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Prazosin binding to human α_1-acid glycoprotein (orosomucoid), human serum albumin, and human serum. Further characterization of the 'single drug binding site' of orosomucoid

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The plasma protein binding of the α_1-adrenergic blocking agent prazosin was investigated by means of circular dichroism (CD) and equilibrium dialysis (ED) measurements. The interaction of prazosin with human α_1-acid glycoprotein (α_1-AGP) results in pronounced negative extrinsic Cotton effects at 255 nm and a smaller negative band at 285 nm which are associated with the binding of prazosin to only one site of the protein. Various basic drugs, and warfarin also, at 50 μM displace prazosin 10 μM from its binding site on α_1-AGP and reduce the CD-spectra at 255 nm by 26% (disopyramide), 52% (mepivacaine), about 70% (verapamil, biperiden), and 90–100% (trihexyphenidyl, warfarin). (±)-Propranolol reduces the CD-spectra by 76%, its (−)-isomer by 89%, and the (+)-isomer by 65%. ED experiments indicated that the binding of prazosin to α_1-AGP is saturable with an association constant of 48 000 M^{-1} and 0·85 binding sites per protein molecule. Displacement of prazosin from α_1-AGP by the same drug as used for the CD experiments at displacer/prazosin ratios of 5 resulted in comparable reductions of the fraction bound as obtained by the CD experiments. Prazosin was also highly bound to human serum albumin (600 μM) with about 80–85% bound at prazosin concentrations from 1–100 μM. Since prazosin binding to human serum is only slightly higher (80–90%) it is concluded that prazosin binding in serum is largely mediated by the albumin fraction. The results indicate that prazosin binds to the single drug binding site of human α_1-AGP, that this site is slightly stereospecific for propranolol, and that anionic as well as cationic drugs bind with high affinity to this drug binding site of α_1-AGP.

α_1-Acid glycoprotein (α_1-AGP) has recently been recognized as a high affinity, but low capacity, binding component in human plasma (Piafsky 1980; Paxton 1983) with high affinity for a variety of basic drugs. The high affinity binding of most of these drugs is mediated by one common site of the glycoprotein molecule, tentatively termed the 'basic drug binding site' (Müller & Stillbauer 1983; Müller et al 1983). Accordingly, the characterization of this binding site has become necessary to understand the molecular basis of drug binding to the acute phase protein α_1-AGP.

The α_1-adrenoceptor antagonist prazosin has recently been reported to bind with high affinity to only one site of α_1-AGP (Dale & Nilson 1984). However, contrary to most basic drugs binding with high affinity to only one site of α_1-AGP, prazosin binds additionally with high affinity to human serum albumin (HSA) with an association constant only one order of magnitude lower than that found for α_1-AGP (Rubin & Blaschke 1980; Dale & Nilsen 1984). Thus, prazosin might be an interesting tool to study common and different molecular aspects of drug binding to α_1-AGP and HSA. Moreover, if the single binding site of prazosin on α_1-AGP is identical with the basic drug binding site mentioned, binding studies with prazosin should give new information about the nature of this binding site for many drugs in human blood.

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MATERIALS AND METHODS

Materials

Human α_1-acid glycoprotein (α_1-AGP, electrophoretic purity 99%) was obtained from Behringwerke, Marburg (FRG) and human serum albumin from Sigma Chem. Comp., St Louis (USA) (A-1887, fatty acid free). Human serum was drawn from several healthy subjects, pooled and stored at −30 °C. Serum albumin was adjusted to 40 g $litre^{-1}$ (600 μM). [^{3}H]prazosin (spec act. 23 Ci $mmol^{-1}$) was obtained from Amersham, Braunschweig (FRG), and had an optical purity >98%. All other drugs were gifts from the manufacturers.

