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SPECTRAL BINDING STUDIES OF THE POLYMORPHICALLY METABOLIZED DRUGS DEBRISOQUINE, SPARTEINE AND PHENFORMIN BY CYTOCHROME P-450 OF NORMAL AND HYDROXYLATION DEFICIENT RAT STRAINS

ADRIAN KÜPFER, SABAH G. AL-DABBAGH,* JAMES C. RITCHIE,* JEFFREY R. IDLE* and ROBERT L. SMITH*

Department of Clinical Pharmacology, University of Berne, Switzerland, and *Department of Pharmacology, St. Mary's Hospital Medical School, London, U.K.

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Abstract—The mechanisms of polymorphic drug hydroxylation of debrisoquine, sparteine and related drugs *in vivo* have been investigated using Cyt P-450 preparations of inbred rat strains as an *in vitro* model of the poor and extensive metabolizer phenotypes found in various rat strains and in man. Optical difference spectroscopy with debrisoquine, sparteine, phenformin and three other drugs (selected test compounds with proven or suspected hydroxylation polymorphisms in man) exhibited Type 1 binding in normal Sprague–Dawley, Fischer and Lewis Cyt P-450, whereas no Type I drug binding was found in the hydroxylation deficient DA rat liver Cyt P-450. Cyt P-450 content and Type II drug binding of metiamide was the same in normal and hydroxylation deficient rat liver microsomes. The pronounced Type I drug binding in extensive hydroxylation Cyt P-450 and the defective Type I binding in DA Cyt P-450 *in vitro*, therefore, closely parallels the polymorphic hydroxylation pattern of these test drugs found in the four rat strains studied *in vivo*. Consequently, missing binding properties of Cyt P-450 or of its micro-environment might represent the enzymatic defect underlying the genetically determined hydroxylation deficiency of polymorphically metabolized drugs in the poor metabolizer phenotype in the DA rat and, by inference, in man.

In recent years, several examples of genetic polymorphism of drug oxidation in man have been described. Thus, the oxidative metabolism of debrisoquine [1–6], sparteine [7] and phenformin [8] have been shown to exhibit polymorphism in the populations investigated. In each case a group of individuals, accounting for 1–9% of the population studied, were identified by their relative inability to effect the oxidative metabolism of these drugs. Impaired oxidative ability in the case of all these drugs has been shown to be a simple Medelian recessive trait. It has been proposed that in the case of debrisoquine metabolism, its oxidation is regulated by a single autosomal gene which is allelomorphic, the two alleles being referred to as the D^H (high hydroxylation) and D^L (low hydroxylation) alleles. Individuals homozygous for the D^L allele exhibit the so-called poor metabolizer (PM) phenotype, whilst heterozygotes and D^H homozygotes constitute a second and major extensive metabolizer (EM) phenotype. The hydroxylation of phenformin is controlled by the same gene regulating debrisoquine oxidation [9] and there are suggestions that this may also apply to sparteine oxidation [10].

The mechanistic basis, however, of the genetically determined impairment of drug oxidation in man is completely unknown. There are indications that it is a function of the microsomal mono-oxygenase system. Thus, liver microsomes obtained from a single PM subject failed to hydroxylate debrisoquine compared to preparations obtained from EM subjects, in spite of the fact that the PM biopsy contained quantitatively normal levels of Cyt P-450. Furthermore, the high-affinity component of phenacetin *O*-deethylation was markedly reduced in the PM liver microsomal preparation [11].

More recently, genetic polymorphism of debrisoquine oxidation, analogous to that found in man, has been described to occur in various rat strains [12]. One of these strains (DA) exhibited an impaired ability to metabolize both debrisoquine and phenacetin and it has thus been proposed that the DA and Lewis inbred rat strains might serve as animal models for the human PM and EM phenotypes, respectively. Additionally, such animal models might provide the basis of mechanistic studies which might be more difficult to achieve in man. This paper describes our studies of the Cyt P-450 spectral binding characteristics of a number of drugs, either known or thought to undergo polymorphic metabolic oxidation in man, using hepatic microsomal preparations from four strains of rat including the hydroxylation deficient DA strain. The findings described herein strongly suggest that the debrisoquine hydroxylation polymorphism observed in the rat, and possibly that observed in man, has its origins in grossly variable binding of so-called Type I binding substrates to Cyt P-450, whereas no apparent binding

Address for correspondence: Adrian Küpfer Ph.D., Dept. of Clinical Pharmacology, University of Berne, Murtenstrasse 35, CH-3010 Berne, Switzerland.

differences could be established with Type II difference spectra of, for example, metiamide between the various rat strains studied.

