Cytochromes P450 mediating the *N*-demethylation of amitriptyline

P. Ghahramani, S. W. Ellis, M. S. Lennard, L. E. Ramsay & G. T. Tucker

Department of Medicine and Pharmacology, University of Sheffield, The Royal Hallamshire Hospital, Sheffield, UK

Aims Using human liver microsomes and heterologously expressed human enzymes, we have investigated the involvement of CYPs 1A2, 2C9, 2C19, 2D6 and 3A4 in the *N*-demethylation of amitriptyline (AMI), with a view to defining likely influences on its clinical pharmacokinetics.

Methods The kinetics of formation of nortriptyline (NT) from AMI were measured over the substrate concentration range 1–500 μM, using liver microsomes from four extensive metabolisers (EM) and one poor metaboliser (PM) with respect to CYP2D6 activity.

Results The data were best described by a two-site model comprising a Michaelis-Menten function for a high affinity site and a Hill function for a low affinity site. The activity at the low affinity site was eliminated by triacetyloleandomycin and ketoconazole, selective inhibitors of CYP3A4, such that the kinetics were then described by a two-site model comprising two Michaelis-Menten functions. A further decrease in activity was associated with the addition of the CYP2C9 inhibitor sulphaphenazole such that the residual kinetics were best described by a single Michaelis-Menten function. The addition of quinidine, a selective inhibitor of CYP2D6, along with triacetyloleandomycin and sulphaphenazole produced an additional decrease in the rate of NT formation in all but the PM liver, but did not completely eliminate the reaction. The remaining activity was best described by a single Michaelis-Menten function. Inhibitors of CYP1A2 (furafylline) and CYP2C19 (mephenytoin) did not impair NT formation. Microsomes from yeast cells expressing CYP2D6 and from human lymphoblastoid cells expressing CYP3A4 or CYP2C9-Arg *N*-demethylated AMI, but those from cells expressing CYPs 1A2 and 2C19 did not.

Conclusions We conclude that CYPs 3A4, 2C9 and 2D6 together with an unidentified enzyme, but not CYPs 1A2 and 2C19, mediate the *N*-demethylation of AMI. Thus, the clinical pharmacokinetics of AMI would be expected to depend upon the net activities of all of these enzymes. However, the quantitative importance of each isoform is difficult to predict without knowledge of the exposure of the enzymes *in vivo* to AMI.

Keywords: cytochrome P450, CYP2D6, amitriptyline, *N*-dealkylation

Introduction

Amitriptyline (AMI) and other tricyclic antidepressants are used widely for the treatment of depression, and have recently found a new role in the control of anxiety states and panic disorders [1]. *In vivo* studies in humans have shown that about 50% of a dose of AMI is *N*-demethylated to nortriptyline (NT), an active metabolite [2, 3]. Both AMI and NT undergo aromatic hydroxylation, largely by CYP2D6 [4–7]. However, the enzymes involved in the *N*-demethylation pathway have not been identified unequivocally. Baumann *et al.* [8] found that plasma concentrations of NT, formed from AMI, correlated with the *O*-demethylation of dextromethorphan, a marker for CYP2D6 activity. Similarly, Mellstrom *et al.* [9] reported a correlation between debrisoquine hydroxylase (CYP2D6) activity and plasma NT concentrations. However, in both of these studies the correlation was weak, suggesting that enzymes in addition to CYP2D6 may be involved in the formation of NT from AMI. Breyer-Pfaff *et al.* [10] observed a relationship *in vivo* between AMI *N*-demethylation and the hydroxylation of S-mephenytoin, a reaction catalysed by CYP2C19. In addition, it has been shown *in vitro* that CYP1A2 and CYP3A4 are involved in the *N*-demethylation of imipramine, which is closely related in structure to AMI [11] (Figure 1).

Using human liver microsomes and heterologously expressed human enzymes, we have investigated the involvement of CYPs 1A2, 2C9, 2C19, 2D6 and 3A4 in the *N*-demethylation of AMI, with a view to defining likely influences on its clinical pharmacokinetics.

Correspondence: Professor G. T. Tucker, Department of Medicine and Pharmacology, University of Sheffield, The Royal Hallamshire Hospital, Sheffield, UK

Methods

Chemicals

Amitriptyline HCl, nortriptyline HCl, desipramine HCl, ketoconazole, 7-ethoxycoumarin, quinidine sulphate and

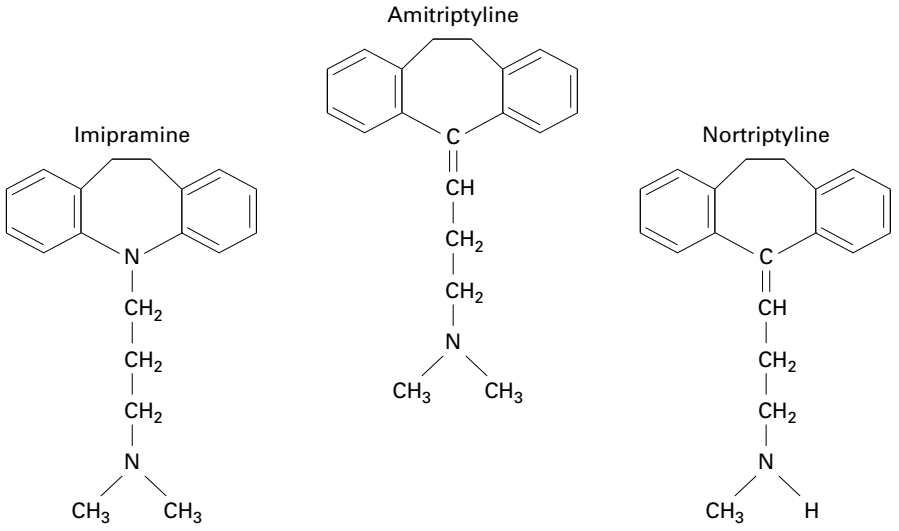

Figure 1 Chemical structures of amitriptyline, nortriptyline and imipramine.

