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ENZYMATIC BASIS OF THE DEBRISOQUINE/SPARTEINE-TYPE GENETIC POLYMORPHISM OF DRUG OXIDATION

CHARACTERIZATION OF BUFURALOL 1′-HYDROXYLATION IN LIVER MICROSOMES OF *IN VIVO* PHENOTYPED CARRIERS OF THE GENETIC DEFICIENCY*

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Abstract—The genetically controlled polymorphic oxidation of debrisoquine and sparteine is caused by the absence or functional deficiency of a cytochrome P-450 isozyme. In order to elucidate the mechanisms underlying the differences in cytochrome P-450 function we have studied the 1′-hydroxylation of the prototype drug bufuralol in human liver microsomes of individuals phenotyped *in vivo* as extensive metabolizers (EM, N = 10), poor metabolizers (PM, N = 5) and in subjects with an intermediate rate of metabolism (IM, N = 4).

PM- as compared to EM-microsomes were characterized by a decreased V_{max} for (+)-bufuralol 1′-hydroxylation (7.51 ± 2.03 nmol × mg^{-1} × hr^{-1} vs 11.95 ± 4.80 nmol × mg^{-1} × hr^{-1}) but not for (−)-bufuralol 1′-hydroxylation (4.72 ± 0.87 nmol × mg^{-1} × hr^{-1} vs 5.55 ± 1.49 nmol × mg^{-1} × hr^{-1}). The apparent K_m for (+)-bufuralol 1′-hydroxylation was increased in PM microsomes (118 ± 84.9 μM vs 17.9 ± 6.30 μM).

Inhibition of bufuralol 1′-hydroxylation by quinidine was biphasic in EM microsomes, providing further support for the involvement of at least two cytochrome P-450 isozymes. Quinidine acted as a competitive inhibitor of only the high affinity/stereoselectivity component of the reaction. Our data suggest that the debrisoquine/sparteine type of oxidation polymorphism is caused by an almost complete loss of a minor cytochrome P-450 isozyme which has a high affinity and stereoselectivity for (+)-bufuralol and a high sensitivity to inhibition by quinidine.

The intensity and duration of the effect of many clinically useful drugs depends on the activities of drug metabolizing enzymes in the liver. Of primary importance, among them, are the microsomal polysubstrate monooxygenase, a family of hemoprotein isozymes collectively known as cytochrome P-450 [1–4]. Marked interindividual differences in the rate of drug oxidation by cytochrome P-450 isozymes occur. These isozymes are distinguishable by differences in physical, chemical and immunological properties which frequently are based on only minor differences in aminoacid sequence but may have major effects on substrate specificity [1–4]. Efforts to elucidate the mechanisms underlying differences in cytochrome P-450 function have been hampered by the extreme difficulty to separate the influence of each isozyme on a defined metabolic reaction.

The recent identification of several genetic polymorphisms of drug oxidation related to cytochrome P-450 function has offered the opportunity to discover more specific substrates for given cytochrome P-450 isozymes and to study in more detail the mechanisms causing interindividual variations in drug metabolism. Independently occurring polymorphic drug oxidations with monogenic inheritance have been described for the metabolism of numerous drugs [5–10].

One of the best studied examples of a genetically controlled polymorphism of hepatic cytochrome P-450 function is the debrisoquine/sparteine-type polymorphism [6, 11–13]. The metabolism of debrisoquine and sparteine is impaired in 7–10% of the white population in Europe and North America [11, 14]. Family studies have indicated that "poor metabolizers (PM)" of debrisoquine and sparteine are homozygous for an autosomal recessive gene [11]. PM-subjects also exhibit an impaired oxidation of numerous other drugs including other antiarrhythmic agents [14–16], beta-adrenoceptor blocking drugs [17–26], antidepressants [27–30], and other clinically useful drugs [31–35]. There is convincing evidence that PMs represent a singular subgroup of the population with a propensity to develop adverse drug effects [36, 37]. Recent studies also have indicated that a link might exist between the debrisoquine/sparteine-type polymorphism and some forms of cancer [38] or with Parkinson's disease [39] presumably related to environmental chemicals.

