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BINDING OF ANTIARRHYTHMIC DRUGS TO PURIFIED HUMAN α_1-ACID GLYCOPROTEIN*

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Abstract—The binding of lidocaine, verapamil, propafenone and propranolol to isolated, purified human α_1-acid glycoprotein was studied using equilibrium dialysis. Lidocaine and verapamil bound to a single class of binding sites which was characterized by high affinity (k_{d1} for lidocaine was $5.79 \times 10^{-6}\,M^{-1}$ and for verapamil $3.43 \times 10^{-6}\,M^{-1}$) and low capacity ($n = 0.40$ for lidocaine and 0.62 for verapamil). The binding of propafenone revealed two classes of binding sites, both with high affinity (k_{d1} was $7.62 \times 10^{-6}\,M^{-1}$ and kd_2 was $6.00 \times 10^{-8}\,M^{-1}$) and low capacity ($n_1 = 0.79$ and $n_2 = 0.20$). Propranolol bound to at least two classes of binding sites (k_{d1} was $2.56 \times 10^{-6}\,M^{-1}$; $n_1 = 0.58$). Complete characterization of the binding parameters of the second site was not possible due to failure to achieve saturation.

The binding of basic drugs to α_1-acid glycoprotein (α_1AGP) has received considerable attention in recent years. Much of the inter-individual variability in the extent of plasma protein binding of basic drugs is due to variability in plasma levels of this protein [1]. The binding of several drugs has been shown to increase following surgical interventions, inflammation and stress, and this increased binding is due to an increase in the plasma concentration of α_1AGP [1]. Plasma α_1AGP levels fall during pregnancy, during the use of oral contraceptives, hepatic disease, nephrotic syndrome and malnutrition [2, 3], and one can anticipate subsequent reductions in drug binding.

While several antiarrhythmic drugs including aprindine, disopyramide, lidocaine, propranolol, quinidine and verapamil have been reported to bind to α_1AGP, much of the evidence is indirect and in most cases the characteristics of this binding have not been elucidated [4–11]. We have studied the binding of four antiarrhythmic drugs, lidocaine, verapamil, propafenone and propranolol, to purified α_1AGP, and this report describes the characteristics of the binding of these drugs.

MATERIALS AND METHODS

Chemicals. [^{14}C]Propafenone HCl (17.5 mCi/mmole) and [^{14}C] verapamil HCl (3.3 mCi/mmole) were provided by the Knoll Pharmaceutical Co. [^{14}C] Lidocaine HCl (30.0 mCi/mmole) and [^{3}H]*d,l*-propranolol HCl (35 mCi/mmole) were purchased from New England Nuclear. Aquasol also was purchased from New England Nuclear.

Protein preparation. Human α_1AGP (99% pure) was purchased from the Sigma Chemical Co. The protein was dissolved in deionized distilled water, placed in a dialysis bag, and dialyzed against 3 vol. of 1000 ml of deionized distilled water for 2.5 hr. The protein then was dried by lyophilization and recrystallized by the method described by Ganguly *et al.* [12]. Two hundred milligrams of α_1AGP was dissolved in 50 ml of 0.1 M acetate buffer (pH 4.1) to which 200 ml of an alcohol–acetone mixture (9:1, v/v) was added. This was maintained for 2 hr at 5° with occasional shaking, then centrifuged at 6000 *g* for 30 min, washed with cold ethanol and centrifuged for another 30 min. The precipitate then was washed with cold ether and centrifuged for a final 30 min. The purified protein was collected and dried overnight in a vacuum dessicator at 5°.

Binding studies. Binding to α_1AGP was determined by equilibrium dialysis using a Dianorm apparatus (Spectrum Medical Industries) with 1 ml Teflon cells. One side of the dialysis cell contained various concentrations of drug dissolved in 1 ml of isotonic phosphate buffer (pH 7.4), and the other side contained α_1AGP (1×10^{-6} moles/liter) in 1 ml of isotonic buffer. The two compartments were separated by a semi-permeable membrane (Spectraphor membranes). Dialysis was carried out for 4 hr at 37°. At the end of the experiment, 500-μl aliquots from each compartment were added to scintillation vials containing 12 ml of Aquasol, and disintegration rates were measured by scintillation counting.