MATERIALS AND METHODS

Experimental animals. All rats weighed 200–250 g and were obtained from the following sources. Male and female Sprague–Dawley (Tuttlingen, F.R.G.); female Fischer (St. Mary's Hospital Medical School colony); female Lewis (Bantin and Kingman, Hull, U.K.); and female DA (Bantin and Kingman). The *in vivo* metabolism of debrisoquine has been previously described for a number of rat strains [12]. The ability to effect the benzylic 4-hydroxylation of debrisoquine was determined after the oral administration of debrisoquine hemisulfate (equivalent to 5 mg/kg free base) and by subsequent gas chromatographic analysis [13] of debrisoquine and 4-hydroxy-debrisoquine in the 0–24 hr urine.

Preparation of liver microsomes. Livers were perfused *in situ* with 10 ml of ice-cold saline (0.15 M) through the portal vein. The livers were homogenized in 5 vol. of 0.25 M sucrose with six up-and-down-strokes in a Dounce glass homogenizer. The homogenate was centrifuged at 900 *g* for 10 min, 9000 *g* for 15 min and at 19,000 *g* for 15 min. The supernatants were then centrifuged at 100,000 *g* for 60 min and the microsomal pellet was washed in potassium chloride (0.15 M) and recentrifuged at 100,000 *g* for 1 hr. The final pellet was resuspended in potassium phosphate buffer (0.1 M, pH 7.4) to give a final protein concentration of 2 mg/ml corresponding to 1.5 nmole Cyt P-450/ml approximately.

Protein was measured by a modified Lowry method [14] and Cyt P-450 concentrations by the Cyt P-450 carbon monoxide complex [15].

Spectral binding studies. An Aminco Chance DW-2 a u.v./vis spectrophotometer equipped with an Aminco Midan TM T microprocessor data analyser and an Aminco X-Y Plotter were used. The test compounds and their solvents were added to the sample and reference cuvettes, respectively, in 5 μl aliquots to reach the various drug concentrations given in Results. One ml disposable polystyrene semi-micro cuvettes (1 cm pathlength) were used and difference spectra were recorded between 350 and 500 nm wavelength in the split-beam mode. The spectra were recorded at a scanning speed of 10 nm/sec with a slit width of 3.0 nm band pass. After baseline subtraction by the Midan TM T microprocessor data analyser the difference spectra were plotted with a sensitivity of $E = 0.05$ full scale.

Spectral binding was quantitated for Types I and II difference spectra by the sum of absolute absorbances E at peak and through wavelengths of the two types of spectra observed. The ΔE values were plotted against substrate concentrations in the sample cuvette and the spectral dissociation constant ($K_d \pm$ S.D.) and the $\Delta E_{max} \pm$ S.D. values were calculated [16] according to the Michaelis–Menten equation. Linear transformation of these spectral binding data for each experiment yielded one single slope in the Scatchard plot and the respective linear correlation coefficients (r) were calculated by least square analysis.

Chemicals. The following drugs and metabolites were used to acquire binding spectra: debrisoquine hemisulfate, 4-hydroxy-debrisoquine (Hoffmann–La Roche, Basle, Switzerland), sparteine (Fluka Chemicals, Buchs, Switzerland), guanoxan hemisulfate (Pfizer Pharma Ltd, U.K.), perhexiline maleate (Richardson-Merrell Ltd, Slough, U.K.), metiamide (St. Mary's Hospital, London, U.K.) and hexobarbitone sodium (Evipan®, Bayer, Leverkusen, F.R.G.). 5-Dehydro-sparteine was obtained by the courtesy of Prof. M. Eichelbaum, Medizinische Universitäts-Klinik, Bonn, F.R.G. and phenformin hydrochloride was kindly donated by Dr L. J. King, University of Surrey, U.K.

Solvents for drugs. Unless otherwise stated, all compounds were freely soluble in 0.9% saline with a final concentration of 0.05 M HCl or NaOH for basic and acidic drugs, respectively. Perhexiline and metiamide were dissolved in 20% ethanolic HCl (0.15 M). Identical solvents were also prepared to be added to the reference cuvette.

