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Phenacetin *O*-deethylase: an activity of a cytochrome P-450 showing genetic linkage with that catalysing the 4-hydroxylation of debrisoquine?

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1 Phenacetin *O*-deethylase activity was impaired, both *in vivo* and *in vitro*, in poor metabolisers of debrisoquine, consistent with the work of others. No impairment was observed in the oxidation of acetanilide, amylobarbitone or antipyrine in the PM phenotype.
2 There was a good correlation ($r = 0.804$) between the high affinity component of phenacetin *O*-deethylase and debrisoquine 4-hydroxylase activities. No such correlation was observed with the low affinity component of phenacetin *O*-deethylase activity.
3 Although debrisoquine was a competitive inhibitor of phenacetin *O*-deethylase activity, phenacetin was without effect on debrisoquine 4-hydroxylation. There were also marked differences in the effects of sparteine, guanoxan and α-naphthoflavone on the two activities.
4 Cigarette smoking was associated with a significant, two-fold, increase in phenacetin *O*-deethylase activity whilst debrisoquine 4-hydroxylase activity was not affected.
5 It is concluded that the high affinity component of phenacetin *O*-deethylase and debrisoquine 4-hydroxylase activities are catalysed by different isozymes of cytochrome P-450 but that these are most probably regulated by closely linked genes.

Keywords human liver phenacetin *O*-deethylase debrisoquine 4-hydroxylase oxidation polymorphism genetic linkage

Introduction

In the eight years since the genetic polymorphism in debrisoquine 4-hydroxylation was first reported (Mahgoub *et al.*, 1977) the oxidation of over 14 other drugs has been shown to be impaired in the poor metaboliser phenotype (reviewed in Eichelbaum, 1982; Smith, 1985). Ten years prior to the discovery of the debrisoquine oxidation polymorphism, however, Shahidi (1968) had already described a deficiency in the *O*-deethylation of phenacetin that appeared to be genetically mediated. Soon after the debrisoquine oxidation polymorphism was reported, Sloan *et al.* (1978) demonstrated that poor metabolisers of debrisoquine also had an impairment in the *O*-deethylation of phenacetin.

Subsequently, studies *in vitro* (Davies *et al.*, 1981) established that the basis for the debrisoquine oxidation polymorphism was the absence or functional deficiency of a specific form of hepatic cytochrome P-450 which catalyses the 4-hydroxylation of debrisoquine. In the same study it was demonstrated that a biopsy sample from a PM subject also lacked the high affinity component of phenacetin *O*-deethylase activity, thus

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supporting earlier work (Sloan *et al.*, 1978) indicating a common defect in the two reactions.

Several groups (Otton *et al.*, 1982; Boobis *et al.*, 1983; von Bahr *et al.*, 1985) have now demonstrated, by competitive inhibition studies, that the form of cytochrome P-450 catalysing the 4-hydroxylation of debrisoquine is active in the oxidation of many of the substrates with impaired metabolism in the PM phenotype. It was concluded that there is a single form of cytochrome P-450 impaired in the PM phenotype and that this form is responsible for the oxidation of all such substrates.

Guengerich and his colleagues (Distlerath *et al.*, 1985) have purified a form of cytochrome P-450 from human liver that is active in the 4-hydroxylation of debrisoquine. Antibodies against the analogous form of cytochrome P-450 in the rat inhibit the microsomal reaction with human liver by 90%. The inevitable conclusion was that the form of cytochrome P-450 purified represents the major form catalysing the 4-hydroxylation of debrisoquine, and that it is this form that is impaired in PM subjects. Interestingly, this form of cytochrome P-450 does not catalyse the *O*-deethylation of phenacetin and, in addition, the anti-debrisoquine 4-hydroxylase antibodies do not inhibit this activity (Distlerath *et al.*, 1985).

The present paper reports the results of a study into the association between phenacetin *O*-deethylase activity and debrisoquine 4-hydroxylase activity in man.

