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Enantiomer/Enantiomer Interaction of (*S*)- and (*R*)-Propafenone for Cytochrome P450IID6-Catalyzed 5-Hydroxylation: *In Vitro* Evaluation of the Mechanism

HEYO K. KROEMER, CHRISTINE FISCHER, CLAUS O. MEESE, and MICHEL EICHELBAUM

Dr. Margarete Fischer Bosch Institut für Klinische Pharmakologie, 7000 Stuttgart 50, Germany

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SUMMARY

Many drugs are used as racemates, and the enantiomers may differ in terms of pharmacological properties and disposition. Stereoselective disposition of the enantiomers can arise from metabolism of the enantiomers via different routes catalyzed by different enzymes. In contrast, the enantiomers may be metabolized by the same enzyme at different rates. In the latter case, the enantiomers can compete for this metabolic step, giving rise to the possibility of an enantiomer/enantiomer interaction. We have chosen the antiarrhythmic propafenone, for which *in vivo* data indicated an interaction between (*S*)- and (*R*)-propafenone, as a model substance to study the mechanism underlying that interaction in human liver microsomes. We used the cytochrome P450IID6-mediated 5-hydroxylation of propafenone as a model pathway, because this metabolic step constitutes the major route of biotransformation of propafenone. The Michaelis-Menten kinetics for 5-hydroxylation were determined after incubation of (*R*)- and (*S*)-propafenone and a pseudoracemate consisting of (*S*)-[$^2H_4$]propafenone and (*R*)-propafenone. Inhibition experiments were performed using (*S*)-[$^2H_4$]propafenone as an inhibitor of the 5-hydroxylation of (*R*)-propafenone, and vice versa. The kinetic model of mixed alternative substrates was used to simulate inhibition experiments. Experimental data were compared with those predicted by this model. We observed a substantial stereoselectivity after incubation of the individual enantiomers [(*S*)-propafenone: V_{max}, 10.2 pmol/μg/hr, and K_m, 5.3 μM; (*R*)-propafenone: V_{max}, 5.5 pmol/μg/hr, and K_m, 3.0 μM]. In contrast, no substrate stereoselectivity was observed after incubation of the pseudoracemate [3.1 pmol/μg/hr for (*S*)-[$^2H_4$]propafenone and 3.3 pmol/μg/hr for (*R*)-propafenone]. Application of the model revealed K_i values of 2.9 and 5.2 μM for the inhibition of 5-hydroxylation of (*S*)-[$^2H_4$]-propafenone by (*R*)-propafenone and for inhibition of 5-hydroxylation of (*R*)-propafenone by (*S*)-[$^2H_4$]-propafenone, respectively. The predicted and the experimental data were in good agreement, and both indicated the mode of inhibition to be competitive. In conclusion, the enantiomers of propafenone interact with respect to 5-hydroxylation, with (*R*)-propafenone being a more potent inhibitor than the *S*-enantiomer with respect to cytochrome P450IID6-mediated 5-hydroxylation. Because β-blocking properties of propafenone reside in the *S*-enantiomer, inhibition of metabolism of this enantiomer by (*R*)-propafenone may have therapeutic consequences.

Propafenone is an antiarrhythmic agent that is clinically used as a racemic mixture of (*S*)- and (*R*)-propafenone (1). The enantiomers are equipotent in terms of sodium channel-blocking activity (2, 3). In addition to the effect on sodium conductance, the drug has β-adrenoreceptor-blocking properties, which are mediated via the *S*-enantiomer (2–4), and, therefore, the disposition of the individual isomers in humans is of relevance. After separate administration of (*S*)- and (*R*)-propafenone to healthy volunteers, (*S*)-propafenone was more rapidly cleared than (*R*)-propafenone (*S*/*R* ratio of 0.5) (5). Stereoselectivity in protein binding did not account for the differences observed (5). In contrast, administration of the clinically used racemate revealed the opposite situation (2). Thus, the *R*-enantiomer was more rapidly cleared after administration of the racemate. These controversial findings may be the result of an enantiomer/enantiomer interaction. Although a vast number of drugs are used as racemic mixtures, the question of interactions between enantiomers with respect to metabolism has been rarely addressed (6). Such interactions may be of clinical relevance if, as in the case of propafenone, the enantiomers differ in terms of pharmacological potency and, therefore, inhibition of metabolism of one enantiomer by its optical antipode may modulate the net pharmacological effect of the drug. Because cytochrome P450IID6-mediated 5-hydroxylation constitutes the major oxidative pathway for propafenone (7), we have chosen this metabolic step as a model, to investigate the general problem of enantiomer/enantiomer interactions *in vitro*. A threefold approach was undertaken. 1) The rate of 5-hydroxylation was determined for the individual enantiomers of pro-

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ABBREVIATIONS: HPLC, high performance liquid chromatography; GC, gas chromatography; MS, mass spectrometry; SIM, selected ion monitoring.

pafenone, as well as for a pseudoracemate consisting of (*S*)-[$^2H_4$]propafenone and unlabeled (*R*)-propafenone. 2) Based on experimental findings, a theoretical model was adapted to predict the rate of 5-hydroxylation of one enantiomer in the presence of variable concentrations of the corresponding antipode. 3) Inhibition experiments using (*S*)-propafenone as an inhibitor of (*R*)-propafenone 5-hydroxylation, and vice versa, were both experimentally performed and simulated using the theoretical model. The results of simulations and actual experiments were compared.