triacetyloleandomycin were purchased from Sigma Chemicals (Poole, UK). Furafylline was a gift from Professor W. Pfleiderer (University of Konstanz, Germany). S-mephenytoin was a gift from SmithKline Beecham Pharmaceuticals (Welwyn, UK) and sulphaphenazole was a gift from Ciba-Geigy (Basel, Switzerland). All other reagents were obtained commercially and were of the highest grade of purity.

Preparation of microsomes

Samples of five livers (HL3, 5, 6, 9 and 10) were obtained from renal transplant donors or from patients undergoing partial hepatectomy, with the approval of the local ethics committee and the Coroner. HL3 was phenotyped and genotyped as being from a poor metaboliser (PM) with respect to CYP2D6 activity; the other four were from extensive metabolisers (EMs). Each liver was characterised with respect to the specific activities of CYPs 2D6, 3A4, 1A2, 2C19 and 2C9 using reactions selective for these isoforms (metoprolol α-hydroxylation, testosterone 6β-hydroxylation, phenacetin *O*-deethylation, S-mephenytoin 4-hydroxylation and tolbutamide hydroxylation, respectively). Microsomes were prepared as described by Otton *et al.* [12].

Microsomes from yeast expressing CYP2D6 or CYP1A2 were prepared as described previously [13, 14]. Microsomes from human lymphoblastoid cells expressing CYP3A4, CYP2C9-Arg or CYP2C19 were purchased from Gentest Corporation (Woburn, USA).

Incubation conditions

Microsomal incubations (1 ml) were carried out in triplicate as described previously [15]. Preliminary experiments established that the *N*-demethylation of AMI in human liver microsomes was linear with respect to time over 20 min, and with respect to protein concentration up to 1.8 mg ml^{-1}. The reaction was linear for 10 min and with respect to protein concentration up to 3.8 mg ml^{-1} in microsomes from yeast expressing CYP2D6. The reaction was stopped by adding 6% (w/v) perchloric acid (125 μl). After the addition of internal standard (desipramine, 200 μl 1 μg ml^{-1}) and NaOH (100 μl, 5M), the incubates were extracted with butan-1-ol in hexane (2 : 98 v/v, 5 ml) by vortex-mixing for 1 min. Following centrifugation for 5 min at 2000 *g*, the organic phase was removed and evaporated to dryness at 40° C using a vacuum-vortex evaporator. The residue was reconstituted in the h.p.l.c. mobile phase (200 μl) and 50 μl was injected onto the h.p.l.c. column.

Assay

NT was measured by h.p.l.c. with u.v. detection [16]. The lower limit of assay was 6.7 nM with a coefficient of determination (within-batch) of 5.7% ($n = 6$).

Metabolite formation

The kinetics of *N*-demethylation of AMI were characterised by measuring the formation of NT after incubation of 1–500 μM substrate with microsomes from each liver for 15 min, and after incubation with 1–400 μM substrate with microsomes from yeast expressing CYP2D6 (100 pmol P450 mg^{-1} protein) for 10 min. Formation of NT was also assessed after incubation of 200 μM AMI with microsomes from yeast expressing CYP1A2 (40 pmol P450 mg^{-1} protein) for 20–60 min, and after incubation of 200 μM AMI with microsomes from human lymphoblastoid cells expressing CYP3A4 (24 pmol P450 mg^{-1} protein), CYP2C9-Arg (16 pmol P450 mg^{-1} protein) or CYP2C19 (23 pmol P450 mg^{-1} protein) for 20–60 min. Control experiments were carried out with microsomes prepared from cells lacking the cDNA for the specific CYP. Preliminary experiments established that the expression systems were catalytically active with respect to metabolism of known substrates of the relevant CYP.

Inhibition experiments

The formation of NT from AMI (1–500 μM) was measured after pre-incubation of microsomes from each liver for 10 min with triacetyloleandomycin (40 μM), a selective,

mechanism-based inhibitor of CYP3A4 [17, 18]. In a separate experiment, ketoconazole (5 μM), also a selective inhibitor of CYP3A4 at relatively low concentrations [19], was co-incubated with AMI (1–500 μM).

The experiment with triacetyloleandomycin was repeated with the further addition of sulphaphenazole (100 μM), a selective inhibitor of CYP2C9 [18] and, again, with the addition of triacetyloleandomycin, sulphaphenazole and quinidine (2 μM), the latter being a selective inhibitor of CYP2D6 [18].

The kinetics of NT formation from AMI (1–500 μM) were also investigated with microsomes from each liver after preincubation for 5 min with furafylline (2–10 μM), a selective inhibitor of CYP1A2 [18]. In a separate experiment, the effect of 7-ethoxycoumarin (100 μM), also an inhibitor of CYP1A2 [20], was investigated. The formation of NT from AMI (1–500 μM) was also investigated with microsomes from each liver in the presence of S-mephenytoin (200 μM), a CYP2C19 substrate.