Methods

In vivo *studies*

Seven healthy volunteers (four male, three female), who gave their written informed consent, participated in this phase of the study, which had Local Research Ethics Committee permission. The subjects refrained from the ingestion of any drugs in the 7 days prior to and during each study period. Their average age was 29 ± 4 years (± s.d.), with average body weight 70 ± 12 kg and average height 1.76 ± 0.08 m. Each period of the study was separated by at least 2 weeks. A single oral dose of the following drugs was taken on separate occasions:

Amylobarbitone sodium as a tablet of 200 mg of the sodium salt, last thing at night.
Antipyrine 2 × 300 mg tablets.
Phenacetin as a tablet of 900 mg.
Acetanilide as a capsule of 900 mg.
Debrisoquine hemisulphate as a tablet of 10 mg.

In every case, except for amylobarbitone, the drug was taken at 9.00 h. For all drugs except debrisoquine, blood samples were collected over a period of four to five half-lives. For amylobarbitone and antipyrine, urine was also collected for this period of time, in 12-hourly collections. Urine for the antipyrine study was collected over sodium metabisulphite (Boobis *et al.*, 1981a), 1 g/12 h collection, to prevent oxidative degradation of antipyrine metabolites, particularly 4-hydroxyantipyrine. With phenacetin and acetanilide, urine collections were made for 24 h, as the half-life of paracetamol exceeds that of the parent drugs. With debrisoquine, only urine was collected, for the first 6 h following dosing. Plasma was immediately separated from the blood samples by centrifugation and, together with the urine samples, was stored at −20°C until analysis.

Amylobarbitone in plasma was determined by the g.l.c. method of Barrett (1971), with pentobarbitone as internal standard. The compounds were derivatised on column with 0.1M trimethylanilinium hydroxide. The stationary phase was 3% OV-17 with an oven temperature of 135°C. The assay was performed on a Hewlett Packard 5750 Research Chromatograph with a nitrogen detector (Hewlett Packard, Wokingham, Berks).

3-Hydroxyamylobarbitone in urine was assayed by a modification of the method of Grove & Toseland (1971) with 3-hydroxypentobarbitone as internal standard. Derivatives were formed using freshly prepared diazomethane. Analysis was performed on the instrumentation described above with a stationary phase of 3% OV-1 heated to 200°C.

Antipyrine in plasma and its three oxidative metabolites, norphenazone, 4-hydroxyantipyrine and 3-hydroxymethylantipyrine in urine were analysed as previously described (Toverud *et al.*, 1981).

Phenacetin in plasma was assayed by h.p.l.c. To 2 ml of plasma were added 2 μg of 4-bromoacetanilide in methanol as internal standard. The samples were alkalinised with 0.5 M sodium hydroxide and extracted with 10 ml of diethyl ether by vortex mixing for 1 min, followed by centrifugation at 2500 × g_{av} for 5 min. The organic phase was dehydrated over sodium sulphate and then taken to dryness in a stream of nitrogen at 40°C. The residue was transferred to a reactivial with 2 × 0.5 ml redistilled ethyl acetate which was evaporated to dryness under nitrogen at 50°C. Samples were finally reconstituted in 50 μl of methanol and injected on to a Zorbax C18 μBondapak h.p.l.c. column (Dupont Instruments, Stevenage, Herts), 25 cm × 4.6 mm i.d. The mobile phase was a mixture of

methanol: water in the ratio of 70:30 and this was delivered by a model 6000A chromatographic pump from Waters Associates (Hartford, Northwich, Cheshire) at a flow rate of 1.7 ml/min. Absorbance was monitored at 254 nm.

Acetanilide in plasma was determined by a modification of the method of Kellermann & Luyten-Kellermann (1978). 4-Bromoacetanilide was used as internal standard. H.p.l.c. separation was performed on a Zorbax C18 μBondapak column utilising the chromatographic instrumentation above. The mobile phase was methanol:water in the ratio of 70:30 with a flow rate of 1.7 ml/min. Absorbance was monitored at 242 nm.

