Stereoselective Hydrolysis of *O*-Acetyl Propranolol as Prodrug in Rat Tissue Homogenates

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Abstract □ The stereochemical characteristics of the hydrolysis of *O*-acetyl propranolol were studied using phosphate buffer (pH 7.4), rat plasma, and rat tissue homogenates. In the phosphate buffer, no difference was observed in the hydrolysis rate between the esters of (*R*)- and (*S*)-propranolol. In rat plasma and tissue homogenates, hydrolysis of the ester was both accelerated and stereoselective. Hydrolysis of *O*-acetyl (*R*)-propranolol was five times faster than that of the (*S*)-isomer in rat plasma. However, in the liver and intestine homogenates, the (*S*)-isomer was hydrolyzed faster than the (*R*)-isomer. Interconversion between the (*R*)- and (*S*)-isomers was not observed under the experimental conditions. The same stereoselective hydrolysis was also observed with racemic *O*-acetyl propranolol. However, observed rate constants for the hydrolysis were lower than those for the pure isomers. These results indicate that enzymatic hydrolysis of *O*-acetyl propranolol occurred stereoselectively and the selectivity of the plasma enzyme was different from those of liver and intestine enzymes.

Propranolol (PL) is a nonselective β-adrenergic receptor blocker that is well absorbed from the gastrointestinal tract but undergoes substantial presystemic metabolism before reaching the general circulation.[1,2] Since one of the major metabolites is the *O*-glucuronide of PL,[3] esterification of PL may be a potentially useful way to reduce presystemic metabolism, as has already been demonstrated.[4,5]

Propranolol has two optical isomers, dextro-PL [(*R*)-PL] and levo-PL [(*S*)-PL]. The (*S*)-PL is ~100 times more potent as a β-adrenoreceptor blocker than the (*R*)-isomer.[6] A number of studies have demonstrated the stereoselective disposition of this drug.[7-12] It is therefore expected that hydrolysis of esterified PL prodrugs may proceed stereoselectively after their administration.

In our preceding report,[13] an HPLC method for determining the enantiomers of PL acetate was described, and the stereoselective hydrolysis of PL acetate was demonstrated with human serum. In the present study, stereoselective hydrolysis of PL acetate has been studied with phosphate buffer, rat plasma, and rat tissue homogenates.

Experimental Section

Animals—Male Wistar rats (180–220 g) were used. All rats were fasted overnight (for 16 h) prior to sacrifice.

Materials—Racemic propranolol hydrochoride was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). The (*R*)- and (*S*)-propranolol (PL) hydrochlorides were obtained from Aldrich Chemical Company (Milwaukee, WI). All other reagents used were of analytical grade. The racemic mixture of PL acetate hydrochloride was prepared by a previously published procedure.[14] The (*R*)- and (*S*)-PL acetate hydrochorides were prepared by the same procedure from (*R*)- and (*S*)-PL hydrochoride, respectively. The synthesized (*R*)- and (*S*)-PL acetates showed a single peak on HPLC, and (*R*)- and (*S*)-PL acetates were hydrolyzed to (*R*)- and (*S*)-PL, respectively.

Preparation of Mucosal Cells from Small Intestine—Rats were killed by decapitation. The upper two-thirds of the small intestine was removed rapidly, immersed in ice-cold 0.9% NaCl, and cut into tubes ~5 cm long. All subsequent manipulations were carried out at ~4 °C. Fat and omentum were removed, and the lumen was washed with saline. Each tube was cut open longitudinally. The mucosal layer was collected by scraping with a glass slide.

Enzyme Preparations—The mucosal cells and the liver were homogenized in 5 volumes of 1.15% KCl with a Potter-Elvehjem glass homogenizer equipped with a Teflon pestle and then centrifuged at 9000 × *g* for 20 min at 4 °C. To adjust the protein content (1.3 mg/mL in liver sample and 0.5 mg/mL in intestine sample), the supernatant fraction was diluted with 1.15% KCl solution. This solution was further diluted nine times with phosphate buffer (pH 7.4, $\mu = 0.5$). Blood was collected in heparinized tubes, and the plasma was separated by centrifugation (10 000 × *g*) for 15 min at 4 °C.

Hydrolysis of Propranolol Acetate—One hundred microliters of PL acetate (150 μM for the pure isomer or 300 μM for the racemic compound) was added to 900 μL of the enzyme preparation and incubated at 37 °C. Then, 100 μL of the sample was withdrawn from the reaction mixture at predetermined intervals and mixed with 200 μL of acetonitrile. The mixture was agitated and centrifuged at 10 000 × *g* for 10 min. The supernatant (100 μL) was mixed with 200 μL of the mobile phase for HPLC (see below) and stored in an ice-water bath prior to assay. Hydrolysis of PL acetate in phosphate buffer (pH 7.4, $\mu = 0.5$) was assayed in the same way as above, using 900 μL of buffer instead of the enzyme preparation.

