing it at a rate of 10° min^{-1} to a final temperature of 250°C. Injector and detector temperatures were set at 270°C; the carrier gas flow rate was 25 mL min^{-1}. Standard curves were obtained by adding different amounts of the analytes to microsomal preparations obtained from control rats.

Inhibition studies were performed by pre-incubating microsomes obtained from untreated rats with sulphaphenazole, quinidine, fluvoxamine or ketoconazole (50 μM) and NADPH-GS at 37°C. After 10 min chlorimipramine or chlorpromazine (100 μM) was added and the reaction followed for 30 min. The samples were then processed as described above.

Rat-liver microsomal proteins were estimated by the Bradford procedure (1976) using bovine serum albumin as standard.

Statistical analysis

All data are reported as means ± s.e.m. The significance of the differences between means from two treatment groups was established by means of Student's *t*-test.

Results

Treatment with the four drugs had no effect on body and liver weights (results not shown) but increased liver P450 content and related enzyme activity, as also happened after treatment with phenobarbital and β-naphthoflavone (Table 1). NADPH-cytochrome P450 reductase activity was increased by chlorimipramine, chlorpromazine and phenobarbital treatment only. When the activity of the two P450-dependent enzymes, ethylmorphine *N*-demethylase, associated with CYP3A isoenzymes (Wrighton et al 1985) and aniline hydroxylase, characteristic of CYP2E1 isoenzymes (Ko et al 1987) was investigated, only chlorimipramine and phenobarbital elicited a significant increase in *N*-demethylation of ethylmorphine, whereas β-naphthoflavone seemed to inhibit it, and chlorpromazine and β-naphthoflavone elicited a significant increase in aniline hydroxylation capacity, whereas chlorimipramine and phenobarbital had no effect.

Preliminary assay of the *N*-demethylase activity of chlorimipramine and chlorpromazine under the conditions described above showed that the quantities of products formed increased linearly with time up to 30–45 min and that the initial rates were proportional to the amounts of microsomal protein added. As shown in Table 2, the rate of *N*-demethylation of chlorimipramine after chlorimipramine treatment was 66% greater than that observed for control microsomes; after chlorpromazine treatment there was an increasing trend which was not statistically significant. The rate of *N*-demethylation of chlorimipramine in microsomes of phenobarbital-treated rats was similar to

Table 1. Effects of repeated administration of chlorimipramine, chlorpromazine, phenobarbital and β-naphthoflavone on cytochrome P450 level and the NADPH-cytochrome P450 reductase, ethylmorphine *N*-demethylase and aniline hydroxylase activity of rat-liver microsomes.

Treatment	Cytochrome P450 level nmol (mg protein)$^{-1}$	%	NADPH-P450 reductase nmol min^{-1} (mg protein)$^{-1}$	%	Ethylmorphine *N*-demethylase nmol min^{-1} (mg protein)$^{-1}$	%	Aniline hydroxylase nmol min^{-1} (mg protein)$^{-1}$	%
Control	0·764 ± 0·093	100	108 ± 6	100	6·08 ± 0·06	100	0·52 ± 0·01	100
Chlorimipramine	0·946 ± 0·061	124	146 ± 6**	136	9·80 ± 0·73**	161	0·53 ± 0·13	102
Chlorpromazine	0·810 ± 0·025	106	130 ± 7*	120	7·79 ± 0·69	128	0·77 ± 0·05**	148
Phenobarbital	1·410 ± 0·166	184	204 ± 18**	189	13·52 ± 1·64**	222	0·51 ± 0·01	97
β-Naphthoflavone	1·190 ± 0·109*	156	103 ± 12	95	3·48 ± 0·86*	57	0·58 ± 0·03*	112

Data are means ± s.e.m. of results from five individual microsomal preparations per group. * $P < 0·05$, ** $P < 0·01$, significantly different from control result.

Table 2. Effects of repeated administration of chlorimipramine, chlorpromazine, phenobarbital and β-naphthoflavone on the chlorimipramine and chlorpromazine *N*-demethylase activity of rat-liver microsomes.

