Cytochrome P450 Isozymes Involved in Aromatic Hydroxylation and Side-Chain *N*-Desisopropylation of Alprenolol in Rat Liver Microsomes

Shizuo NARIMATSU,*[,a] Masaya TACHIBANA,[a] Yasuhiro MASUBUCHI,[a] Susumu IMAOKA,[b] Yoshihiko FUNAE,[b] and Tokuji SUZUKI[a]

Laboratory of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Chiba University,[a] 1–33 Yayoi-cho, Inage-ku, Chiba 263, Japan and Laboratory of Chemistry, Osaka City University Medical School,[b] 4–54 Asahi-machi, Abeno-ku, Osaka 545, Japan. Received January 24, 1995; accepted April 30, 1995

Alprenolol 4-hydroxylation and *N*-desisopropylation in liver microsomes from male Wistar rats were kinetically analyzed to be biphasic. In the 4-hydroxylation at a low substrate concentration (5 μM), significant strain [Wistar > Dark Agouti (DA)] and sex (male > female) differences were observed, and the differences decreased at a high substrate concentration (1 mM). In the *N*-desisopropylation, only a strain difference (Wistar > DA) was observed at the low substrate concentration. Cytochrome P450BTL (P450BTL, corresponding to CYP2D2) in a reconstituted system with 5 μM alprenolol had high 4-hydroxylase activity, which was about 10 times that of P450ml corresponding to CYP2C11, and *N*-desisopropylase activity at a similar extent to P450ml. The two microsomal activities at 5 μM alprenolol were efficiently decreased by antibodies against P450BTL and by sparteine, a typical substrate of the CYP2D subfamily. Polyclonal antibodies against P450ml and P450PB-1 (corresponding to CYP3A2) partially suppressed only *N*-desalkylation at 5 μM, whereas they reduced the two activities at 1 mM. P450ml showed a high *N*-desisopropylase activity at a substrate concentration of 1 mM, where the sex difference was not observed. Furthermore, P450PB-2 corresponding to CYP2C6, which is one of the major P450 isozymes in female rats, also had 4-hydroxylase and *N*-desalkylase activities. These results suggest that a CYP2D isozyme(s) is the primary enzyme in alprenolol 4-hydroxylation and *N*-desisopropylation in a lower substrate concentration range, and that the involvement of some male-specific P450 isozyme(s) other than CYP2C11 or CYP3A2 may cause the sex difference in the 4-hydroxylation. In a higher substrate concentration range, CYP2C11 is thought to play a major role particularly in *N*-desisopropylation in male rats. In female rats, some major constitutive P450 isozyme(s) with a relatively high K_m value (*e.g.*, CYP2C6) may be involved in the metabolism of alprenolol, resulting in the disappearance of the sex difference.

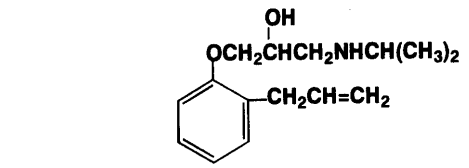
Key words alprenolol 4-hydroxylation; *N*-desisopropylation; CYP2D2; strain difference; sex difference; Wistar > Dark Agouti rat

It has been reported that oxidative metabolism of various adrenoceptor β-blocking agents (β-blockers) is catalyzed by the cytochrome P450 (CYP) 2D subfamily, particularly CYP2D6 in the human.[1,2] This isozyme is the key enzyme for the genetic polymorphism in the oxidative metabolism of over 30 drugs.[3] Alprenolol is one of the β-blockers often prescribed for the treatment of arrhythmias and hypertension. The oxidative metabolism of alprenolol was extensively studied in rats,[4-7] dogs,[4,5,7] guinea pigs,[5,6] and humans.[4,5,7] Its primary metabolic pathways consist of aromatic 4-hydroxylation of the phenyl ring, desisopropylation of the side chain at the 1-position (*N*-desalkylation) (Fig. 1), and 1″-hydroxylation and 2″-epoxidation at the allyl side chain at the 2-position.

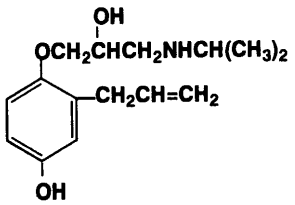
Alvan *et al.*[8] reported high plasma concentrations of alprenolol after its oral administration in debrisoquine poor metabolizers. They further found that 4-hydroxyalprenolol was not detected in the plasma from a subject who received the β-blocker, while plasma concentration of the parent drug was at a high level.[8] Recently, we reported that 4-hydroxylation of bunitrolol, a β-blocker whose chemical structure is similar to that of alprenolol, is mediated by the CYP2D subfamily in rats[9] and humans.[10] These results suggest that major isozyme mediating alprenolol 4-hydroxylation is CYP2D6 in human liver. However, there has been no *in vitro* evidence confirming this speculation. We thus conducted the present study to probe the possibility of the involvement of the CYP2D subfamily in the oxidative pathways of alprenolol such as 4-hydroxylation of the aromatic ring and *N*-desalkylation of the side chain in rat liver microsomes.

