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Rat Tissue Concentrations of Chlorimipramine, Chlorpromazine and their *N*-Demethylated Metabolites after a Single Oral Dose of the Parent Compounds

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Abstract

A single oral dose of 90 mg kg^{-1} chlorimipramine or chlorpromazine, corresponding to 54.5 or 55.9 μmol, respectively, was given to male Sprague-Dawley rats and concentrations of parent drugs and their *N*-desmethyl metabolites measured by gas chromatography in plasma and major organs (brain, liver, lung, kidney, heart, spleen and peritoneal fat).

In the case of chlorimipramine, *N*-desmethyl metabolite levels were consistently higher than those of the parent drug for the entire observation period of 24 h in all tissues except fat, while lower *N*-desmethyl metabolite/parent compound ratios were observed for chlorpromazine. *N*-Desmethyl metabolite kinetics of chlorimipramine appeared to be elimination-rate limited, while those of chlorpromazine were formation-rate limited. In all analysed organs, the maximum detectable drug + metabolite concentrations accounted for only 2·3 and 4·6% of the initial dose of chlorimipramine and chlorpromazine. Chlorpromazine treatment gave rise to an area under the total amount-time curve (AUC_{0-24}) for parent drug + metabolites, 3·9-fold that for chlorimipramine. Closer scrutiny discloses a conversion ratio of parent compound to *N*-desmethyl metabolite of 1·1 for chlorpromazine and of 2·2 for chlorimipramine, indicating the greater efficiency of chlorimipramine metabolism in all compartments. The expected high conversion index found in the liver (2·3) reaches its maximum of 5·4 in the lung. Fractional data analysis of chlorimipramine and chlorpromazine distribution patterns revealed greater organ transfer for the *N*-desmethyl metabolites than for the more stably-located parent compounds. The *N*-desmethyl metabolites of chlorimipramine apparently moved from liver to lung, kidney and spleen, whereas *N*-desmethylchlorpromazine moved preferentially to the brain and lung tissue.

This single dose study of chlorimipramine and chlorpromazine kinetics, highlights the two distinct dispositional processes at work in the rat in all likelihood, attributable to different absorption patterns, to a slower metabolism and, thus, to the longer persistence of chlorpromazine.

The study of metabolism and pharmacokinetics of psychotropic drugs has been an active aspect of their evaluation. So far, however, few generalizations of clinical relevance can be made. This is especially true of the tricyclic antidepressants. Both chlorpromazine and chlorimipramine have a long-established clinical history as psychoactive agents and have been extensively studied in experimental psychopharmacology. Although chlorpromazine is an antipsychotic agent and chlorimipramine is an antidepressant, the two drugs share several pharmacological features. Moreover they exhibit great similarity of structure and metabolism patterns. In mammals, metabolic transformations include reactions at the ring system, at the C-2 (chlorpromazine) or C-3 (chlorimipramine) substituent, at the N-10 (chlorpromazine) or N-5 (chlorimipramine) side chain, and combinations of these reactions (Fig. 1).

Removal of one or both of the terminal methyl groups from the di-methylaminopropyl side chain (*N*-demethylation) is a reaction pathway of major importance in the metabolism of chlorpromazine and chlorimipramine, leading to metabolites which are pharmacologically active and capable of crossing the blood-brain barrier (Bickel 1980; Breyer-Pfaff 1980). Mono-*N*-desmethyl- and di-*N*-desmethylchlorpromazine have been shown to share activity with the parent compound in pharmacological and behavioural tests (Posner et al 1962; Kohl et al 1964). In rat brain preparations mono-*N*-desmethylchlorimipramine, in addition to its specific inhibition of 5-hydroxytryptamine uptake (Carlsson et al 1969; Bertilsson et al 1974), is an even more potent inhibitor of noradrenaline uptake (Ross & Renyi 1975; Maj et al 1982).

Although the plasma of psychotics chronically medicated with chlorpromazine contains appreciable concentrations of its mono- and di-*N*-desmethyl metabolites (Phillipson et al 1977; Bailey & Guba 1979; Sgaragli et al 1986; Valoti et al

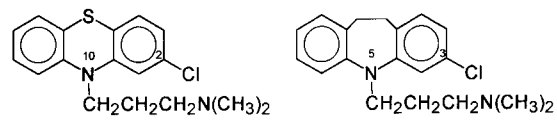

FIG. 1. Structure of chlorimipramine and chlorpromazine.

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1992), there is still some debate whether these compounds directly contribute to the therapeutic efficacy of chlorpromazine. A therapeutic role, however, has been shown for the *N*-desmethyl metabolites of chlorimipramine. Indeed in a chronically depressed population medicated orally with chlorimipramine, steady-state plasma levels of the chlorimipramine *N*-desmethyl metabolites were greater than the parent compound. Furthermore, correlation was shown between plasma levels of these metabolites and overall clinical outcome (Della Corte et al 1979).

Findings from this laboratory suggest that a major distinction in metabolic handling of chlorpromazine and chlorimipramine in chronically-treated patients resides in dechlorination at the C-2 substituent of chlorpromazine, giving rise to promazine (Sgaragli et al 1986; Valoti et al 1992). While this appears to be a pivotal step in human metabolism, dechlorination seems of secondary importance in the rat. In the case of chlorimipramine, no such dechlorination was observed in a depressed population (unpublished data).

The similarities between chlorpromazine and chlorimipramine however far outweigh this difference in metabolic processing and include: bioavailability in man (Dahl & Strandjvrd 1977; Nagy & Johansson 1977; Evans et al 1980), lipophilicity (Westenberg et al 1977; Gigon et al 1983) and plasma protein binding capacity (Campbell & Todrick 1970; Curry 1970).

Given the intensive clinical use and impact of chlorpromazine and chlorimipramine, there is a surprising dearth of comparative pharmacokinetic literature, although chlorimipramine metabolism has attracted some attention (Nagy 1977; Della Corte et al 1979; Friedman & Cooper 1983).

To elucidate the kinetics underlying the empirical difference in prescribed dosage levels of these two structurally similar compounds, we recently investigated the plasma pharmacokinetics of chlorimipramine, chlorpromazine and their *N*-desmethyl metabolites after single oral doses of the parent compounds to healthy volunteers (Della Corte et al 1993). Though restricted to a single dose and to a healthy population, our previous work points to the remarkably different manner in which the two drugs are processed. At the same dosage, chlorimipramine ingestion resulted in a total plasma AUC_{0-24} values fivefold of those after chlorpromazine intake despite a slower apparent absorption rate. Plasma chlorimipramine levels reached a mean peak value three times that of chlorpromazine. In addition, while desmethyl metabolite kinetics of chlorimipramine appeared to be elimination-rate limited, those of chlorpromazine appeared to be formation-rate limited.