Circular dichroism measurements

Circular dichroism measurements (CD) were made at room temperature (20 °C) with a Cary 61 CD spectropolarimeter calibrated with (+)-camphorsulphonic acid. Spectra were recorded in cylindrical cells with 10 mm path length using a full scale deflection of 0·02° and a spectral band width of 2 nm. All measurements were made in 0·05 M phosphate buffer pH 7·4. Results are expressed as molar ellipticity ([θ]) calculated with reference to the α_1-AGP concentration (10 μM, 0·4 mg ml^{-1}).

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 (Diachema, m. w. cutoff: 10 000). Equilibration time was 1½ h in the absence and 2 h in the presence of competitors. The buffer cell was filled with 100 μl [^{3}H]prazosin as tracer (15–20·000 d min^{-1} = 0·5–1 nM final concentration) plus 100 μl cold prazosin of various concentrations (generally 1–100 μM) for saturation experiments. In competition experiments, the buffer compartment contained 10 μl [^{3}H]prazosin (5–10 nM), 90 μl [^{3}H]prazosin at 44 μM (final concn 10 μM) and 100 μl of competitor at 200 μM (final concn 50 μM), yielding a competitor/prazosin ratio of 5. The second half cell was filled with 200 μl of the respective protein solution. Drugs were dissolved in 67 mM phosphate buffer, pH 7·4 whenever possible or in ethanol and diluted with buffer. The final ethanol content never exceeded 5% and was usually less than 1%. Non-specific binding to the dialysis membrane amounted to 15–25% and was neglected in the calculation of prazosin concentrations. 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 × 100 with B being the molar concentration of prazosin in the protein compartment and A in the protein-free compartment.

The binding data derived from the association of prazosin with α_1-AGP were transformed according to Rosenthal (1967).

RESULTS

Circular dichroism measurements

The binding of prazosin to α_1-AGP generates a monophasic negative extrinsic Cotton effect comprising a pronounced maximum at 255 and a lesser one at 285 nm. Fig. 1 shows spectra of 3 selected prazosin/α_1-AGP ratios. The extrinsic Cotton effects depend on the prazosin concentration as shown for the strong negative maximum at 255 nm in Fig. 2. The molar ellipticity increases up to a drug to protein ratio of one. Higher drug/protein ratios have no effect on the intensity of the CD-bands (Fig. 2).

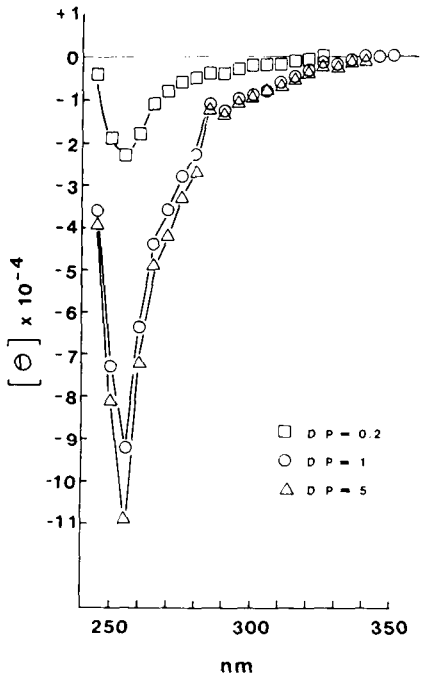

FIG. 1. Induced circular dichroism spectra of prazosin (2, 10 and 50 μM) in the presence of α_1-acid glycoprotein (10 μM), yielding drug/protein ratios of 0·2, 1 and 5. The data are difference values, using the Cotton effects of the protein at each wavelength as blank. The data are means of 2 different runs.

The presence of various other drugs reduces the prazosin-induced Cotton effects due to displacement from the common binding site on the glycoprotein molecule (Table 1). Of the drugs tested, disopyramide and mepivacaine are relatively weak inhibitors while biperiden, bupivacaine, and verapamil are moderately potent and trihexyphenidyl, as well as the acidic warfarin, are highly effective displacers of prazosin.

The (−)-isomer of propranolol is more effective than the (+)-isomer in displacing prazosin (Table 1). This is also evident from CD-measurements at propranolol/prazosin of 0·2–7·5 (Fig. 3). IC50-values of (−)- and (+)-propranolol are 1·3 and 1·9 [M/M], respectively.