Integration of the three parts of our study will allow us to predict how the enantiomers of propafenone interact with regard to 5-hydroxylation. Therapeutic consequences arising from such an interaction are discussed.

Materials and Methods

Microsomal Preparations

Microsomes were prepared from the liver of one kidney transplant donor (16-year-old male, nonsmoker, no chronic intake of drugs), according to a previously described method (7). Protein and cytochrome P-450 contents were estimated according to the methods of Lowry *et al.* (8) and Omura and Sato (9), respectively.

Substrates

Racemic unlabeled propafenone hydrochloride and 5-hydroxypropafenone hydrochloride were a gift from Knoll AG (Ludwigshafen, Germany). Racemic [$^2H_4$]propafenone hydrochloride was synthesized by aminolysis of 2-(2′,3′-epoxypropoxy)-ω-phenylpropiophenone using *n*-[2,2,3,3-$^2H_4$]propylamine, followed by hydrochloride formation according to the method of Meese *et al.*[1] The isotopic composition was $^2H_4$, 97%; $^2H_3$, 3%; and $^2H_0$, <0.2%. Fig. 1 displays the position of the labels. The deuterium label used is not affected by 5-hydroxylation. The individual enantiomers were obtained from both labeled and unlabeled racemic propafenone by a separation method of Blaschke and Walther (10). The optical purity was 96% for both labeled and unlabeled compounds.

Incubation Conditions

Incubations were carried out at 37° at pH 7.4, for 25 min, in the presence of 25 μg of microsomal protein with an NADPH-regenerating system, which is described elsewhere (11). The final volume was 100 μl. Each experiment was carried out in duplicate. For kinetic experiments, (*S*)-propafenone, (*R*)-propafenone, and the pseudoracemate were used in concentrations of 0.5, 0.75, 1.0, 1.25, 2.5, 5, 10, and 20 μM. The reaction was stopped by addition of 10 μl of 30% perchloric acid. After centrifugation at 12,000 × *g*, 50 μl of the supernatant were injected in the HPLC system. If pseudoracemate was used for incubation, the supernatant was divided into two parts. One was injected in the HPLC system, to estimate the total rate of formation of 5-hydroxy propafenone. The other half was adjusted to pH 9 by addition of 500 μl of ammonium hydroxide/ammonium chloride buffer (1 mM, pH 9), extracted with 2 ml of dichloromethane, and subsequently derivatized, as described below, after evaporation of the dichloromethane.

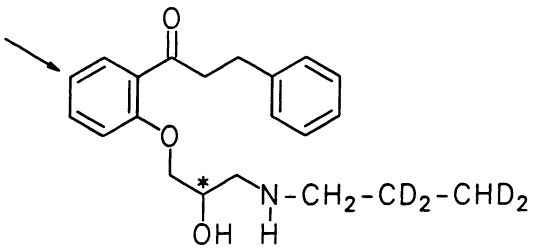


Fig. 1. Structure of propafenone. *D*, Site of deuteration; *arrow*, place of 5-hydroxylation; *, chiral center.

[1]Meese C. O. and G. Heinkele. Synthesis of isotopically labelled enantiomers of propafenone. Manuscript in preparation.

Inhibition Experiments

Inhibition experiments were carried out to estimate 5-hydroxylation of (*S*)-[$^2H_4$]propafenone, at concentrations of 5, 10, and 20 μM, in the presence of the inhibitor (*R*)-propafenone (2.5, 5, 10, 20, and 40 μM). Identical experiments were carried out using (*S*)-[$^2H_4$]propafenone as an inhibitor of the 5-hydroxylation of (*R*)-propafenone.

HPLC Assay for 5-Hydroxypropafenone

5-Hydroxypropafenone was quantified after incubation of the individual enantiomers and the pseudoracemate, by means of HPLC with UV detection (λ = 220 nm). A 5-μm C18 reverse phase column (15 × 0.46 cm) was used, with a flow rate of 1.0 ml/min. The mobile phase consisted of aqueous 0.01 M tetrabutylammonium sulfate and methanol (25:20, v/v). The retention time for 5-hydroxypropafenone averaged 8.5 min (Fig. 2). Quantification was performed by external standardization, using samples spiked with 5-hydroxypropafenone. Calibration curves were linear from 20 to 500 pmol. The interassay variability, as estimated from the slope of the calibration curve, averaged 9.5%. After incubation of the pseudoracemate, the ratio of labeled and unlabeled 5-hydroxypropafenone was determined by combined GC/MS.

