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Inhibitory effect of phenothiazines on the binding of [^{3}H]perazine to α_1-acid glycoprotein

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A system is described which allows the determination of the afffinity constant of unlabelled drugs to α_1-acid glycoprotein (α_1-AGP) by displacing [^{3}H]perazine from the binding protein with equilibrium dialysis. All drugs investigated appear to bind to only one site at the α_1-AGP molecule. From experiments, in which the chemical structure of the displacers was varied, the fragment 21–31 of the amino acid sequence appears to be a candidate for hydrophobic interactions. The glutamic acids 177 and 178 of the α_1-AGP molecule could be involved in ionic interactions with the side chain of phenothiazine derivatives. The relevance of α_1-AGP for drug binding, distribution, and the possible reasons for insufficient correlation between psychotropic plasma concentration and therapeutic response is discussed.

Serum albumin is the most frequently studied plasma protein in relation to the binding of drugs and other small molecules; however, α_1-acid glycoprotein (α_1-AGP) has shown a particularly high affinity for tricyclic antidepressants and neuroleptics. Acidic (anionic) drugs are assumed to be bound mainly to human serum albumin in plasma (Tillement et al 1974). In comparison, α_1-AGP is reported as a plasma protein which binds predominantly basic (cationic) drugs (Piafsky 1980). The binding forces involve both hydrophobic and electrostatic interactions (Sharples 1976). In the ligand protein interactions with α_1-AGP, the nature of the binding forces is not well established. Lemaire & Tillement (1982) expected hydrophobic interactions between α_1-AGP and drugs, electrostatic interactions being negligible. Urien et al (1982) investigated the binding of some acidic (anionic) drugs and found that most of those studied bind to α_1-AGP very weakly. Anionic drugs which exhibit a higher or intermediate α_1-binding do not possess a carboxylic moiety. Those workers expected hydrophobic interactions and neglected electrostatic interactions, too.

The present communication reports the effects of different substituents on phenothiazine binding. α_1-AGP appears to be a convenient model to study the molecular basis of specific ligand protein interactions in varying solvent environments.

Materials and methods

Materials. α_1-Acid glycoprotein was obtained from Behring-Werke (Marburg, FRG). All other drugs were generous gifts of the German manufacturers or were obtained from Ega-Chemie (Steinheim, FRG). [^{3}H]Perazine was synthesized in our laboratory; specific activity was 52·6 mCi mmol^{-1}. Radiochemical purity was checked by TLC and found to be 99%.

Methods. The strong affinity and high specificity of drug binding to α_1-AGP are the basis for a convenient and sensitive competitive drug binding assay. A binding system which obeys the law of mass action and in which the binding sites are assumed to be independent, can be treated similarly to enzyme substrate kinetics. Where such analogy is appropriate, the use of the Scatchard plot and the 'single reciprocal plot' (used in enzyme inhibition studies) supposes that labelled and unlabelled compounds possess affinity for the binding protein and are able to displace one another from the binding site of the protein. Under conditions of saturation, the affinity constant (1/K) can be calculated from the slope and the intercept of the straight line in the 'single reciprocal plot' (Wombacher & Körber 1972). To minimize the variation in the results, three concentrations of [^{3}H]perazine were combined with four concentrations of the displacers. The affinity constant was calculated from 1/K, the point of intersection of the three calculated straight lines of the equilibrium experiments with three [^{3}H]perazine concentrations. A specification of the assay procedure and data of precision and accuracy have been reported recently (Schley & Müller-Oerlinghausen 1986). The concentration of α_1-AGP in the experiments was $1{\cdot}75 \times 10^{-5}$ M, the concentration of [^{3}H]perazine varied between $0{\cdot}5$–$2{\cdot}5 \times 10^{-6}$ M and that of the drugs between 0 and about $0{\cdot}5 \times 10^{-4}$ M, respectively. The binding of the investigated compounds to α_1-AGP was assessed by competition experiments and measured by means of equilibrium dialysis at pH 7·4 in 66 mM phosphate/50 mM NaCl buffer using a Dianorm apparatus from Bachofer (Reutlingen, FRG). Some competitive experiments were also performed with phosphate buffer (50–250 mM), or at different pH values and with Tris buffer in the same range. In addition, various amino acids were investigated as possible competitors of the binding of perazine to α_1-AGP.