Paracetamol in urine was assayed after hydrolysis of the sulphate and glucuronide conjugates. To 1 ml urine was added 100 μg 4-bromoacetanilide in 100 μl methanol as internal standard, followed by 20 μl 4% (w/v) EDTA, and 1 ml of 1.0 M sodium acetate buffer, pH 4.8 containing 29 units of sulphatase (type H1 containing β-glucuronidase). The conjugates were hydrolysed by incubating at 37°C for 3 h. The samples were then neutralised by the addition of 2 ml of 1.0 M sodium acetate buffer pH 7.0, followed by extraction with 10 ml of diethyl ether. The organic phase was evaporated under reduced pressure in a vortex evaporator at 40°C and the residue reconstituted in 100 μl methanol. Chromatography was on Zorbax C18 μBondapak using the chromatograph described above. The mobile phase was 80% (v/v) methanol in water at a flow rate of 2.0 ml/min. Absorbance was determined at 254 nm.

Debrisoquine and 4-hydroxydebrisoquine in urine were determined as described previously (Davies *et al.*, 1981).

Total oral clearance of the drugs and partial clearance to their oxidative metabolites were calculated as described previously (Boobis *et al.*, 1981a).

In vitro *studies*

Human liver samples were obtained, with Local Research Ethics Committee permission, as previously described (Boobis *et al.*, 1980). Microsomal fractions were prepared and stored at −80°C until required. Under these conditions of preparation and storage, there was no deterioration of oxidative activity.

Protein concentration (Boobis *et al.*, 1980) debrisoquine 4-hydroxylase activity (Kahn *et al.*, 1982), and phenacetin *O*-deethylase activity (Boobis *et al.*, 1981b) were all determined by previously published methods. Phenacetin *O*-deethylation exhibits biphasic kinetics, with two components of activity with widely separate K_m values distinguishable. In some studies, therefore, phenacetin *O*-deethylase activity was determined at concentrations of 20 μM and 2.5 mM, at which concentrations the high affinity component of activity contributes 81% and 31% respectively to total activity. Each component of activity was then corrected for the contribution of the other as previously described (Boobis & Davies, 1984). Components of activity thus estimated have been designated high affinity and low affinity components, respectively.

The effects of inhibitors on monooxygenase activity were determined by adding the compounds in aqueous solution, except for α-naphthoflavone, which was added in a minimum volume of methanol, shown not to affect activity itself with the concentration used, prior to addition of the substrate and preincubating at 37°C for 1 min.

Results

In a preliminary study *in vivo* debrisoquine oxidation phenotype was determined in a group of healthy volunteers. This revealed that one subject belonged to the PM phenotype (Table 1). Total oral clearance of amylobarbitone, antipyrine and acetanilide were normal in this subject but the clearance following an oral dose of phenacetin was less than half of the mean value for the EM subjects, and was less than 60% of the value for the subject with the next lowest clearance.

Values in the PM subject for metabolic clearance to the oxidative metabolites of antipyrine, to 3-hydroxyamylobarbitone from amylobarbitone and to paracetamol from acetanilide were all close to or within the range of EM subjects (Table 2). However, clearance to paracetamol from phenacetin was only 34% of the mean value for the EM subjects and was only 52% of the value for the subject with the next lowest clearance to paracetamol.