GC/MS Assay for Determination of 5-Hydroxypropafenone

Derivatization. An aliquot of the organic extract was evaporated to dryness with a gentle stream of nitrogen, and the dried residue was

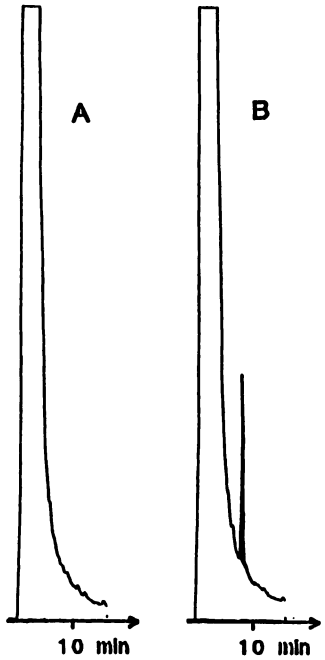


Fig. 2. HPLC chromatograms used for quantification of 5-hydroxypropafenone formed in the presence of human liver microsomes (see Materials and Methods for experimental conditions). A, Blank (incubation of 5 μM propafenone with heat-inactivated microsomes). B, HPLC chromatogram after incubation of 5 μM propafenone in the presence of human liver microsomes. The retention time of 5-hydroxypropafenone is 8.5 min.

dissolved by addition of 10 μl of acetonitrile. Derivatization consisted of trifluoroacetylation, which was achieved by addition of 20 μl of *N*-methyl-bis(trifluoroacetamide) (CS Chromatography Service GmbH, Langerwehe, FRG). The mixture was allowed to react for 30 min at 60°.

GC/MS conditions. A Hewlett Packard 5985A instrument was used to determine the ratio of deuterated versus undeuterated 5-hydroxypropafenone. A 25-m chemically bonded SE-52 fused-silica capillary column (0.25-μm film thickness, 0.32-mm internal diameter; CS Chromatography Service GmbH) was installed and directly inserted into the ion source. The following GC conditions were used: helium as a carrier gas and splitless injection at 280° of 1–3-μl aliquots of the samples. A temperature program was started at 110°, held for 1 min, and then heated at a rate of increase of 15°/min. Retention time for 5-hydroxypropafenone averaged 10.6 min (Fig. 3). The following MS conditions were selected: methane as reactant gas, negative ion chemical ionization mode, electron energy of 110–150 eV, transfer line at 320°, and ion source at 200°. SIM mode was used to trace the molecular ions of unlabeled and labeled tris-(trifluoroacetyl)-5-hydroxypropafenone, at m/z 645 and m/z 649, respectively. Spectra obtained in the negative ion chemical ionization mode and representative SIM tracings obtained after incubation of 20 μM (*S*)-[$^2H_4$]propafenone in the presence of 20 μM (*R*)-propafenone are displayed in Figure 3. The isotope pattern of the molecular ion, m/z 649, of the deuterated metabolite was identical to that of the unlabeled compound, which indicated that the deuterium label was retained under the experimental conditions used.

Theoretical Model for the Interaction of (*S*)- and (*R*)-Propafenone

We used a model for competitive inhibition and total velocity with mixed alternative substrates, according to the method of Segel (12). The model was adapted to the 5-hydroxylation of (*S*)- and (*R*)-propafenone (Fig. 4) and applied to estimate the total rate of 5-hydroxylation of a racemic mixture on the basis of V_{max} and K_m values obtained from experiments using the individual enantiomers. Moreover, assuming a lack of substrate selectivity, inhibition experiments were simulated using (*R*)-propafenone as an inhibitor of 5-hydroxylation of (*S*)-[$^2H_4$] propafenone, and vice versa. The results were plotted as $1/V$ versus the inhibitor concentration (Dixon plots). Results obtained in these simulations were compared with those derived from experiments.

Determination of V_{max} and K_m

V_{max} and K_m were determined by an iterative curve-fitting program (13). K_i was determined mathematically and graphically from the Dixon plots. V_{max} was expressed in pmol/μg of protein/hr and K_m and K_i in μM.

Results

Pronounced substrate enantioselectivity for 5-hydroxylation was observed after incubation of the individual enantiomers, with the major difference occurring for V_{max} [(*S*)-propafenone: V_{max}, 10.2 pmol/μg/hr, and K_m, 5.3 μM; (*R*)-propafenone: V_{max}, 5.5 pmol/μg/hr, and K_m, 3.0 μM]. In contrast, no substrate selectivity was observed after incubation of the pseudoracemic mixture. The rate of formation of 5-hydroxypropafenone was decreased, compared with values obtained after incubation of the individual enantiomers [3.1 pmol/μg/hr for (*S*)-[$^2H_4$]propafenone and 3.3 pmol/μg/hr for (*R*)-propafenone]. Representative curves are displayed in Fig. 5.

Data obtained after incubation of (*S*)-[$^2H_4$]propafenone were identical to those obtained after incubation of the unlabeled *S*-enantiomer. Therefore, an isotope effect could be excluded.