Assay Methods—The enantiomers of PL acetate were determined by HPLC with an ovomucoid-conjugated column (Ultron ES-OVM, Shinwa Kako Company Ltd., Kyoto, Japan) as described previously.[13] The concentration of protein in the enzyme solution was determined by the method of Lowry et al.[15]

Results and Discussion

The disappearance of PL acetate obeyed first-order kinetics. Figure 1 shows the typical first-order plots for the hydrolysis of (*S*)- and (*R*)-isomers of PL acetate. Apparent first-order rate constants were determined from the slopes of the plots. The rate constants for PL acetate with various tissue preparations are summarized in Table I. There was no significant difference between the hydrolysis rates of (*R*)- and (*S*)-PL acetate in the phosphate buffer without tissue preparations, as previously reported.[13] The hydrolysis was accelerated by the addition of plasma or tissue homogenate, and stereoselectivity was observed for the accelerated hydrolysis. In plasma, the hydrolysis of (*R*)-PL acetate was five times faster than that of the (*S*)-isomer when the individual enantiomers were used as substrates (Table I). In our preceding report,[13] the same stereoselective hydrolysis of PL acetate was observed with human serum. However, the stereoselectivity of the hydrolysis was reversed when the liver or intestine homogenate was used as the enzyme preparation [i.e., the hydrolysis of the (*S*)-isomer was faster than that of the (*R*)-isomer (Table I)]. Although the stereoselectivity for PL acetate of the intestine and liver homogenates was the same, a higher value of *S*:*R* was obtained for liver than for intestine, suggesting a higher stereoselectivity for PL acetate in liver.

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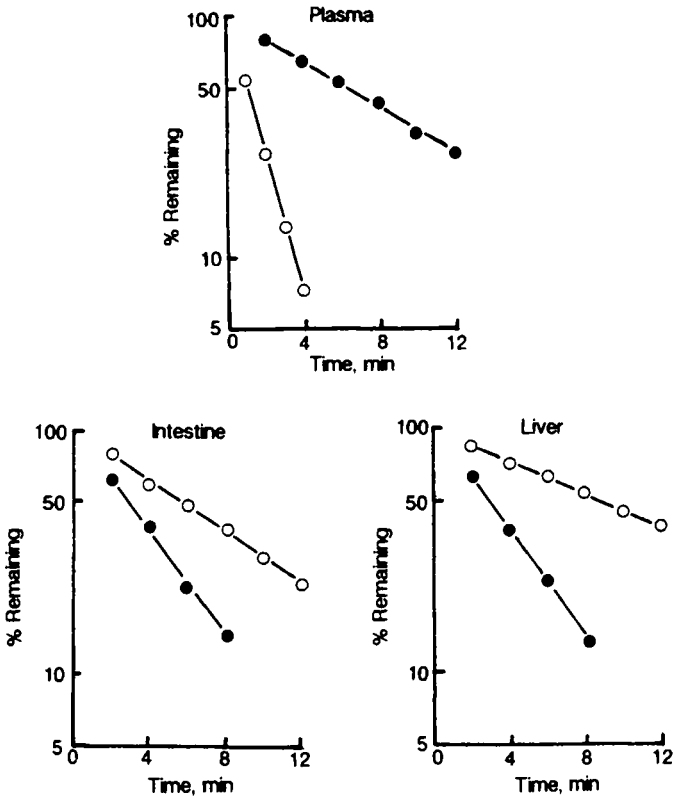

Figure 1—First-order plots for the hydrolysis of *O*-acetyl propranolol in rat tissue homogenates. Key: (○) *O*-acetyl (*S*)-propranolol; (●) *O*-acetyl (*R*)-propranolol.

Table I—Hydrolysis Rate Constant of *O*-Acetyl Propranolol[a]

Drug	Rate Constant (× 10 min^{-1} × mg $protein^{-1}$)		
	Plasma	Intestine	Liver
Enantiomer			
R	0.159 ± 0.005	5.12 ± 0.11	1.10 ± 0.04
S	0.029 ± 0.001	10.20 ± 0.20	3.44 ± 0.06
S:R	0.18	2.00	3.13
Racemic compound			
R	0.067 ± 0.003	4.07 ± 0.20	0.70 ± 0.02
S	0.020 ± 0.001	9.40 ± 0.51	1.98 ± 0.04
S:R	0.30	2.31	2.83

[a] Each value represents mean ± SD (n = 3).

The same stereoselective hydrolysis was observed with the racemic compound (Table I). However, the observed rate constants were lower than those obtained with the individual isomers, although the final concentration of substrate used was 15 μM for the isomers and 30 μM for racemic compound. This suggests the possibility that each isomer may inhibit the hydrolysis of another isomer in the study using racemic compounds.

Judging from the hydrolysis rates shown in Table I, PL acetate may be hydrolyzed mainly by esterases in the intestine and liver. These esterases consist of several isozymes, and esterase activity differs among various tissues. Salmona et al.[16] reported that oxazepam succinate was stereoselectively hydrolyzed and the selectivity differed between brain and liver. In this study, we demonstrated that hydrolysis of PL acetate by plasma, liver, and intestine showed different stereoselectivity. It is therefore generally considered that many esterases catalyze the enantioselective hydrolysis of optically active prodrugs and that stereoselectivity differs among tissues.

The (*S*)-isomer is known to reduce hepatic blood flow, which will influence metabolism of the drug.[17,18] Anderson et al.[5] suggested that the oral bioavailability of PL was significantly increased when PL hemisuccinate and acetate were used as the prodrugs. They also suggested that the prodrugs were absorbed from the gastrointestinal tract in the intact form, since the prodrugs were detected in blood. The bioavailability of the (*S*)-isomer is important when considering the activity of PL after oral administration, because the (*S*)-isomer is ~100 times more potent as a β-adrenoreceptor blocker than the (*R*)-isomer.[6] However, they did not detect enantiomers of the prodrugs and PL. In the present study, we demonstrated that the (*S*)-isomer of PL acetate was more extensively converted to PL by the esterases of intestine and liver.

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