The formation of NT was investigated with microsomes from yeast expressing CYP2D6 co-incubated with AMI (1–400 μM). The recombinant CYP2D6 was also co-incubated with AMI (20 μM) in the presence of quinidine in concentrations up to 50 μM.

Data analysis

The kinetics of NT formation were characterised in the absence of selective inhibitors and then, sequentially, in the presence of one, two and three isoform inhibitors. The relationships between substrate concentration and rate of formation of NT (mean values from three determinations) were described by various functions using non-linear least squares regression (SPSS software, V6.0). These functions varied in complexity depending upon the presence and number of selective inhibitors, and ranged from combinations of Michaelis-Menten and/or Hill functions to simple single-site Michaelis-Menten or Hill functions. The most parsimonious model for each data set was selected on the basis of the dispersion of residuals and whether an *F*-test showed significant reduction ($P<0.05$) in the residual sum of squares.

To determine the kinetic parameters for each isoform blocked by selective inhibition, the difference in NT formation caused by the addition of the inhibitor was calculated over the full substrate concentration range and fitted by a single Michaelis-Menten or Hill function. These parameters were then used to construct plots of the contribution of each isoform to the net formation of NT over the full substrate concentration range in microsomes from each liver.

Results

The kinetics of the *N*-demethylation of AMI by microsomes from each of the five livers, including the CYP2D6 poor metaboliser liver, were best described by a two-site model comprising a Hill function (low affinity site) and a Michaelis-Menten function (high affinity site) (Figure 2, Table 1a). The Hill component was eliminated when microsomes were incubated with triacetyloleandomycin, such that the kinetics were best described by a two-site model comprising two Michaelis-Menten functions (Figure 3, Table 1b). Similarly, the Hill component was eliminated when microsomes were incubated with ketoconazole. In the presence of both triacetyloleandomycin and sulphaphenazole the data were best described by a single Michaelis-Menten function (Figure 4, Table 1c). Finally, when triacetyloleandomycin, sulphaphenazole and quinidine were present there was a further inhibition of amitriptyline *N*-demethylation in microsomes from CYP2D6 extensive metaboliser livers only, but with residual activity described by a single Michaelis-Menten function (Figure 5, Table 1d). This activity was not abolished by doubling the concentrations of each of the three added inhibitors. Neither furafylline, 7-ethoxycoumarin nor S-mephenytoin affected the kinetics of NT formation.

Table 1 Functions and parameters describing NT formation from AMI in human liver microsomes

	Parameters				
(a) *Uninhibited reaction*					
	K_{Hill}[a] (μM)	$V_{max.Hill}$[b] (pmol min^{-1} mg^{-1} protein)	N[c]	K_m[d] (μM)	V_{max}[e] (pmol min^{-1} mg^{-1} protein)
HL10	181	849	2.0	25	239
HL9	250	799	1.7	22	162
HL6	190	139	2.1	26	61
HL5	201	443	1.7	24	117
HL3	231	484	2.1	25	190
(b) *In the presence of TAO*[f] *(40 μM)*					
	K_{m1}	V_{max1}		K_{m2}	V_{max2}
HL10	26	205		122	150
HL9	22	130		102	137
HL6	23	38		110	65
HL5	21	85		109	91
HL3	23	120		113	145
(c) *In the presence of TAO (40 μM) and SP*[g] *(100 μM)*					
	K_m	V_{max}			
HL10	26	201			
HL9	22	129			
HL6	22	40			
HL5	20	82			
HL3	22	117			
(d) *In the presence of TAO (40 μM), SP (100 μM) and Q*[h] *(2 μM)*					
	K_m	V_{max}			
HL10	24	65			
HL9	22	59			
HL6	25	16			
HL5	20	24			
HL3	21	110			

[a]:Hill constant; [b]:Maximum velocity—Hill function; [c]:Hill exponent; [d]:Michaelis-Menten constant; [e]:Maximum velocity—Michaelis-Menten function; [f]:Triacetyloleandomycin; [g]:Sulphaphenazole; [h]:Quinidine.

Microsomes from yeast expressing CYP2D6 *N*-demethylated AMI according to a single Michaelis-Menten function ($K_m = 19.5 \pm 4.2$ μM; V_{max} 1.34 ± 0.06 pmol min^{-1} mg^{-1} protein, $n=3$). This activity was abolished by quinidine at concentrations above 1 μM. Microsomes from human lymphoblastoid cells expressing CYP3A4 or CYP2C9-Arg were capable of *N*-demethylating AMI. NT formation was 18.6 ± 0.6 μM mg^{-1} protein and 14.2 ± 0.8 μM mg^{-1} protein ($n=3$), respectively, after incubation with 200 μM AMI for