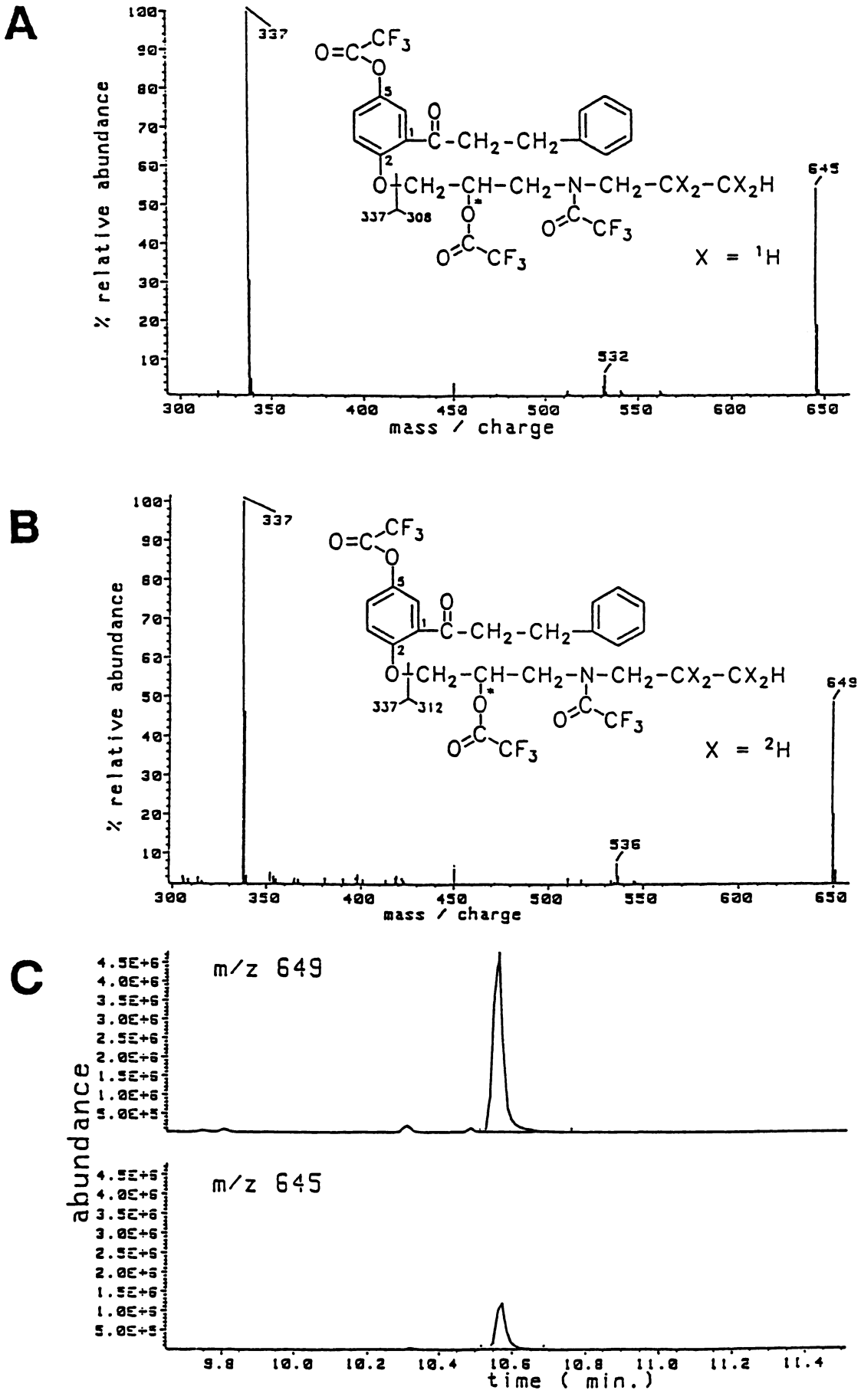
Based on the model described in Materials and Methods, the V_{max} and K_m data for the individual enantiomers were used to calculate the total velocity after incubation of the racemic mixture. Data predicted by the model were compared with those experimentally obtained. As can be seen in Fig. 6, the experimental data (V_{max}, 6.9 pmol/μg/hr; K_m, 3.8 μM) were almost superimposable on those obtained by simulation (V_{max}, 7.2 pmol/μg/hr; K_m, 3.9 μM).

Using the model, inhibition experiments were simulated using (*R*)-propafenone to inhibit the 5-hydroxylation of (*S*)-[$^2H_4$] propafenone, and vice versa. The resulting Dixon plots, which were indicative of a competitive mechanism, are shown in Fig. 7. The K_i values for inhibition of 5-hydroxylation of (*S*)-[$^2H_4$] propafenone by (*R*)-propafenone and for inhibition of 5-hydroxylation of (*R*)-propafenone by (*S*)-[$^2H_4$]propafenone were 2.9 and 5.2 μM, respectively.

The same concentrations used in the simulations were used for actual incubation experiments. The calculated $1/V$ values were compared with those experimentally obtained (Fig. 8). A highly significant correlation was obtained for inhibition of both enantiomers [inhibition of 5-hydroxylation of (*S*)-[$^2H_4$] propafenone by (*R*)-propafenone: slope = 1.19, y-intercept = −0.02, r = 0.98; inhibition of 5-hydroxylation of (*R*)-propafenone by (*S*)-[$^2H_4$]propafenone: slope = 1.06, y-intercept = −0.01, r = 0.98).

