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The stereoselectivity of the 'single drug binding site' of human α_1-acid glycoprotein (orosomucoid)

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The stereoselective binding of six pairs of basic, one pair of acidic drug enantiomers, and one pair of diastereomers to human α_1-acid glycoprotein was investigated by means of competition experiments against [^{3}H]propranolol- or [^{14}C]nicardipine-labelled binding sites using equilibrium dialysis to separate free from bound marker ligand. The affinity constants (K_a) for association of [^{3}H]propranolol and [^{14}C]nicardipine with α_1-AGP were $1{\cdot}2 \pm 0{\cdot}6 \times 10^5\ M^{-1}$ and $3{\cdot}4 \pm 1{\cdot}4 \times 10^5\ M^{-1}$, respectively, and control binding amounted to 57 ± 7 and $91 \pm 2\%$, respectively. The following selectivity factors, calculated as the ratio of the higher over the lower enantiomer concentrations displacing 15% of control radiomarker binding (IC15-value), were obtained against propranolol and nicardipine: (−)-/(+) propranolol: 1·9 and 1·7.; (+)-/(−)-disopyramide: 2·8 and 1·4; (+)-/(−)-verapamil: 1·6 and 1·9; (+)-(*S*)-/(−)-(*R*)-202-791, a dihydropyridine derivative: 2·6 and 2·0; (−)-/(+)-asocainol: 1·7 and 3·0; (+)-/(−)-tilidine: 1·1 and ≈2; (−)-(*S*)-/(+)-(*R*)- warfarin: 1·6 and 2·4; (±)-*cis*/(±)-*trans*-*trans*-tilidine: 1·7 and 1·8. When the calculation of radioligand-free fractions is also taken into account, it is apparent that only the tilidine isomers show no selectivity at propranolol-marked, and the disopyramide isomers at nicardipine-marked α_1-AGP-binding sites, in all other cases, a weak selectivity is detectable, which is, however, far below the values obtained for most neurotransmitter receptors. It is concluded that the single drug binding site of α_1-AGP is only slightly stereoselective and that the stereoselective binding of the drugs investigated is probably of no clinical consequence.

Protein binding by α_1-acid glycoprotein (α_1-AGP) is recognized for a number of basic and some acidic drugs (Piafsky 1980; Paxton 1983), all of which seem to bind to a single high affinity site on the glycoprotein molecule which has tentatively been termed the 'basic drug binding site' (Müller & Stillbauer 1983; Brunner & Müller 1985; for a recent review see Müller et al 1986). Although the binding to α_1-AGP has originally been considered to be non-stereoselective, some recent reports have demonstrated stereoselective binding of propranolol (Albani et al 1984; Walle et al 1983), disopyramide (Lima et al 1984) and verapamil (Vogelsang & Echizen 1985). The stereoselectivity factors are generally only around 2. It seems that stereoselective binding to plasma proteins may contribute to stereospecific pharmacokinetics (Wilkinson & Shand 1975), e.g. stereospecific protein binding has been implicated in the first-pass metabolism of verapamil (Vogelsang et al 1984) and propranolol (Von Bahr et al 1982). We have therefore decided to characterize the stereoselectivity of the α_1-AGP binding site with a method commonly used in receptor-radioligand binding studies. The binding site was labelled with either (±)-[^{3}H]propranolol or (±)-[^{14}C]nicardipine and the degree of stereoselectivity was determined in terms of the various drugs' ability to displace the radioligands. Propranolol has long been known to bind with high affinity to α_1-AGP (Glasson et al 1980) and nicardipine has recently been described to bind with even higher affinity (Urien et al 1985). The application of this indirect method using two specific ligands of the single binding site has the advantage that all binding data observed for the isomers can be attributed to binding to this site.

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MATERIALS AND METHODS

Materials

Human α_1-acid glycoprotein was obtained from Behringwerke (Marburg, FRG) (electrophoretic purity 99%). (±)-4-[^{3}H]Propranolol hydrochloride (20 Ci mmol^{-1}) was purchased from Amersham, Braunschweig (FRG), (±)-[^{14}C]nicardipine hydrochloride (24·4 mCi mmol^{-1}) and non-radioactive nicardipine hydrochloride were a generous gift of Yamanouchi Pharmaceutical Co., Tokyo (Japan). Drug enantiomers were obtained from the following manufacturers; Goedecke AG, Freiburg (FRG): (−)-tilidine hydrochloride (Gö 1261-M), (+)-tilidine hydrochloride (Gö 1261-P), (−)-asocainol hydrochloride (Gö 4474-A), (+)-asocainol hydrochloride (Gö 3764-A), (±)-*cis*-tilidine hydrochloride, (±)-