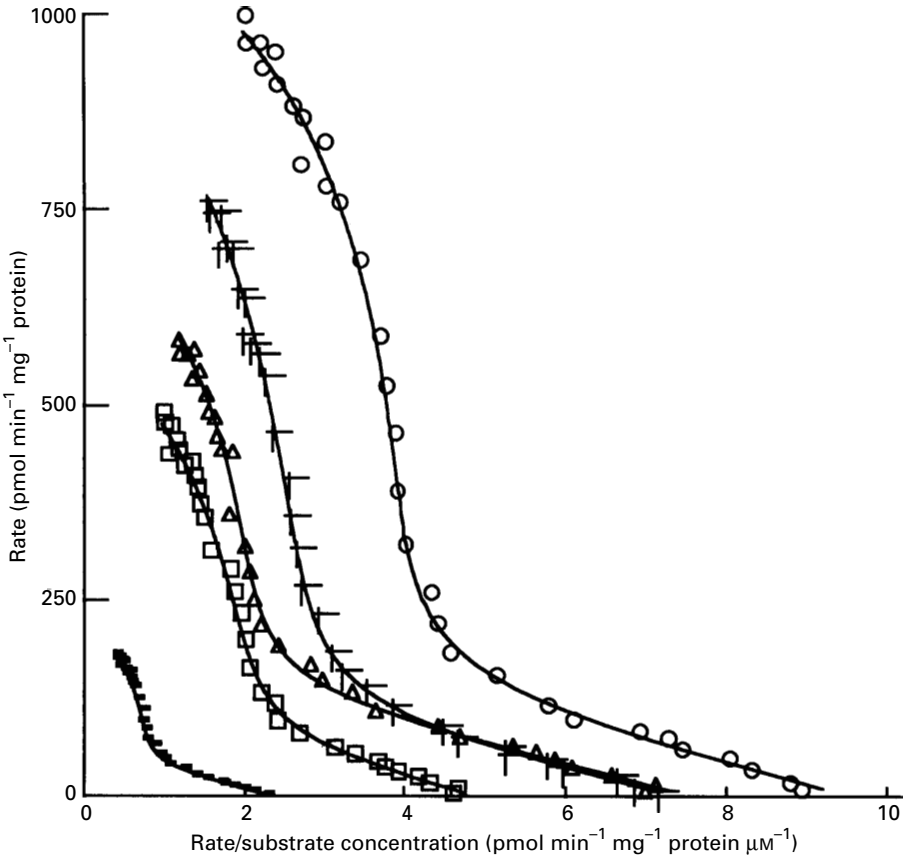

Figure 2 Eadie-Hofstee plots for NT formation in microsomes from five human livers (HL; ○ HL10; † HL9; △ HL3; □ HL5; – HL6) co-incubated with a range of AMI concentrations. Each point represents the mean result of three replicate experiments (CV < 7%).

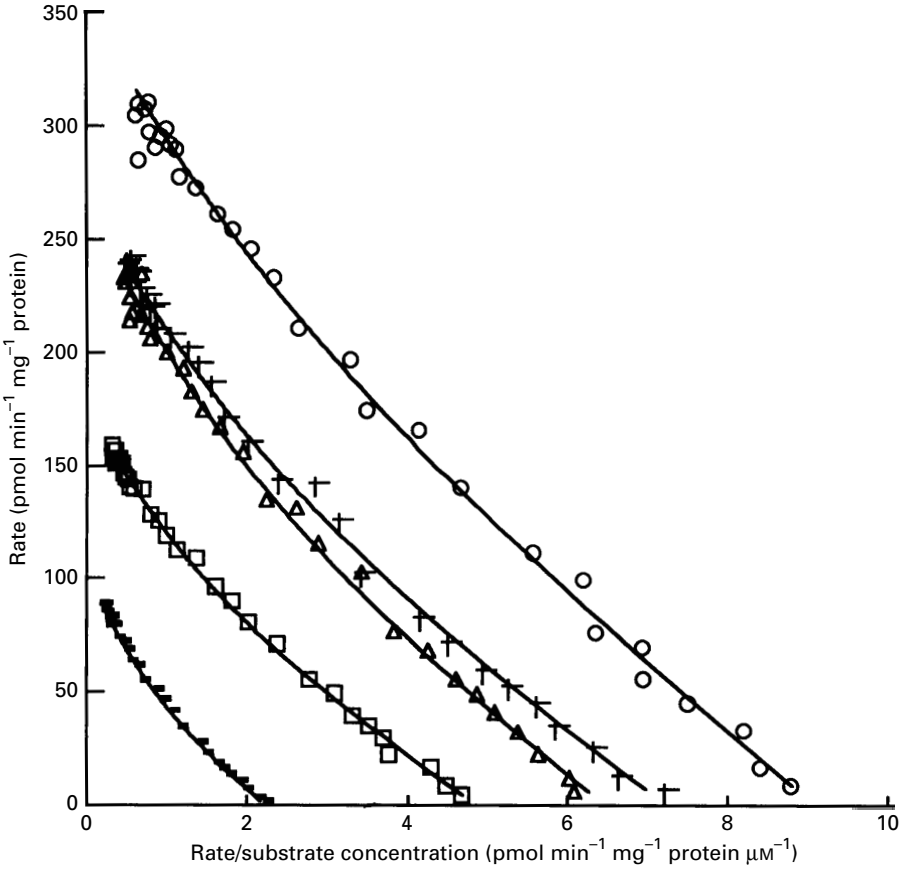

Figure 3 Eadie-Hofstee plots for NT formation in microsomes from five human livers (HL; ○ HL10; † HL9; △ HL3; □ HL5; – HL6) co-incubated with a range of AMI concentrations in the presence of triacetyloleandomycin (40 μM). Each point represents the mean result of three replicate experiments (CV < 7%).