MATERIALS AND METHODS

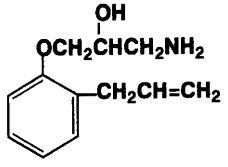
General Alprenolol, 4-hydroxyalprenolol and *N*-des-



Alprenolol



4-Hydroxyalprenolol



N-Desisopropylalprenolol

Fig. 1. Chemical Structures of Alprenolol and Its Oxidative Metabolites Used

* To whom correspondence should be addressed.

isopropylalprenolol as hydrochlorides were obtained from A. B. Hassle (Molndal, Sweden). 7-Hydroxypropranolol was synthesized from propranolol by the method of Oatis *et al.*[11] Debrisoquine hemisulfate was obtained from Hoffmann-La Roche (Basel, Switzerland); sparteine sulfate was from Sigma Chemical Co. (St. Louis, Mo). Other reagents and organic solvents were of the highest quality commercially available.

Animal Adult Wistar (8 week-old) and Dark Agouti (DA, 10 week-old) rats of both sexes were obtained from Takasugi Experimental Animals (Kasukabe, Japan) and SLC (Shizuoka, Japan), respectively, and were allowed food and water *ad libitum*. The animals were killed by decapitation, and liver microsomes were prepared as described previously.[12] Protein concentrations were determined by the method reported by Lowry *et al.*[13]

Determination of Oxidative Activities of Alprenolol in Rat Liver Microsomes A reaction mixture (1.0 ml) contained microsomes (1.0 mg), G-6-P (10 nmol), $MgCl_2$ (10 nmol), G-6-P dehydrogenase (2 U), alprenolol (0.5 nmol to 2 μmol) and Tris–HCl (pH 7.4, 154 mmol) to make a final volume of 1.0 ml. After preincubation at 37 °C for 5 min, incubation was started by adding NADPH (5 nmol) and continued for 0.5 min. The reaction was terminated by adding 1 ml of 1 N NaOH. After adding 7-hydroxypropranolol as internal standard, the reaction medium was extracted with ethyl acetate (5 ml) by vigorous shaking and centrifugation. The organic layer was evaporated with a rotary evaporator, and the residue was dissolved in 100 μl of the mobile phase for HPLC as described below. 4-Hydroxyalprenolol and *N*-desisopropylalprenolol were determined by HPLC on the basis of calibration curves, which were made by adding known amounts of synthetic standards to an ice-cold reaction medium.

Purified Enzymes and Antibodies P450BTL,[14] P450-ml[15] and P450PB-2,[16] which are thought to correspond to CYP2D2, CYP2C11 and CYP2C6, respectively, from their N-terminal amino acid sequences, and their antibodies were obtained as reported previously.[14–16] Preparations of antibodies against CYP2C12[17] and P40PB-1 corresponding to CYP3A2[18] were described elsewhere. Alprenolol oxidation activities were reconstituted in the system consisting of CYP isozyme (25 pmol), NADPH-CYP reductase (0.25 units), dilauroylphosphatidylcholine (5 μg), G-6-P (10 nmol), G-6-P dehydrogenase (2 U), $MgCl_2$ (10 nmol), alprenolol (5 nmol or 1 μmol), and Tris–HCl buffer (pH 7.4, 154 μmol) to make a final volume of 1.0 ml. After preincubation at 37 °C for 5 min, reaction was started by adding NADPH (5 nmol) and terminated 5 min later by adding 1 ml of 1 N NaOH. Metabolites formed were extracted, and 4-hydroxyalprenolol and *N*-desisopropylalprenolol were determined by HPLC as described above.

HPLC Conditions The HPLC apparatus consisted of an LC-3A liquid chromatograph equipped with an RF 535 fluorescence detector and a C-R6A Chromatopac integrator (Shimadzu, Kyoto, Japan). Other conditions were: a column, Inertsil ODS (4.6 mm i.d. × 250 mm, GL Science Co., Tokyo, Japan); mobile phase, methanol–water–acetonitrile–acetic acid (22 : 58 : 20 : 2.5, v/v); flow rate, 1.2 ml/min; detection, excitation/emission wavelength, 280 and 330 nm.

Data Analysis Enzyme kinetic parameters (K_m and V_{max}) were analyzed according to a nonlinear least-square regression analysis based on a simplex method.[19] Statistical significance was calculated by Student's *t*-test.