Discussion

Questions related to the administration of racemic drugs have received considerable attention over the past decade (14). A difference observed in *in vitro* potency of enantiomers may be modulated by enantioselective drug metabolism, depending on which of the enantiomers is preferentially metabolized. Substrate enantioselectivity in drug metabolism, which results from metabolism of the enantiomers mediated via the same enzyme at different rates, appears to be the rule rather than the exception. Enantiospecificity, however, which would be indicative of the exclusive use of an enzymatic pathway by one enantiomer but not the other, has been rarely reported (15). Therefore, the scenario of two enantiomers competing for the active site of the same enzyme is a common phenomenon. A racemate can be regarded as a combination of two drugs that differ in metabolic and pharmacodynamic properties and, therefore, possible drug/drug interactions arising from such combinations should be taken into account. Surprisingly, the question of metabolic enantiomer/enantiomer interactions has been the subject of only a few detailed investigations. Wright *et al.* (16) reported the rate of metabolism of (*R*)-(−)-amphetamine in the 9000 × *g* supernatant of rabbit liver. The rate of metabolism of racemic amphetamine was similar to that of (*S*)-(+)-amphetamine. This study did not determine the contributions of the individual enantiomers after incubation of the racemate. Ames and Frank (17) reported an interaction of the enantiomers of *para*-chloramphetamine for the active site of the metabolizing enzyme. This investigation, however, reported only the disappearance rate of the substrate and, thus, no kinetic parameters for the formation of metabolites could be calculated. A recent review on clinical consequences of drug chirality states that "although such possibilities abound it is disappointing that there have been so few demonstrations of these [enantiomer/enantiomer] interactions" (6). This lack of



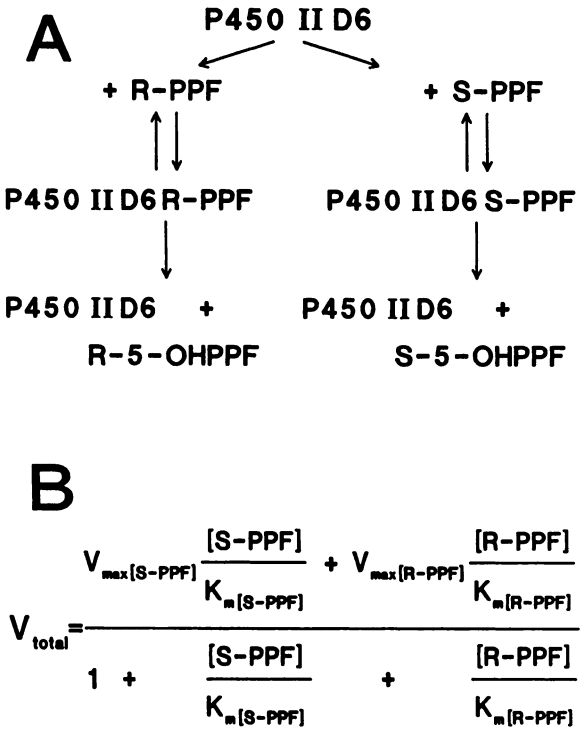


Fig. 4. 5-Hydroxylation of (*R*)- and (*S*)-propafenone by cytochrome P450IID6, adapted from the mixed alternative substrate approach (12). A, Scheme of formation of 5-hydroxypropafenone (*5-OHPPF*) via cytochrome P450IID6, using (*S*)- and (*R*)-propafenone (*PPF*) as substrate. B, Equation for estimation of the total rate of 5-hydroxylation of propafenone.

data may be due to analytical problems. Direct separation of chiral metabolites from chiral parent compounds via chiral chromatography, with subsequent UV detection, is either difficult or not sensitive enough to detect the small amounts of metabolites formed *in vitro*. The approach used in our investigation employed pseudoracemates for chiral discrimination and a combination of HPLC and GC/MS, which provides a specific and sensitive method of detection.

We addressed in detail the question of an enantiomer/enantiomer interaction, selecting the example of propafenone and using *in vitro* techniques. The oxidative step investigated, 5-hydroxylation, comprises the major pathway of propafenone biotransformation; the other known metabolite, -*N*-desalkyl propafenone, contributes to only a minor extent (18). The starting point of this investigation was the *in vivo* observation of a higher clearance of (*S*)-propafenone, compared with (*R*)-propafenone, after separate administration of the individual enantiomers (5), whereas after administration of the racemate the opposite situation was observed (2, 20). The present *in vitro* study showed, in fact, that the V_{max} of (*S*)-propafenone was twice that of (*R*)-propafenone, which is in agreement with *in vivo* data, assuming 5-hydroxylation to be the major stereoselective step of oxidative metabolism in humans. Incubation of pseudoracemate, however, resulted in a reduction of the rate of 5-hydroxylation for both enantiomers and a loss of substrate stereoselectivity. The reduction in the rate of 5-hydroxylation

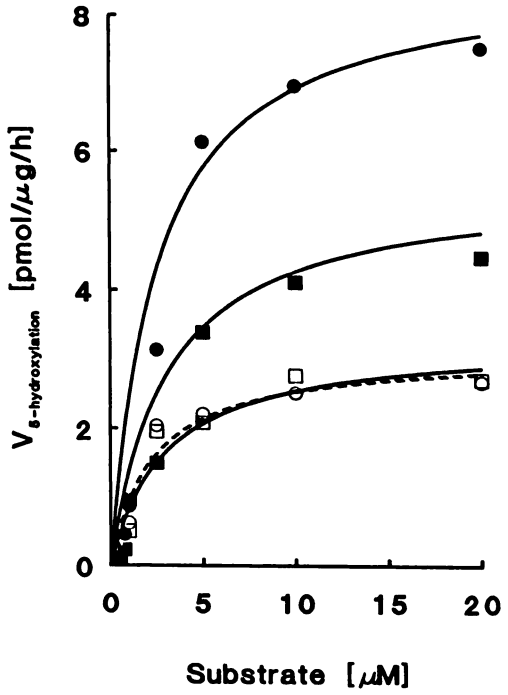


Fig. 5. Substrate dependency of propafenone 5-hydroxylation after incubation of (*S*)-propafenone (●), (*R*)-propafenone (■), or a pseudoracemic mixture consisting of (*S*)-[$^2H_4$]propafenone (○) and (*R*)-propafenone (□) with human liver microsomes.