Treatment	Substrate					
	Chlorpromazine		Chlorimipramine		NOR_1-chlorimipramine	NOR_1-chlorpromazine
	Formation of:					
	mono-*N*-demethyl-chlorimipramine	di-*N*-demethyl-chlorimipramine	mono-*N*-demethyl-chlorpromazine	di-*N*-demethyl-chlorpromazine	di-*N*-demethyl-chlorimipramine	di-*N*-demethyl-chlorpromazine
Control	3·10 ± 0·33	0·090 ± 0·010	0·94 ± 0·06	ND	0·160 ± 0·025	0·015 ± 0·003
Chlorimipramine	4·65 ± 0·25†	0·087 ± 0·026	1·27 ± 0·15	ND	0·160 ± 0·018	0·035 ± 0·010
Chlorpromazine	4·08 ± 0·40	0·100 ± 0·037	1·11 ± 0·16	ND	0·190 ± 0·044	0·038 ± 0·013
Phenobarbital	2·93 ± 0·40	0·250 ± 0·040†	0·98 ± 0·13	ND	0·510 ± 0·120‡	0·144 ± 0·080‡
β-Naphthoflavone	1·53 ± 0·20†	0·077 ± 0·016	0·65 ± 0·07‡	ND	0·280 ± 0·019†	0·162 ± 0·030†

ND = not detectable. Data (nmol min^{-1} (mg protein)$^{-1}$) are means ± s.e.m. of enzyme activities measured in five individual microsomal preparations per group. ‡ $P < 0·05$, † $P < 0·01$, significantly different from control result.

that for controls; treatment with β-naphthoflavone inhibited the activity of this enzyme. The rate of formation of di-*N*-demethyl-chlorimipramine from chlorimipramine was much greater with phenobarbital-treated microsomes—with the other microsomes values were similar to those observed for control microsomes. The overall metabolic efficiency of microsomes was much greater towards chlorimipramine than towards chlorpromazine. The rate of formation of mono-*N*-demethyl-chlorpromazine by control and other microsomal preparations was one third that observed for formation of mono-*N*-demethyl-chlorimipramine. The rate of formation of mono-*N*-demethyl-chlorpromazine was not increased by any of the previous treatments but it was slightly but significantly reduced by treatment with β-naphthoflavone. Di-*N*-demethyl-chlorpromazine was never detected in the reaction mixture when chlorpromazine was used as substrate.

Table 3 gives a more general account of the metabolic changes to chlorimipramine and chlorpromazine caused by microsomes. During 30-min incubation of 100 nmol chlorimipramine with the microsomal suspension, more than 60% was metabolized, with no differences between control, chlorimipramine-, chlorpromazine- and phenobarbital-treated rats. β-Naphthoflavone microsomes metabolized chlorimipramine to a lesser extent—only 33% of the initial amount of chlorimipramine disappeared from the incubation mixture. Chlorimipramine and chlorpromazine treatment, however, modified the metabolic pattern of chlorimipramine by channelling it towards the *N*-dealkylation pathway and the amount of mono-*N*-demethyl-chlorimipramine formed accounted for almost 90% of the chlorimipramine utilized. In contrast, phenobarbital treatment stimulated chlorimipramine metabolism to the greatest extent, but it did so in

Table 3. Effects of repeated administration of chlorimipramine, chlorpromazine, phenobarbital and β-naphthoflavone on the overall metabolism of chlorimipramine and chlorpromazine and on the formation of the respective demethyl metabolites.

Treatment	Chlorimipramine metabolism	Mono-*N*-demethyl-chlorimipramine formation	Di-*N*-demethyl-chlorimipramine formation	Chlorpromazine metabolism	Mono-*N*-demethyl-chlorpromazine formation
Control	64·7 ± 3·3	46·5 ± 4·9	1·3 ± 0·2	46·4 ± 9·0	14·1 ± 0·9
Chlorimipramine	74·5 ± 5·3	69·7 ± 3·75*	1·4 ± 0·4	49·8 ± 4·5	19·5 ± 2·2
Chlorpromazine	65·4 ± 5·1	61·2 ± 6·0	1·5 ± 0·5	49·7 ± 8·1	16·5 ± 2·4
Phenobarbital	78·7 ± 2·1*	43·9 ± 6·0	4·8 ± 0·9*	31·2 ± 10·5	14·7 ± 1·9
β-Naphthoflavone	33·3 ± 9·4**	22·9 ± 3·0*	1·1 ± 0·2	18·4 ± 5·8	10·0 ± 1·0

The parent drugs (100 nmol) were incubated for 30 min with different microsomal preparations (0·5 mg protein). Data (nmol) are means ± s.e.m. of results from five individual preparations per group. * $P < 0.05$, ** $P < 0.01$, significantly different from control result.

favour of metabolites other than mono-*N*-demethyl-chlorimipramine, the formation of mono-*N*-demethyl-chlorimipramine accounting for only ca 50% of the chlorimipramine utilized, an amount even lower than that observed for control microsomes.

