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Stereoselective binding of β-blockers to purified rat α_1-acid glycoprotein

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Abstract—The stereoselectivity of binding of four β-blockers, pindolol, propranolol, oxprenolol and acebutolol, to purified rat α_1-acid glycoprotein (AAG) was examined using equilibrium dialysis. Pindolol and propranolol were bound stereoselectively to AAG, whereas binding of oxprenolol was non-stereospecific. Neither of the enantiomers of acebutolol bound to either AAG or any other plasma protein. The affinity of (+)-pindolol was 25 times that of (−)-pindolol, as determined in a single enantiomer experiment. Both enantiomers of propranolol demonstrated two classes of binding sites in AAG, the total binding for the high affinity site for (+)-propranolol being double that of (−)-propranolol, which could explain the higher binding of the (+)-enantiomer in racemate experiments. These results further showed that stereoselective binding to a rat AAG is not a property common to all β-blockers.

A number of basic as well as some acidic drugs have been shown to specifically bind to human α_1-acid glycoprotein (AAG) (Piafsky 1980). This latter is known to increase in stressful situations such as infection, inflammation, cancer, trauma (Schmid 1975) and in patients after surgery (Fremstad et al 1976). Stereoselective binding of propranolol to human AAG has been clearly demonstrated (Walle et al 1983; Albani et al 1984).

Belpaire et al (1984) suggested that oxprenolol and propranolol might bind mainly to AAG in dog and rat plasma, as tris(2-butoxyethyl)phosphate, a displacer for human AAG, effectively depressed the binding of these drugs in plasma. In addition Yasuhara et al (1985) showed that increase of plasma protein binding of propranolol was accompanied by elevated levels of plasma AAG in rats after laparotomy. Bai et al (1983a, b) showed that the binding of (−)-propranolol was higher than that of (+)-propranolol in dog plasma. More recently, Takahashi & Ogata (1990) and Takahashi et al (1990) reported stereoselective plasma binding to produce distinct differences between the distribution patterns of (+)- and (−)-propranolol in rats. However, there have been no animal studies on the stereoselectivity of binding of β-blockers to AAG itself. Because pre-clinical studies of all drugs containing racemic components have been conducted in experimental animals and the concentration of free drug in the plasma might affect not only pharmacological function but also hepatic uptake and distribution to the tissues, stereoselective binding properties and changing of binding protein levels should be examined in-vivo under the same experimental conditions.

Recently, we found strong stereoselective binding of pindolol, one of the widely used β-blockers, to rat plasma protein and indicated that an increase of plasma binding to both enantiomers after endotoxin treatment was accompanied by increased AAG (Hasegawa et al 1989).

The purpose of the present study was to determine whether stereoselective binding of pindolol to purified rat AAG occurs and to examine whether stereoselective binding to rat AAG might be a common property of all β-blockers.

Materials and methods

Materials. (±)-Pindolol and (±)-acebutolol hydrochloride were generous gifts of Sandoz Pharmaceuticals, Ltd (Tokyo, Japan). (±)-Propranolol hydrochloride and (±)-oxprenolol were purchased from Sigma Chemical Company (St Louis, MO USA). (±)-Pindolol, (±)-acebutolol and (±)-oxprenolol were resolved into their enantiomers by fractional crystallization of diastereoisomers obtained by reaction with di-*p*-toluoyl-l-(+)-tartaric acid (Howe & Shanks 1966). (+)- and (−)-Propranolol hydrochloride were kindly supplied by ICI Pharmaceuticals. 2,3,4,6-Tetra-*O*-acetyl-D-glucopyranosyl isothiocyanate (GITC), used to derivatize the diastereomers of β-blockers, was prepared from α-acetobromoglucose as described by Nimura et al (1981). All other chemicals used were of analytical or HPLC grade.

Purification of rat AAG. Wistar male rats, 200–250 g, were injected with 0·4 mL turpentine oil subcutaneously two days before drawing blood. AAG was purified by the method of Charlwood et al (1976).