In a previous study (Boobis *et al.*, 1981b) it was observed that the *O*-deethylation of phenacetin *in vitro* by microsomal fractions of human liver is biphasic, with a high affinity-low capacity component of activity and a low affinity-high capacity component of activity. We have already reported that there was a relative impairment in the capacity of the high affinity component of phenacetin *O*-deethylase activity in a liver sample obtained from a subject phenotyped as PM *in vivo* (Davies *et al.*, 1981). The low affinity component of activity was unimpaired in the sample. Correcting the results obtained in the earlier study for the contribution of the low

Table 1 Total oral clearance of four drugs in healthy volunteers

	Debrisoquine oxidation		*Total oral clearance (ml/min) of*			
Subject	*Metabolic ratio*	*Phenotype*	*Amylobarbitone*	*Antipyrine*	*Phenacetin*	*Acetanilide*
1	8.5	EM	33.0	33.6	4040	287
2	5.5	EM	32.3	43.1	2410	904
3	1.0	EM	26.7	28.8	2420	367
4	2.4	EM	39.2	24.8	3910	242
5	0.3	EM	62.6	45.8	4310	442
6	0.7	EM	46.7	51.6	2600	374
7	> 120	PM	36.7	33.8	1400	289
Mean of EM Subjects	3.1		40.1	38.0	3280	436

*EM, extensive metaboliser and PM, poor metaboliser as defined in Mahgoub *et al.* (1977).

affinity component of activity (Boobis & Davies, 1984) revealed that the PM sample had no detectable high affinity *O*-deethylase activity (Table 3).

The activity of the two components of phenacetin *O*-deethylase activity and debrisoquine 4-hydroxylase activity were determined in 14 samples of human liver. Debrisoquine 4-hydroxylase activity showed a highly significant correlation with the high affinity component of phenacetin *O*-deethylase activity (Figure 1), but there was no correlation with the low affinity component of this activity. When the smoking history of the patients from whom these samples were obtained was examined it was found that cigarette smoking (more than 10 cigarettes/day) was associated with a significant increase in the high affinity component of phenacetin *O*-deethylase activity, by over two-fold, and a small but significant increase in the low-affinity component of activity, by 44% (Figure 2). However, cigarette smoking had no significant effect on debrisoquine 4-hydroxylase activity.

The effects of competitive inhibitors of debrisoquine 4-hydroxylase activity on the two components of phenacetin *O*-deethylase activity were investigated (Figure 3). Debrisoquine itself, sparteine and guanoxan all inhibited the high affinity component of *O*-deethylase activity relatively selectively, compared with the low affinity

Table 2 Partial clearance of four drugs to their oxidative metabolites in healthy volunteers

		Partial clearance (ml/min)					
Subject	*Debrisoquine oxidation phenotype*	*AB→3OHAB**	*3OHMeAP*	*AP→NP*	*4OHAP*	*Phen→Para*	*Acet→Para*
1	EM+	13.5	9.6	6.8	11.3	2140	305
2	EM	18.7	6.5	6.5	ND**	1180	1236
3	EM	20.7	10.9	2.6	18.6	1310	311
4	EM	29.2	6.4	4.2	20.1	2280	329
5	EM	27.5	8.9	6.9	13.9	1950	376
6	EM	26.8	5.4	6.9	37.1	1760	473
7	PM	12.8	12.9	6.0	ND	610	310
Mean of EM Subjects		22.7	8.0	5.7	20.2	1770	505

*Abbreviations used are AB, amylobarbitone, 3OHAB, 3-hydroxyamylobarbitone, AP, antipyrine, 3OHMeAP, 3-hydroxymethylantipyrine, NP, norphenazone, 4OHAP, 4-hydroxyantipyrine, Phen, phenacetin, Para, paracetamol, Acet, acetanilide.
+EM, extensive metaboliser and PM, poor metaboliser determined as described by Mahgoub *et al.* (1977).
**ND, Value not determined.

Table 3 Phenacetin *O*-deethylase activity of microsomal fractions of human liver

Sample	*Phenacetin O-deethylase activity (pmol mg^{-1} protein min^{-1})*	
	High affinity component	*Low affinity component*
Non-phenotyped (n = 25)	79 (0–230)+	1060 (450–2290)
EM1**	93	1250
EM2	38	510
PM3	< 5	580

*The activity of the high affinity component has been corrected for the contribution of the low affinity component as previously described (Boobis & Davies, 1984).
+Values for non-phenotyped samples are means, with ranges in parentheses.
**Patients 1–3 were phenotyped for debrisoquine oxidation *in vivo* as previously described (Davies *et al.*, 1981).

component of activity. The kinetics of inhibition of phenacetin *O*-deethylase activity by debrisoquine were investigated (Table 4). Debrisoquine was a competitive inhibitor of the high affinity component of activity (K_m increased almost four-fold), with a K_i value of 170 μM. There was no significant inhibition of the low affinity component.