The total amount of chlorpromazine metabolized in 30 min ranged from 31% with phenobarbital-treated microsomes to 50% for those treated with chlorimipramine; β-naphthoflavone-treated microsomes metabolized only 19% of the chlorpromazine. The mono-*N*-demethyl-chlorpromazine formed accounted for less than 20% of the chlorpromazine added to the different microsomes (range 10% with β-naphthoflavone-treated microsomes and 19% with those treated with chlorimipramine).

As shown in Table 2, the rate of di-*N*-demethyl formation from both mono-*N*-demethyl substrates was much slower, by more than one order of magnitude, than the rate observed with chlorimipramine or chlorpromazine as substrates.

The formation of di-*N*-demethyl metabolites accounted for the whole quantity of mono-*N*-demethyl metabolites which disappeared during incubation. This ranged from 0·3–2·5% for mono-*N*-demethyl-chlorpromazine and 2·5–8% for mono-*N*-demethyl-chlorimipramine. For both substrates the highest rate of accumulation of di-*N*-demethyl was observed when mono-*N*-demethyl derivatives were incubated in the presence of microsomes obtained from phenobarbital- or β-naphthoflavone-treated rats, the rate of formation of di-*N*-demethyl-chlorpromazine being one order of magnitude higher than that observed with control microsomes.

As shown in Table 4, the formation of mono-*N*-demethyl-chlorimipramine was inhibited when control microsomes were incubated with sulphaphenazole or quinidine. Inhibition by sulphaphenazole was approximately 70% and that by quinidine was ca 40%. Di-*N*-demethyl-chlorimipramine formation was inhibited when the microsomes were incubated with chlorimipramine in the presence of sulphaphenazole or ketoconazole. Quinidine inhibited the rate of formation of mono-*N*-demethyl-chlorpromazine by approximately 50%.

When chlorpromazine was incubated with microsomes under reductive conditions, the dehalogenation product promazine was not detected and chlorpromazine was not consumed. Furthermore, promazine was not detected under any of the oxidative conditions described above.

Table 4. In-vitro effects of different CYP inhibitors (50 μM) on the formation of mono-*N*-demethyl-chlorimipramine, di-*N*-demethyl-chlorimipramine and mono-*N*-demethyl-chlorpromazine by control rat liver microsomes after incubation with 100 nmol of either chlorimipramine or chlorpromazine.

Inhibitor	Rate of formation (nmol min^{-1} (mg protein)$^{-1}$)		
	Mono-*N*-demethyl-chlorimipramine	Di-*N*-demethyl-chlorimipramine	Mono-*N*-demethyl-chlorpromazine
None	3·67 ± 0·44	0·035 ± 0·004	0·79 ± 0·13
Sulphaphenazole	1·11 ± 0·41†	0·015 ± 0·007*	0·91 ± 0·11
Quinidine	2·10 ± 0·23*	0·032 ± 0·005	0·41 ± 0·01*
Fluvoxamine	3·13 ± 1·14	0·031 ± 0·013	0·62 ± 0·09
Ketoconazole	2·61 ± 1·17	0·014 ± 0·006*	0·56 ± 0·11

Data are means ± s.e.m. of enzyme activities measured for four individual microsome preparations. * $P < 0.05$, † $P < 0.01$, significantly different from control result.

Discussion

Tricyclic antidepressants and phenothiazine neuroleptics have been associated with modulation of P450 activity. It has been suggested that they interact with other drugs by inducing or inhibiting CYP isoenzymes (Murray 1992; Murray & Field 1992).

In the current study in-vivo treatment of rats with chlorimipramine or chlorpromazine did not inhibit CYP activity assayed in-vitro with isolated microsomes. This suggests that the inhibition of CYP activity reported by others might have been a result of chlorimipramine and chlorpromazine acting in-vivo as competitive inhibitors in the metabolism of other drug substrates.