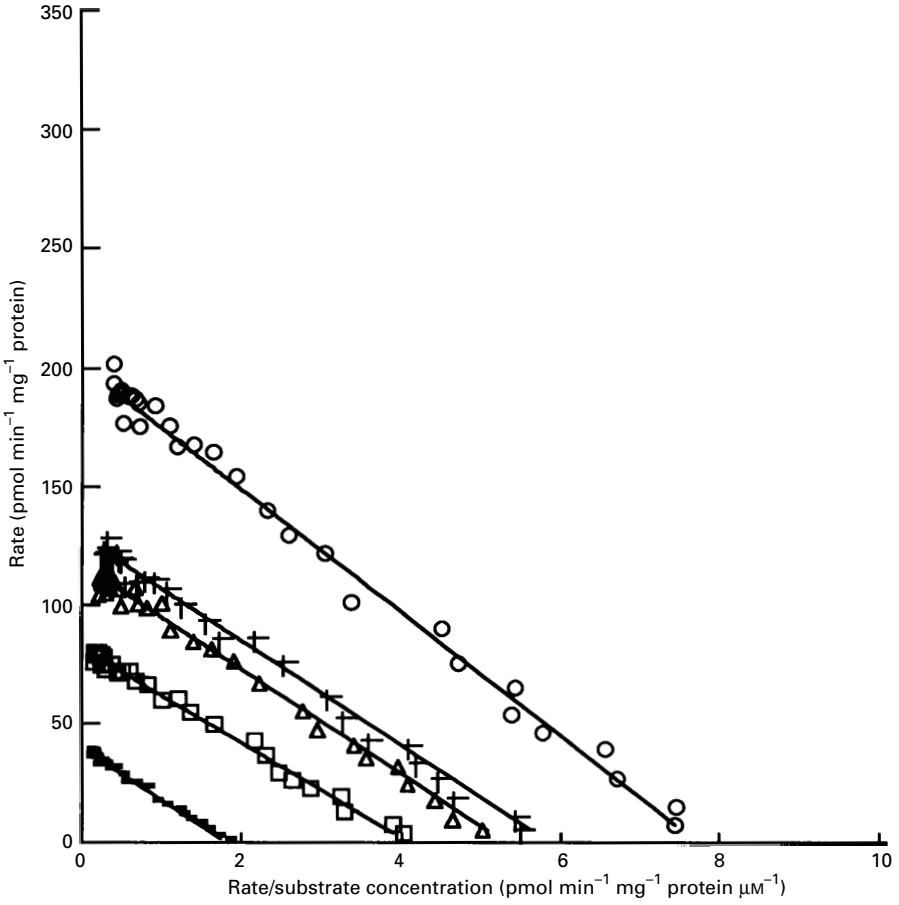

Figure 4 Eadie-Hofstee plots for NT formation in microsomes from five human livers (HL; ○ HL10; † HL9; △ HL3; □ HL5; – HL6) co-incubated with a range of AMI concentrations in the presence of both triacetyloleandomycin (40 μM) and sulphaphenazole (100 μM). Each point represents the mean result of three replicate experiments (CV < 7%).

20 min. In contrast, microsomes from yeast expressing CYP1A2 and human lymphoblastoid cells expressing CYP2C19 did not *N*-demethylate AMI even after incubation for 60 min.

The estimated parameters describing NT formation by CYP3A4, CYP2C9, CYP2D6 and the unknown enzyme in liver microsomes are shown in Table 2. Spearman rank correlations between estimated V_{max} values for NT formation by CYPs 3A4, 2C9 and 2D6 in each liver and the specific activities of the livers with respect to each of these enzymes were 0.90 ($P<0.04$), 0.70 ($P<0.18$) and 0.67 ($P<0.22$), respectively. Spearman rank correlations between estimated Vmax values for NT formation by the unknown enzyme in each liver and the specific activities of the livers with respect to CYPs 2C19 and 1A2 were 0.40 ($P<0.51$) and 0.30 ($P<0.62$), respectively.

Based on the derived kinetic parameters, the percentage contribution of each isoform to net NT formation by microsomes from each liver over the substrate concentration range 1–500 μM is shown in Figure 6. Thus, CYP3A4 was seen to be the dominant enzyme at high concentrations of AMI. However, at therapeutic total plasma AMI concentrations (0.15–1 μM) [21], CYPs 2C9, 2D6 and the unidentified enzyme were more important. The contribution of the latter isoforms to net NT formation at 'therapeutic levels' varied significantly from liver to liver. Although CYP2D6 was found to contribute the greatest activity in all but the PM liver, the relative contribution of CYP2C9 and the unidentified enzyme was not constant.

Discussion

Collectively, the results indicate the involvement of at least four enzymes in the *N*-demethylation of AMI by human liver microsomes. A contribution of CYP3A4, as a low affinity site, is indicated by inhibition of NT formation by triacetyloleandomycin and ketoconazole, a significant correlation between inhibitable activity and specific CYP3A4 activity across the livers, and by the ability of the heterologously expressed enzyme to carry out the reaction. Inhibition of NT formation by quinidine at lower substrate concentrations in microsomes from EM livers only, together with quinidine-inhibitable *N*-demethylation by the expressed enzyme, is evidence for a role of CYP2D6. This finding corroborates *in vivo* results indicating an association between debrisoquine hydroxylase (CYP2D6) and *N*-demethylation of AMI [9]. A contribution from CYP2C9 is indicated by sulphaphenazole inhibition of the liver microsomal reaction, and by the ability of the expressed enzyme to effect *N*-demethylation. Correlations between activity inhibitable by quinidine and sulphaphenazole and specific liver microsomal activities of CYPs 2D6 and 2C9, respectively, were appreciable but did not reach statistical significance. NT formation by a fourth, unidentified enzyme was suggested by the finding of residual *N*-demethylase activity in liver microsomes in the combined presence of selective inhibitors of CYPs 3A4, 2C9 and 2D6. This enzyme did not appear to be either CYP1A2 or CYP2C19 as inhibitors of these isoforms were without effect on *N*-demethylase activity. Furthermore, heterologous expression