The effects of phenacetin on debrisoquine 4-hydroxylase activity were determined. Phenacetin produced virtually no inhibition of debrisoquine 4-hydroxylase activity, even at concentrations 200 times the K_m of the high affinity component of phenacetin *O*-deethylase activity (Table 5). The selective inhibitor ANF was also without inhibitory effect on debrisoquine 4-hydroxylase activity and indeed caused a modest stimulation of this activity (Figure 4).

Discussion

Evidence from both *in vivo* (Sloan *et al.*, 1978; this study) and *in vitro* (Davies *et al.*, 1981; Distlerath *et al.*, 1985) studies strongly suggest that the PM phenotype for debrisoquine 4-hydroxylase activity has impaired capacity to *O*-deethylate phenacetin. More than one form of cytochrome P-450 in man can catalyse this reaction (Boobis *et al.*, 1981b) but the absence of the high affinity component of activity in the PM phenotype would suggest that a single isozyme is responsible for this component. From the present study it appears that this enzyme is inducible by cigarette smoking.

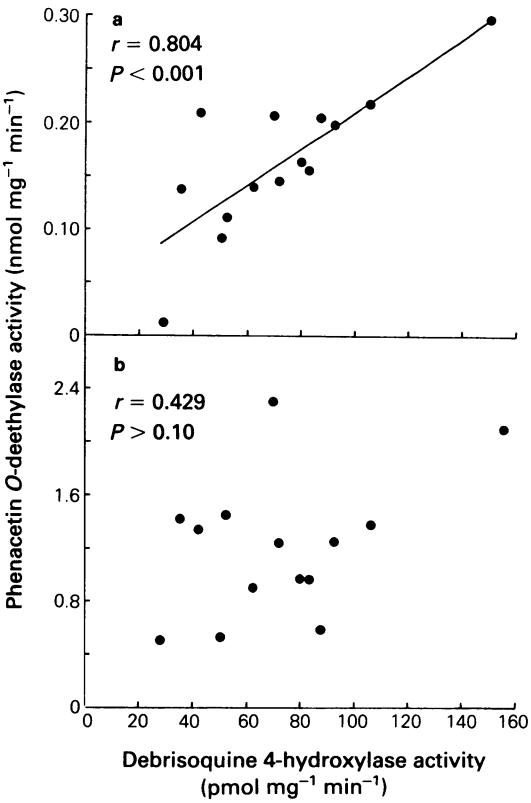

Figure 1 Correlation between debrisoquine 4-hydroxylase and **a**) the high affinity component and **b**) the low affinity component of phenacetin *O*-deethylase activities. Values of r were determined by least squares linear regression analysis.

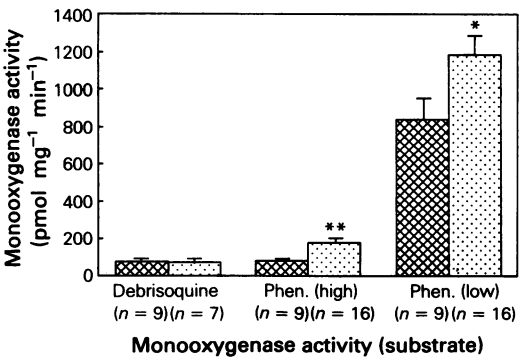

Figure 2 Effects of cigarette smoking on debrisoquine 4-hydroxylase and phenacetin *O*-deethylase activities. Results are ± s.e. mean *$P < 0.05$, **$P < 0.01$, smokers (▨) compared with non-smokers (▩) by paired Student's t-test. Abbreviations used are: Phen. (high) high affinity component and Phen. (low) low affinity component respectively of phenacetin *O*-deethylase activity.