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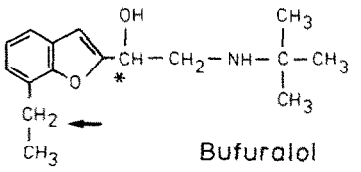

Fig. 1. The prototype drug bufuralol is a beta-adrenoceptor blocking agent. The arrow points to the site of 1'-hydroxylation. The asterisk indicates the asymmetric carbon center generating the two optical isomers.

Studies in this and other laboratories have suggested that the absence or functional deficiency of a particular cytochrome P-450 isozyme may cause this polymorphism [40–43]. In an attempt to define further this enzymatic deficiency we undertook *in vitro* metabolic studies in human liver microsomes from subjects phenotyped *in vivo* as PM or "extensive metabolizers (EM)". The prototype reaction we used to monitor this cytochrome P-450 function *in vitro* is bufuralol* 1'-hydroxylation [42–46] (Fig. 1).

As an additional tool to characterize the variant enzyme we used inhibition studies with the specific inhibitor quinidine [46–49].

MATERIALS AND METHODS

Subjects. The experimental protocol for *in vivo* testing and use of material from liver biopsies was approved by the ethical review boards (Department of Medicine, Universities of Zurich and Bonn). Human liver samples were obtained as wedge biopsies at laparotomy for diagnostic or therapeutic reasons. During their hospitalization patients were phenotyped and characterized *in vivo* as extensive or poor metabolizers as described below. None of them had abnormal liver histology and liver or kidney function tests. For the present study 19 subjects were selected. Their mean age was 54.6 years (range 25–71 years). Ten were classified as extensive metabolizers, 5 as poor metabolizers and 4 subjects were selected because they had an "intermediate" pattern of metabolism as defined below.

Phenotyping procedure. The *in vivo* phenotype was defined by means of the sparteine urinary metabolic ratio (17 subjects) and by the debrisoquine urinary metabolic ratio (2 subjects—Table 2). Patients received (after an overnight fast) either a 100 mg oral dose of sparteine sulfate or 10 mg of debrisoquine. Urine was collected for the next 8 hr (debrisoquine) or 12 hr (sparteine) and stored at −20° until assayed [11, 50]. A metabolic ratio of above 20 for sparteine (sparteine/2- and 5-dehydrosparteine [50]) or above 12.6 for debrisoquine (debrisoquine/4-hydroxydebrisoquine [11]) has been reported to be indicative of the PM phenotype which corresponds in pedigree studies to the homozygous genotype for the recessive allele. This was the case for 5 subjects (3 PM for sparteine and 2 for debrisoquine).

Ten subjects had a metabolic ratio below 1, characteristic of the EM phenotype. Four subjects with an "intermediate" rate of sparteine metabolism (IM), a rare occurrence, with metabolic ratios ranging from 1.91 to 18.4 were also included in this study.

Subcellular fractionation. Wedge biopsies were immediately frozen in liquid nitrogen and stored at −80°. For preparation of microsomes, small samples (50–150 mg wet weight) were homogenized in 5 vol. of 0.25 M sucrose, with a Polytron homogenizer (Kinematica, Kriens, Switzerland) three times for 30 sec at 17,000 rpm with 1 min cooling intervals in between. The homogenate was centrifuged in an Eppendorf 5414 centrifuge at 10,000 *g* for 5 min. The pellet was resuspended in 0.5 ml sucrose and again centrifuged. Both supernatants were combined, fractionated in 180 μl aliquots and centrifuged for 12 min at 148,000 *g* (Airfuge, Beckman, Palo Alto, CA). The pellets were resuspended in 0.1 M Na-pyrophosphate, pH 7.4, and centrifuged for 12 min at 148,000 *g*. This washing step was repeated again and the final pellets resuspended in 0.1 M $NaPO_4$, pH 7.4, and stored at −80°. The whole procedure was carried out in a cold room (<5°). The protein content, estimated with the Lowry method [51], ranged from 2.21 to 5.66 mg/ml and the content of spectrally measured cytochrome P-450 [52] from 0.099 to 0.366 nmole cytochrome P-450/mg protein. The yield of total cytochrome P-450 and specific bufuralol 1'-hydroxylase activity are, with this micromethod, comparable to conventional preparations: in 6 EM livers from kidney donors and using a classical method of preparation of microsomes [41], we measured a specific activity of 0.329 ± 0.055 nmol cytochrome P-450/mg protein (mean ± SD) and a V_{max} for (+)-bufuralol 1'-hydroxylation of 10.96 ± 6.00 nmol × mg protein^{-1} × hr^{-1} as compared to 0.271 ± 0.137 nmol cytochrome P-450/mg protein and a V_{max} of 11.95 ± 4.80 nmol × mg protein^{-1} × hr^{-1}, respectively, for the method used in this study.