RESULTS

Kinetic Analysis of Alprenolol Oxidation in Rat Liver Microsomes Figure 2B shows a chromatogram of metabolites formed from alprenolol (5 μM) for 0.5 min of incubation with liver microsomes from male Wistar rats. Retention times were: 4-hydroxyalprenolol, 3.55 min; *N*-desisopropylalprenolol, 6.95 min; alprenolol, 11.63 min; 7-hydroxypropranolol (internal standard), 5.67 min. Peaks with retention times of 2.61, 4.03 and 4.54 min were not identified because of the unavailability of synthetic standards. The lowest detection limits defined as three times the levels of baseline noise were 2 and 5 nM for 4-hydroxyalprenolol and *N*-desisopropylalprenolol, respectively. We thus determined amounts of 4-hydroxyalprenolol and *N*-desisopropylalprenolol by HPLC in the following experiments.

Both alprenolol 4-hydroxylation and *N*-desisopropylation were drawn as typical Eadie–Hofstee plots (Fig. 3). These reactions were analyzed to be biphasic, and the kinetic parameters are listed in Table 1. From the results, it was thought that alprenolol 4-hydroxylation consists of high and low affinity enzymes with similar capacities, whereas *N*-desisopropylation is mediated by at least two enzymes, *i.e.*, one with high affinity and low capacity, and the other with low affinity and high capacity in rat liver

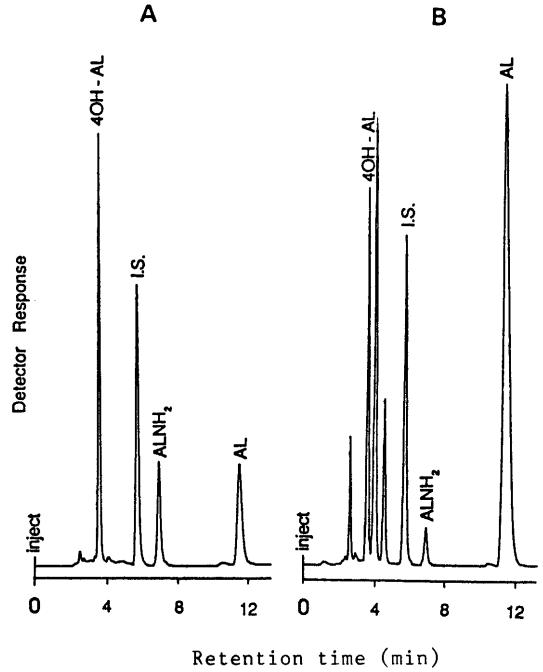


Fig. 2. High-Performance Liquid Chromatograms of Alprenolol and Its Metabolites

A, alprenolol and its metabolites (0.5 nmol each) and 7-hydroxypropranolol (2 μg as internal standard) were added to ice-cold incubation medium containing rat liver microsomes and an NADPH-generating system, and were extracted into ethyl acetate immediately after the addition without incubation. B, Alprenolol (5 nmol) was incubated in the reaction medium as described above, and incubated at 37 °C for 1 min. Metabolites were extracted into ethyl acetate, and examined by HPLC under the conditions given in Materials and Methods.

microsomes. On the basis of these results, we proceeded with further experiments using two substrate concentrations of 5 μM and 1 mM for the high and low affinity enzymes, respectively.

Sex and Strain Differences in Alprenolol 4-Hydroxylation and *N*-Desisopropylation Alprenolol 4-hydroxylase and *N*-desisopropylase activities were assayed in liver microsomes from Wistar and DA rats of both sexes. As shown in Fig. 4, significant strain differences were observed in both indices at a lower substrate concentration (5 μM), *i.e.*, alprenolol 4-hydroxylase and *N*-desisopropylase activities were significantly higher in Wistar than in DA rats of corresponding sex. In addition, a significant sex difference (male > female) was observed in alprenolol 4-hydroxylase activity but not in *N*-desisopropylase activity at the low substrate concentration. On the contrary, a strain but not sex difference was shown in alprenolol 4-hydroxylation (Wistar > DA) at a high substrate concentration (1 mM).

Alprenolol Oxidation in Reconstituted Systems Alprenolol (5 μM or 1 mM) was incubated in a reconstituted system containing purified P450BTL, P450 ml or P450PB-2 probably corresponding to CYP2D2, CYP2C11 and CYP2C6, respectively, on the basis of their N-terminal amino acid sequences, NADPH-CYP reductase and dilauroylphosphatidylcholine. As listed in Table 2, P450BTL showed alprenolol 4-hydroxylase and *N*-desisopropylase activities to a similar extent at both substrate concentrations, whereas P450 ml exhibited much higher activities in *N*-desalkylation than in 4-hydroxylation. In addition, alprenolol 4-hydroxylase activity of P450BTL was 10 times higher than that of P450 ml at a low substrate