In preliminary experiments the approximate K_d values were established in order to determine the appropriate concentrations for each drug for the quantitative spectral binding analysis. At least 6 data points were obtained from each compound (in triplicates) in the concentration range of $0.1 \times K_d$ to $10 \times K_d$.

RESULTS

Drug binding by male Sprague–Dawley Cyt P-450 (extensive hydroxylation microsomes)

Seven drugs, three of which are polymorphically metabolized in man and four for which there exists an indication of human polymorphic metabolism, have been analysed for their spectral binding characteristics with both extensively hydroxylating (normal) rat microsomes (Sprague–Dawley, Lewis and Fischer strains) and defective hydroxylation microsomes (DA strain). With normal rat liver microsomes, all compounds showed either a blue shift (Type I spectrum) or a red shift (Type II spectrum) clearly indicating a positive interaction with Cyt P-450. Six substrates exhibited Type I binding (debrisoquine, sparteine, guanoxan, hexobarbitone, perhexiline and phenformin) whilst metiamide displayed a Type II spectrum (Table 1). In all analyses, spectral changes were concentration dependent and showed saturability with increasing amounts of drug added. Thus the spectral dissociation constant (K_d) could be calculated as the concentration at which half of the maximum spectral binding occurred per μmole Cyt P-450. Table 1 summarizes the results obtained with 7 substrates using liver microsomes from the normal extensive hydroxylation Sprague–Dawley rat strain.

In Sprague–Dawley microsomes, the range of K_d values of polymorphically metabolized Type I substrates ranged from 0.5 μM using sparteine to 8.5 μM using debrisoquine (Table 1), whereas for metiamide, the only Type II substrate found, the K_d value was 58 μM. The ΔE_{max} values varied by a factor of 1.2 between the Type I substrates sparteine and debrisoquine, whereas ΔE_{max} of the Type II substrate

Table 1. Spectral dissociation constants (K_d) and maximal spectral binding (ΔE_{max}) of some known and putative polymorphically metabolized drugs in extensive hydroxylator male Sprague–Dawley rat liver microsomes

Drug	Peak nm	Trough nm	Type	K_d (μM)	ΔE_{max}/μmole Cyt P-450	r*
Debrisoquine	390	428	I	8.5 ± 1.4	11.079 ± 0.665	0.9801
Sparteine	389	426	I	0.49 ± 0.04	13.513 ± 0.405	0.9947
Guanoxan	390	428	I	1.0 ± 0.06	11.953 ± 0.299	0.9992
Hexobarbitone	390	428	I	50.5 ± 8.4	18.006 ± 0.580	0.9989
Perhexiline	392	428	I	2.7 ± 0.23	10.380 ± 0.156	0.9848
Phenformin	390	426	I	1.6 ± 0.2	13.952 ± 0.279	0.9897
Metiamide	438	398	II	58.0 ± 1.3	28.763 ± 0.863	0.9996

* r = correlation coefficient (see text).

metiamide was approximately twice of that found with Type I ligands (Tables 1 and 3). Linear transformation of the binding data for each drug showed that only one binding site was involved in the drug–enzyme interaction, as judged by graphical analysis of the Scatchard plots and by the correlation coefficients (r; see Table 1 *et seq.*) [16].

Drug binding to Lewis and Fischer Cyt P-450 (extensive hydroxylation microsomes)

Similar experiments were performed with microsomal liver fractions from female Lewis and Fischer rats. Both strains are extensive hydroxylators of debrisoquine *in vivo* [12]. Table 2 gives the K_d and ΔE_{max}/μmole Cyt P-450 values for these two strains. In agreement with the known debrisoquine *in vivo* metabolic data for three rat strains, little difference was observed in the K_d values for Type I substrates. Maximal spectral binding of the 5 drugs studied was up to 2-fold higher for sparteine and phenformin in the Lewis rat, whereas maximal binding of guanoxan and perhexiline was 1.5 times greater in the Fischer rat. The largest difference between Sprague–Dawley, Lewis and Fischer strains was observed in the K_d value of Type II binding of metiamide (Table 3).

The K_d value for male Sprague–Dawley Cyt P-450–metiamide interaction was 58 μM, whilst for female Fischer and Lewis Cyt P-450 the corresponding values were 213 and 251 μM, respectively. The K_d value for female DA Cyt P-450 (*vide infra*) was even higher at 280 μM. Little variation between any of the four strains was discernible in maximum Type II drug binding as expressed by ΔE_{max} per μmole Cyt P-450 (Fig. 3, Table 3).