Estimation of binding to purified rat AAG. Equilibrium dialysis was carried out as described previously (Hasegawa et al 1989). The concentrations of both enantiomers in both sides of the membranes were determined by HPLC after derivatization with GITC (Hasegawa et al 1989).

Calculation of binding parameters. In the single enantiomer experiment, initial estimates of association constants and numbers of binding sites were obtained from Scatchard plots. Binding parameters were then obtained by non-linear regression analysis using MULTI (Yamaoka 1984) and the following equation:

$$\gamma = \frac{n_1 k_1 f}{1 + k_1 f} + \frac{n_2 k_2 f}{1 + k_2 f}$$

where γ is the number of drug molecules bound per AAG molecule, f is the free drug concentration, n_1 and n_2 are the binding capacities of high (1)- and low (2)-affinity binding sites and k_1 and k_2 are the respective association constants of the two groups of binding site.

Results

Binding properties of β-blockers to rat plasma. In this study, rat plasma containing high concentration of AAG was obtained by pretreatment with turpentine oil. After equilibrium dialysis of four racemic β-blockers with normal or turpentine oil-pretreated rat plasma samples, enantiomer binding ratios were estimated on the plasma side (Table 1).

The results indicate stereoselective binding of pindolol and propranolol, and non-stereoselective binding of oxprenolol to plasma proteins. Neither acebutolol enantiomer was bound to rat plasma. Turpentine oil pretreatment effectively increased the level of plasma binding proteins.

Binding properties of racemic β-blockers to purified rat AAG. AAG was purified from plasma of rats pretreated with turpentine oil and identified. In a preliminary experiment, 10 μg mL^{-1} of the four racemic β-blockers was dialysed against 1 or 2 mg mL^{-1} of purified AAG (Table 2). At 1 mg mL^{-1} AAG, pindolol and propranolol demonstrated definite stereoselective binding while oxprenolol showed nearly equal values of binding for both enantiomers. Increasing the concentration of AAG (2 mg mL^{-1})

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Table 1. Stereoselective binding of β-blockers to rat plasma.

	Normal rat		Turpentine-treated rat	
	(−)-Enantiomer	(+)-Enantiomer	(−)-Enantiomer	(+)-Enantiomer
Pindolol	18·6±2·8	48·0±1·2	72·2±1·1	88·7±0·5
Propranolol	57·0±0·9	78·4±1·2	94·4±0·6	101·3±2·5
Oxprenolol	57·0±11·9	60·0±10·3	95·4±1·2	96·0±0·9
Acebutolol	*	*	*	*

Turpentine oil was injected subcutaneously (0·2 mL/100 g) 2 days before bleeding. Initial concentration of each drug was 10 μg mL^{-1} on the buffer side. All binding percentage values (plasma side) are expressed as means ± s.d. for four experiments. *No binding.

resulted in higher binding ratios for all three drugs but the differences of binding ratios between enantiomers in the cases of pindolol and propranolol diminished. Acebutolol did not bind to AAG at either concentration, consistent with the results of the plasma binding experiment in this study.

Dose-dependent binding study of β-blockers. In this experiment, the AAG level was set at 1 mg mL^{-1} in view of the good stereoselective binding obtained for pindolol and propranolol; it also corresponds to therapeutic concentrations of these drugs. (±)-Pindolol in the range of 0·3–150 μg mL^{-1} (1·2–600 μM) on the buffer side was dialysed, and total and bound concentrations of both enantiomers on the AAG side were estimated by stereoselective determination. Fig. 1a illustrates the strong differences in binding for the enantiomers of pindolol, observed throughout the range of concentrations investigated.