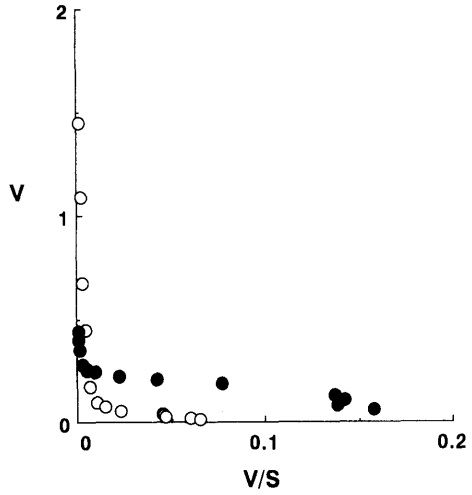


Fig. 3. Eadie–Hofstee Plots Showing Alprenolol 4-Hydroxylation and *N*-Desisopropylation in Rat Liver Microsomes

Alprenolol oxidation activities were determined in liver microsomes from male Wistar rats using a substrate concentration range from 0.5 μM to 2 mM with 13 points. V, nmol/min/mg protein; V/S, ml/min/mg protein. Open circles, *N*-desisopropylation; closed circles, 4-hydroxylation. A typical plotting is shown whose kinetic parameters were: 4-hydroxylation; K_{m1} (0.88 μM), V_{max1} (0.238 nmol/min/mg protein), K_{m2} (452.5 μM) and V_{max2} (0.305 nmol/min/mg protein); *N*-desisopropylation; K_{m1} (0.98 μM), V_{max1} (0.072 nmol/min/mg protein), K_{m2} (521.2 μM) and V_{max2} (2.067 nmol/min/mg protein)

Table 1. Michaelis–Menten Parameters for Alprenolol 4-Hydroxylase and *N*-Desisopropylase Activities in Liver Microsomes from Male Wistar Rats

Reaction	K_{m1}	V_{max1}	K_{m2}	V_{max2}
4-Hydroxylation	0.83±0.14	0.303±0.074	336.4±46.6	0.298±0.026
N-Desisopropylation	0.89±0.25	0.087±0.001	551.1±85.0	2.28 ±0.31

K_m, μM; V_{max}, nmol/min/mg of protein. Each value represents the mean±S.E. of three animals.

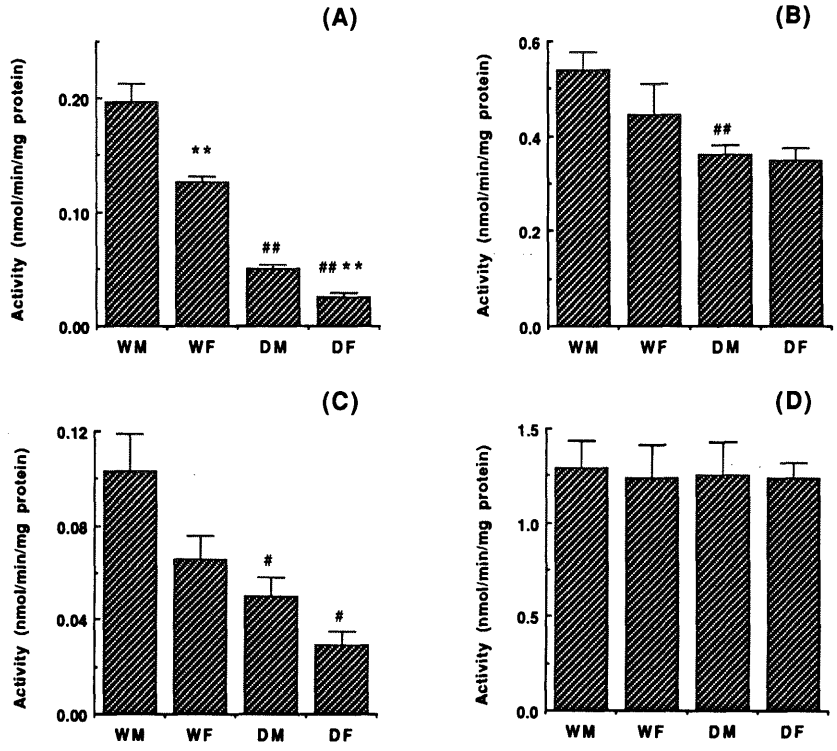


Fig. 4. Sex and Strain Differences in Alprenolol 4-Hydroxylase (A and B) and *N*-Desisopropylase (C and D) Activities in Rat Liver Microsomes

Substrate concentrations were 5 μM (A and C) and 1 mM (B and D). W, D, M and F represent Wistar, Dark Agouti, male and female, respectively. Each value is the mean±S.E. of four animals. ** Significantly different from male rats in the same strain ($p<0.01$). #,## Significantly different from Wistar rats in the same sex ($p<0.05$ and 0.01, respectively).

concentration, whereas the activities of the two enzymes were almost the same at a high substrate concentration. In contrast, P450ml showed alprenolol *N*-desisopropylase activity about 12 times higher than P450BTL at a substrate concentration of 1 mM, whereas the activities of the enzymes were similar at 5 μM. Although only a high substrate concentration (1 mM) was employed, P450PB-2 showed a moderate alprenolol *N*-desisopropylase activity; that is, the activity was about double that of P450BTL but one-seventh that of P450ml, whereas its alprenolol 4-hydroxylase activity was about one-fourth the values of the other two enzymes.