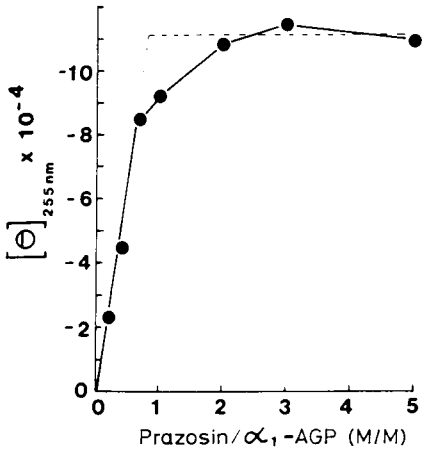

FIG. 2. Relation between the molar prazosin/α_1-acid glycoprotein ratio and the intensity of the induced circular dichroism band at 255 nm ($[\theta]_{255}$). Extrapolation (dotted line) yields 0·8 binding sites/α_1-acid glycoprotein molecule.

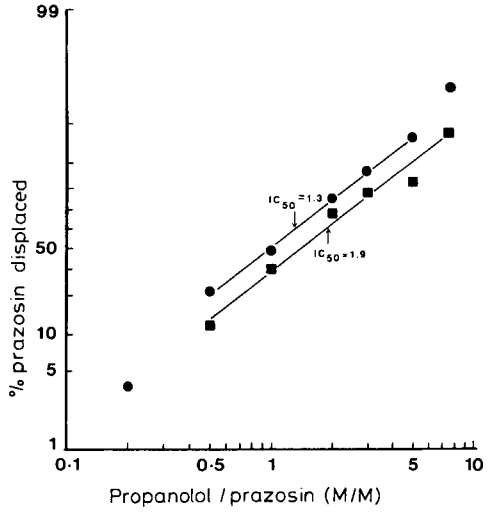

FIG. 3. Stereoselectivity of the prazosin binding site on the α_1-acid glycoprotein molecule. % prazosin displaced refers to the reduction of the molar ellipticity ($[\theta]$) of the prazosin/α_1-acid glycoprotein (10 μM) complex in presence of 2–75 μM (−)-propranolol or (+)-propranolol. Mean values of 2–4 runs.

Equilibrium dialysis experiments

Equilibrium dialysis experiments complement the characterization of the prazosin binding site. The transformation of the saturation data according to Rosenthal (1967) (Fig. 4) yield 0·85 binding sites/α_1-AGP molecule and an association constant of 48000 M^{-1}. Dale & Nilsen (1984) also found 1 binding site for prazosin on human α_1-AGP but a 10-times higher affinity.

Their dialysis experiments, however, were carried out at 23 ± 1 °C. Rubin & Blaschke (1980) also found an affinity constant 10-times higher (dialysis at 37 °C). The different temperature (37 °C in our studies) therefore cannot explain the 10-times higher affinity found in other studies, although experiments at 22 °C indicate association constants about 4-times higher than at 37 °C (data not shown). Both Dale & Nilsen (1984) and Rubin & Blaschke (1980) however, calculated prazosin affinity according to the Romer & Bickel (1979) modification of the Scatchard relationship (α_1-AGP-concentration varied between 15–70 and 6·8–20·4 μM, respectively, and prazosin-concentrations used were 90 and 78 μM, respectively), which could contribute to this discrepancy. Moreover the method used is only valid if binding constants have been shown to be independent of protein concentration (Aarons 1979).

Table 1. Displacement of prazosin (10 μM) by basic drugs and warfarin (all 50 μM) from its binding to α_1-AGP (10 μM) as evident from CD-measurements. % Reduction refers to the decrease of the induced Cotton effect at 255 nm (quantified as molar ellipticity) of the prazosin/α_1-AGP complex in the presence of the competitors. The data are means ± s.d. of three to four determinations.