The binding percentage of (+)-pindolol reached nearly 100% but (−)-pindolol did not bind more than 70% even at a concentration of less than 0·14 μM. The biggest difference in the binding was obtained at around 1·6–160 μM. In the case of propranolol, similar stereoselective binding was observed at concentrations greater than 10 μM (Fig. 1b). However, binding of (−)-propranolol increased to 100% at a concentration of 3 μM, in clear contrast to a maximum of 70% binding observed for (−)-pindolol. In the study of oxprenolol, no clear stereoselective binding was recognized, the maximum binding of both enantiomers reaching 100% at 2 μM (Fig. 1c). Almost the same binding properties to purified rat AAG were demonstrated by both, although the (+)-enantiomer bound slightly more strongly than the (−)-enantiomer at all concentrations. No binding was observed in the same experiment with (±)-acebutolol (data not shown).

Binding parameters of pindolol and propranolol enantiomers. Binding of single enantiomers to AAG was examined to obtain accurate binding parameters because the binding of both enantiomers was affected by the presence of the opposite enantiomers in racemate experiments (data not shown). Single enantiomers of pindolol or propranolol in the range of 0·6–600 μM were dialysed against 1 mg mL^{-1} AAG. On Scatchard plots, both enantiomers of pindolol showed a linear relationship, indicating a single class of binding site. The binding parameters, n, k and nk, for each pindolol enantiomer were calculated by the least squares method on Scatchard plots (Table 3). The association constant (k) and the binding affinity value (nk) of (+)-pindolol were respectively 40 and 25 times that of (−)-pindolol, indicating a large difference in binding site affinity. In this single enantiomer experiment, binding percentage of (−)-pindolol reached 70% as in the racemate study. Binding profiles of (+)- and (−)-propranolol showed a non-linear relationship on Scatchard plots. On the assumption of two classes of binding sites, binding parameters (n_1, n_2, k_1, k_2) were calculated by computer simulation. As shown in Table 3, the high-affinity constant, n_1k_1 for (+)-propranolol was double that of (−)-propranolol but the low-affinity constants were similar in both cases.

Discussion

The disposition of (±)-pindolol has been studied in both man and experimental animals (Schwarz 1982) and the binding properties to human serum protein, and especially AAG, has been demonstrated to be characterized by one binding site (Lemaire & Tillement 1982). Hsyu & Giacomini (1985) reported that (±)-pindolol was stereoselectively eliminated by renal clearance but stereoselectivity in plasma protein binding was not observed. It might be possible that human AAG shows stereoselective binding of pindolol with opposite stereoselectivity for human albumin binding, as well as the binding property of propranolol as shown by Walle et al (1983). We have already shown that stereoselective binding of (±)-pindolol to plasma affected the distribution volume in rats and that an endotoxin-induced increase in plasma binding protein resulted in lower levels of free enantiomers. This increment was accompanied by elevated levels of AAG, suggesting that the latter is in fact a stereospecific binding protein for pindolol in rats (Hasegawa et al 1989).

Although AAG appears to have a single binding site for both

Table 2. Stereoselective binding % of β-blockers to α_1-acid glycoprotein (AAG) purified from plasma of rats pretreated with turpentine oil.

	1 mg mL^{-1} AAG		2 mg mL^{-1} AAG	
	(−)-Enantiomer	(+)-Enantiomer	(−)-Enantiomer	(+)-Enantiomer
Pindolol	17·8	63·8	43·0	83·3
Propranolol	35·4	80·8	75·6	92·7
Oxprenolol	63·3	67·9	82·2	85·2
Acebutolol	0·0	0·0	0·0	0·0

AAG was purified from plasma of turpentine oil-pretreated rats. The initial concentration of each drug was 10 μg mL^{-1} on the buffer side. All binding percentage values (AAG side) are expressed as the means of triplicate determinations.