Effects of Antibodies against CYP Isozymes on Alprenolol Oxidation Preincubation of rat liver microsomes with anti-P450BTL IgG caused a concentration-dependent suppression of alprenolol 4-hydroxylase and *N*-desalkylase activities at the low substrate concentration, with the extent of the inhibition being larger in 4-hydroxylase than in *N*-desalkylase activity (Fig. 5A). At the high substrate concentration (1 mM), only 4-hydroxylase activity was suppressed by the antibodies (Fig. 5B). The inhibition of the reactions by anti-P450ml IgG was shown more clearly in the higher substrate concentration range than in the lower (Fig. 5C and 5D). When anti-P450ml IgG was added to the reaction medium, both oxidation activities were decreased with *N*-desalkylation being suppressed to a greater extent than 4-hydroxylation at the substrate concentration of 1 mM (Fig. 5D).

Table 2. Alprenolol Metabolism Activities of Purified Cytochromes P450BTL, P450ml and P450PB-2

Enzyme	Speculated CYP name	Metabolic activity (nmol/min/nmol P450) 4-Hydroxylation Substrate concentration 5 μM	4-Hydroxylation 1 mM	*N*-Desisopropylation 5 μM	*N*-Desisopropylation 1 mM
P450BTL	CYP2D2	0.491	1.30	0.230	1.65
P450ml	CYP2C11	0.0495	1.33	0.316	19.4
P450PB-2	CYP2C6	N.D.	0.38	N.D.	2.90

Each value represents the mean of two determinations. CYP names were speculated from their N-terminal amino acid sequences (See ref. 14—16). N.D., not detected.

Figure 6 shows effects of antibodies against P450PB-1 corresponding to CYP3A2 on the oxidative reactions. The antibodies suppressed only 4-hydroxylation at the lower substrate concentration (Fig. 6A) but reduced both 4-hydroxylase and *N*-desisopropylase activities at the higher alprenolol concentration (Fig. 6B), although the potencies of the anti-P450PB-1 IgG were much lower than those of the antibodies raised against P450BTL and P450ml. On the other hand, addition of antibodies against CYP2C12 (a female-specific isozyme) showed no effect on alprenolol oxidation activities under the conditions used (data not shown).

Effect of Sparteine on Alprenolol Oxidation Activities To further confirm the involvement of CYP2D isozyme(s)

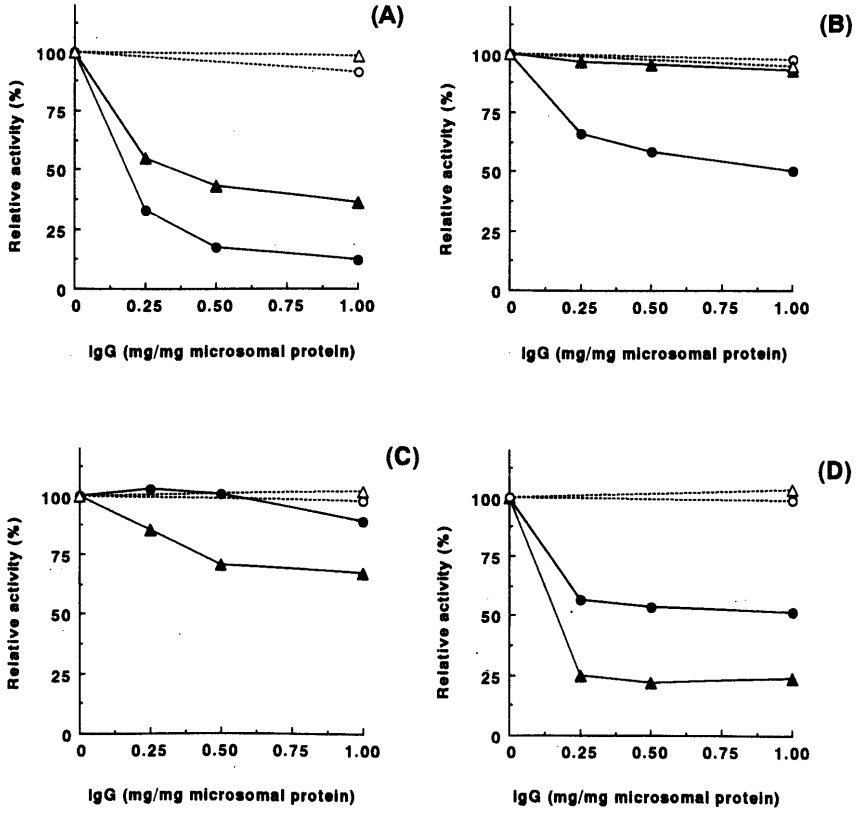


Fig. 5. Effects of Antibodies Raised against P450BTL and P450ml on Alprenolol 4-Hydroxylase and *N*-Desisopropylase Activities in Liver Microsomes from Male Wistar Rats