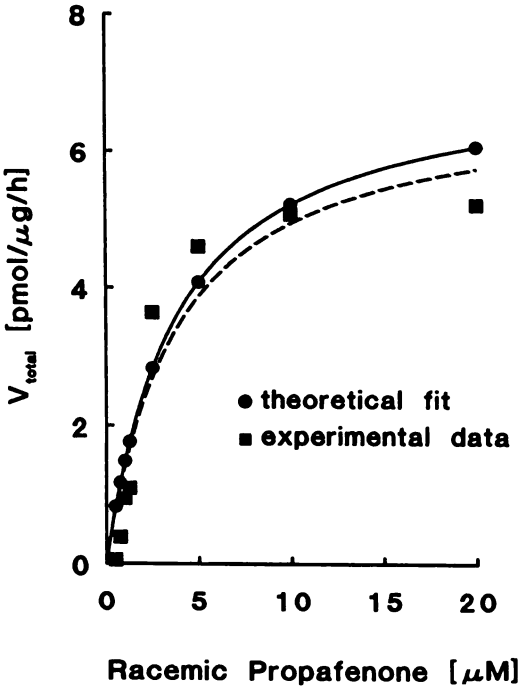


Fig. 6. Total rate of 5-hydroxylation as a function of racemic propafenone concentration. (●, Data simulated using the model shown in Fig. 4A; ■, experimentally obtained values).

Fig. 3. A, Mass spectrum of 5-hydroxypropafenone (reference compound) as its tris-(trifluoroacetyl)-derivative. B, Mass spectrum of enzymatically formed [$^2H_4$]5-hydroxypropafenone as its tris-(trifluoroacetyl)-derivative. C, SIM tracings of an incubation with human liver microsomes [40 μM (*S*)-[$^2H_4$]propafenone and 10 μM (*R*)-propafenone; for extraction and derivatization, see Materials and Methods]; *m/z* 645 represents unlabeled derivatized 5-hydroxypropafenone and *m/z* 649 represents tetradeuterated derivatized 5-hydroxypropafenone.