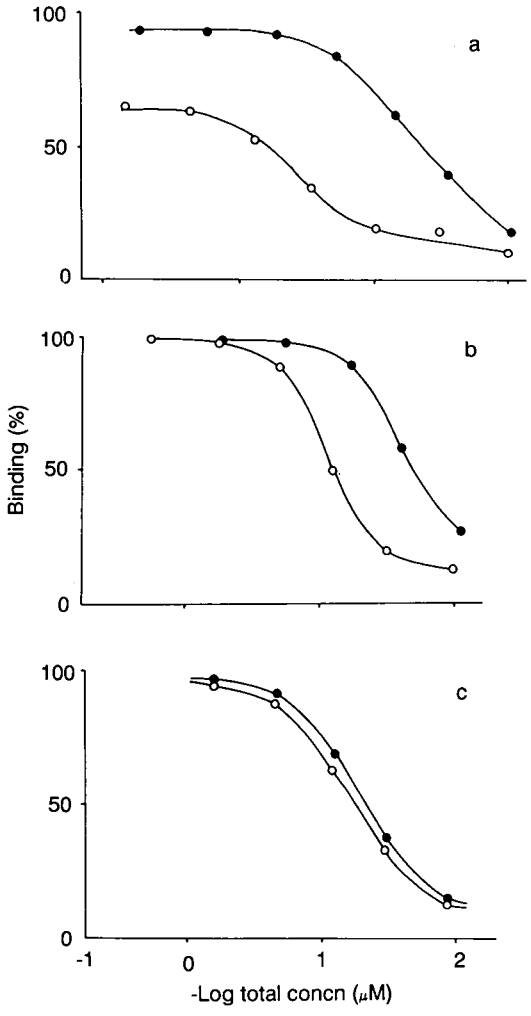

FIG. 1. Dose-dependent and stereoselective binding of pindolol (a), propranolol (b) and oxprenolol (c) to purified rat AAG. Racemate on the buffer side (0·4–400 μM) was dialysed against purified rat AAG (1 mg mL^{-1}) and the free and binding concentrations of both enantiomers were analysed on the AAG side by stereoselective determination. Each point represents the binding percentage of the total concentration (free and bound) of the (−)-enantiomer (○) or (+)-enantiomer (●).

(+)- and (−)-pindolol, the binding affinity value (nk) of the (+)-enantiomer was approximately 25 times higher than that of the (−)-enantiomer, suggesting a good reason for the stereospecific disposition of (±)-pindolol observed in our previous in-vivo study. However, it still remains to be clarified why the highest binding ratio of (−)-pindolol to AAG was only around 70% even in the present single enantiomer study.

The present experiment clearly showed stereoselectivity in binding to purified rat AAG, related to a difference in the high binding affinity values (n_1k_1) for propranolol enantiomers. Compared with human AAG (Oravcová et al 1989), k_1 values of both enantiomers to rat AAG were similar to that of the (−)-enantiomer and higher than that of the (+)-enantiomer to human AAG, but the n_1 values of both enantiomers were similar to that of the (+)-enantiomer to human AAG. On the other hand, since oxprenolol demonstrated non-stereospecific binding to rat AAG and acebutolol did not show any binding activity to rat AAG or any other plasma protein, stereoselectivity is just as clearly not a common property of all β-blockers.

It has been suggested that the lipophilic character of a drug molecule is an important factor in the binding to AAG (Gillis et al 1985; Pervaiz & Brew 1987). Furthermore, Walle et al (1988) reported that stereoselectivities in plasma and non-specific tissue binding and hepatic metabolism could be found only for lipophilic β-blockers such as propranolol. Distribution coefficients in n-octanol phosphate buffer (pH 7·4) for propranolol, pindolol, oxprenolol and acebutolol were reported to be 20·2, 0·82, 2·28 and 0·68, respectively (Woods & Robinson 1981). In the present study, stereoselective binding to rat AAG was observed not only for the most lipophilic drug, propranolol, but also for pindolol, with a much lower distribution coefficient. Furthermore, no significant stereoselectivity was found in the AAG binding of oxprenolol, which is more lipophilic and binds more extensively to AAG than does pindolol. These findings therefore suggest that lipophilicity is not the only factor determining the extent and stereoselectivity of binding of β-blockers to rat AAG.