trans-trans-tilidine hydrochloride (diastereoisomers); Sandoz Ltd, Basel (Switzerland): (+)-(*S*)-202-791 and (−)-(*R*)-202-791 (isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-nitro-3-pyridine-carboxylate) (courtesy Dr Robert Hof). (−)-Disopyramide (SC29200) and (+)-disopyramide (SC29199) were obtained from Searle Laboratories, (+)- and (−)-propranolol hydrochloride from ICI, Plankstadt (FRG), (+)- and (−)-verapamil hydrochloride from Knoll AG, Ludwigshafen (FRG) and (−)-(*S*)- and (±)-(*R*)-warfarin from Dr E. Jähnchen, Bad Krozingen (FRG). Stock solutions of all compounds (1 mM) were made in distilled water except 202-791 and warfarin (ethanol).

Equilibrium dialysis

All experiments were performed with an equilibrium dialysis system (Dianorm) at 37 °C using Teflon cells of 0·2 mL half cell volume under constant stirring at 20 rev min^{-1}. The two chambers were separated by a semipermeable membrane (Thomopor; mol. wt cut off: 12 000). Dialysis time was 2·5 h for saturation experiments and 3–4 h in the presence of enantiomers. When [^{3}H]propranolol was used as radiotracer, the buffer cell was filled with 25 μL [^{3}H]propranolol (≈15–20 000 d min^{-1}) dissolved in water plus 75 μL [^{1}H]propranolol dissolved in 130 mM Sørensen phosphate buffer, pH 6·5 + 0·9% NaCl (initial concn: 3·2 μM, final concn: 0·6 μM) plus 100 μL of distilled water (control) or enantiomer dissolved in water to give various enantiomer/propranolol-concentration ratios. When [^{14}C]nicardipine was used, 25 μL of a 9·6 μM solution (initial concentration; final concentration: 0·6 μM = 12–14 000 d min^{-1}) plus 75 μL Sørensen buffer plus 100 μL water or enantiomer were added to the protein-free cell. For saturation experiments, the buffer cell was filled with 25 μL [^{3}H]propranolol (15–20 000 d min^{-1}) plus 100 μL distilled water plus 75 μL 'cold' propranolol of various concentrations (0·6–50 μM final concentrations) dissolved in buffer or 25 μL [^{14}C]nicardipine (10–15 000 d min^{-1}) plus 75 μL 'cold' nicardipine dissolved in buffer (0·6–100 μM final concentrations) plus 100 μL distilled water. The protein side was always filled with 200 μL α_1-AGP (0·4 mg mL^{-1} ≃ 10 μM) dissolved in distilled water. Non-specific binding to the Teflon chambers and the dialysis membrane amounted to 10–20% ([^{3}H]propranolol) and 20–40% ([^{14}C]nicardipine), but did not influence the calculation of per- cent binding. The reduction of total drug concentrations was, however, taken into account in saturation isotherms. The radioactivity in both cells was determined by counting 100 μL of each cell. Binding percentages were calculated according to the formula % bound = (B − A)/B with B being the molar concentration of propranolol or nicardipine in the protein compartment and A in the buffer compartment. The difference between total and bound drug concentration was considered as free concentration (fu). Due to the small volumes of the cells (0·2 mL) and the short dialysis times (4 h) no volume shift was observed.

Analysis of data

Saturation isotherms were analysed using a non-linear least-squares computer curve-fitting program (Wiemer et al 1982). The capacity of the tested enantiomers to displace the marker ligands from the glycoprotein binding site was expressed as the percentage of control binding (57 ± 7% for propranolol; 91 ± 2% for nicardipine, $\bar{x}$ ± s.d., n > 35) remaining in the presence of 4–5 concentrations of enantiomer (= competitor) spanning competitor radiotracer-ratios between 5 and 150 (propranolol) and 10–200 (250) (nicardipine). From such linearized inhibition curves, IC15-values (i.e. the enantiomer concentrations corresponding to 85% control binding) were determined and selectivity factors calculated by division of IC15-values in the order of lower affinity (higher IC15-value) through higher affinity. In addition, free fractions (fu) of radiotracer were determined for all competitor concentrations and the ratio between fu for the two enantiomers calculated (±s.d.).