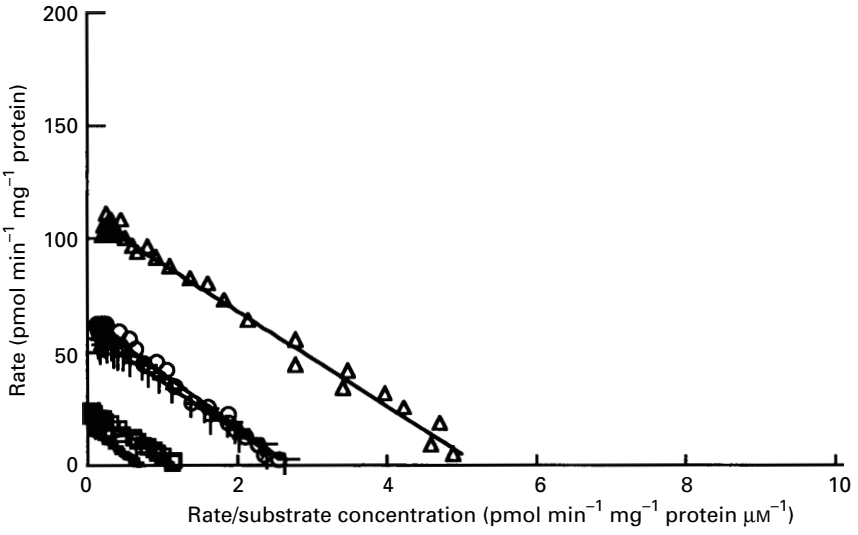

Figure 5 Eadie-Hofstee plots for NT formation in microsomes from five human livers (HL; ○ HL10; † HL9; △ HL3; □ HL5; – HL6) co-incubated with a range of AMI concentrations in the presence of a mixture of triacetyloleandomycin (40 μM), sulphaphenazole (100 μM) and quinidine (2 μM). Each point represents the mean result of three replicate experiments (CV < 7%).

Table 2 Parameters describing NT formation from AMI by single enzymes in microsomes from human livers.

	Kinetic parameters of the P450 isoenzymes involved in NT formation								
	CYP3A4			*CYP2C9*		*CYP2D6*		*Unknown enzyme*	
	K_{Hill}[a] (μM)	$V_{max.Hill}$[b] (pmol min^{-1} mg^{-1} protein)	N[c]	K_{md} (μM)	V_{max}[e] (pmol min^{-1} mg^{-1} protein)	K_m (μM)	V_{max} (pmol min^{-1} mg^{-1} protein)	K_m (μM)	V_{max} (pmol min^{-1} mg^{-1} protein)
HL10	183	739	2.1	116	153	27	136	24	65
HL9	251	666	1.9	101	138	22	70	22	59
HL6	195	99	2.5	120	64	20	24	25	16
HL5	204	385	1.8	107	94	20	58	20	24
HL3	236	424	2.0	113	148	–	–	21	110

[a]:Hill constant; [b]:Maximum velocity—Hill function; [c]:Hill exponent; [d]:Michaelis-Menten constant; [e]:Maximum velocity—Michaelis-Menten function.

systems for CYP1A2 and CYP2C19 did not generate detectable concentrations of NT. Given the close structural resemblance of AMI and imipramine (Figure 1), which has been shown to be *N*-demethylated by CYP1A2 [11], the finding that this enzyme did not metabolise AMI was surprising.

An alternative explanation for the residual *N*-demethylase activity is that the inhibitors used were incompletely effective. No further impairment of activity was observed when the concentrations of the inhibitors were doubled. However, in the case of triacetyloleandomycin it is particularly difficult to obtain complete inhibition owing to its slow rate of enzyme inactivation and low potency. Typically, only about 80% inhibition of CYP3A4 by triacetyloleandomycin is observed [22–24]. Nevertheless, doubling the concentration of ketoconazole to 10 μM also failed to cause a significant decrease in residual *N*-demethylase activity, suggesting that this is not due to CYP3A4.

Schmider *et al.* [25, 26] have recently also reported the involvement of CYP3A4 and CYP2C9 in the *N*-demethylation of AMI by human liver microsomes. Their data regarding CYP2C9 were based on experiments using microsomes from a single human liver [25]. They excluded any involvement of CYP2D6 on the basis of a lack of inhibition by quinidine of NT formation from 100μM AMI in microsomes from apparently a single liver of undefined CYP2D6 genotype [26]. Our data at this substrate concentration indicate a 20% contribution of CYP2D6 to total NT formation in microsomes from EM livers (Figure 6).

The question of what concentrations of AMI might be present at hepatic enzyme sites *in vivo* is difficult to resolve. Under equilibrium conditions, these concentrations might be expected to be similar to unbound plasma drug concentrations. However, the issue is complicated by the possibilities of ion-trapping within the hepatocyte, active hepatic uptake and first-pass exposure to higher drug concentrations following absorption from the gut. Furthermore, a contribution to the oral clearance of AMI of first-pass metabolism by intestinal CYP3A4 cannot be excluded. Ignoring these complexities, and assuming total plasma concentrations of AMI within the 'therapeutic range' as a reference point, our findings suggest that variability in the activities and levels of CYPs 2D6 and 2C9 and of an unidentified enzyme would have the greatest influence on the clinical pharmacokinetics of AMI. A significant contribution of CYP2D6 to the *N*-demethylation reaction would add its major influence on the aromatic hydroxylation of AMI [4–7].