These findings may contribute to an understanding of the remarkably different oral daily clinical doses–up to 200 mg for chlorimipramine and 2 g for chlorpromazine. It is therefore surprising that in the rat their effective oral dosages are reversed–up to 80 mg kg^{-1} for chlorimipramine and 7 mg kg^{-1} for chlorpromazine (Jewett & Norton 1963; Delini-Stula 1980).

Studies of the pharmacodynamics of these two drugs at known cellular target sites in the rat brain have provided convergent evidence that chlorpromazine is effective at much lower concentrations than those required for chlorimipramine (Iversen et al 1976a, b; Koe 1976; Dahl et al 1986). Although these pharmacodynamic data partly explain why chlorpromazine is effective at lower oral doses in the rat, it is reasonable to assume that its kinetics also contribute to its greater efficacy. This study therefore reports concentrations in plasma and other tissues at varying intervals of chlorimipramine, chlorpromazine or their *N*-desmethyl metabolites after oral administration of the parent compounds. Other metabolites, even more polar than *N*-desmethylderivatives, have not been considered in this study due to their limited access to the brain (Manian et al 1971; Gram 1977; Bickel 1980; Breyer-Pfaff 1980).

Materials and Methods

Materials

Chlorimipramine HCl, mono-*N*-desmethylchlorimipramine. HCl and di-*N*-desmethyl-chlorimipramine. HCl were generous gifts from Ciba-Geigy, Milan, Italy. Amitriptyline was kindly supplied by Lepetit SpA, Milan, Italy. Chlorpromazine. HCl was supplied by S. Maria Nuova Hospital, Florence, Italy. Mono- and di-*N*-desmethylchlorpromazine. HCl were gifts from Dr A. A. Manian, National Institute of Mental Health, Rockville, MD, USA, and di-chlorpromazine HCl was donated by Dr A. Heeley, Peterborough District Hospital, UK. *n*-Heptane and *n*-hexane (analytical grade, redistilled before use) were purchased from E. Merck, Darmstadt, Germany, and methanol (Aristar grade) from BDH Chemical Ltd, Poole, UK. All other compounds were analytical grade reagents.

Animals and treatments

Male Sprague-Dawley rats, 200-250 g, were fasted overnight and given a single dose of chlorimipramine or chlorpromazine (90 mg kg^{-1}, dissolved in 2 mL distilled water) by gavage. Blood samples (3–5 mL) were withdrawn by heart puncture from seven groups of five animals under light diethylether anaesthesia, using a heparinized syringe at intervals of 0·5, 1·0, 1·5, 3·0, 6·0, 9·0 and 24 h after drug administration. The animals were subsequently killed by decapitation.

Blood samples were collected into heparinized tubes and immediately centrifuged at 1400 *g*. Red cells were centrifuged twice again to ensure complete removal of buffy coat. The organs (liver, kidney, heart, spleen, brain and peritoneal fat) were removed, weighed, chopped and then suspended in 3 vols of an ice-cold medium containing (mM): 170 NaCl, 3 KCl, 10 $Na_2HPO_4.72\ H_2O$, and 2 KH_2PO_4 adjusted to pH 7·4. The suspension was homogenized in an Ultra-Turrax homogenizer at 30% full power using four 10-s strokes.

Analyses

Drugs and metabolites were analysed by gas chromatography using a nitrogen-phosphorus selective detector as described elsewhere (Ninci et al 1986). A three-step extraction into organic solvent from plasma and homogenates excluded the most polar metabolites (-OH, -NO and -SO derivatives). The *N*-desmethyl metabolites were derivatized with trifluoracetic anhydride (Pierce Eurochemie, Rotterdam, The Netherlands) which allowed for chromatographic separation and nanogram quantification of the parent drugs, and their mono-*N*-desmethyl and bis-*N*-desmethyl

metabolites. For chlorimipramine and its *N*-desmethylmetabolite analysis, stock solutions of the internal standard amitriptyline, chlorimipramine, mono-*N*-desmethylchlorimipramine and di-*N*-desmethylchlorimipramine in methanol (200 ng mL^{-1}) were stored at −20°C and diluted with water immediately before use. Standard curves were obtained for each test by adding varying amounts of the analytes to plasma or tissue homogenates obtained from control rats. Under the experimental chromatographic conditions (see below), mono- and di-desmethylchlorimipramine showed similar retention times, showing a single peak when present in the same sample.

The term *N*-desmethylchlorimipramine as used in this paper refers to the concentration of both demethylated metabolites. Detector response for di-*N*-desmethylchlorimipramine was 84% that of mono-*N*-desmethylchlorimipramine; consequently measured concentrations reflect this deviation. Recovery rates of chlorimipramine and amitriptyline varied between 70 and 80% according to tissue and between 60 and 70% for mono- and di-*N*-desmethylchlorimipramine. Amounts of analytes in different tissues were thus adjusted accordingly.

For the analysis of chlorpromazine and its *N*-desmethyl metabolites, stock solutions of the internal standard dichlorpromazine, chlorpromazine, mono-*N*-desmethylchlorpromazine and di-*N*-desmethylchlorpromazine in methanol (200 ng mL^{-1}) were stored at −20°C and diluted with water immediately before use. Standard curves were derived in each experiment by adding various amounts of the analytes to plasma or tissue homogenates obtained from control rats. Chlorpromazine, mono-*N*-desmethylchlorpromazine and di-*N*-desmethyl-chlorpromazine were identified by comparison of their respective retention times with the internal standard.

Under these conditions (see below), mono- and di-*N*-desmethylchlorpromazine showed similar retention times,