The concentration of drug added to the cells was varied over a broad range. The ranges employed for each drug were:

Lidocaine 3.7×10^{-7} M to 3.7×10^{-4} M
Propafenone 2.65×10^{-8} M to 2.38×10^{-4} M
Propranolol 3.4×10^{-7} M to 8.5×10^{-4} M
Verapamil 1.0×10^{-6} M to 2.0×10^{-4} M

Data analysis. The fraction of unbound drug (α) was calculated from the ratio of disintegrations per

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minute (dpm) in the two compartments of the dialysis cell.

$$\alpha = \frac{\text{dpm buffer side}}{\text{dpm protein side}} \quad (1)$$

The total molar concentration of drug in the protein side (C_t) after equilibrium was determined from equation 2

$$C_t = C_i/(1 + \alpha) \quad (2)$$

where C_i is the initial molar concentration of drug in the buffer compartment.

The concentration of unbound drug (C_u) was calculated from the relationship:

$$C_u = C_t \times \alpha \quad (3)$$

Initial estimates of dissociation constants and number of binding sites were obtained from Scatchard plots. Binding parameters then were determined by nonlinear regression using MLAB [13] to fit the data to equation 4

$$R = \sum_{i=1}^{m} \frac{n_i C_u}{k_{d_i} + C_u} \quad (4)$$

where R is the molar ratio of bound drug (moles bound/mole protein), m is the number of independent classes of binding sites, k_{d_i} is the dissociation constant for the ith site and n_i is the number of sites per mole of protein.

RESULTS

Saturable binding for lidocaine, verapamil and propafenone to α_1AGP was observed. However, the binding isotherm for propranolol did not appear to approach saturation. At the highest concentrations of propranolol studied ($>8.5 \times 10^{-4}$ M) we were unable to obtain reproducible results.

Scatchard analysis of the binding data (Fig. 1) shows a linear relationship for both lidocaine and verapamil, indicating that binding of these drugs to α_1AGP is associated with only one class of binding sites. The Scatchard plots for propafenone and propranolol are nonlinear, suggesting that these two drugs bind to at least two different classes of sites.

Binding parameters for each drug were obtained using nonlinear regression to fit the data. Graphs of the experimental data (moles drug bound per mole of protein versus unbound drug concentration) and the fitted curves are shown in Fig. 2. The best fit parameters are shown in Table 1. Lidocaine and verapamil were best fit to a model with one class of binding sites as predicted by the Scatchard analysis. The propafenone data were best fit to a model with two classes of binding sites. Both sites are characterized by a relatively high binding affinity and low binding capacity. For the propranolol concentration range studied, this drug binds to two classes of sites—one high-affinity site having a dissociation constant of the same order of magnitude as the high-affinity sites of verapamil, lidocaine and propafenone. A second lower affinity class of sites also was indicated. Saturation of this binding site did not occur, and the dissociation and binding constants reported for this second site are only estimates.

DISCUSSION

α_1AGP has been shown to be an important binding protein for basic drugs, and much remains to be learned about the nature of the interaction of drugs with this protein. Müller and Stillbauer [14] have reported that cationic compounds are highly bound

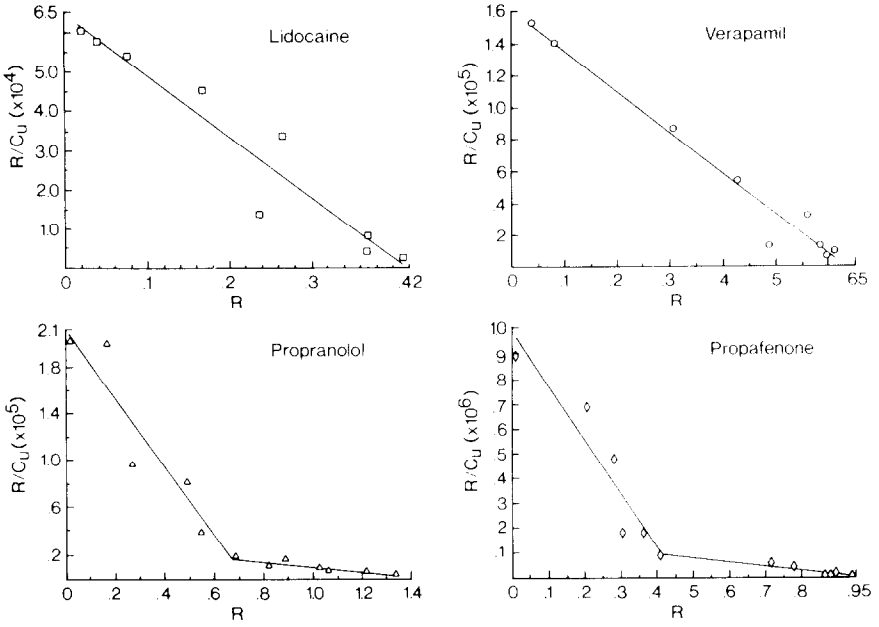

Fig. 1. Scatchard graphs for the interactions of lidocaine, verapamil, propranolol and propafenone with α_1AGP. R = number of moles of drug bound per mole of protein, C_u = molar concentration of unbound drug.