Results

The association constant of [^{3}H]perazine between pH 1·6 and 5 was around 2×10^5, between 5 and 6 around 3×10^5, at pH 7, 4×10^5, at pH 8, 14×10^5, and increased at pH 9 to 18×10^5. With 50–250 mM phosphate or Tris buffer, no dependence of binding on ionic strength could be established. The association constants of the investigated substances given here were all obtained by displacement of [^{3}H]perazine at pH 7·4.

Table 1. Association constants of unlabelled aromatic and cyclic drugs obtained by displacement of [^{3}H]perazine from the binding site of α_1-acid glycoprotein ($1{\cdot}75 \times 10^{-5}$ M).

Phenol	$0{\cdot}5 \times 10^2$
Naphthol	$1{\cdot}2 \times 10^3$
Octahydroacridin	8×10^2
Acridine	8×10^3
Coumarin	4×10^2
Coumarin-3-carboxylic acid	4×10^2
Warfarin	$1{\cdot}9 \times 10^5$
Phenylbutazone	$3{\cdot}4 \times 10^3$
Propanolol	$5{\cdot}3 \times 10^4$
7-Chlortetracycline	1×10^4
Dipyridamole	4×10^5
Phenothiazine	$1{\cdot}2 \times 10^4$
PPED*	$1{\cdot}2 \times 10^4$
3-Methoxyphenothiazine	1×10^4
2-Trifluorphenothiazine	$0{\cdot}4 \times 10^4$
3-Hydroxyphenothiazine	$0{\cdot}4 \times 10^4$
2-Chlorphenothiazine	$0{\cdot}6 \times 10^4$
Perazine	$3{\cdot}8 \times 10^4$
Demethylperazine	$1{\cdot}5 \times 10^5$
Perazine sulphoxide	$1{\cdot}2 \times 10^4$
Promazine	$3{\cdot}8 \times 10^4$

* PPED = *N*-γ[(phenothiazinyl)-10]-propyl ethylenediamine.

In some pilot experiments with added amino acids it could be shown that the aromatic amino acids tyrosine and tryptophan were able to reduce the binding of perazine to α_1-AGP.

Discussion

Scatchard plots for various labelled drugs indicate that the binding to α_1-AGP is associated primarily with one site (Müller & Stillbauer 1983; Schley 1983). This could also be confirmed by calculating the association constant for perazine obtained simultaneously by the method of Scatchard and by the displacement of [^{3}H]-perazine by unlabelled perazine itself (Schley 1983). Displacement experiments with drugs of chemically different structures have shown that a large number of drugs compete with the used marker ligands and that the markers compete with each other (Schley & Müller-Oerlinghausen 1983, 1986). Hence, only one binding area seems to be responsible for the binding to α_1-AGP of drugs (Paxton 1983) and the compounds examined in the present study.

The neuroleptics of the phenothiazine type are derivatives of phenothiazine with a basic sidechain at N_{10} of the phenothiazine nucleus. This sidechain has a strong influence on the neuroleptic potency. By competition experiments with different phenothiazine derivatives, it could be established that the tricyclic skeleton of phenothiazine is the bearer of the main part of the affinity to α_1-AGP. A basic sidechain at N_{10} of the phenothiazine system increases the affinity (Schley 1983). In contrast to El-Gamal et al (1983), we consider the substitution at N_{10} as much more important than the substituents on the phenothiazine skeleton.