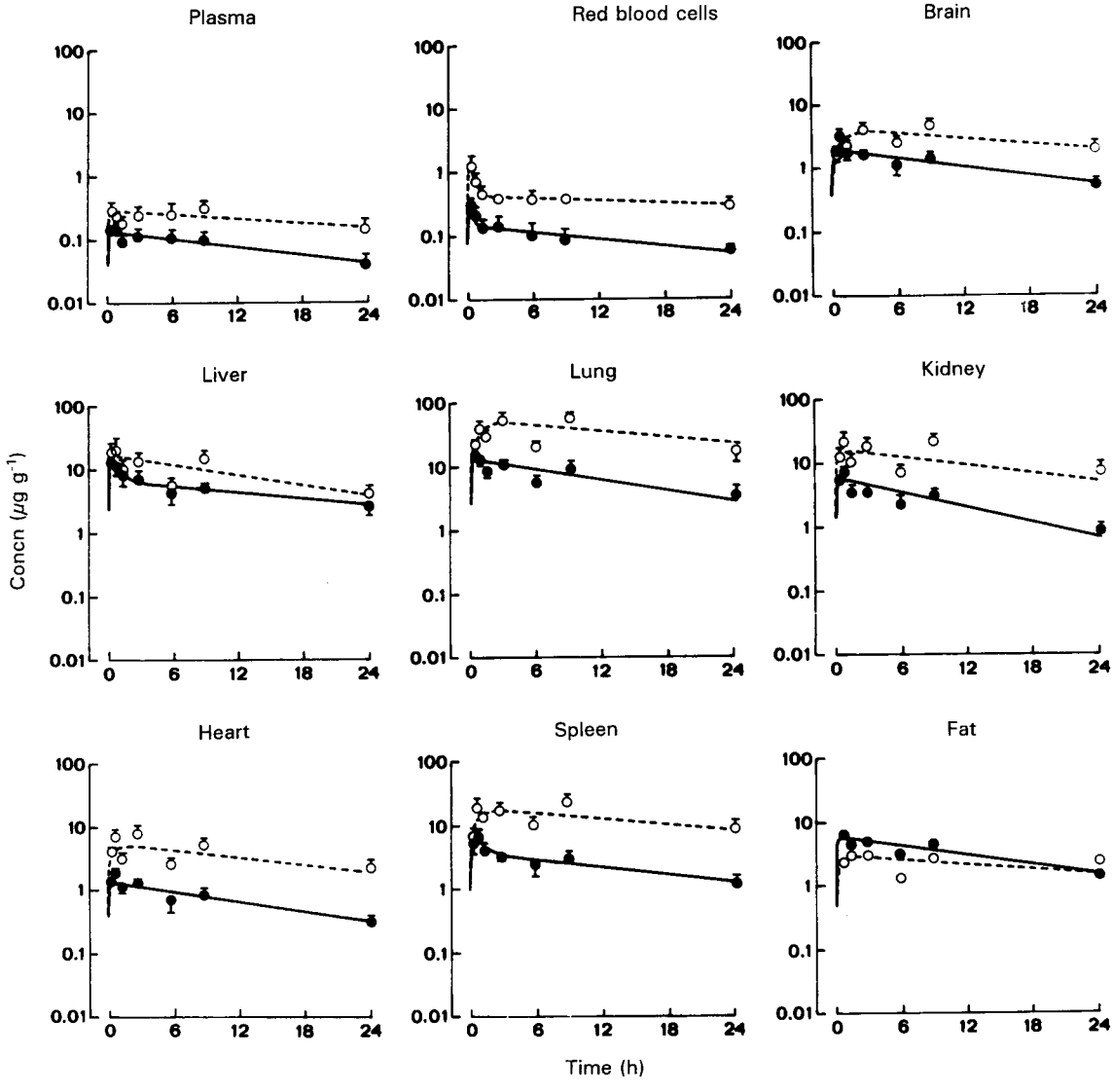

FIG. 2. Concentrations of chlorimipramine (●) and *N*-desmethylchlorimipramine (○) in rat plasma and tissue after a single oral dose of chlorimipramine. Each experimental point represents the mean of five animals and the bars represent the s.e. of the mean. Fat values are expressed as a pool of five animals. Curves denote the best fit.

exhibiting a single peak when present in the same sample. The term *N*-desmethylchlorpromazine as used in this paper refers to the concentration of both *N*-demethylated metabolites. Detector response for di-*N*-desmethylchlorpromazine was 60% that of mono-desmethylchlorpromazine.

Recovery of chlorpromazine from different tissues was 65–80%, except from brain and kidney where it was 44 and 53%, respectively. Recovery of mono and di-*N*-desmethylchlorpromazine was 60–80% in all tissues except brain and kidney, where it was 40 and 45%, respectively. Recovery of di-chlorpromazine was 50–70% in all tissues except brain, kidney and liver, where it was between 35 and 40%, respectively.

Analyses were performed on a Perkin Elmer Sigma 1 series gas chromatograph equipped with electronic integrator and coiled glass column (1·8 m; length; 2 mm, i.d.) containing 3% OV-17 on Chromosorb W HP, 100-120 mesh (Supelco Inc. Bellefonte, PA, USA).

Chromatographic conditions were: column temperature, 275°C for chlorimipramine and its *N*-desmethyl metabolites and 285°C for chlorpromazine and *N*-desmethyl metabolites; detector temperature, 300°C; carrier gas (N_2) flow rate, 20 mL min^{-1}.

Pharmacokinetic analysis

The best fit of plasma and tissue drug concentrations vs time was obtained according to a procedure described by Gomeni & Gomeni (1978). This method is based on the assumption that the drug has linear disposition kinetics describable by the sum of up to three exponential terms. Pharmacokinetic parameters were evaluated by the exponential stripping method (Foss 1969). The fit was obtained by plotting the mean values of five subjects for each time point. The statistical criterion of goodness of fit was the correlation coefficient.