Microsomes were preincubated with varying amounts of anti-CYP IgG (closed symbols) or preimmune IgG (open symbols) at 25 °C for 30 min, and then were incubated with alprenolol [5 μM (left panel) or 1 mM (right panel)] and an NADPH-generating system at 37 °C for 2 min. Closed circles, 4-hydroxylation; closed triangles, *N*-desisopropylation. A and B, anti-P450BTL; C and D, anti-P450ml. The activities of control as 100% mean the activities in the absence of the IgG fraction. Each value represents the mean of two determinations. Alprenolol 4-hydroxylase activities of the control were 0.206 and 0.339 nmol/min/mg protein at substrate concentrations of 5 μM and 1 mM, respectively; *N*-desisopropylase activities were 0.143 and 1.847 nmol/min/mg protein at 5 μM and1 mM, respectively.

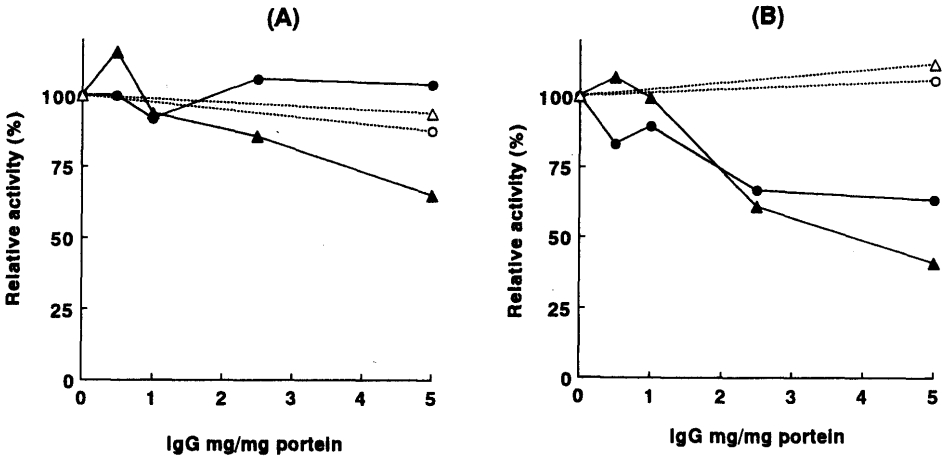


Fig. 6. Effects of Antibodies Raised against P450PB-1 on Alprenolol 4-Hydroxylase and *N*-Desisopropylase Activities in Liver Microsomes from Male Wistar Rats

Microsomes were preincubated with varying amounts of anti-P450PB-1 IgG (closed symbols) or preimmune IgG (open symbols) at 25 °C for 30 min, and then incubated with alprenolol [5 μM (A) or 1 mM (B)] and an NADPH-generating system at 37 °C for 2 min. Closed circles, 4-hydroxylation; closed triangles, *N*-desisopropylation. The activities of control as 100% mean the activities in the absence of the IgG fraction. Each value represents the mean of two determinations. Alprenolol 4-hydroxylase activities of the control were 0.214 and 0.298 nmol/min/mg protein at substrate concentrations of 5 μM and 1 mM, respectively; *N*-desisopropylase activities were 0.112 and 1.322 nmol/min/mg protein at 5 μM and 1 mM, respectively.

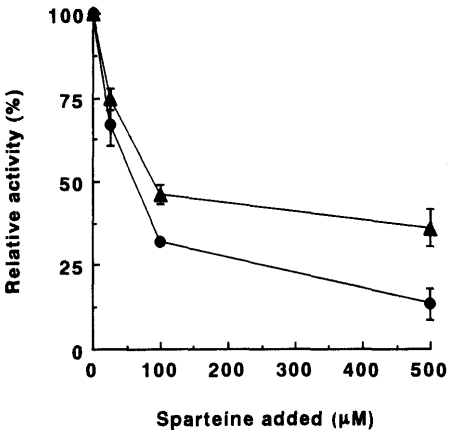


Fig. 7. Inhibitory Effect of Sparteine on Alprenolol 4-Hydroxylase and *N*-Desisopropylase Activities in Liver Microsomes from Male Wistar Rats