Drug binding to DA Cyt P-450 (hydroxylation deficient microsomes)

Female animals of the inbred DA rat strain have been proposed as an animal model for the poor metabolizer phenotype of drug oxidation in man, because, unlike other strains of rat studied, they excrete some 4–5-fold greater quantity of unchanged debrisoquine than 4-hydroxy-debrisoquine [12]. In this latter study, females of other strains of rat, including Lewis and Fischer, eliminated 2–10 times more 4-hydroxy metabolite than unchanged debrisoquine after an oral dose of 5 mg/kg debrisoquine, which is similar to the findings for the human extensive metabolizer (EM) phenotype. Accordingly, it was of interest to examine the spectral binding characteristics of debrisoquine and several other drugs with Cyt P-450 fractions obtained from female DA rat livers.

Figure 1 shows a comparison between Lewis (on top) and DA Cyt P-450 (on the bottom) with respect to debrisoquine binding. Even at concentrations of debrisoquine up to 10 times those used when attaining a Type I interaction with Lewis Cyt P-450, only a modest spectral blue shift could be seen with DA Cyt P-450, allowing a tentative assignment of a Type I interaction. No quantitative analysis could be performed with such weak difference spectra and the issue of the relative affinity comparison by K_d calculations thus remains unaccomplished. However, the ΔE values per nmole Cyt P-450 at these excessive substrate concentrations might be considered as ΔE_{max} values, in which case only 14–16% of the Lewis ΔE_{max} values could be recorded with DA Cyt P-450 for debrisoquine (Fig. 1), sparteine (Fig. 2),

Table 2. Spectral dissociation constants (K_d) and maximal spectral binding (ΔE_{max}) of some known and putative polymorphically metabolized drugs in microsomes of female Lewis and Fischer rats

Drug	Type	K_d (μM)		ΔE_{max}/μmole Cyt P-450		r*	
		Lewis	Fischer	Lewis	Fischer	Lewis	Fischer
Debrisoquine	I	9.8 ± 1.8	10.9 ± 1.4	6.783 ± 0.339	7.656 ± 0.383	0.9974	0.9954
Sparteine	I	0.56 ± 0.03	0.37 ± 0.13	8.201 ± 0.246	3.190 ± 0.298	0.9997	0.9511
Guanoxan	I	0.74 ± 0.14	0.25 ± 0.04	6.278 ± 0.188	10.276 ± 0.424	0.9991	0.9843
Perhexiline	I	2.9 ± 0.4	1.4 ± 0.3	5.101 ± 0.102	7.512 ± 0.353	0.9870	0.9424
Phenformin	I	3.6 ± 0.2	2.6 ± 0.4	7.849 ± 0.157	3.785 ± 1.140	0.9976	0.9824

* r = correlation coefficient (see text).

Table 3. Type II binding parameters of metiamide and Cyt P-450 content in liver microsomes of various rat strains

Strain	Cyt P-450 content (nmole/mg microsomal protein)	K_d metiamide (μM)	ΔE_{max}/μmole Cyt P-450 metiamide	r*
Sprague–Dawley	0.688 ± 0.117	58.0 ± 1.3	28.763 ± 0.863	0.9999
Lewis	0.882 ± 0.146	251.0 ± 15.0	25.851 ± 0.776	0.9980
Fischer	0.911 ± 0.016	212.6 ± 6.2	22.968 ± 0.255	0.9985
DA	0.667 ± 0.291	280.0 ± 16.5	28.516 ± 0.855	0.9899

* r = correlation coefficient (see text).

guanoxan, hexobarbitone, perhexiline and phenformin (Table 4). By contrast and as stated earlier, the Type II binding characteristics of metiamide to DA, Lewis, Fischer and Sprague–Dawley Cyt P-450 were comparable (Fig. 3, Table 3). None of the differences found in the various rat strains in respect to Type I and Type II spectral binding of drugs could be explained by their total microsomal Cyt P-450 contents since they were essentially the same (Table 3) with a tendency for female Lewis and Fischer rats to be slightly higher than male Sprague–Dawley microsomes.