However, a drastic lowering of the affinity is observed by the oxidation of the sulphur in the phenothiazine system (perazine-sulphoxide), since the electrons of the sulphur are withdrawn from the π-electron system. This is in accordance with the low affinity of the hydrated systems (octahydroacridin) to α_1-AGP. These results demonstrate that a single aromatic system is not sufficient for hydrophobic interactions. Two coupled aromatic rings raise the affinity considerably (naphthol); an increase is also caused by a basic sidechain (propranolol). Urien et al (1982) described some compounds with carboxylic groups which bind poorly to α_1-AGP. Regarding coumarin and coumarin-3-carboxylic acid, we could not find any difference in binding. Drugs which showed only weak binding in the experiments of Urien et al do not possess suitable aromatic systems for hydrophobic interaction with α_1-AGP. It is conspicuous that these substances do not have coupled aromatic rings. We would, therefore, expect that binding of acidic drugs to α_1-ACP does not involve the carboxylic groups. This hypothesis is in accordance with our own findings. Compounds with single aromatic rings could displace perazine from its α_1-AGP binding only weakly and had low affinities (Schley & Müller-Oerlinghausen 1983). The relatively low affinity of phenylbutazone is also in accordance with this hypothesis. The astonishingly high affinity of warfarin is possibly caused by a quasi tetracyclic conformation of warfarin, due to the binding to α_1-AGP, and resulting in a structure like tetracycline. Possibly there is a similar explanation for the high affinity of dipyridamole with its six-ring structure (El-Gamal et al 1982).

Preliminary experiments suggest that the binding of perazine to α_1-AGP is reduced by aromatic amino acids. In these experiments perazine, α_1-AGP and aromatic amino acids influenced each other indicating that an area with aromatic amino acids at the α_1-AGP molecule is involved in the binding of that drug by hydrophobic interactions. In fact, the amino acid sequence 21–31 (-Ile-Thr-Gly-Lys-Trp-Phe-Tyr-Ile-Ala-Ser-Ala-) has been proposed as the binding site of the α_1-AGP molecule (Kute & Westphal 1976).

The larger association constant in the basic range points to relevant ionic interactions. The possibility of lower binding in the acid pH range by decomposition of α_1-AGP during the dialysis in the experiments was excluded by testing the sample after dialysis by gel electrophoresis. No cleavage of α_1-AGP due to a decreased pH was observed. Aspartic acid and/or glutamic acid could be involved in ionic interactions, but they must be associated since the piperazine ring is bound, but the cleaved ring (PPED) is not bound. For this second 'fix point' of the binding site the amino acids 177 and 178, two glutamic acids (Schmid et al 1973), are possible candidates. The amino acid sequence of the α_1-AGP molecule allows the speculation that the hydrophobic region of the phenothiazine derivative,

perazine (tricyclic skeleton of phenothiazine) may interact with the region 21–31 of the amino acid sequence, whilst the piperazine side chain may cause ionic interactions with the glutamic acids 177 and 178 at the other end of the α_1-AGP molecule.

It has been shown that a variety of drugs, both basic and acidic, bind to the α_1-AGP molecule, albeit with differing affinities (Urien et al 1982; Schley & Müller-Oerlinghausen 1986). The potential physiological significance of α_1-AGP as a drug binder was indicated by calculations that α_1-AGP was capable of binding 30–50% of serum perazine or amitriptyline (Brinkschulte & Breyer-Pfaff 1980). It could be demonstrated that the presence of two competing drugs may lead to the displacement of the more weakly bound drug from its protein-binding site. The displaced drug, depending upon its properties, may penetrate into other body compartments or it may be redistributed among other haematological binding sites (Lima et al 1981). In addition to serum proteins, red cell membranes (Hahn et al 1973; Bickel 1975), and platelets (Briley et al 1979) have been reported to bind certain psychotropic drugs. These displacements and redistributions will be relevant for psychotropic drugs, if an additional medication with other drugs also binding to that site would lead to micromolar plasma levels. A correlation between the plasma concentration of α_1-AGP and the free fraction of perazine has been demonstrated (Schley et al 1980). This has been shown also for other drugs. However, it has to be considered that α_1-AGP variations under drug treatment, different polymorphic forms of α_1-AGP with different binding properties and their genetic dependence (Tinguely et al 1985), could contribute to insufficient correlations between psychotropic drug plasma concentrations and therapeutic response.

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