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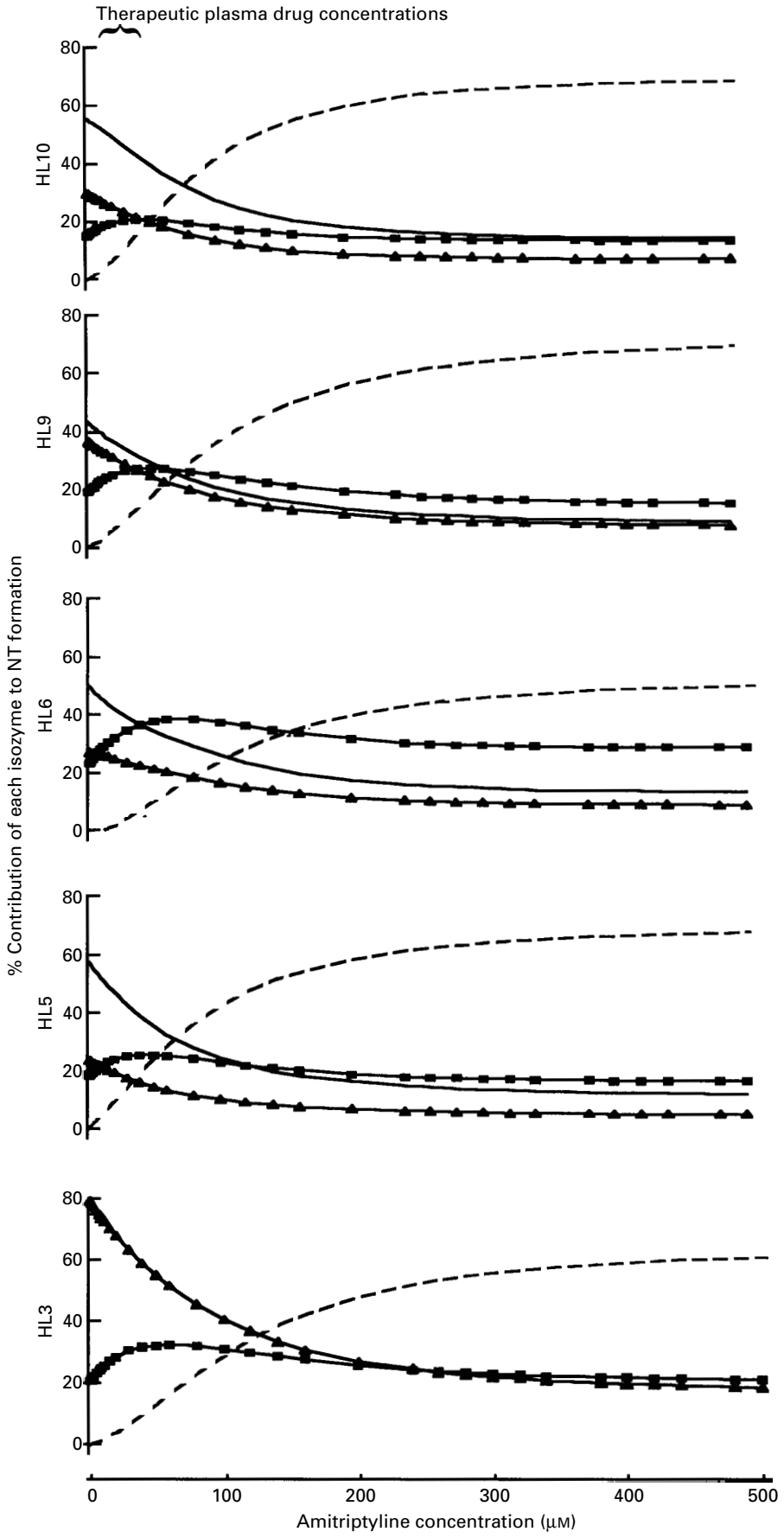

Figure 6 The percentage contribution of CYP isoenzymes to NT formation by microsomes from five human livers. --- 3A4; ■ 2C9; — 2D6; ▲ unknown enzyme.

References

1 Ballenger JC. Pharmacotherapy of panic disorders. *J Clin Psychiatr* 1986; **47**(suppl.6): 27–32.

2 Rollins DE, Alvan G, Bertilsson L, *et al*. Interindividual differences in amitriptyline demethylation. *Clin Pharmacol Ther* 1988; **32**: 664—667.

3 Mellstrom B, Alvan G, Bertilsson L, *et al*. Nortriptyline formation after single oral and intramuscular doses of amitriptyline. *Clin Pharmacol Ther* 1982; **32**: 664–667.

4 Nordin C, Siwers B, Benitez J, Bertilsson L. Plasma concentrations of nortriptyline and its 10-hydroxy metabolite in depressed patients- Relationship to the debrisoquine hydroxylation metabolic ratio. *Br J Clin Pharmacol* 1985; **19**: 832–835.

5 Mellstrom B, Bertilsson L, Sawe J, Schulz HU, Sjoqvist F. E- and Z-10-hydroxylation of nortriptyline: Relationship to polymorphic debrisoquine hydroxylation. *Clin Pharmacol Ther* 1981; **30**: 189–193.