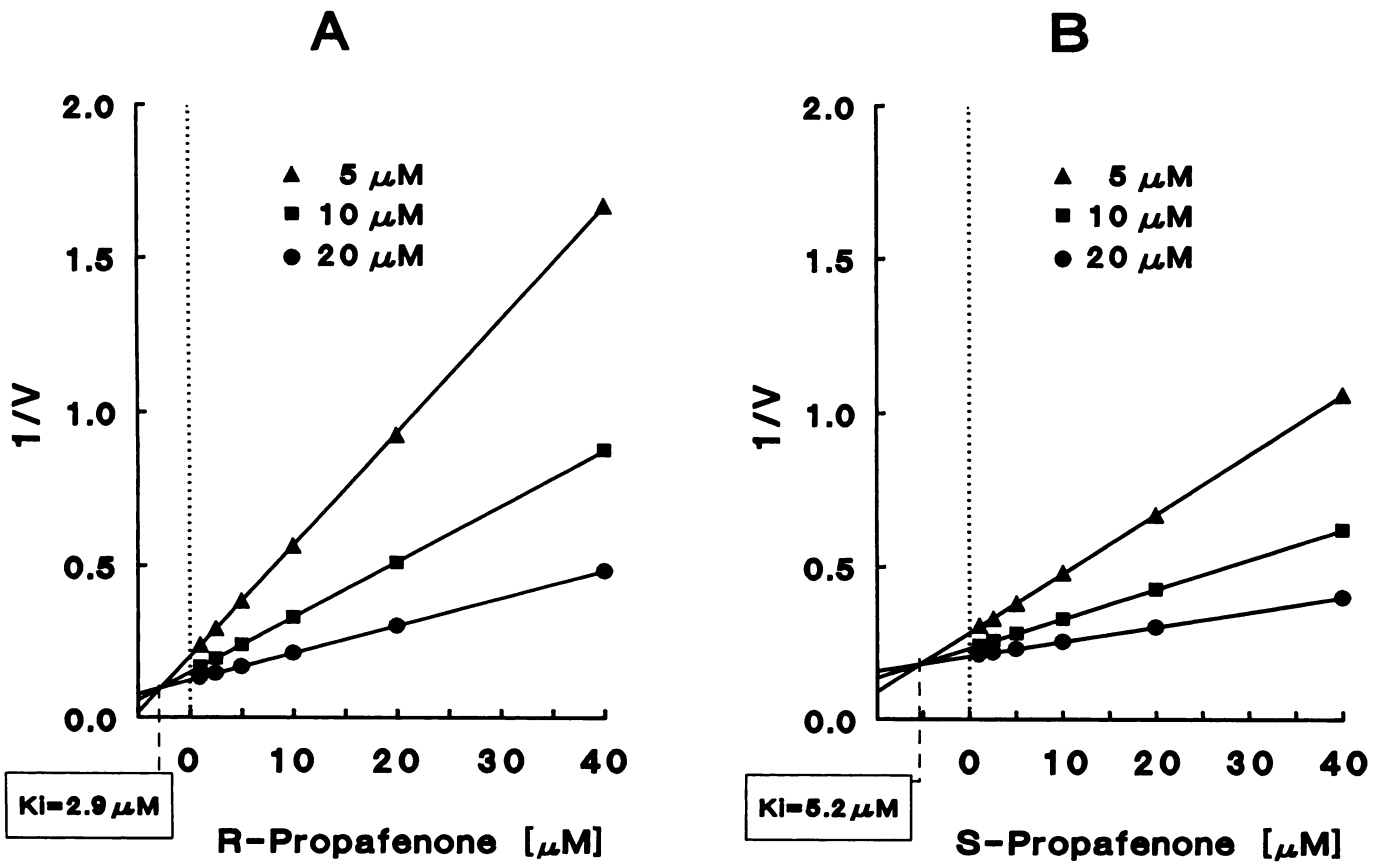


Fig. 7. Simulation of inhibition of 5-hydroxylation of (*S*)-[$^2H_4$]propafenone in the presence of (*R*)-propafenone (A) and inhibition of 5-hydroxylation of (*R*)-propafenone by (*S*)-[$^2H_4$]propafenone (B).

was more pronounced for (*S*)-propafenone (−70%) than for (*R*)-propafenone (−40%), indicating that the higher affinity of the *R*-enantiomer for the enzyme led to a more pronounced inhibition of (*S*)-propafenone 5-hydroxylation than vice versa. These data are in agreement with those obtained in inhibition experiments with bufuralol, in which (*R*)-propafenone proved to be a more potent inhibitor (K_i, 21 nM) than (*S*)-propafenone (K_i, 56 nM) of bufuralol 1′-hydroxylation (7), a reaction that is catalyzed by the same cytochrome P450 isoenzyme as propafenone 5-hydroxylation.

After incubation of the pseudoracemate, the formation rate of 5-hydroxypropafenone was virtually identical for the two enantiomers. Thus, one would expect an *S*/*R* ratio around unity if the racemate is administered in humans. We observed, however, that the apparent clearance of (*R*)-propafenone is higher than that of (*S*)-propafenone, leading to an *S*/*R* ratio of about 1.7 (2, 20), after either a single or chronic oral administration of propafenone. Therefore, the enantioselective disposition observed *in vivo* after administration of the racemate can be explained only partially by 5-hydroxylation. Formation of 5-hydroxypropafenone in humans exhibits genetic polymorphism, cosegregating with polymorphic sparteine/debrisoquine oxidation (7, 19). Five to ten percent of a caucasian population, who are designated poor metabolizers, lack P450IID6, which is the cytochrome catalyzing 5-hydroxylation of propafenone, and, thus, do not form this pharmacologically active metabolite. As a consequence, clearance of propafenone is markedly reduced in these patients. The stereoselective disposition observed after chronic administration of racemic propafenone is similar in patients who form 5-hydroxypropafenone (2, 20), when compared with poor metabolizers. Thus, both *in vivo* and *in vitro* lines of evidence indicate that routes of biotransformation other than 5-hydroxylation contribute to stereoselective disposition of propafenone after administration of the racemate. A possible pathway that could account for the difference may be hepatic or extrahepatic glucuronidation, because formation of the ether glucuronide comprises the major metabolic pathway of propafenone in the poor metabolizer subset.

Different scenarios can arise from the setting in which two enantiomers of a racemate are biotransformed. Both enantiomers can compete for the active site of the same enzyme, being metabolized at different rates. In this case, a mutual competitive interaction can arise. On the other hand, if enantiomers are metabolized by two distinct enzymes (different routes), no interaction will be observed. Moreover, metabolism of both enantiomers could be catalyzed by the same enzyme, with only one enantiomer acting as competitive inhibitor of the other's metabolism. A unidirectional interaction would be the result. A nice example for such an interaction was demonstrated by Wilson and Thompson (21) for glucuronidation of propranolol by dog liver microsomes, with (*S*)-propranolol being a noncompetitive inhibitor of (*R*)-propranolol glucuronide formation. Finally, only one of the enantiomers could be oxidized by an enzyme, with the other acting as an inhibitor but not being a substrate of that process. In order to decide which of these theoretical possibilities applied to the propafenone enantiomer/enantiomer interaction, we compared data experimentally obtained with those simulated by a model. It was known from