Drug metabolite binding to Sprague–Dawley Cyt P-450

There is some evidence in certain rat strains that 4-hydroxy-debrisoquine may be further metabolized in part to a dihydroxy derivative, possibly a 4,6-dihydroxy-debrisoquine [12, 17]. In addition, urinary and plasma pharmacokinetic studies of sparteine disposition in man would seem to suggest the presence of hitherto unrecognized metabolites, possibly arising from secondary metabolism of 2- and 5-dehydro-sparteine metabolites [7, 17]. It was therefore of interest to investigate whether or not both 4-hydroxy-debrisoquine and 5-dehydro-sparteine showed any major interaction with Cyt P-450. Table 5 gives the K_d and ΔE_{max} values for these two metabolites with the extensively metabolizing male Sprague–Dawley Cyt P-450 microsomal preparations. In the case of debrisoquine, the metabolite has some 7-fold lower affinity with a Type I spectrum of comparable ΔE_{max}, whilst for sparteine the metabolite had approximately 15-fold lower affinity than the parent compound with a Type I spectrum of about half ΔE_{max}. One possible interpretation of these findings would be a confirmatory view that both primary metabolites of sparteine and debrisoquine may be further metabolized to a minor extent in certain rat strains.

DISCUSSION

Among four strains of rats with variable degrees of drug hydroxylation capabilities, a nearly total lack of Type I spectral binding of some known and putative polymorphically metabolized drugs could be detected in liver microsomes of the hydroxylation

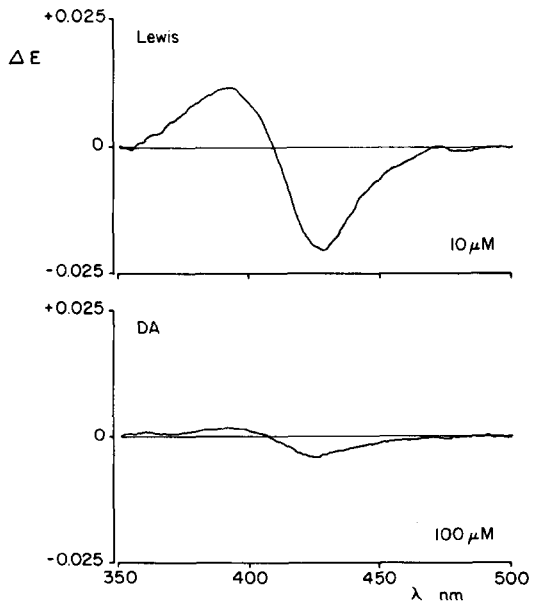

Fig. 1. Type I binding spectra of the polymorphically metabolized drug debrisoquine in rat liver microsomes (approx. 1 μM Cyt P-450 each). Top panel: drug binding at 10 μM drug concn (K_d debrisoquine) by extensive hydroxylation Lewis microsomes. Bottom panel: drug binding at 10 × K_d drug concn by hydroxylation deficient DA microsomes.

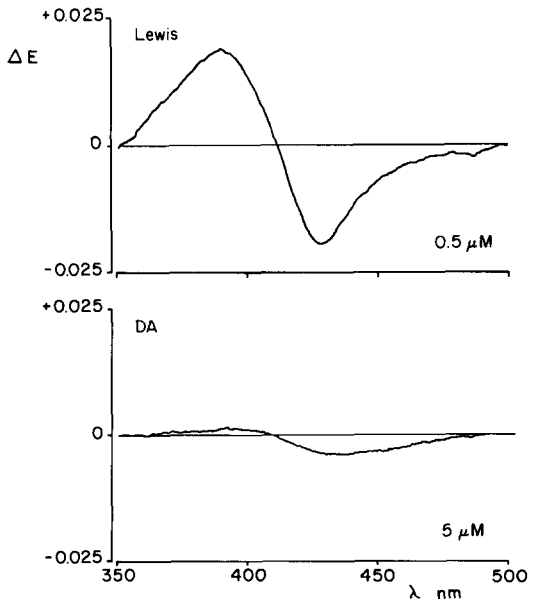

Fig. 2. Type I binding spectra of the polymorphically metabolized drug sparteine in rat liver microsomes (approx. 1 μM Cyt P-450 each). Top panel: drug binding at 0.5 μM drug concn (K_d sparteine) by extensive hydroxylation Lewis microsomes. Bottom panel: drug binding at 10 × K_d drug concn by hydroxylation deficient DA microsomes.