The area under the experimental concentration curve

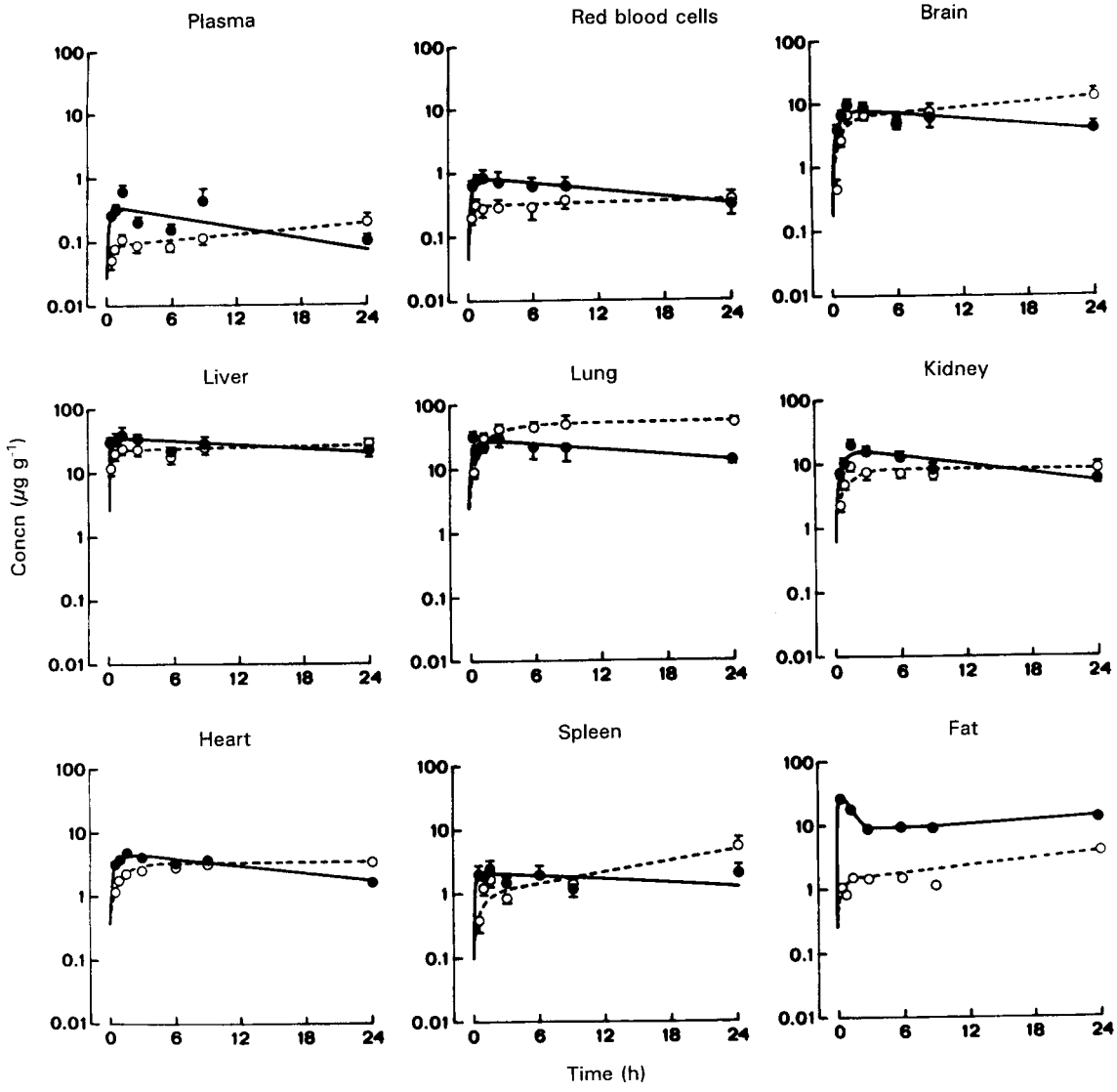

FIG. 3. Concentrations of chlorpromazine (●) and *N*-desmethylchlorpromazine (○) in rat plasma and tissue after a single oral dose of chlorpromazine. Each experimental point represents the mean of five animals and the bars represent the s.e. of the mean. Fat values are expressed as a pool of five animals. Curves denote the best fit.

(AUC) was calculated by a combined logarithmic trapezoidal method (Shumaker 1986).

Statistical analysis and methods

Data are expressed as mean values ± s.e. of the mean. Calculations of total drug concentration in plasma and tissues of animals at 0·5, 1·0, 1·5, 3, 9 and 24 h post dose were obtained by multiplying tissue concentrations by tissue weight. Plasma totals were obtained by assuming an average plasma volume of 3·5 mL/100 g body weight (Waynforth 1980), while red blood cells were estimated using a haematocrit of 0·49. Fractional compartmentation trends were described by normalizing individual organ concentrations as percentages of the total.

Results

Figs 2 and 3 show mean tissue concentrations of drugs and metabolites in rats treated with a single oral dose of the parent compounds while C_{max}, t_{max} and the calculated AUC_{0-24} values are reported in Table 1. As may be seen in Fig. 2, concentrations of the *N*-desmethyl metabolites of chlorimipramine were higher in all tissues over the entire observation period with the exception of the omentum. The opposite was observed in fat tissue except for the sample taken at the 24 h interval. Furthermore, the behaviour of chlorimipramine metabolites parallels the parent compound, suggesting that elimination occurs very rapidly, concentrations being limited by the rate of production.

As reported in Fig. 3, the concentrations of chlorpromazine's *N*-desmethyl metabolites were lower than the parent compound in most tissues for the greater part of the experiment. In fat tissue this held true for the entire experiment. Furthermore, in all tissues the concentration vs time curve of desmethyl metabolites exhibited a positive slope. This indicates that they were eliminated at a much slower rate than the parent drug, their concentrations being elimination-rate limited.

Taking the AUC_{0-24} ratios of desmethyl metabolites to their parent compound (Table 1) as an indication of the relative tissue accumulation of both primary and secondary *N*-desmethylated metabolites, a marked difference was observed between the two drugs. In all tissues, in fact, the chlorimipramine ratio was much higher than chlorpromazine, suggesting that tissues either produce or receive and retain more *N*-desmethylchlorimipramine than *N*-desmethylchlorpromazine. This indicates that chlorimipramine was *N*-demethylated at a faster rate than chlorpromazine. This trend becomes even more apparent when total detected quantities of drugs and metabolites (in different tissues and at various times) are compared (Fig. 4A). Even at 0·5 h after administration, *N*-desmethylchlorimipramine was present in greater quantities than the parent drug, while *N*-desmethylchlorpromazine values overtook parent concentrations only after 9 h. Fig. 4B and C compare the accumulation of both parent compounds and their *N*-desmethyl metabolites in the two major compartments of liver and lung. *N*-Demethylation for both drugs proceeds serially while lung tissue, as the obligatory second first-pass waystation, contributed substantially to the transformation process initiated in the liver. In fact, the ratio between the organ AUC_{0-24} of metabolite and parent drug, taken as a *N*-demethylation index, increases from 1·0 in the liver to 2·3 in the lung for chlorpromazine, and from 2·1 to 5·4 in the

Table 1. C_{max}, t_{max} and AUC_{0-24} mean values for drugs and metabolites according to tissue.

Tissue	t_{max} (h)		C_{max} ($\mu g\ g^{-1}$)		AUC_{0-24} ($\mu g\ h\ g^{-1}$)		Metabolite/drug	
	CI	CZ	CI	CZ	CI	CZ	CI	CZ
Plasma								
Drug	1·0	1·5	0·162	0·670	2·1	7·0		
Metabolite	0·5	24·0	0·31	0·21	5·6	3·3	2·7	0·5
Red blood cells								
Drug	0·5	1·5	0·31	0·97	2·2	14·2		
Metabolite	0·5	24·0	1·36	0·38	9·2	8·6	4·2	0·6
Brain								
Drug	1·0	1·5	3·50	10·40-	29·7	146·6		
Metabolite	9·0	24·0	5·10	14·00	85·1	213·9	2·9	1·5
Liver								
Drug	0·5	1·5	14·90	44·30	127·6	684·0		
Metabolite	1·0	24·0	23·90	33·20	270·7	691·1	2·1	1·0
Lung								
Drug	0·5	0·5	17·20	36·90	191·2	506·7		
Metabolite	3·0	24·0	62·20	53·50	1019·0	1141·0	5·3	2·3
Heart								
Drug	1·0	1·5	2·0	5·4	18·5	78·6		
Metabolite	3·0	24·0	8·70	3·80	104·4	81·7	5·6	1·0
Kidney								
Drug	1·0	1·5	7·60	22·00	62·1	234·3		
Metabolite	1·0	1·5	23·00	9·3	362·5	176·6	5·8	0·8
Spleen								
Drug	1·0	1·5	6·80	2·70	62·9	43·9		
Metabolite	1·0	24·0	20·70	6·00	389·6	69·8	6·2	1·6
Fat								
Drug	1·0	0·5	7·30	28·60	89·7	285·2		
Metabolite	1·5	24·0	3·30	4·10	63·9	52·7	0·7	0·2

CI = Chlorimipramine, CPZ = chlorpromazine.