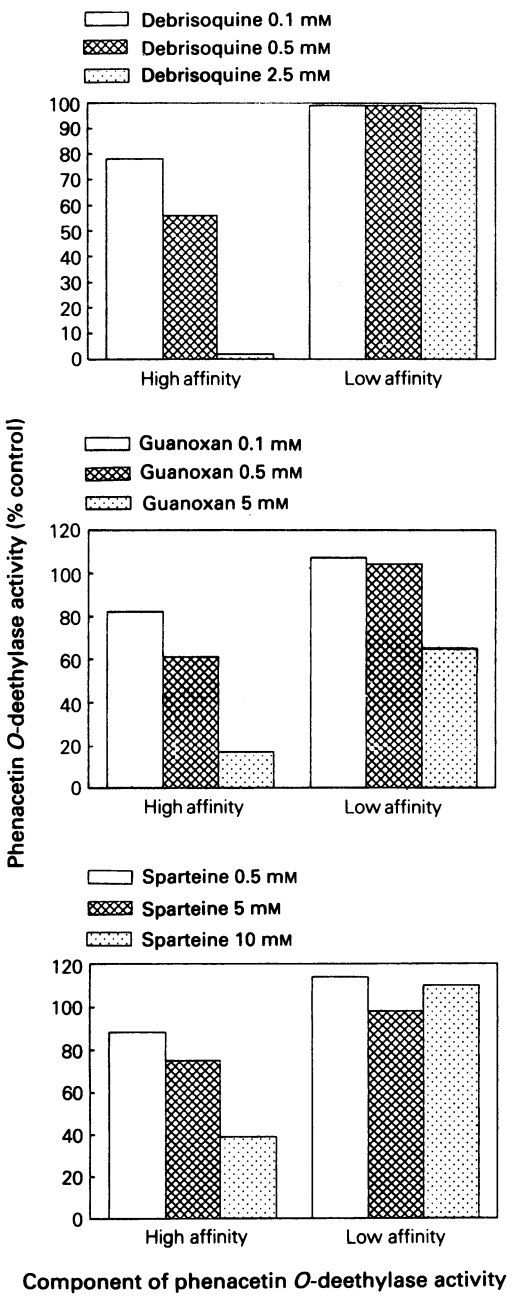

Figure 3 Effects of debrisoquine, guanoxan and sparteine on the two components of phenacetin *O*-deethylase activity. Results are mean of duplicate determinations, which were always within 10% of each other, on two different liver samples.

The oxidation of over 14 compounds has now been shown to be impaired in the PM phenotype. The possibility that there are multiple linked polymorphisms has largely been discounted as a result of a number of studies on mutual inhibition conducted *in vitro* (Boobis *et al.*, 1983, 1985; Otton *et al.*, 1982, 1983, 1984; Spina *et al.*, 1984; von Bahr *et al.*, 1985). All the substrates tested, with the exception of phenacetin in the present report, have been shown to be potent competitive inhibitors of the oxidation of debrisoquine, sparteine or desmethylimipramine. Where investigated, these compounds have been shown to be inhibitors of all these activities. It thus seems likely that one form of cytochrome P-450, impaired in the PM phenotype, is responsible for the metabolism of most, if not all, of these compounds. The drugs which fall into this category include debrisoquine, sparteine, guanoxan, phenformin, nortriptyline, desmethylimipramine, bufuralol, metoprolol and encainide. Studies by Distlerath *et al.* (1985) on the purification of the form of cytochrome P-450 in man which catalyses debrisoquine 4-hydroxylation revealed that this isozyme is also active in the metabolism of encainide and propranolol. However, it does not catalyse the *O*-deethylation of phenacetin.