Albumin is another candidate plasma-binding protein. The differences (Tables 1, 2) observed for β-blocker binding to total plasma proteins and purified AAG might indeed suggest some for albumin in the plasma, as well as human albumin as shown by Walle et al (1983). However, the plasma concentration of albumin was decreased by turpentine oil-pretreatment (control: 34·3 ± 1·7 mg mL^{-1}, turpentine: 23·9 ± 1·1 mg mL^{-1}, $P < 0.01$) without change in total protein levels (control: 42·7 ± 2·1 mg mL^{-1}, turpentine: 41·4 ± 1·1 mg mL^{-1}) and we therefore conclude that AAG is the most important plasma binding protein in this inflammatory condition.

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Table 3. Binding parameters of single pindolol and propranolol enantiomers to purified rat AAG.

	n_1	$k_1(M^{-1})$	$n_1k_1(M^{-1})$	n_2	$k_2(M^{-1})$	$n_2k_2(M^{-1})$
(+)-Pindolol	0·642	$2·82 \times 10^6$	$1·5 \times 10^6$			
(−)-Pindolol	1·04	$5·96 \times 10^4$	$6·20 \times 10^4$			
(+)-Propranolol	0·904	$2·82 \times 10^6$	$2·55 \times 10^6$	1·74	$1·07 \times 10^4$	$1·86 \times 10^4$
(−)-Propranolol	0·607	$2·12 \times 10^6$	$1·29 \times 10^6$	2·81	$4·28 \times 10^3$	$1·20 \times 10^4$

Initial concentration of each enantiomer was 0·6–600 μM on the buffer side. After dialysis against 1 mg mL^{-1} of AAG, free and bound concentrations were estimated on the AAG side. Binding parameters for pindolol enantiomers were calculated by the least square method on Scatchard plots and the parameters for propranolol enantiomers were obtained by computer simulation, assuming two classes of binding site.

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Centpropazine affinity to cortical noradrenergic receptors and effect on their responsiveness in the rat*

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Abstract—We have studied the in-vitro effect of centpropazine on cerebral cortical noradrenergic receptors measured as the accumulation of second messengers, cyclic AMP and inositol phosphate, stimulated by noradrenaline, and the binding to α_1- and β-adrenoceptors. Centpropazine inhibited inositol phosphate, but not the cyclic AMP accumulation in the cerebral cortical slices of the rat. It moderately antagonized the specific binding of [^{3}H]prazosin, but did not affect the specific binding of the β-adrenoceptor ligand, [^{3}H]CGP 12177, to cerebral cortical membranes.

Centpropazine, 1-(*p*-propionylphenoxy)-3-(N^4-phenylpiperazynyl)-propan-2-ol, has been described as a putative antidepressant with a pharmacological profile resembling that of amitriptyline and imipramine (Rastogi et al 1972). In male volunteers centpropazine given in single oral doses of 10–160 mg was well tolerated and did not affect vital parameters (Gupta et al 1989). Phase II clinical studies with depressed patients gave promising results (Srivastava et al 1991).

In animal experiments, centpropazine counteracted reserpine-induced depression, potentiated amphetamine-induced activity in mice and rats, and augmented amphetamine toxicity in mice. In higher doses the drug displayed anti-amphetamine properties (Rastogi et al 1972).

Given chronically to rats, centpropazine, like imipramine, depressed the density of 5-HT_1 and 5-HT_2 receptors in the cortex, but differed from imipramine in producing no β-down-regulation (Hussain et al 1988). As β-down-regulation is regarded as one of the important characteristics of antidepressant drugs (Vetulani 1984), further studies on effects of centpropazine on adrenergic receptor responses were warranted.

No biochemical data concerning the action of centpropazine on the second messenger systems related to adrenergic receptors

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