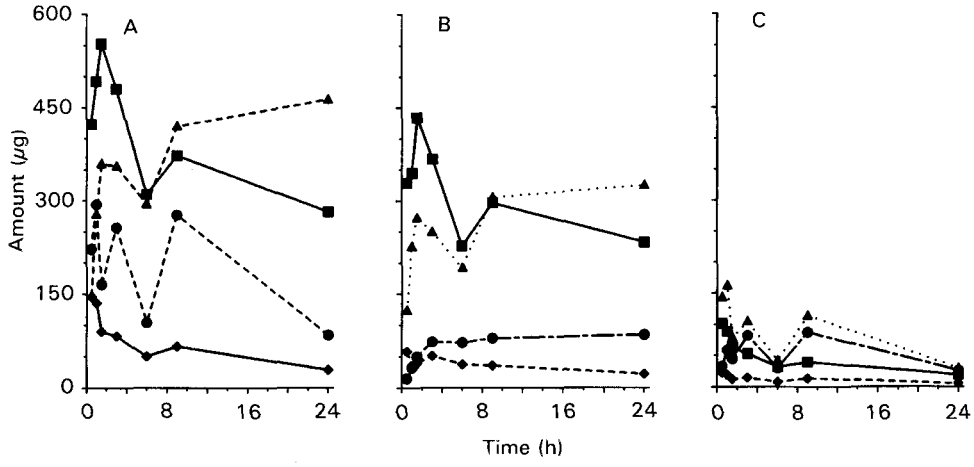

FIG. 4. Distribution over time of parent and metabolite in tissues of rat. A. Total amounts measured of chlorimipramine (◆), chlorpromazine (■), *N*-desmethylchlorimipramine (●) and *N*-desmethylchlorpromazine (▲) in all tissues. B. Chlorpromazine (■) and *N*-desmethylchlorpromazine (▲) in liver; chlorpromazine (◆) and *N*-desmethylchlorpromazine (●) in lung. C. Chlorimipramine (■) and *N*-desmethylchlorimipramine (▲) in liver; chlorimipramine (◆) and *N*-desmethylchlorimipramine (●) in lung.

case of chlorimipramine. In the case of chlorimipramine, the *N*-demethylation pathway seemed to continue beyond these two metabolic stations through the kidney and spleen (see, M/D ratios in Table 1).

AUC_{0-24} values also indicate a longer persistence of chlorpromazine and its *N*-desmethyl metabolites, compared with chlorimipramine and its *N*-desmethyl metabolites. Although the most representative, the compartments under investigation accounted for only a small part of the original dose. In fact, the highest total concentration of drug + metabolites found after chlorimipramine treatment was 439 μg (about 1·3 μmol) i.e. 2·3% of the administered dose. In contrast, the highest total amount of chlorpromazine + metabolites was 912 μg (about 2·6 μmol) i.e. 4·6% of the administered dose. The preponderance of parent drug and metabolite alike, consistently accumulated in the liver, with the remainder mostly in the lung. In the liver, chlorimipramine concentrations (parent and *N*-desmethyl metabolites) reached maximum levels 1-h post dose and recovery from this compartment represented 58% of the total. Throughout the experimental period the *N*-desmethylmetabolite was present in significantly higher concentrations than the parent chlorimipramine. From a 1-h post dose peak, parent and metabolite concentrations declined sharply with similar slopes. In contrast, chlorpromazine concentrations (parent and *N*-desmethyl metabolites) were much

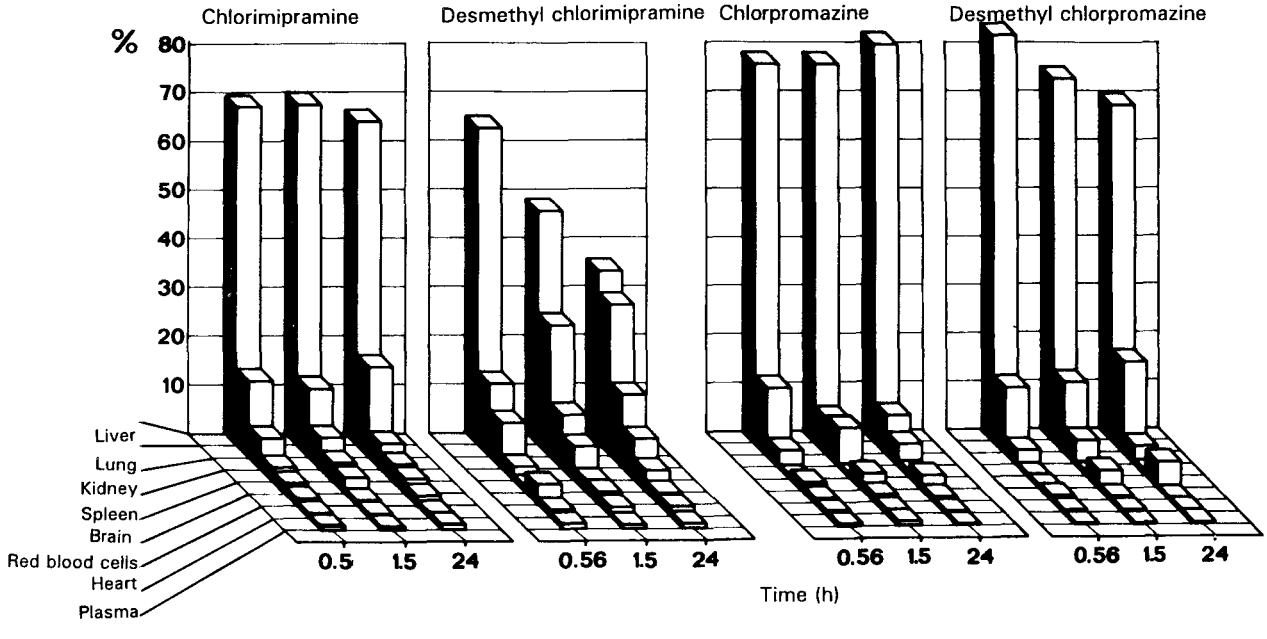

FIG. 5. Fractional distribution in tissues of chlorimipramine, *N*-desmethylchlorimipramine, chlorpromazine and *N*-desmethylchlorpromazine. Tissue concentrations are expressed as percentages of totals found at various time intervals.