6 Balant-Gorgia AE, Schulz P, Dayer P, *et al*. Role of oxidation polymorphism on blood and urine concentrations of amitriptyline and its metabolites in man. *Arch Psychiatr Nervenkr* 1982; **232**: 215–222.

7 Woolhouse NM, Adjepon-Yamoah KK, Mellstrom B, *et al*. Nortriptyline and debrisoquine hydroxylations in Ghanaian and Swedish subjects. *Clin Pharmacol Ther* 1984; **36**: 374–378.

8 Baumann P, Meyer JW, Amey M, *et al*. Dextromethorphan and mephenytoin phenotyping of patients treated with thioridazine or amitriptyline. *Ther Drug Monit* 1992; **14**: 1–8.

9 Mellstrom B, Sawe J, Bertilsson L, Sjoqvist F. Amitriptyline metabolism: Association with debrisoquine hydroxylation in nonsmokers. *Clin Pharmacol Ther* 1986; **39**: 369–371.

10 Breyer-Pfaff U, Pfandl B, Nill K, *et al*. Enantioselective amitriptyline metabolism in patients phenotyped for two cytochrome P450 isoenzymes. *Clin Pharmacol Ther* 1992; **52**: 350–358.

11 Lemoine A, Gautier JC, Azoulay D, *et al*. Major pathway of imipramine is catalysed by cytochromes P-450 1A2 and P-450 3A4 in human liver. *Mol Pharmacol* 1993; **43**: 827–832.

12 Otton SV, Crewe HK, Lennard MS, Tucker GT, Woods HF. Use of quinidine inhibition to define the role of the sparteine/debrisoquine cytochrome P450 in metoprolol oxidation by human liver microsomes. *J Pharmacol Exp Ther* 1988; **247**: 242–247.

13 Ellis SW, Ching MS, Watson PF, *et al*. Catalytic activity of human debrisoquine 4-hydroxylase cytochrome P450 (CYP2D6) expressed in yeast. *Biochem Pharmacol* 1992; **44**: 617–620.

14 Rowland K, Ellis SW, Lennard MS, Tucker GT. Variable contribution of CYP2D6 to the *N*-dealkylation of S-(-)-propanolol by human liver microsomes. *Br J Clin Pharmacol* 1996; **42**: 390–393.

15 Otton SV, Brinn RU, Gram LF. *In vitro* evidence against the oxidation of quinidine by the sparteine/debrisoquine monooxygenase of human liver. *Drug Metab Dispos* 1988; **16**: 15–17.

16 Ghahramani P, Lennard MS. Quantitative analysis of amitriptyline and nortriptyline in human plasma and liver microsomal preparations by high-performance liquid chromatography. *J Chromatogr* 1996; **685**: 307–313.

17 Chang T, Gonzalez FJ, Waxman DJ. Evaluation of triacetyloleandomycin, alpha-naphthoflavone and diethyldithiocarbamate as selective chemical probes for inhibition of human cytochromes P450. *Arch Biochem Biophys* 1994; **311**: 437–442.

18 Newton DJ, Wang RW, Lu A. Cytochrome P450 inhibitors: Evaluation of specificities in the in vitro metabolism of therapeutic agents by human liver microsomes. *Drug Metab Dispos* 1995; **23**: 154–158.

19 von Moltke LL, Greenblatt DJ, Cotreau-Bibbo MM, Harmatz JS, Shader RI. Inhibitors of alprazolam metabolism *in vitro*: Effect of serotonin- reuptake-inhibitor antidepressants, ketoconazole and quinidine. *Br J Clin Pharmacol* 1994; **38**: 23–31.

20 Tassaneeyakul W, Birkett DJ, Veronese ME, *et al*. Specificity of substrate and inhibitor probes for human cytochromes P450 1A1 and 1A2. *J Pharmacol Exp Ther* 1993; **265**: 401–407.

21 Schulz P, Dick P, Blaschke TF, Hollister L. Discrepancies between pharmacokinetic studies of amitriptyline. *Clin Pharmacokin* 1985; **10**: 257–268.

22 Silvers KJ, Chazinski T, McManus ME, *et al*. Cytochrome P-450 3A4 (nifedipine oxidase) is responsible for the C-oxidative metabolism of 1-nitropyrene in human liver microsmal samples. *Cancer Res* 1992; **52**: 6237–6243.

23 Jurima-Romet M, Crawford K, Cyr T, Inaba T. Terfenadine metabolism in human liver–*in vitro* inhibition by macrolide antibiotics and azole antifungals. *Drug Metab Dispos* 1994; **22**: 849–857.

24 Jonsson G, Astrom A, Andersson P. Budenoside is metabolized by cytochrome-P450 3A (CYP3A4) enzymes in human liver. *Drug Metab Dispos* 1995; **23**: 137–142.

25 Schmider J, Greenblatt DJ, Harmatz JS, Shader RI. Enzyme kinetic modelling as a tool to analyse the behaviour of cytochrome P450 catalysed reactions: application to amitriptyline *N*-demethylation. *Br J Clin Pharmacol* 1996; **41**: 593–604.

26 Schmider J, Greenblatt DJ, von Moltke LL, Harmatz JS, Shader RI. *N*-demethylation of amitriptyline in vitro: role of cytochrome P-4503A (CYP3A) isoforms and effect of metabolic inhibitors. *J Pharmacol Exp Ther* 1995; **275**: 592–597.

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