Incubations. Twenty-five to 50 μg of microsomal protein (2.5–10 pmoles of total cytochrome P-450) were incubated in a final volume of 250 μl of 0.1 $NaPO_4$ containing an NADPH regenerating system (1 mM NADP, 5 mM isocitrate, 5 mM $MgCl_2$ and 1 unit of isocitrate dehydrogenase-type IV (Sigma, St. Louis, MO)). The system was preincubated at 37° for 5 min before addition of chemically pure (+)- or (−)-bufuralol (F. Hoffman-La Roche Ltd., Basel, Switzerland) at final concentrations ranging from 6 to 800 × 10^{-6} M. For the inhibition experiments, quinidine sulfate (Aldrich Chemical Company Inc., Milwaukee, WI) dissolved in 0.1 M $NaPO_4$ was added at the same time as (+)-bufuralol, at final concentrations ranging from 10^{-8} to 10^{-5} M. Under these conditions the rate of 1'-hydroxybufuralol formation was linear with time for up to 1 hr. The reaction was stopped after 40 min by cooling on ice and addition of 20 μl of 60% $HClO_4$. Protein was sedimented by centrifugation at 10,000 *g* for 1 min and supernatants stored at −80° until analyzed.

Assay of 1-hydroxybufuralol. The production of 1'-hydroxy-bufuralol was determined in $HClO_4$ supernatants by reverse-phase HPLC as previously described [53].

* Bufuralol (7-ethyl-α(ter-butylamino)-methyl-2-benzofuran methanol).

Data analysis and statistics. Untransformed kinetic data were analyzed by means of a non-linear least square curve fitting program which allows an observer-independent weighting of the data [54]. All the data from the kinetic study were treated according to Michaelis–Menten type kinetics which offered adequate description of the results in the substrate range ($6–800 \times 10^{-6}$ M). However, in 4 of 10 EM microsomes a statistically significant improvement of the curve fit was achieved by the application of a biphasic kinetic model with a high and low affinity component of the enzymatic reaction. For the comparison between the simple and the double enzyme activity models we used the full versus restricted model hypothesis [54]. An improvement of the fit, when using a 2 enzyme kinetics model, was considered significant when the difference between the goodness of the fit parameters (2 log likelihood) of each model evaluation reached a significant value using the Chi^2 distribution [54]. The inhibition kinetics required consistently a two enzyme component model to reach adequate curve fitting. The most appropriate model for this purpose is described by the following relationship

$$V\frac{V_{max_a} \cdot S}{(S + K_{m_a})} + \frac{V_{max_b} \cdot S}{S + K_{m_b} \cdot (1 + I/K_i)} \quad (1)$$

where V_{max} is the maximal velocity of the reaction, K_m the Michaelis constant, K_i the dissociation constant of the enzyme + inhibitor complex, S the substrate concentration, I the inhibitor concentration, and a or b the low or high affinity component respectively. In this model only the high affinity component is competitively inhibited by quinidine and the K_m of the low affinity component was not influenced by the inhibitor.

For the correlations (*in vivo* data versus *in vitro* results) and in view of the unknown distribution of the data, the non-parametric Spearman rank correlation was used [55]. For group comparisons the non-parametric Wilcoxon–Mann–Whitney test was used [55].