Table 2. Reported plasma AUC_{0-12} values of drug and *N*-desmethyl metabolites after a single dose of chlorimipramine and chlorpromazine.

Drug (mg kg^{-1})	Route	AUC_{0-12}			Ratio	References
		Parent	*N*-Desmethyl (ng h mL^{-1})	*N*-Desmethyl + parent	*N*-Desmethyl/parent	
Chlorimipramine						
15	i.p.	909	286	1195	0·3	Friedman & Cooper (1983)
15	p.o.	nd	nd	–	–	Nagy (1977)
90	p.o.	1320[a]	2966[a]	4286[a]	2·3	This study
Chlorpromazine						
10	i.p.	909[a]	–	–	–	Curry et al (1970)
10	p.o.	1375[a]	–	–	–	Curry et al (1971)
90	p.o.	3622[a]	1172[a]	4794[a]	0·3	This study

[a]These data were calculated by interpolating values obtained at 8 and 24 h after administration. nd, not detectable.

higher and more stable than chlorimipramine over the entire observation period. At maximum values (1·5 h), the amount of chlorpromazine and metabolite concentrated in the liver was approximately 80% of the total distribution.

Fractional compartmentation patterns for chlorimipramine and chlorpromazine were fairly constant over time, while those of both metabolites shifted markedly according to tissue (Fig. 5). This suggests a redistribution process from the liver to other compartments, especially obvious for *N*-desmethylchlorimipramine and, less so, for *N*-desmethylchlorpromazine. In particular, hepatic *N*-desmethylchlorimipramine decreased from 65%, at 0·5 h, to 36%, at 24 h, and rose simultaneously in the lung, kidney, spleen and brain tissue (from 15, 9, 3 and 1% respectively at 0·5 h, to 31, 15, 8 and 4%, at 24 h). A similar pattern of redistribution was observed for desmethylchlorpromazine. Hepatic *N*-desmethylchlorpromazine fell from 84%, at 0·5 h to 69%, at 24 h, with a concurrent accumulation in brain tissue, from 0·6 to 5%; in the lung, from 10 to 19%; and in the spleen, from 0·2 to 1%.

Discussion

Our findings show the remarkably different way in which the two drugs are processed by the rat. The experimental oral doses, while close to the ED50 for chlorimipramine (Maitre et al 1980), were considerably higher than the ED50 for chlorpromazine (Kornetsky & Bain 1965; Cook & Davidson 1978) reported in behavioural pharmacology tests; however, for reasons of pharmacokinetic comparison, the same dose was used for both drugs.

The recovery-to-dose ratio of drugs and metabolites was very low, although chlorpromazine concentrations were consistently double those of chlorimipramine. These modest recovery rates either indicate poor absorption, rapid transformation into metabolites other than those measured, or a high rate of excretion. These drugs are highly extracted by liver and considerable amounts of non-conjugated hydroxylated metabolites are excreted with the faeces. The source of this material is biliary excretion which is a major excretory pathway for imipramine-like drugs (Bickel & Weder 1968; von Bahr & Borga 1971; Ryrfeld & Hannson 1971; Beaubieu & Pakuts 1979). As Table 2 shows, the literature on rat absorption and bioavailability of the two drugs is at best fragmentary and rarely comparative. At 12 h post-dose the present study found comparable plasma AUC values for parent and *N*-desmethyl metabolites for both chlorimipramine and chlorpromazine (Table 2) despite the former's well-known greater antimuscarinic activity (Weinstock & Cohen 1976; Snyder & Yamamura 1977; Shein & Smith 1978; Dahl et al 1986; Della Corte et al 1993). The antimuscarinic rationale, invoked to explain the poor absorption of psychoactive drugs in man (Rivera-Calimlin & Hershey 1984) was contradicted by a previous study where higher plasma concentrations were reported for chlorimipramine, the stronger of the two antimuscarinics (Della Corte et al 1993). This apparent contradiction in man, however, is tempered by the smaller zero input duration of chlorpromazine (1·5 h as against 2·5 h for chlorimipramine). Although our present study did not expressly address this issue, absorption parameters in the rat might be fruitful ground for future investigation.

When attention is directed away from the shaky assumptions of overall absorption behaviour to a closer consideration of metabolic patterns, an interesting trend emerges; analysis of the plasma AUC_{0-12} reveals a much slower *N*-demethylation rate for chlorpromazine than for chlorimipramine. Parent/metabolite ratios differed markedly: 0·3 for chlorpromazine and 2·3 for chlorimipramine. A comparison between hepatic concentrations of parent and metabolite reveals a clear difference in *N*-demethylation and overall elimination rates. This is consistent with the in-vitro observation that rat liver microsomal preparations *N*-demethylate chlorimipramine at a faster rate than chlorpromazine (Valoti et al 1989). Other rat organs–namely lung, kidney, spleen and brain–have been shown to *N*-dealkylate xenobiotics very efficiently by cytochrome P450-dependent oxidases (Imaoka & Funae 1990; Imaoka et al 1990; Snoz & Dmitrenko 1993). It is conceivable that these organs behave like liver towards chlorimipramine and chlorpromazine as substrates. We can conclude therefore that chlorimipramine is the preferred substrate for P450-dependent *N*-demethylating systems and this explains the preponderance of its *N*-demethylated form in all major organs.

The discrepancy between the findings of this study and the only other reported chlorimipramine *N*-demethylation rates (Friedman & Cooper 1983) may be explained as a function of the experimental dose used. Whereas our oral dose of 90 mg kg^{-1} gave rise to a metabolite/parent ratio of 2·3, a 15-mg intraperitoneal dose administered by Friedman &

Cooper (1983) yielded a mere 0.3 ratio. We can only speculate that in-vivo the saturation of the liver enzyme systems involved in the *N*-demethylation pathway and hence in the conversion rate exhibits a certain dose-dependent proportionality. Table 2 also suggests a reliable correlation between doses and plasma concentrations for chlorimipramine + *N*-desmethylchlorimipramine.

Chlorimipramine and chlorpromazine differ with respect to both metabolic performance and tissue uptake. While as a consequence of first-pass metabolism both compounds and their metabolites initially accumulated mostly in the liver, redistribution to other tissue reservoirs suggests selective affinity and perhaps a differential binding capacity according to organ and analyte. *N*-Desmethylchlorpromazine, for instance, preferentially accumulated in the brain, while *N*-desmethylchlorimipramine accumulated mostly in the spleen.