Table 4. Maximal spectral binding of some known and putative polymorphically metabolized drugs in microsomes of hydroxylation deficient female DA rats

Drug	Peak nm	Trough nm	Type	Concentration (μM)	ΔE_{max}/μmole Cyt P-450	% ΔE_{max} of Sprague–Dawley
Debrisoquine	390	430	I	75	1.577	14
Sparteine	390	420	I	10	1.936	14
Guanoxan	390	430	I	10	1.864	16
Hexobarbitone	390	430	I	1550	2.294	17
Perhexiline	390	430	I	80	1.577	15
Phenformin	390	420	I	625	2.940	21
Metiamide	440	400	II	1000	28.516	99

deficient DA rat, whereas the microsomes of the normally hydroxylating Sprague–Dawley, Lewis and Fischer rats exhibited extensive drug binding to variable extents. The defective drug binding in DA liver microsomes closely ressembles the *in vivo* hydroxylation pattern of this rat strain which has been proposed as an animal model for the poor metabolizer phenotype in human pharmacogenetics [12]. With six drugs, extensive Type I spectral binding was found in normal Lewis, Fischer and Sprague–Dawley rats (Tables 1 and 2), whereas the same compounds failed to show Type I spectral binding in the DA rat. The Type I spectral binding defect compared to apparently normal Type II binding of metiamide, expresses itself with all drugs with established polymorphic hydroxylation in man (debrisoquine, sparteine and phenformin) and might, therefore, reflect the molecular mechanism underlying to the defective hydroxylation of these substrates in the poor metabolizer phenotype in the DA rat and—by inference—in man. Therefore, a genetically determined defect at the level of the Cyt P-450 containing membrane structures seems to be directly responsible for the hydroxylation deficiencies observed in two different species *in vivo*.

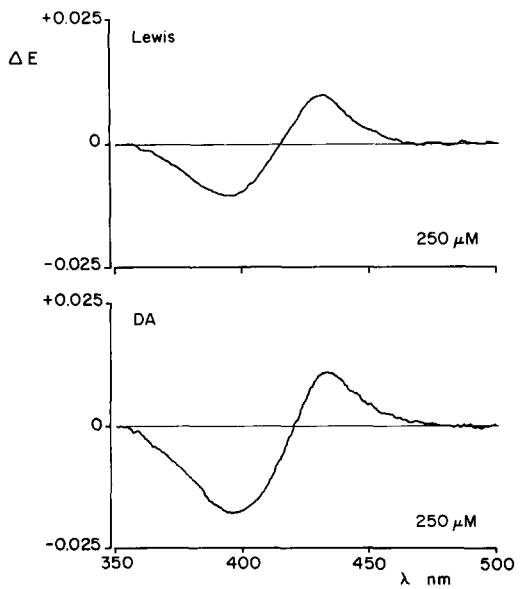

Fig. 3. Type II binding spectra of metiamide in rat liver microsomes (approx. 1 μM Cyt P-450 each). Top panel: drug binding at 250 μM drug concn (K_d metiamide) by extensive hydroxylation Lewis microsomes. Bottom panel: drug binding at 250 μM drug concn by hydroxylation deficient DA microsomes.

Binding of a drug to Cyt P-450 represents the first step in the whole catalytic cycle of substrate mono-oxygenation. This first step of drug binding depends only on the presence of oxidized Cyt P-450 and the respective ligand. After drug–Cyt P-450 complex formation several additional steps are required prior to metabolite formation, including the rate limiting reduction of the drug–Cyt P-450 complex by NADPH Cyt P-450 reductase (Cyt *c* reductase). Oxidized Cyt P-450 changes its spin state from low to high spin following the addition of a suitable ligand to the microsomal suspension [18]. Several techniques including nuclear magnetic resonance spectroscopy [19], electron spin resonance spectroscopy [20, 21] and circular dichroism [22] have been used to measured the low to high spin conversion of Cyt P-450. Since the changes in spin state are also visible by slightly different absorbance characteristics of the Soret band of the octahedral iron atom in protoporphyrin IX at around 420 nm wavelength, the drug–Cyt P-450 complex formation can also be monitored by optical difference spectroscopy.