Within groups of a defined phenotype, intra-subject comparisons were assessed by the paired *t*-test.

RESULTS

Kinetics of bufuralol 1'-hydroxylation in extensive metabolizer microsomes

The microsomal formation of 1'-hydroxy-bufuralol from the (+)- and the (−)-isomer of bufuralol are shown in Fig. 2 and in Tables 1 and 2. As judged by a mean ratio of 0.5 for the V_{max} of (−)-bufuralol hydroxylation to the V_{max} of (+)-bufuralol hydroxylation the (+)-enantiomer is oxidized twice as fast as the (−)-enantiomer, an observation in accordance with *in vivo* data [56]. Interestingly, the apparent overall Michaelis constant is not significantly different between both isomers. In the four microsomal preparations where a biphasic enzymatic reaction was detected, the difference between K_m values of the high and of the low affinity component was 10-fold in the absence of significant differences in the V_{max} values (Table 2).

Table 1. Enzymatic characteristics of (+)- and (−)-bufuralol 1'-hydroxylation in microsomes of extensive and poor metabolizers and in microsomes of patients with an intermediate rate of sparteine metabolism *in vivo* (mean ± SD (range), data analyzed according to Michaelis–Menten kinetics)

In vivo phenotype	Number	Substrate	V_{max} (nmol × mg^{-1} × hr^{-1})	K_m* (μM)	(−)/(+) ratio†
Extensive metabolizers (MR: 0.18–0.81)‡	10	(+)-bufuralol	11.95 ± 4.80 (7.17–22.3)	17.9 ± 6.30 (9.56–27.5)	
		(−)-bufuralol	5.55 ± 1.49 (3.79–8.45)	21.8 ± 11.0 (7.33–39.5)	0.49 ± 0.09 (0.37–0.65)
			P < 0.0005§	NS	
Poor metabolizers (MR: 52–>200)	5	(+)-bufuralol	7.51 ± 2.03 (5.42–9.93)	118 ± 84.9 (38.0–218)	
		(−)-bufuralol	4.72 ± 0.87 (3.58–5.92)	93.0 ± 64.3 (29.6–166)	0.65 ± 0.12 (0.50–0.81)
			P < 0.05	NS	
"Intermediate" metabolizers (MR: 1.91–18.4)	4	(+)-bufuralol	5.03 ± 1.29 (3.77–6.81)	83.4 ± 48.6 (40.4–151)	
		(−)-bufuralol	3.28 ± 0.87 (2.57–4.46)	101 ± 67.6 (48.3–190)	0.65 ± 0.07 (0.56–0.71)
			NS	NS	

* Measured at substrate concentrations between 6.25 and 800 μM.
† Ratio of (−)-bufuralol V_{max}/(+)-bufuralol V_{max}.
‡ MR sparteine urinary metabolic ratio, two poor metabolizers were phenotyped by means of the debrisoquine urinary metabolic ratio and had MRs of 34.4 and 66.
§ P relates to (+)-versus (−)-isomer comparison.

Table 2. Enzymatic characteristics of (+)-bufuralol 1'-hydroxylation in microsomes of 4 extensive metabolizers with biphasic kinetics

	V_{max} (nmol × mg $P^{-1} \cdot hr^{-1}$)*	K_m (μM)*
High affinity component	4.64 ± 1.03 (3.12–5.38)	7.34 ± 2.45 (3.95–9.26)
Low affinity component	5.65 ± 1.83 (3.83–7.67)	204 ± 154 (76.9–428)

* K_m was measured at substrate concentrations between 6.25 and 800 μM. The data represent means ± SD (range), the data are fitted according to equation 1 (see Methods).