In the present study, despite competitive inhibition of phenacetin *O*-deethylation by debrisoquine, it was not possible to inhibit the 4-hydroxylation of debrisoquine with phenacetin. This was despite adding concentrations of phenacetin several hundred times the K_m of the high affinity component of activity. Debrisoquine, sparteine and guanoxan were selective inhibitors of the high affinity component of phenacetin *O*-deethylase activity relative to the low affinity component. When the kinetics of inhibition of debrisoquine were determined it was found that inhibition was competitive and that the K_i value for debrisoquine was similar to its K_m for 4-hydroxylation. In marked contrast, the estimated K_i values for guanoxan and sparteine were substantially greater than their K_i values for inhibition of debrisoquine 4-hydroxylation (Table 6). This is in contrast to the results of an earlier study in which the inhibition of bufuralol 1′-hydroxylase and debrisoquine 4-hydroxylase activities by a range of compounds was investigated. In all cases competitive inhibitors of one reaction inhibited the other reaction with a K_i value similar to that for the first reaction (Table 7).

ANF has been widely used as an inhibitor of cytochrome P-448 catalysed activities (Wiebel & Gelboin, 1975). Although it is not implied in the

Table 4 Effects of debrisoquine on the two components of phenacetin *O*-deethylase activity of human liver

Concentration of debrisoquine (mM)	V_{max1} *(pmol mg^{-1} min^{-1})*	K_{m1} *(μM)*	V_{max2} *(pmol mg^{-1} min^{-1})*	K_{m2} *(μM)*
0	158*	6.1	1320	537
	(22)	(2.5)	(200)	(133)
0.5	184	23.8	1360	660
	(16)	(3.1)	(130)	(98)

*Michaelis-Menten parameters were estimated by iterative non-linear least squares regression analysis as previously described (Boobis *et al.*, 1981b). Values in parentheses are the radex errors. Data are mean of determinations with two different liver samples.

present study that the high affinity component of phenacetin *O*-deethylase in man is necessarily catalysed by cytochrome P-448, nevertheless ANF does provide a useful selective inhibitor of certain monooxygenase activities. Although it does inhibit the high affinity component of phenacetin *O*-deethylase (Boobis *et al.*, 1981b), if anything, it stimulates debrisoquine 4-hydroxylase activity of human liver. In addition, cigarette smoking was shown to cause a significant induction of the high affinity component of phenacetin *O*-deethylase activity, whereas debrisoquine 4-hydroxylase activity of cigarette smokers was not different to that of non-smokers.

As discussed above, there is ample evidence that phenacetin *O*-deethylase activity is impaired in the PM phenotype. However, equally there is considerable evidence that the two activities, phenacetin *O*-deethylase and debrisoquine 4-hydroxylase, are not catalysed by the same isozyme of cytochrome P-450 in man. The corollary of this, therefore, is that the PM phenotype possesses an impairment of two different isozymes of cytochrome P-450, the products of two separate, but closely linked genes. This would resolve the anomaly that phenacetin appears to be the only substrate for the debrisoquine 4-hydroxylating form of cytochrome P-450 which is not a polar base (Smith, 1985).

How, then, do we explain the inhibition of phenacetin *O*-deethylase activity by debrisoquine. At present it is only possible to speculate. It might be that the isozyme of cytochrome P-450 catalysing the high affinity component of phenacetin *O*-deethylase activity can also catalyse a small proportion, perhaps less than 10%, of debrisoquine 4-hydroxylase activity. Debrisoquine would thus be a substrate for this isozyme and hence one would predict it would be a

Table 5 Effect of phenacetin on debrisoquine 4-hydroxylase activity of human liver

*Experiment**	*Concentration of phenacetin (μM)*	*Concentration of debrisoquine (μM)*	*Debrisoquine 4-hydroxylase activity (% control)*
1	10	100	90.2
	100	100	93.5
	1000	100	97.7
2	10	200	108.0
	100	200	109.5
	1000	200	108.0
3	500	200	91.1
	1000	200	75.5
	2500	200	60.0

*Each experiment was performed on a separate occasion with a liver sample from a different patient.