Therefore, missing binding properties of the respective Cyt P-450 should be clearly visible in form of a defective spectral binding of a given substrate. To test this hypothesis, a set of circumstances had to be found where *in vivo* differences in drug metabolism were large enough to overcome the methodological difficulties inherent to the *in vivo–in vitro* comparison of drug metabolism. In the systematic search of an animal model for the genetically determined deficiency of debrisoquine and sparteine hydroxylation, the female DA rat emerged as a possible candidate for spectral binding studies because of its relative inability to hydroxylate debrisoquine, a situation which closely resembles the condition encountered in the poor metabolizer phenotype in man. Three out of seven substrates (Table 1) have already been reported to exhibit polymorphic metabolism in man and a number of others are suspected [1, 2, 7, 23]. These should therefore represent the drugs of choice to detect any abnormalities of spectral binding in DA liver microsomes, providing the underlying mechanisms of defective debrisoquine and sparteine hydroxylation are the same in human poor metabolizer phenotypes and in DA rats. With six substrates, extensive Type I spectral binding was found in normal Fischer, Lewis and Sprague–Dawley rats (Tables 1 and 2) whereas the

Table 5. Spectral dissociation constants (K_d) and maximal spectral binding (ΔE_{max}) of 4-hydroxy-debrisoquine and 5-dehydro-sparteine in extensive hydroxylator male Sprague–Dawley rat liver microsomes

Drug	Peak nm	Trough nm	Type	K_d (μM)	ΔE_{max}/μmole Cyt P-450	r*
4-Hydroxy-debrisoquine	388	425	I	57.9 ± 5.5	12.076 ± 0.483	0.9938
5-Dehydro-sparteine	385	426	I	7.8 ± 0.2	6.754 ± 0.135	0.9996

* r = correlation coefficient (see text).

same drugs failed to exhibit Type I binding in DA rats (Table 4, Figs. 1 and 2).

The fact that all Type I spectral binding substrates exhibit binding deficiencies in DA rats compared to normal Sprague–Dawley, Fischer, and Lewis rats is highly suggestive that drug binding indeed is the major determinant for the initiation of the catalytic cycle. Also the fact that deficiencies occur so far with Type I binding substrates only is highly indicative that the binding defect might be located at a specific molecular interaction site of the micro-environment of the heme moiety [24, 25]. From these considerations and from the selection criteria for the substrates used it is not surprising that the only Type II binding substrate (metiamide) used in these studies did not show interstrain binding spectral differences, even in the DA rat (Table 3).

Of interest is the observation that the total content of the Cyt P-450 is obviously not the explanation for the inter-strain differences observed in Type I binding. DA and Lewis microsomes contained 0.67 ± 0.29 and 0.88 ± 0.15 nmole Cyt P-450/mg protein (mean ± S.D.) respectively, yet showed remarkable differences in Type I binding for all six Type I substrates studied, whereas the Type II binding of metiamide was not significantly different between these two strains. In addition, no major sex difference could be found in respect to Type I binding of debrisoquine and sparteine between male Sprague–Dawley microsomes (Table 1) and female Lewis and Fischer microsomes (Table 2), respectively. It is possible, therefore, that the interstrain *in vivo* metabolic differences observed in the rat [12] are the reflection of qualitative (polymorphic) differences in a form of Cyt P-450 which is *in toto* or in part responsible for the metabolic oxidation of Type I binding substrates such as debrisoquine. This, by inference, may also be the case in man, where some 1–9% of various ethnic populations have an inherited relative deficiency of debrisoquine, sparteine and phenformin metabolism. In these persons, it might be viewed that the gene product of the homozygous recessives (D^LD^L) is a variant Cyt P-450 with little binding capacity for Type I substrates, such as those described in this paper. The single study to date [11] of debrisoquine metabolism in human EM and PM liver biopsies would seem to support this view. Nevertheless, alternative explanations for the deficient Type I binding in DA rat microsomes might involve a tightly bound endogenous ligand preventing the interaction with Type I substrates. Consequently, modifications of the lipophilic micro-environment [26], by, for example, delipidation procedures [25] could possibly answer these questions. In conclusion, it would seem a potentially attractive concept that relatively simple spectral binding investigations, using, for example, DA in combination with Sprague–Dawley, Lewis or Fischer microsomal preparations, might reveal additional candidates for polymorphic drug oxidation in the human population, due to the close parallelism between the interstrain metabolic handling of drugs like debrisoquine and the interphenotype metabolic differences seen in man.

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