RESULTS

Fig. 1 shows the saturation isotherms of the two marker ligands used in this study. The affinity constants, derived from individual curves, were 1·2 ± 0·6 × 10^5 M^{-1} (n = 5) for propranolol and 3·4 ± 1·4 × 10^5 M^{-1} (n = 5) for nicardipine. Based on the glycoprotein concentration used (10 μM), the calculated number of tracer binding sites per molecule of protein (n-value) was 1·25 ± 0·25 for propranolol and 1·6 ± 0·9 for nicardipine. The ability of seven pairs of enantiomers and one pair of diastereomers to displace the radiolabels from their binding site was determined as outlined in Methods. Representative examples of linearized radiotracer inhibition curves are shown in Figs. 2 and 3. IC15-values, determined from such graphs, the resulting selectivity factors and ratios of free fractions of radiotracer in the presence of enantiomers are compiled in Table 1. All drugs investigated show some degree of stereoselectivity, at least with one of the two radioligands. (−)-Propranolol, (+)-disopyramide, (−)-asocainol and (−)-(*S*)-

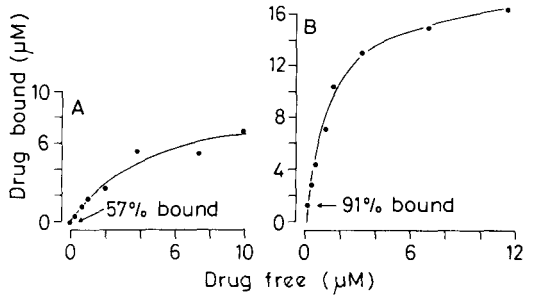

FIG. 1. Binding of (A) (±)-[^{3}H]propranolol (0·6–50 μM total concentration) and (B) (±)-[^{14}C]nicardipine (0·6–100 μM) to human α_1-AGP (10 μM) (representative experiment). The curves were analysed by nonlinear regression and the following parameters obtained from 5 experiments: K_a = 1·2 ± 0·6 × 10^5 M^{-1}, n = 1·25 ± 0·25 (propranolol); K_a = 3·4 ± 1·4 × 10^5 M^{-1}, n = 1·6 ± 0·9 (nicardipine). Binding at 0·6 μM amounted to 57 ± 7 and 91 ± 2%, respectively.

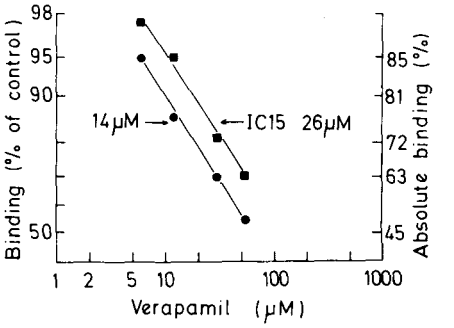

FIG. 3. Displacement of (±)-[^{14}C]nicardipine by (●) (+)- and (■) (−)-verapamil (probability scale). The left ordinate shows marker ligand binding as % of the control value (relative axis), the right ordinate as absolute binding values (%). IC15-values are indicated.

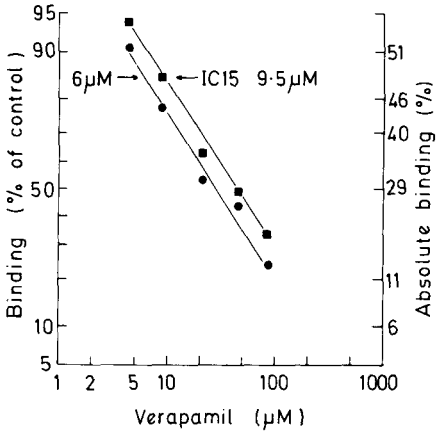

FIG. 2. Displacement of (±)-[^{3}H]propranolol by (●) (+)- and (■) (−)-verapamil (probability scale). The left shows marker ligand binding in % of the control value (relative axis), the right ordinate as absolute binding values (%). IC15-values are indicated.

warfarin displace more potently than the respective (+)-isomers and for verapamil, 202-791 and tilidine it is the converse (for disopyramide the selectivity factor is only 1·4). There is also a preference of (±)-*cis*-tilidine over the *trans-trans* form, whereas the stereoisomers (+)- and (−)-tilidine show the same affinity towards propranolol binding sites, but not nicardipine binding sites. To complement these data, the ratios of free fractions of radioligands were also calculated (Table 1): the highest value is 1·7 ((−)-(*S*)-warfarin, (+)-202-791), indicating that this analysis leads to somewhat lower indices of stereoselectivity than the comparison of percentages of radiotracer binding.