Previous research with healthy volunteers (Nielsen et al 1994) and in-vitro systems expressing cytochromes P450 from man (Nielsen et al 1996) have implicated several forms of CYP in the metabolism of chlorimipramine. Among these, CYP2C18, CYP2C19, CYP3A4 and CYP1A2 have all been reported to be responsible for chlorimipramine *N*-demethylation. In the current study we have shown that the rate of *N*-demethylation is markedly inhibited by sulphaphenazole, an inhibitor of the CYP2C subfamily in rats and man (Cribb et al 1995), but unaffected by fluvoxamine and β-naphthoflavone, inhibitor and inducer, respectively, of CYP1A2 (Brøsen et al 1993). This provides additional support for the hypothesis that a member of the CYP2C, rather than CYP1A2, subfamily (Härter et al 1995) plays a role in chlorimipramine *N*-demethylation in the rat. Further work is necessary to identify the form(s) of CYPs induced by chlorimipramine treatment and hence the forms involved in chlorimipramine *N*-demethylation in the rat. In contrast with the results obtained from the in-vitro metabolism of chlorimipramine, the *N*-demethylation of chlorpromazine could not be induced by treating rats with phenobarbital, β-naphthoflavone, chlorimipramine or even chlorpromazine itself, although chlorpromazine treatment significantly stimulated aniline hydroxylase activity. Because this reaction is associated with the induction of CYP2E1, the possibility that chlorpromazine might act as an inducer of this isoform should be explored. In an in-vitro study with liver microsomes from man Inaba et al (1985) demonstrated that chlorpromazine strongly inhibits the oxidation of sparteine, a fairly specific substrate of the CYP2D subfamily. Its role in chlorpromazine *N*-demethylation is compatible with the current finding that the reaction is inhibited by quinidine, an inhibitor of CYP2D-dependent reactions in rats and in man (Boobis et al 1990).

The different effects of phenobarbital and β-naphthoflavone on the metabolism of chlorimipramine, chlorpromazine, mono-*N*-demethyl-chlorimipramine and mono-*N*-demethyl-chlorpromazine have two possible explanations. Either the rate of the first reaction is rate-limiting and when the mono-*N*-demethyl derivative is used this step is by-passed and the stimulatory effect of the inducers becomes apparent, or an inhibitory effect is exerted by the di-*N*-demethyl derivative acting as a ligand for CYP which is displaced in the presence of large amounts of the mono-*N*-demethyl so that the relevant CYP remains active. Moreover, the stimulation of the rate of formation of di-*N*-demethyl-chlorimipramine and di-*N*-demethyl-chlorpromazine by phenobarbital and β-naphthoflavone suggests the involvement of CYP1A and CYP2B subfamilies in these metabolic pathways. The different CYP isoenzymes involved in the *N*-demethylation pathway of chlorimipramine and chlorpromazine, as suggested by the current results, are depicted in Figure 1.

The dechlorination of chlorpromazine has been demonstrated to be effective in a population of chronic schizophrenics and in rats (Sgaragli et al 1986; Valoti et al 1992), as revealed by the detection of promazine in plasma. To assess whether P450 is involved in this pathway, in-vitro experiments were performed under oxidative and reductive conditions. The negative results obtained in the current study suggest that other organs or enzyme systems, or both, are involved in the dehalogenation reaction of chlorpromazine,

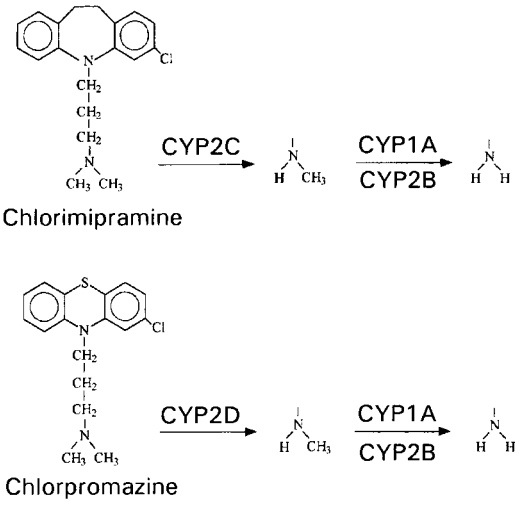

Figure 1. Sequential pathway for *N*-demethylation of chlorimipramine and chlorpromazine with the possible involvement of various CYP isoenzymes.

although the role of gut microflora cannot be discounted.

In conclusion, the involvement of the CYP2C and CYP2D subfamilies in the *N*-monodemethylation of chlorimipramine and chlorpromazine could have important clinical implications in view of the genetic polymorphism of these two cytochromes in man.

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