Chlorpromazine and its *N*-desmethyl metabolites as well as chlorimipramine and its *N*-desmethyl metabolites share an amphipathic, amine-containing structure which allows them to bind to biomembranes through both hydrophobic and electrostatic interactions with phospholipids (Frenzel et al 1978; Forrest et al 1984). Furthermore, they can be bound with high affinity to plasma orosomucoid (α_1-acid glycoprotein) and to tissue proteins structurally related to it (Kremer et al 1988). We can speculate that the enhanced affinity mentioned above may be a reflection of binding-site localization and density. In turn, these distribution dynamics may represent the fundamental processes underlying the complex multiphasic kinetics often posited for psychotropic drugs (Baldessarini 1990).

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References

Bailey, D. N., Guba, J. J. (1979) Gas-chromatographic analysis for chlorpromazine and some of its metabolites in human serum, with use of a nitrogen detector. Clin. Chem. 25: 1211–1215

Baldessarini, R. J. (1990) Drugs and the treatment of psychiatric disorders. In: Gilman, A G., Rall, T. W., Nies, A. S., Taylor, P. (eds) Goodman and Gilman's The Pharmacological Basis of Therapeutics. 8th Edn, Pergamon Press, New York, pp 383–435

Beaubieu, A. R., Pakuts, A. P. (1979) Influence of dose on first-pass kinetics of ^{14}C-imipramine in the isolated perfused rat liver. Drug Met. Dispos. 7: 34–39

Bertilsson, L., Esberg, M., Thoren, P. (1974) Differential effect of chlorimipramine and nortriptyline on cerebrospinal fluid metabolites of serotonin and norepinephrine in depression. Eur. J. Clin. Pharmacol. 7: 365–368

Bickel, M. H. (1980) Metabolism of antidepressants. In: Born, G. V. R., Farah, A., Herken, H., Welch, A. D. (eds) Handbook of Experimental Pharmacology. Heidelbergh, Berlin, Springer-Verlag, New York, pp 551–572

Bickel, M. H., Weder, H. J. (1968) The total fate of a drug: kinetics of distribution, excretion and formation of 14 metabolites in rats treated with imipramine. Arch. Int. Pharmacodyn. Ther. 173: 433–463

Breyer-Pfaff, U. (1980) Metabolism and kinetics. In: Born, G. V. R., Farah, A., Herken, H., Welch, A. D. (eds) Handbook of Experimental Pharmacology. Heidelbergh, Berlin, Springer-Verlag, New York, pp 287–304

Campbell, I. C., Todrick, A. (1970) Plasma protein binding of tricyclic antidepressive drugs. J. Pharm. Pharmacol. 22: 226–227

Carlsson, A., Corrodi, H., Fuxe, K., Hvkfelt, T. (1969) Effect of antidepressant drugs on the depletion of intraneuronal brain 5-hydroxytryptamine stores caused by 4-methyl-alpha-ethyl-metatyramine. Eur. J. Pharmacol. 5: 357–366

Cook, L., Davidson, A. B. (1978) Behavioral pharmacology: animal models involving aversive control of behaviour. In: Lipton, M. A., Di Mascio, A., Killam, K. F. (eds) Psychopharmacology: a Generation of Progress. Raven Press, New York, pp 563–567

Curry, S. (1970) Plasma protein binding of chlorpromazine. J. Pharm. Pharmacol. 22: 193–197

Curry, S. H., Derr, J. E., Maling, H. M. (1970) The physiological disposition of chlopromazine in the rat and dog. Proc. Soc. Exp. Biol. Med. 134: 314–318

Curry, S. H., D'Mello, A., Mould, G. P. (1971) Destruction of chlorpromazine during absorption in the rat in vivo and in vitro. Br. J. Pharmacol. 42: 403–411

Dahl, S. G., Strandjvrd, R. E. (1977) Pharmacokinetics of chlorpromazine after single and chronic dosage. Clin. Pharmacol. Ther. 21: 437–448

Dahl, S. G., Hough, E., Hals, P. A. (1986) Phenothiazine drugs and metabolites: molecular conformation and dopaminergic, alpha adrenergic and muscarinic cholinergic receptor binding. Biochem. Pharmacol. 35: 1263–1269

Delini-Stula, A. (1980) Drug-induced alterations in animal behaviour as a tool for the evaluation of antidepressants, correlation with biochemical effects. In: Born, G. V. R., Farah, A., Herken, H., Welch, A. D. (eds) Handbook of Experimental Pharmacology. Raven Press, Heidelbergh, Berlin, New York, pp 505–526

Della Corte, L., Heeley, A. F., Broadhurst, A. D., James, H. D., Sgaragli, G. P., Faravelli, C., Filippini, S., Pazzagli, A. (1979) Clinical response and tricyclic plasma level during treatment with clomipramine. Br. J. Psychiatry 134: 390–400

Della Corte, L., Valoti, M., Palmi, M., Giovannini, M. G., Sgaragli, G. P. (1993) Pharmacokinetics of chlorimipramine, chlorpromazine and their *N*-dealkylated metabolites in plasma of healthy volunteers after a single oral dose of the parent compounds. J. Pharm. Pharmacol. 45: 825–829

Evans, L. E. J., Bett, J. H. N., Cox, J. R., Dubois, J. P., Van Hees, T. (1980) The bioavailability of oral and parenteral chlorimipramine (Anafranil). Prog. Neuropsychopharmacol. 4: 293–302

Forrest, B. J., Linehan, P. T., Mattai, J. (1984) Interactions of a phenothiazine tranquilizer with phosphatidylcholine and phosphatidylcholine/cholesterol membranes. Biochemistry 23: 2288–2293

Foss, S. D. (1969) A method for obtaining initial estimates of the parameters in exponential curve fitting. Biometrics 25: 580–584

Frenzel, J., Arnold, K., Nuhn, P. (1978) Colorimetric ^{13}C NMR and ^{31}P NMR studies on the interaction of some phenothiazines derivatives with dipalmitoyl phosphatidylcholine model membranes. Biochim. Biophys. Acta 507: 185–197

Friedman, E., Cooper, T. B. (1983) Pharmacokinetics of chlorimipramine and its demethylated metabolite in blood and brain regions of rats treated acutely and chronically with chlorimipramine. J. Pharmacol. Exp. Ther. 225: 387–390

Gigon, P. L., Van Hees, H., Bickel, M. H. (1983) A technique for the comparison of biological distribution and solvent partition of drugs. Experientia 39: 801–804

Gomeni, C., Gomeni, R. (1978) IGPHARM: Interactive graphic package for pharmacokinetics analysis. Computers Biomed. Res. 11: 345–361

Gram, L. F. (1977) Plasma level monitoring of tricyclic antidepressant therapy. Clin. Pharmacokinet. 2: 237–251

Imaoka, S., Funae, Y. (1990) Purification and characterization of rat pulmonary cytochrome P-450. J. Biochem. (Tokyo) 108: 33–36