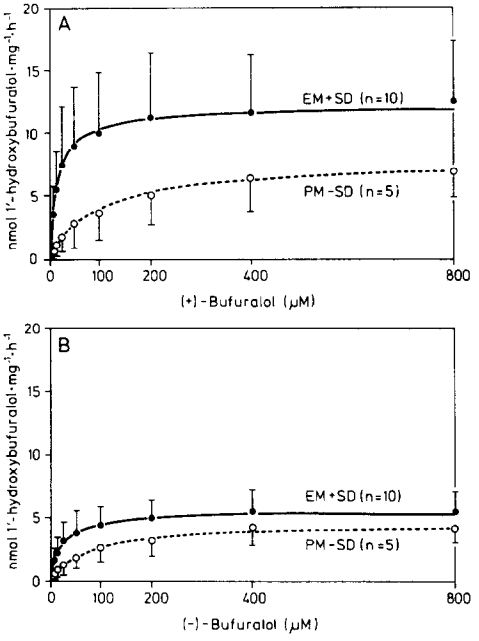

Fig. 2. Kinetics of (+)-bufuralol (A) and (−)-bufuralol (B) 1'-hydroxylation in 10 extensive (●) and 5 poor (○) metabolizer subjects phenotyped *in vivo* (debrisoquine/sparteine type of oxidation polymorphism). Values are expressed as mean ± SD.

Influence of the poor metabolizer (PM) phenotype on microsomal kinetics of bufuralol 1'-hydroxylation

PM- microsomes (N = 5) in comparison to EM-microsomes (N = 10) were characterized by a decreased V_{max} for (+)-bufuralol hydroxylation (7.51 nmoles 1'-hydroxybufuralol × mg protein^{-1} × hr^{-1} vs 11.95 nmoles 1'-hydroxy-bufuralol × mg protein^{-1} × hr^{-1} in EM, $P < 0.05$) but showed no decrease of the V_{max} for hydroxylation of (−)-bufuralol (4.72 nmol 1'-hydroxybufuralol × mg protein^{-1} × hr^{-1} vs 5.55 nmol 1'-hydroxybufuralol × mg protein^{-1} × hr^{-1}).

This resulted in a decrease stereoselectivity of the reaction as indicated by an increase in the (−)/(+) ratio. Another major characteristic of the PM microsomes is a 5-fold increase in the apparent Michaelis constant (mean of 118 μM vs 17.9 μM for (+)-bufuralol in EM microsomes, $P < 0.005$ and 93 μM vs 21.8 μM, for (−)-bufuralol $P < 0.005$ (Table 1)). In PM microsomes, no biphasicity of the kinetics was identifiable (in the absence of quinidine, see below). As in EM microsomes, the isomer configuration of the substrate did not influence significantly the Michaelis constant of the PM microsomes.

Studies in intermediate metabolizers (IM)

From *in vivo* population studies, the *antimode* of the frequency distribution of the sparteine urinary metabolic ratio, which allows segregation between EM and PM, was tentatively defined after logarithmic transformation at a value of 20 [50]. PM and EM subjects are easily identified as most of them have a metabolic ratio above or below 1, respectively. A few individuals, however, have a metabolic ratio between these two values. Four patients selected here cannot easily be assigned to either phenotype, displaying metabolic ratios ranging from 1.91 to 18.4. Their *in vitro* parameters (K_m, V_{max}, (−)/(+) ratio—Table 1) are indistinguishable, however, from those of the PM phenotype,* as illustrated also in Fig. 3.

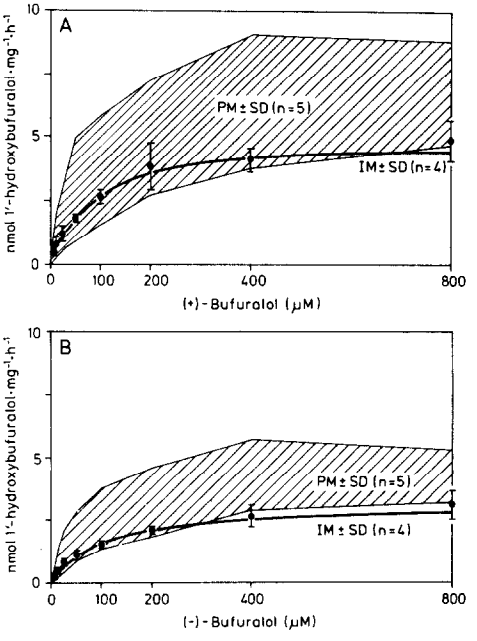

Fig. 3. (+)-Bufuralol (A) and (−)-bufuralol (B) 1'-hydroxylation kinetics in liver microsomes from subjects with "intermediate" rates of sparteine metabolism *in vivo* (IM). Values are means ± SD, the shaded area corresponds to ±1 SD of the values observed in microsomes of 5 poor metabolizers (PM).