DISCUSSION

In both the pharmacodynamic action of drugs and their pharmacokinetic fate the importance of stereoselective phenomena is increasingly being recognized (for recent reviews see Williams & Lee 1985; Walle & Walle 1986; Simonyi et al 1986). Binding of predominantly basic, but also some acidic drugs to plasma-borne α_1-acid glycoprotein has been known for several years (Paxton 1983; Müller et al 1986), but the question whether these drugs bind stereoselectively to α_1-AGP has only recently been addressed. To judge the selectivity data obtained in this work, it is necessary first to comment on the radioligand binding data. For propranolol, an affinity constant of 1·2 × 10^5 M^{-1} was determined, which is within the range of association constants reported by others (Soltes et al 1985). The affinity of racemic nicardipine has been reported as 3 × 10^6 M^{-1} (Urien et al 1985) which is nine times higher than the value obtained by us. Nicardipine avidly binds to the dialysis membrane and the Teflon chambers, thus competing with α_1-AGP-bound drug and necessitating correction which was, however, apparently not necessary in the experiments of Urien et al (personal communication). The number of binding sites per molecule of glycoprotein was not significantly different from 1, with, however, a marked tendency towards 2 binding sites in some experiments with nicardipine. Additionally, in competition studies, linearized inhibition curves with nicardipine were not always strictly parallel which may indicate that nicardipine binds to a second site on α_1-AGP of unknown nature. Urien et al (1985), however, have reported an n-value of 0·84 lending no support to a second binding site.

Table 1. Displacement of (±)-[^{3}H]propranolol and (±)-[^{14}C]nicardipine (0·6 μM) from α_1-acid glycoprotein (10 μM) by 7 pairs of enantiomers and the diastereomeric pair of *cis*- and *trans-trans*-tilidine (***) at 4–5 enantiomer- to radiotracer-ratios as described in Methods. Tracer binding (%) was calculated for each competitor/tracer-ratio and plotted on logit/log (probability) paper as exemplified in Figs 2 and 3. IC15-values were obtained from such graphs at 85% radiotracer binding. The selectivity factor was obtained by division of the 2 IC15-values. Radiotracer free fractions (fu) were determined for each competitor/radiotracer ratio and fu-values for the more potent enantiomer divided by the value of the corresponding less potent enantiomer ($\bar{x} \pm$ s.d. of 4–5 pairs). Every data point was determined 4–8 times with a coefficient of variation between 5 and 10 ([^{14}C]nicardipine) and 15–20% ([^{3}H]propranolol).

Enantiomeric pair (competitors)	(±)-[^{3}H]Propranolol			(±)-[^{14}C]Nicardipine		
	IC15 (μM)	Selectivity factor	Ratio of fu ($\bar{x} \pm$ s.d.)	IC15 (μM)	Selectivity factor	Ratio of fu ($\bar{x} \pm$ s.d.)
(−)-/(+)-Propranolol	3·4 and 6·5	1·9	1·4 ± 0·19	11 and 19	1·7	1·4 ± 0·16
(+)-/(−)-Disopyramide	0·65 and 1·8	2·8	1·3 ± 0·10	70 and 100	1·4	1·1 ± 0·28
(+)-/(−)-Verapamil	6·0 and 9·5	1·6	1·3 ± 0·26	14 and 26	1·9	2·1 ± 0·64
(+)-(*S*)-/(−)-(*R*)-202-791	5·0 and 13*	2·6	1·2 ± 0·05	30 and 60	2·0	1·7 ± 0·34
(−)-/(+)-Asocainol	1·5 and 2·6	1·7	1·2 ± 0·17	6·0 and 18	3·0	1·6 ± 0·27
(+)-/(−)-Tilidine	2·4 and 2·6	1·1	1·0 ± 0·14	≈300 and ≈600**	≈2	1·5 ± 0·15
(−)-(*S*)/(+)-(*R*)-Warfarin	2·2 and 3·6	1·6	1·3 ± 0·11	8 and 19	2·4	1·7 ± 0·93
(±)-*cis*/*trans-trans*-Tilidine***	1·2 and 2·0	1·7	1·4 ± 0·15	170 and 300	1·8	1·8 ± 0·20

* IC30-values. ** Extrapolated. *** Diastereoisomers.