Drug	pK_a	% Reduction ($\bar{x}$ ± s.d.)
Disopyramide	8·36	26·4 ± 21·8
Biperiden	—	74·7 ± 6·7
(±)-Propranolol	9·45	76·5 ± 7·8
(−)-Propranolol	9·45	89·1 ± 4·6
(+)-Propranolol	9·45	64·7 ± 3·9
Bupivacaine	8·1	87·6 ± 6·6
Mepivacaine	7·7	51·8 ± 23·8
Verapamil	8·9	72·0 ± 4·9
Trihexyphenidyl	9·78	95·0 ± 1·4
Warfarin	5·05	97·1 ± 1·6

Displacement experiments were carried out with the same drugs as used in CD-measurements. Results are shown in Table 2. The ability to displace prazosin from its binding site on α_1-AGP (Table 2) corresponds well with the data obtained by CD-measurements (Table 1) $y = 1·2 \times -1·4$; $R = 0·85$). Because of the high potential to displace prazosin in some cases (trihexyphenidyl, warfarin) and because of the low binding of prazosin in the presence of five times more displacing drug (5–10% prazosin bound, Table 2), the experimental error in dialysis experiments is of significance.

To evaluate the contribution of α_1-AGP binding to the serum binding in-vivo, ED-experiments were made with human serum albumin and serum, respectively. Representative results are given in Table 3. HSA-binding is around 80% and serum binding

Table 2. The binding of [^{3}H]prazosin (10 μM) to α_1-AGP (10 μM) in the presence of 50 μM inhibitors. Prazosin binding in the absence of inhibitors is 23·1 ± 8%. The data are means ± s.d. of 8–10 determinations.

Drug	Prazosin bound (%)	Reduction in binding (%)
Disopyramide	14·7 ± 2·60	36
Biperiden	7·7 ± 8·80	67
(−)-Propranolol	10·3 ± 1·96	55
(+)-Propranolol	10·6 ± 1·19	54
Bupivacaine	5·0 ± 4·11	78
Mepivacaine	11·9 ± 3·98	48
Verapamil	6·7 ± 3·84	71
Trihexyphenidyl	2·4 ± 2·44	89
Warfarin	5·4 ± 3·95	77

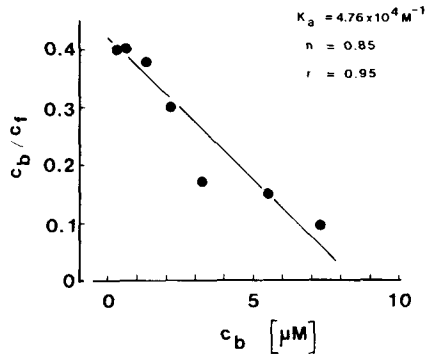

FIG. 4. Rosenthal plot of the interaction of [^{3}H]prazosin with α_1-acid glycoprotein (equilibrium dialysis experiments). K_a = association constant; n = number of binding sites per α_1-acid glycoprotein molecule, and r = correlation coefficient of the calculated regression line. Data of 3 or more experiments.

around 90% at prazosin concentrations between 1–100 μM. Neither the HSA nor the serum binding capacities are saturable due to low prazosin solubility (100–200 μM at pH 7·4).

DISCUSSION

As indicated by CD as well as by ED experiments, prazosin binds only to one site of α_1-AGP, which agrees with many other observations indicating the presence of only one high-affinity binding site of this protein for nearly all drugs so far investigated (Müller & Stillbauer 1983; Paxton 1983). Prazosin binding to its single binding site can be inhibited by various drugs known to interact with the 'basic drug binding site' of α_1-AGP with a rank order of potencies similar to that previously found for the binding of three other drugs to the common site of α_1-AGP (Müller & Stillbauer 1983). Thus, prazosin binding to α_1-AGP is also mediated through the 'basic drug binding site' of this protein.