The substrate concentration used was 5 μM. Closed circles, 4-hydroxylation; closed triangles, *N*-desisopropylation. Each value represents the mean ± S.E. of three determinations. Alprenolol 4-hydroxylase activity of the control was 0.188 ± 0.021 nmol/min/mg protein; *N*-desisopropylase activity was 0.079 ± 0.010 nmol/min/mg protein.

in these alprenolol oxidation activities, a known typical substrate of the CYP2D subfamily sparteine was added to the reaction mixture. When a substrate concentration of 5 μM was employed, alprenolol 4-hydroxylation and *N*-desalkylation were suppressed by the inhibitor in a concentration-dependent manner (Fig. 7).

DISCUSSION

We examined the oxidative metabolism of alprenolol in rat liver microsomes in the present study to probe the possible involvement of the CYP2D subfamily using P450BTL purified from rat liver microsomes,[14] antibodies against P450BTL and a substrate (sparteine) of the CYP2D subfamily as inhibitor. As reported by Skanberg *et al.*,[5] alprenolol 4-hydroxylation and *N*-desisopropylation were also analyzed to be biphasic in rat liver microsomes in the present study. We thus employed two substrate concentrations of 5 μM and 1 mM for high and low affinity enzymes, respectively, in further experiments.

A strain difference (Wistar > DA) was observed in alprenolol 4-hydroxylation at a low substrate concentration. The female DA rat is known to be deficient in catalytic activity of the CYP2D subfamily in the liver, and has been proposed as an animal model of debrisoquine poor metabolizers.[20,21] Interestingly, a clear strain difference was also observed in alprenolol *N*-desisopropylation at low substrate concentration. These strain differences tended to decrease at high substrate concentration. Hence, it seems likely that a CYP2D enzyme(s) may catalyze rat liver microsomal alprenolol 4-hydroxylation and *N*-desisopropylation in a low substrate concentration range.

This speculation was supported by the reconstitution experiment with purified P450BTL corresponding to CYP2D2, which had been purified from rat liver microsomes by pursuing bunitrolol 4-hydroxylase activity.[14] At low substrate concentration, the reconstituted alprenolol 4-hydroxylase activity of the purified enzyme was over 1.5-fold that of microsomal fractions. Possible involvement of the CYP2D subfamily in the reactions was further supported by the immunoinhibition experiments using antibodies against the CYP2D isozyme and an inhibitor. That is, anti-P450BTL (probably corresponding to CYP2D2) IgG suppressed both alprenolol 4-hydroxylation and *N*-desalkylation at the lower substrate concentration range in a concentration-dependent manner. Sparteine, a typical substrate of the CYP2D subfamily, also effectively inhibited both reactions at low substrate concentration. These results indicated that alprenolol *N*-desisopropylation as well as 4-hydroxylation is primarily mediated by the CYP2D subfamily in the rat in a lower substrate concentration range.

A sex difference was observed in alprenolol 4-hydroxylation at low substrate concentration, and the difference was reduced by increasing the substrate concentration. We

have observed no sex difference in other metabolic activities mediated by the CYP2D subfamily such as lidocaine 3-hydroxylation[22] or propranolol ring 4-, 5- and 7-hydroxylations[23] in liver microsomes from Wistar rats. Considering these results and findings, it seems likely that not CYP2D enzyme(s) but a male-specific CYP isozyme may be responsible for the appearance of the sex difference.

We thus examined the oxidative metabolism of alprenolol in a reconstituted system containing P450ml (corresponding to CYP2C11) as a typical male-specific CYP isozyme, and compared the activity with that of P450BTL. Although both isozymes mediated alprenolol 4-hydroxylation in the reconstituted systems, P450ml had 10 times lower activity than P450BTL at a substrate concentration of 5 μM. The polyclonal antibodies against P450ml did not affect alprenolol 4-hydroxylation and partially suppressed *N*-desalkylation at 5 μM.

P450PB-1 corresponding to CYP3A2 is another constitutive male-specific CYP isozyme[18] which may be involved in alprenolol metabolism. But anti-P450PB-1 IgG did not suppress microsomal alprenolol 4-hydroxylation at 5 μM substrate concentration. As a possible explanation for the sex difference observed in the lower substrate concentration range, some constitutive male-specific CYP isozyme(s) other than CYP2C11 or the CYP3A subfamily may be involved in rat liver microsomal alprenolol 4-hydroxylation.

P450ml exhibited an alprenolol 4-hydroxylase activity comparable to that of P450BTL and a very high *N*-desalkylation activity (*ca.* 12 times that of P450BTL) at high substrate concentration. The anti-P450ml antibodies effectively decreased both reactions at 1 mM, suggesting that CYP2C11 was a major isozyme catalyzing alprenolol *N*-desalkylation in a higher substrate concentration range in male rat liver. In spite of the results, no significant sex difference was observed in *N*-desisopropylation at the higher substrate concentration in rat liver microsomes (Fig. 4).