Imaoka, S., Yamaguchi, Y., Funae, Y. (1990) Induction and regulation of cytochrome P-450 K-56 (lauric acid hydroxylase) in rat renal microsomes by starvation. Biochim. Biophys. Acta 1036: 18–23

Iversen, L. L., Horn, A. S., Miller, R. J. (1976a) Structure-activity

relationship for interactions of agonist and antagonist drugs with dopamine-stimulated adenylate cyclase of rat brain: a model for CNS dopamine receptors? In: Sedvall, G., Uvnas, B., Zotterman, Y. (eds) Antipsychotic Drugs. Raven Press, Oxford, New York, pp 285–303

Iversen, L. L., Horn, A. S., Miller, R. J. (1976b) Comparison of the effects of neuroleptic drugs on pre- and post-synaptic dopaminergic mechanism in the rat striatum. Mol. Pharmacol. 12: 251–262

Jewett, R., Norton, S. (1963) Drug effects on behaviour of rat under chronic isolation. Pharmacologist 5: 240

Koe, K. G. (1976) Molecular geometry of inhibition of the uptake of catecholamines and serotonin in synaptosomal preparations of rat brain. J. Pharmacol. Exp. Ther. 199: 649–661

Kohl, H. H., Brune, G. G., Himwick, H. E. (1964) A comparative study of chlorpromazine and its demethylated derivates: potency and tissue distribution. Biochem. Pharmacol. 13: 539–541

Korňetsky, C., Bain, G. (1965) The effects of chlorpromazine and pentobarbital on sustained attention in the rat. Psychopharmacologia (Berlin) 8: 277–284

Kremer, J. M. M., Wilting, J., Janssen, L. H. M. (1988). Drug binding to human alpha-1-acid glycoprotein in health and disease. Pharmacol. Rev. 40: 1–47

Maitre, L., Maser, P., Baumann, P. A., Waldmeir, P. C. (1980) Amine uptake inhibitors: criteria of selectivity. Acta Psychiatr. Scand. 61 (Suppl. 280): 97–110

Maj, J., Stala, L., Gorka, Z., Adamus, A. (1982) Comparison of the pharmacological actions of desmethylclomipramine and clomipramine. Psychopharmacology 78: 165–169

Manian, A. A., Efron, D. H., Harris, S. R. (1971) Appearance of monohydroxylated chlorpromazine metabolites in the central nervous system. Life Sci. 10: 679–684

Nagy, A. (1977) Blood and brain concentrations of imipramine, clomipramine and their monomethylated metabolites after oral and intramuscular administration in rats. J. Pharm. Pharmacol. 29: 104–107

Nagy, A., Johansson, R. (1977) The demethylation of imipramine and chlorimipramine as apparent from their plasma kinetics. Psychopharmacology 54: 125–131

Ninci, R., Giovannini, M. G., Della Corte, L., Sgaragli, G. P. (1986) Isothermal gas chromatographic determination of nanogram amounts of chlorimipramine, chlorpromazine and their *N*-desmethyl metabolites in plasma using nitrogen-selective detection. J. Chromatogr. 381: 315–322

Phillipson, O. I., Mckeawn, J. M., Baker, J., Heeley, A. F. (1977) Relationship between plasma chlorpromazine and its metabolites and clinical ratings in patients with acute relapse of schizophrenic and paranoid psychosis. Br. J. Psychiatry 131: 172–184

Posner, H. S., Hearst, H., Taylor, W. L., Cosmides, G. J. (1962) Model metabolites of chlorpromazine and promazine: relative activities in some pharmacological and behavioural test. J. Pharmacol. Exp. Ther. 137: 84–90

Rivera-Calimlin, L., Hershey, L. (1984) Neuroleptic concentrations and clinical response. Ann. Rev. Pharmacol. Toxicol. 24: 361–386

Ross, S., Renyi, A. (1975) Tricyclic antidepressant agents. II. Effect of oral administration on the uptake of ^{3}H-noradrenaline and ^{14}C-5 hydroxytryptamine in slices of the mid-brain hypothalamus region of the rat. Acta Pharmacol. Toxicol. 36: 395–408

Ryrfeld, A., Hansson, E. (1971) Biliary excretion of quaternary ammonium compounds and tertiary amines in the rat. Acta Pharmacol. Toxicol. 30: 59–68

Sgaragli, G. P., Ninci, R., Della Corte, L., Valoti, M., Nardini, M., Andreoli, V., Moneti, G. (1986) Promazine: a major plasma metabolite of chlorpromazine in a population of chronic schizophrenics. Drug Metab. Dispos. 14: 263–266

Shein, K., Smith, S. E. (1978) Structure activity relationship for the anticholinoceptor action of tricyclic antidepressant. Br. J. Pharmacol. 62: 567–571

Shumaker, R. C. (1986) PKCALC: a basic interactive computer program for statistical and pharmacokinetic analysis of data. Drug Metab. Rev. 17: 331–348

Snoz, S. V., Dmitrenko, N. P. (1993) *N*-Demethylation and denitrosation activity of the rat liver, thymus lymphocytes and spleen after exposure to simazine and sodium nitrite. Ukr. Biokhim. Zh. 65: 94–98

Snyder, S. H., Yamamura, H. I. (1977) Antidepressant and the muscarinic acetylcholine receptor. Arch. Psychiatr. 34: 236–239

Valoti, M., Palmieri, R., Palmi, M., Della Corte, L., Sgaragli, G. P. (1989) A comparison of chlorimipramine and chlorpromazine as inducers of *N*-demethylase activities in rat liver microsomes. 6th Conference on Neuroscience, Villasimius, Italy. 4–9 June. Abstract book, p. 34

Valoti, M., Palmi, M., Della Corte, L., Nardini, M., Corti, P., Sgaragli, G. P. (1992) Dehalogenation and *N*-dealkylation of chlorpromazine as revealed by plasma concentrations of metabolites in a population of chronically medicated schizophrenics. Methods Find. Exp. Clin. Pharmacol. 14: 445–450

von Bahr, C., Borga, O. (1971) Uptake, metabolism and excretion of desmethylimipramine and its metabolites in the isolated perfused rat liver. Acta Pharmacol. Toxicol. 29: 359–374

Waynforth, H. B. (1980) Experimental and Surgical Technique In the Rat. Academic Press, New York, p. 240

Weinstock, M., Cohen, D. (1976) Tricyclic antidepressant drugs as antagonists of muscarinic receptors in sympathetic ganglia. Eur. J. Pharmacol. 40: 321–328

Westenberg, H. G., De Zeeuw, R. A., Decuyper, H., Van Praag, H. M., Korf, J. (1977) Bioanalysis and pharmacokinetics of clomipramine and desmethylclomipramine in man by means of liquid chromatography. Postgrad. Med. J. 53 (Suppl.4): 124–130