* A similar observation with one debrisoquine "intermediate" metabolizer (debrisoquine urinary metabolic ratio of 2.79) was recently made in our laboratory.

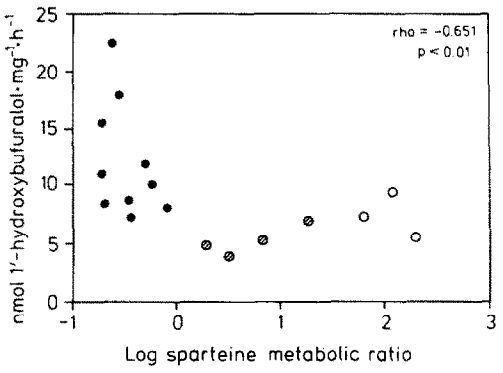

Fig. 4. Relationship between *in vivo* determined sparteine metabolic ratio and *in vitro* estimated maximal velocity (V_{max}) of (+)-bufuralol 1'-hydroxylation in microsomes of subjects phenotyped as: ● = extensive metabolizers; ○ = metabolizers; ⊘ = subjects with intermediate rates of sparteine metabolism.

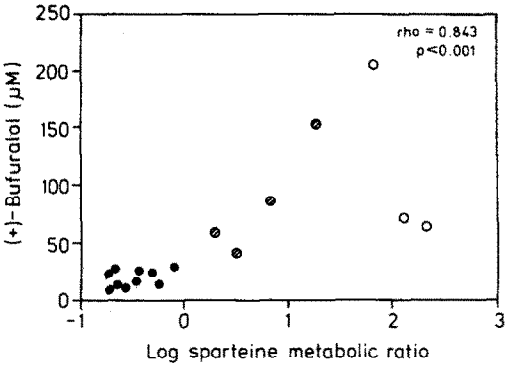

Fig. 5. Relationship between *in vivo* determined sparteine metabolic ratio and *in vitro* estimated apparent Michaelis constant (K_m) for (+)-bufuralol 1'-hydroxylation in microsomes from the same subjects. ● = extensive metabolizers; ○ = poor metabolizers; ⊘ = subjects with intermediate rates of sparteine metabolism.

In vivo/in vitro *correlations*

There was a significant negative correlation between the *in vivo* sparteine metabolic ratio and the microsomal maximal velocity of (+)-bufuralol 1'-hydroxylation (Fig. 4) or affinity constant (Fig. 5). A significant correlation was obtained between the sparteine metabolic ratio and the (−)/(+)-bufuralol 1'-hydroxylation V_{max} ratio used as an index of the stereoselectivity of each microsomal preparation (rho = 0.678, $P < 0.01$). It should be noticed that among these three parameters (V_{max}, K_m and (−)/(+) ratio) only K_m values did not overlap between the two phenotypes.

Inhibition of (+)-bufuralol 1'-hydroxylation by quinidine

Quinidine has recently been shown to be a powerful inhibitor of microsomal sparteine oxidation [47]. We have studied the influence of quinidine on bufuralol hydroxylation *in vitro* and on the metabolism