The antibodies raised against CYP2C12, a female-specific isozyme, did not affect either of the reactions. However, P450PB-2 corresponding to CYP2C6, which is one of the major constitutive isozymes in female rats,[17] had a high activity for alprenolol *N*-desisopropylation at 1 mM. The participation of a major isozyme(s) in female rat liver (*e.g.*, CYP2C6), which may have a relatively high K_m value, might cause the disappearance of the sex difference in the alprenolol *N*-desalkylation in the higher substrate concentration range. From these results and speculation, it seems likely that CYP2C isozymes such as CYP2C6 and CYP2C11 may contribute to these reactions at the high substrate concentration range.

Unfortunately, polyclonal antibodies raised against CYP2C6 or CYP2C11 in our hand cross-reacted with other constitutive isozymes belonging to the CYP2C subfamily so that we could not distinguish the individual roles of CYP2C6 and CYP2C11. Specific monoclonal antibodies against these isozymes are necessary to further understand the roles of the CYP2C isozymes in the oxidative metabolism of alprenolol in rat liver microsomes. We are now proceeding with further studies to confirm CYP isozymes mediating alprenolol 4-hydroxylation and *N*-desisopropylation in liver microsomes from rats and humans.

Acknowledgments We thank Misses N. Fujino and K. Hayashi and Mr. M. Mochida for their valuable technical assistance. This work was supported in part by a grant from the Japan Research Foundation for Clinical Pharmacology to S.N.

REFERENCES

1) Otton S. V., Inaba T., Kalow W., *Life Sci.*, **34**, 73 (1984).
2) Kalow W., *Eur. J. Clin. Pharmacol.*, **31**, 633 (1987).
3) Murray M., *Clin. Pharmacokinet.*, **23**, 132 (1992).
4) Bodin N.-O., *Life Sci.*, **14**, 685 (1974).
5) Skanberg I., Borg K. O., Fellenius E., Hoffmann K.-J., Bahr C.v., Moldeus P., *Acta Pharmacol. Toxicol.*, **44**, 28 (1979).
6) Hoffmann K.-J., Skanberg I., Borg K. O., *Xenobiotica*, **9**, 79 (1979).
7) Hoffmann K.-J., Arfwidsson A., Borg K. O., Skanberg I., *Xenobiotica*, **9**, 93 (1979).
8) Alvan G., Bahr C.v., Seideman P., Sjoqvist F., *Lancet*, i, 333 (1982).
9) Suzuki T., Narimatsu S., Fujita S., Masubuchi Y., Umeda S., *Biochem. Pharmacol.*, **42**, 2241 (1991).
10) Narimatsu S., Masubuchi Y., Hosokawa S., Ohmori S., Kitada M., Suzuki T., *Biol. Pharm. Bull.*, **17**, 803 (1994).
11) Oatis Jr. J. E., Russel, M. P., Knapp D. R., Walle T., *J. Med. Chem.*, **24**, 309 (1983).
12) Omura T., Sato R., *J. Biol. Chem.*, **239**, 2370 (1964).
13) Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., *J. Biol. Chem.*, **193**, 265 (1951).
14) Suzuki T., Narimatsu S., Fujita S., Masubuchi Y., Umeda S., Imaoka S., Funae Y., *Drug Metab. Dispos.*, **20**, 367 (1992).
15) Fujita S., Morimoto R., Chiba M., Kitani K., Suzuki T., *Biochem. Pharmacol.*, **38**, 2925 (1989).
16) Imaoka S., Terano Y., Funae Y., *J. Biochem.*, **105**, 939 (1989).
17) Imaoka S., Terano Y., Funae Y., *Biochim. Biophys. Acta*, **916**, 358 (1987).
18) Imaoka S., Terano Y., Funae Y., *J. Biochem.*, **104**, 481 (1988).
19) Yamaoka K., Tanigawara Y., Nakagawa T., Uno T., *J. Pharmacobio-Dyn.*, **4**, 879 (1981).
20) Al-Dabagh S. G., Idle J. R., Smith R. L., *J. Pharm. Pharmacol.*, **33**, 161 (1981).
21) Kahn G. C., Rubenfield M., Davies D. S., Murray S., Boobis A. R., *Drug Metab. Dispos.*, **13**, 510 (1985).
22) Masubuchi Y., Umeda S., Chiba M., Fujita S., Suzuki T., *Biochem. Pharmacol.*, **42**, 693 (1991).
23) Masubuchi Y., Kagimoto N., Narimatsu S., Fujita S., Suzuki T., *Drug Metab. Dispos.*, **21**, 1012 (1993).