It is evident from the data reported in Table 1 that prazosin binding can be inhibited by drugs of widely different chemical structures, which is in agreement with other reports about the binding of drugs to α_1-AGP (Kornguth et al 1981; Pike et al 1981; Lemaire & Tillement 1982; Nyberg & Martensson 1982; Abramson 1982; Javaid et al 1983; Schley & Müller-Oerlinghausen 1983; Müller & Stillbauer 1983; Müller et al 1983). It seems premature to draw any conclusions about possible relations between chemical structure and high affinity for the single binding site of this protein. On the other hand, our observation that the anionic drug warfarin also inhibits the binding of prazosin agrees with the high affinity of warfarin for this protein (Urien et al 1982), but raises doubts about the importance of the basic character of the drugs as mandatory for high affinity to this site. This is supported by the lack of any relation between the pK_a values and the inhibition of prazosin binding for the drugs listed in Table 1. Moreover, while prazosin, which binds only with intermediate affinity to α_1-AGP, will be present largely as the cationic form at pH 7·4 (pK_a = 6·5), most of the drugs in Table 1 to a large extent will be present in the unionized form at pH 7·4. Thus, we interpret our findings that ionic forces are not playing a crucial role for the binding of drugs to this site. This supports previous conclusions that this site might represent a remote hydrophobic area within the protein part of the glycoprotein molecule (El-Gamal et al 1982).

The displacement of prazosin by the isomers of the

Table 3. Binding of [^{3}H]prazosin to α_1-AGP (10 μM), human serum albumin (600 μM) and human serum (HSA = 600 μM) at selected prazosin concentrations. The data are means ± s.d. of 3 determinations.

Prazosin-concn (μM)	α_1-AGP			HSA			Human Serum		
	Prazosin (μM)		Bound (%)	Prazosin (μM)		Bound (%)	Prazosin (μM)		Bound (%)
	Free	Bound		Free	Bound		Free	Bound	
1	0·7 ± 0·16	0·3 ± 0·12	29 ± 12	0·3 ± 0·03	1·4 ± 0·05	84 ± 2	0·2 ± 0·04	1·7 ± 0·19	90 ± 2
5	3·6 ± 0·25	1·3 ± 0·17	27 ± 10	1·3 ± 0·23	7·3 ± 0·14	85 ± 2	1·1 ± 0·04	8·4 ± 0·70	88 ± 1
50	37·7 ± 3·40	5·5 ± 4·20	13 ± 9	13·8 ± 1·50	66·6 ± 1·70	83 ± 2	12·6 ± 2·50	73·1 ± 3·30	84 ± 4
100	75·9 ± 2·8	7·2 ± 0·8	9 ± 5	31·5 ± 0·00	145·4 ± 13·00	81 ± 0	27·2 ± 6·20	131·4 ± 13·00	79 ± 7

β-adrenoceptor antagonist propranolol is slightly but reproducibly (Walle et al 1983) different and indicates a degree of stereoselectivity of this major binding site of α_1-AGP, which has also been recently demonstrated using a different technique (Hermansson 1983). Obviously, the main drug binding site of α_1-AGP also has dualistic properties similar to drug binding sites of HSA, with stereoselectivity on the one hand and high affinity binding for drugs of different chemical structure on the other hand (Müller & Wollert 1979).

Prazosin is more than 90% bound in human serum, a finding consistent with earlier reports (Hobbs et al 1978; Grahnen et al 1981, Rubin & Blaschke 1980; Dale & Nilsen 1984) and both albumin and acid glycoprotein contribute to serum binding. Our results are in accordance with these reports. At concentrations of prazosin below 1 μM, serum binding amounts to 90%, binding to HSA to 84% and glycoprotein binding to 25–30% (Table 3). Serum binding is far from saturated at 100 μM prazosin, while binding to α_1-AGP is then almost complete. In-vivo prazosin binding is therefore largely mediated by serum albumin (Table 3). Unexpected drug reactions due to prazosin displacement are therefore very unlikely. In conclusion, prazosin binding to α_1-AGP might be less important for clinically significant changes of the serum protein binding of this drug (Rubin and Blaschke 1980; Schulz et al 1983).

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