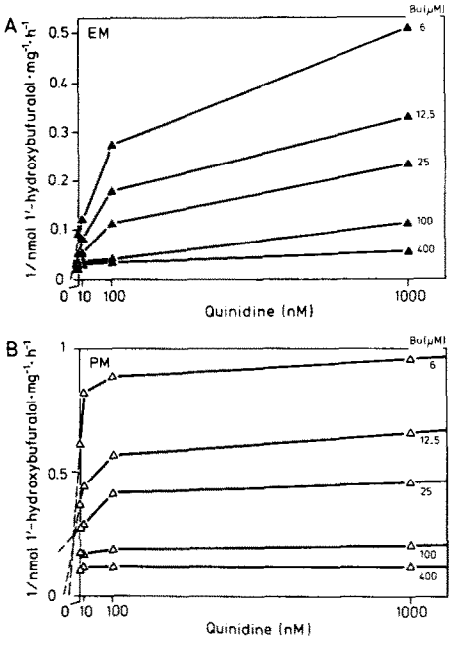

Fig. 6. Quinidine inhibition kinetics of (+)-bufuralol 1'-hydroxylation in extensive (A) and poor (B) metabolizer microsomes (Dixon plots). Both types of microsomes display biphasic kinetics. Bu = bufuralol.

of the related compound metoprolol *in vivo* [49]. In microsomes prepared from EM biopsies, quinidine concentrations between 10^{-7} and 10^{-5} M lowered the V_{max} of (+)-bufuralol 1'-hydroxylation, abolishing almost completely the stereoselectivity of the reaction. Moreover, quinidine also increased the apparent K_m, mimicking the decreased affinity observed in the PM microsomes. At various bufuralol and quinidine concentrations in EM and PM microsomes (+)-bufuralol 1'-hydroxylation characteristically exhibited biphasic inhibition curves (Fig. 6). At low substrate and inhibitor concentrations, a high affinity component was competitively inhibited by quinidine. The use of a two component activity model indicated (Table 3) a high affinity component, inhibited at very low quinidine concentrations and a low affinity component, non-competitively inhibited only at high quinidine concentrations. The major difference between EM and PM (or IM) microsomes was a reduction of about 95% of the high affinity and stereoselective component in the latter.

DISCUSSION

In vivo/in vitro *correlations*

We have demonstrated that *in vivo* bufuralol hydroxylation is under identical or linked genetic control as debrisoquine 4-hydroxylation [18, 19]. In human liver microsomes bufuralol and debrisoquine are competitive inhibitors of their reciprocal hydroxylations, a strong argument in favor of the concept that the same cytochrome P-450 isozyme(s) is (are) responsible for both bufuralol 1'-hydroxylation and debrisoquine 4-hydroxylation [45]. The

Table 3. Enzymatic characteristics (range) of (+)-bufuralol 1′-hydroxylation and inhibitory effect of quinidine in microsomes of 2 extensive, 2 poor and 1 intermediate metabolizer (see also Fig. 6)

	K_m (μM)	K_i (nM)	V_{max} (nmol × mg^{-1} × hr^{-1})
High affinity component	6–19	3–13	*
Low affinity component	55–196	†	5–26

* V_{max} was 0.51 and 1.5 nmol × mg^{-1} × hr^{-1} for two PM, 1.3 for IM, 7.0 and 22 for two EM microsome samples.

† The low affinity component is non-competitively inhibited by quinidine only at concentrations > 10^{-5} M.

present data demonstrate that the *in vivo* sparteine metabolic ratio correlates with bufuralol 1′-hydroxylation *in vitro* lending further support to the postulate that the metabolic pathways studied *in vivo* and *in vitro* are catalyzed by the same cytochrome P-450 isozyme. The sole discrepancy in the *in vivo/in vitro* correlation is the case of the subjects with intermediate rates of metabolism *in vivo*. Such IM cases cannot be classified as heterozygous for the recessive allele without pedigree studies. Moreover, heterozygous individuals must be more frequent (40% in European Caucasians) [11] and usually have a metabolic capacity which is overlapping in frequency distribution curves with that of homozygous extensive metabolizers. Nor is it possible to classify these individuals with the present enzyme probes. Using the 1′-hydroxylation reaction as *in vitro* probe IM microsomes are undistinguishable from the poor metabolizer group, whatever criteria of the reaction (V_{max}, K_m, (−)/(+) ratio) are analyzed. One explanation of this observation could be that these individuals are genotypically homozygote PM but possess additional enzyme(s) activity(ies) able to oxidize sparteine or debrisoquine to some extent, but unable to metabolize bufuralol. A definitive answer will be possible only when more specific probes are available.