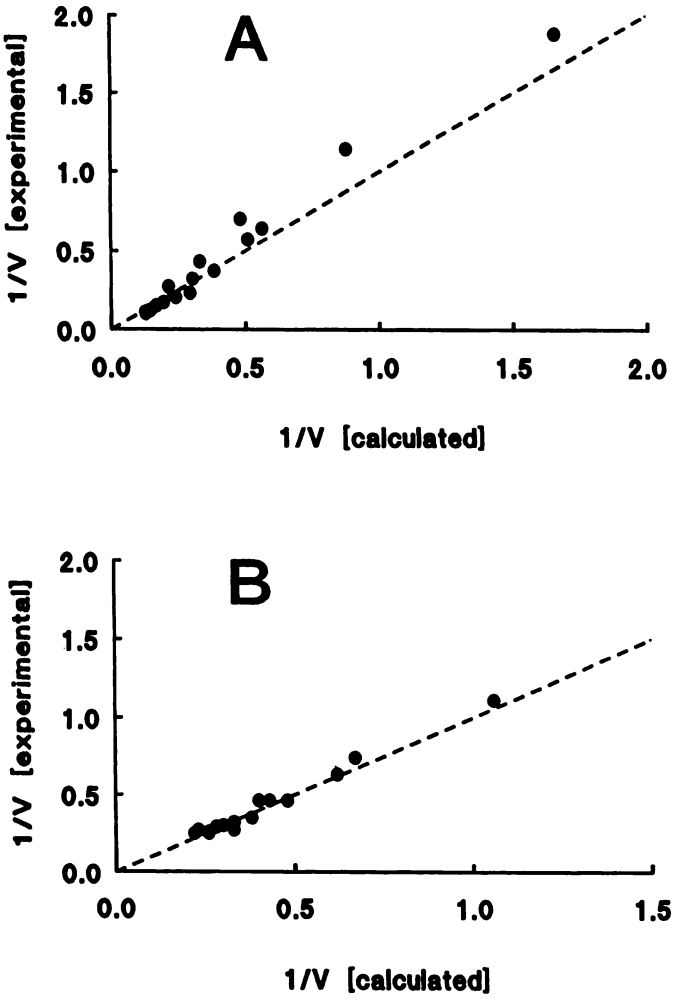


Fig. 8.. 1/V values obtained from simulations shown in Fig. 6, in comparison with those obtained experimentally. A, Inhibition of 5-hydroxylation of (*S*)-[$^2H_4$]propafenone in the presence of (*R*)-propafenone; B, inhibition of 5-hydroxylation of (*R*)-propafenone by (*S*)-[$^2H_4$]propafenone. The line of identity is indicated.

earlier experiments that 5-hydroxylation of propafenone can be completely inhibited by an antibody directed against cytochrome P450IID6, indicating the involvement of only one enzyme (7). Moreover, both enantiomers of propafenone were competitive inhibitors of the cytochrome P450IID6-mediated 1′-hydroxylation of bufuralol. Our present investigation shows both (*R*)- and (*S*)-propafenone to be 5-hydroxylated. These findings are evident of a system consisting of a single enzyme acting on propafenone enantiomers as two different substrates, which, in turn, both are competitive inhibitors with respect to their optical antipode. Consequently, the model suggested by Segel (12) for the situation of a mixed alternative substrate approach was adapted. As shown in Figs. 6 and 8, experimental data correlate well, in fact, with those estimated by means of the model. The inhibition studies shown in Figs. 7 and 8 used different concentrations of a substrate in the presence of variable concentrations of an inhibitor. These experiments may reflect an *in vivo* situation, in which stereoselective first-pass metabolism leads to a *S*/*R* ratio different from unity. As a consequence, the ratio of substrate to inhibitor in this system will vary with time. Thus, pharmacokinetic properties of a racemic compound that exerts an enantiomer/enantiomer interaction consist of a dynamic system in which achievement of the steady state depends on the individual parameters of the Michalis-Menten kinetics.

Another aspect of these theoretical considerations deserves attention; the K_m of a racemic mixture represents, in our model, an apparent affinity of two substrates for an enzyme in the presence of competitive inhibitors. In the case of propafenone, this value gives at least a rough estimate concerning the magnitude of affinity, because the K_m of 5-hydroxylation is not markedly different for (*S*)- and (*R*)-propafenone after incubation of the individual enantiomers. If, however, only one enantiomer is metabolized via an enzyme and the other enantiomer acts as a competitive inhibitor, then K_m values may be completely misleading. Thus, affinity data obtained from *in vitro* experiments with racemic mixtures should be interpreted with caution, as long as the kinetic properties of the individual enantiomers are unknown.

In view of the fact that propafenone enantiomers differ in terms of pharmacological properties (β-blockade resides in the *S*-enantiomer), the inhibition of (*S*)-propafenone clearance, via 5-hydroxylation, by the *R*-enantiomer may be of therapeutic relevance. β-Blocking properties are more pronounced in the subset of poor metabolizers (22), due to accumulation of the parent compound. They may contribute to the overall antiarrhythmic activity but may, as well, provoke side effects in patients who are intolerant of β-blockade. This effect should be more evident after administration of 150 mg of (*S*)-propafenone in the presence of an equimolar dose of (*R*)-propafenone than after administration of 150 mg of (*S*)-propafenone alone, although an identical dose of β-blocking agent was administered.

In conclusion, we have shown an enantiomer/enantiomer interaction of (*S*)- and (*R*)-propafenone for 5-hydroxylation. Both *in vitro* experiments and simulations using a model of mixed alternative substrates indicate the mechanism to be competitive. This way of addressing the question of enantiomer/enantiomer interactions should be applicable to other drugs as well.

Acknowledgments

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Send reprint requests to: Dr. H. K. Kroemer, Dr. Margarete Fischer-Bosch Institut für Klinische Pharmakologie, Auerbachstr. 112, 7000 Stuttgart 50, Germany.