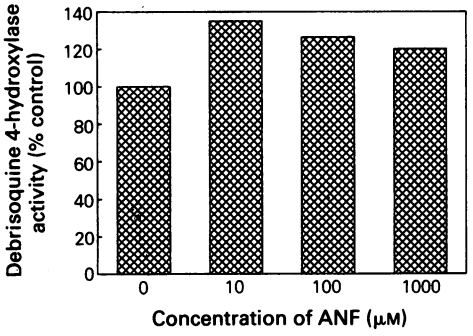

Figure 4 Effects of α-naphthoflavone on debrisoquine 4-hydroxylase activity. Results are mean of duplicate determinations, which were always within 10% of each other, on two different liver samples. The concentration of debrisoquine was 200 μM.

competitive inhibitor. With such a small percentage of debrisoquine 4-hydroxylation catalysed by this isozyme, it would not be possible to observe inhibition by phenacetin of debrisoquine 4-hydroxylase activity. Curiously, the K_i for inhibition by debrisoquine of phenacetin *O*-deethylase activity is similar to the K_m for the 4-hydroxylation reaction. If our hypothesis for this inhibition is correct, it would be necessary to postulate that the K_ms of the two isozymes for the 4-hydroxylation of debrisoquine were coincidentally almost identical. However, there is evidence that this might be so from studies by Thorgeirsson (1985) on the oxidation of 2-acetylaminofluorene by purified forms of rabbit cytochrome P-450. He found that there are populations of cytochromes P-450 with similar K_m values catalysing any one oxidation reaction. A similar situation might obtain for debrisoquine 4-hydroxylase activity in man. An alternative explanation for the effects of debrisoquine on phenacetin *O*-deethylase activity is that debrisoquine might bind to the active site of the enzyme but not serve as a substrate.

Table 6 Inhibition of phenacetin *O*-deethylase and debrisoquine 4-hydroxylase activities of human liver

Inhibitor	K_i (μM) *for inhibition of* *Phenacetin O-deethylase activity (high affinity component)*	*Debrisoquine 4-hydroxylase activity**
Guanoxan	170	30
Sparteine	2100	85
Debrisoquine	128	130+

*Data for inhibition of debrisoquine 4-hydroxylase activity are taken from Boobis *et al.* (1984).
+The K_m for debrisoquine 4-hydroxylase activity is shown for comparison.
Values of K_i for phenacetin *O*-deethylase activity were calculated from the corresponding IC_{50} values for the inhibitors and the K_m value for phenacetin *O*-deethylase activity.

Table 7 Inhibition of debrisoquine 4-hydroxylase and bufuralol 1′-hydroxylase activities of human liver

Inhibitor	K_i (μM) *for inhibition of* *Bufuralol 1′-hydroxylase activity*	*Debrisoquine 4-hydroxylase activity**
Bufuralol	12.8**	15.6
Debrisoquine	60	140**
Phenformin	59	205
Guanoxan	30	30
Sparteine	60	85
Acetanilide	8.1mM+	1.23mM+
Amylobarbitone	Stimulation	Stimulation

*Data for debrisoquine 4-hydroxylase activity are taken from Boobis *et al.* (1984).
**The values shown are for the respective K_ms, for comparison.
+Inhibition of both activities by acetanilide was non-competitive. In all other instances inhibition was competitive.

It is concluded, therefore, that the high affinity component of phenacetin *O*-deethylase activity is catalysed by an isozyme of cytochrome P-450 separate from that catalysing debrisoquine 4-hydroxylase activity. This isozyme might have modest ability to catalyse the oxidation of debrisoquine and perhaps sparteine and guanoxan. The activity is inducible by cigarette smoking in man. It appears to be regulated by a gene showing close linkage to that gene regulating the 4-hydroxylation of debrisoquine.

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