Enzymatic characteristics of bufuralol 1′-hydroxylation in extensive metabolizers

In vivo, bufuralol 1′-hydroxylation is stereoselective for the (+)-isomer [56]. A role of substrate configuration has been observed, *in vivo*, also with perhexiline [57], nortriptyline [58] and metoprolol [49, 59]. Our *in vitro* data thus confirm this characteristic as illustrated by the difference in V_{max} for the (+)- and the (−)-bufuralol 1′-hydroxylation. The fact that the substrate steric conformation does not play a role on the apparent affinity constant (K_m) may suggest that the domain of the enzyme which binds the substrate (probably the positively charged nitrogen center) is not stereoselective, contrary to the catalytic site of the enzyme. Studies at the microsomal level have obvious limitations since the enzymatic activity measured is the resultant of several isozyme activities with overlapping substrate selectivity and various affinities. Initial studies suggested that bufuralol 1′-hydroxylation was characterized by simple Michaelis–Menten type kinetics [43, 45].

The findings of biphasic kinetics suggest that a low affinity cytochrome P-450 isozyme is detectable in some if not all microsomes. The quinidine inhibition kinetics provide more compelling evidence that (+) bufuralol 1′-hydroxylation in all microsomes is biphasic with a 10-fold difference in the apparent K_m of the high and low affinity isozymes (Table 2). More direct evidence for a two isozyme system is derived from the purification of cytochrome P-450 isozymes from human liver. At the same time as these *in vivo/in vitro* studies were performed our group purified from several human livers two cytochrome P-450 isozymes with a high capacity for 1′-bufuralol hydroxylation [46]. The two forms differ in their mobility on ion-exchange chromatography, in their affinity for bufuralol 1′-hydroxylation (low and high K_m), in their substrate stereoselectivity (high and low selectivity) and in their sensitivity to quinidine inhibition (low and high K_i). They do not differ in their molecular weight (50 kD) and polyclonal rabbit antibodies raised against either the high affinity/stereoselectivity isozyme (cytochrome P-450 buf I) or the low affinity/stereoselectivity isozyme (cytochrome P-450 buf II) recognize both forms. It seems thus a reasonable speculation that the two purified isozymes may correspond to the two kinetic component of bufuralol 1′-hydroxylation identified at the microsomal level.

Enzymatic characteristics of poor metabolizer microsomes

In vivo data from PM subjects show that they excrete less 1′-hydroxybufuralol than the EM subjects and also display a marked decrease in the stereoselectivity of bufuralol hydroxylation [55]. As indicated by the V_{max} values, the *in vivo* observations can be explained by the present *in vitro* data. In the substrate concentration range used throughout this study, no biphasic hydroxylation was detected in PM. However, the quinidine inhibition kinetics (Fig. 6) suggest that PM microsomes may still contain an isozyme of high affinity for bufuralol and high sensitivity to quinidine inhibition. This would partly explain why some degree of stereoselectivity is yet present in poor metabolizer microsomes as well as in poor metabolizers *in vivo*. Thus, in PM microsomes bufuralol 1′-hydroxylation also is catalyzed by two different enzyme components. The high apparent K_m previously noticed in PM microsomes [43] may result from a shift of a mixed contribution of both

isozymes in EM microsomes, to a major contribution of the low affinity isozyme in PM microsomes, the main characteristic of PM microsomes being a dramatic decrease of the high affinity/stereoselectivity isozyme.

On the basis of the present results we therefore propose that the debrisoquine/sparteine type of oxidation polymorphism is caused by an almost total loss of a minor cytochrome P-450 isozyme which is characterized by a high affinity/stereoselectivity for (+)-bufuralol 1'-hydroxylation and an extreme sensitivity to quinidine inhibition. The observation that some activity of the high affinity/stereoselectivity component is yet measurable in PM microsomes could be compatible with a structural mutation causing a catalytically modified enzyme or with defective synthesis of the enzyme protein.

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