When competing with both propranolol and nicardipine, (−)-propranolol was more potent than (+)-propranolol, displacing 2–7% more radiolabel at any propranolol concentration. This is in agreement with all previous reports. Thus, Walle et al (1983) have reported a ratio of 1·16 for the unbound fraction ((+)-/(−)-propranolol), and Albani et al (1984), using a chromatographic method, have reported a fu-ratio of 1·31 for (+)-/(−)-propranolol. In plasma, the binding of the (−)-enantiomer is likewise greater than that of the (+)-enantiomer. In the present work, factors of 1·3 and 1·4 were obtained (Table 1). In circular dichroism measurements, the higher affinity of the (−)-isomer was also evident (Brunner & Müller 1985). In dog plasma, Bai et al (1983) have also found higher binding of the (−)-isomer and speculated that α_1-AGP might be the stereoselective binding protein.

With disopyramide the data are contradictory. When competing against propranolol, (+)-disopyramide is more potent than (−)-disopyramide resulting in a selectivity factor of 2·8. Labelling with nicardipine masks any selectivity. Lima et al (1984) using acid glycoprotein at 100 mg mL^{-1}, i.e. at 250 times the concentration used here and (±)-[^{14}C]disopyramide as marker, have found a preference of *S*(+)-disopyramide for α_1-AGP, as did Cook et al (1982). Huang & Øie (1983), on the other hand, have found no stereoselectivity in the binding of the drug to rabbit serum spiked with human α_1-AGP. Verapamil which shows pronounced differences in the various pharmacokinetic parameters for the two optical isomers (Eichelbaum et al 1984) also binds stereoselectively with a strong preference of (+)-verapamil to α_1-AGP and serum (Vogelsang & Echizen 1985), resulting in a ratio of free fractions of 1·55 ((−)/(+), α_1-AGP). The present results show fu-ratios of 1·3 (propranolol) and 2·1 (nicardipine), confirming the above results.

202-791 is a 1,4-dihydropyridine derivative with enantio specific action. The (−)-(*R*)-isomer is a calcium channel inhibitor whereas (+)-(*S*) 202-791 activates calcium entry into the cell (Williams et al 1985; Hof et al 1985). Again, binding to α_1-AGP of the (+)-isomer is more pronounced than that of the laevorotatory form with selectivity factors between 2 and 2·6 and fu-ratios of 1·2 and 1·7. Since the two isomers exert pharmacologically opposite actions, they should be considered as two distinct drug entities with potentially very different plasma protein binding, making the determination of binding of the racemic drug worthless.

The new antiarrhythmic drug, asocainol, is highly bound to plasma proteins (96%) without apparent stereoselectivity (Vollmer et al 1985). The present study shows that binding of (−)-asocainol to α_1-AGP is more pronounced than that of (+)-asocainol, especially with nicardipine. Ratios of free fractions are, however, not significantly different from unity for propranolol-marked binding sites. As a whole, asocainol binding to α_1-AGP is of borderline stereoselectivity.

The analgesic tilidine was also available both as a pair of enantiomers and diastereomers. Of the stereoisomers, (+)-tilidine displaces nicardipine more potently, but against propranolol there is no selectivity. The diastereomer (±)-*cis*-tilidine, on the other hand, is much more potent than (±)-*trans-trans*-tilidine, resulting in about 2-fold higher free fractions of the *trans-trans* form than the *cis*-form. This selectivity is detected by both tracers. A similar

preference of (±)-*cis*-tilidine was determined from human plasma proteins (Jänicke & Gundert-Remy 1985). α_1-AGP therefore seems to play a major role in tilidine-diastereomer binding. The binding of the acidic drug warfarin to α_1-AGP, first reported by Urien et al (1982), is also stereoselective in that the (−)-(*S*)-isomer binds more strongly. The selectivity factors and ratios of free fractions are, however, in the same range as for all other drugs tested in the present study. Again, nicardipine yields somewhat more pronounced effects.

In conclusion, binding of pharmacologically relevant drugs to human α_1-AGP is moderately stereoselective, the degree of which varies somewhat with the radioligand used to label the glycoprotein binding site.

Despite the use of enantiomer/radiolabel-ratios up to 150 (propranolol) and 200–250 (nicardipine), parallel inhibition curves have been obtained in nearly all cases, and it is concluded that the competition at the binding site has not led to a redistribution of radiolabel to other areas of the glycoprotein molecule. This indirect evidence seems to substantiate further the concept of a single drug binding site on α_1-AGP. As a whole, the results with nicardipine are also compatible with this scheme, although binding to an additional site cannot be ruled out.

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