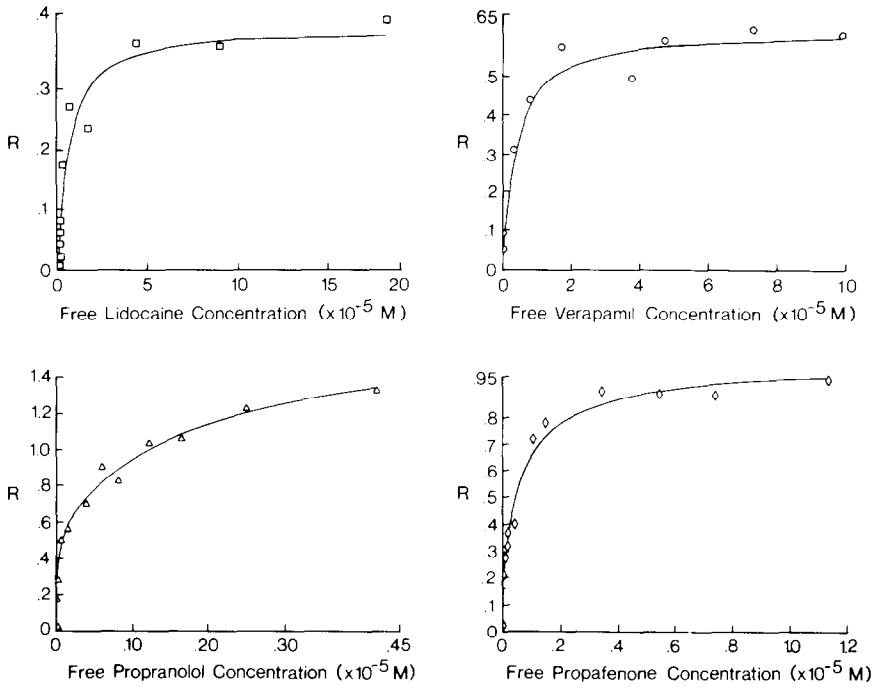

Fig. 2. Binding of lidocaine, verapamil, propranolol and propafenone to α_1AGP. The curves are generated from nonlinear least squares regression. R = number of moles of drug bound per mole of protein.

to a single nonspecific site on α_1AGP. All four drugs that we studied bound to a high-affinity site on α_1AGP; the dissociation constants are of the same order of magnitude for each drug. Other workers have demonstrated that lidocaine is displaced from α_1AGP by propranolol and other basic drugs supporting the idea that this high-affinity site is common to this drug group [15]. The binding of basic drugs to α_1AGP does not appear to fit a simple model, however, as some drugs appear to have only one class of binding sites and others at least two.

Although lidocaine and verapamil have been reported to bind to α_1AGP, the binding characteristics to isolated α_1AGP have not been reported previously. Several investigators have studied the binding of *d,l*-propranolol to α_1AGP. Glasson *et al.* [9] reported one class of binding sites for propranolol ($n = 1$, $k_a = 3.04 \times 10^{-5}\,M^{-1}$). Müller and Stillbauer [14] report similar behaviour of propranolol binding to α_1AGP ($n = 1.05$, $k_a = 11.3 \times 10^5\,M^{-1}$) as do Wong and Hsia [16]. However, Belpaire *et al.* [17] recently reported that propranolol binds to two classes of sites on α_1AGP. They were unable to saturate the second site because of insolubility of the drug at higher concentrations. Our findings support the existence of at least one additional binding site. The propranolol binding isotherm did not show evidence of saturation. Results at concentrations of propranolol higher than those reported were not reproducible. While this may have been due to insolubility of the drug in the buffer, the inability of our method to accurately measure the small increases in bound propranolol concentration which occur at higher concentrations is also a likely factor.

Others have reported that propranolol demonstrates stereoselective binding to α_1AGP. L-Propranolol preferentially binds to α_1AGP, and there appears to be greater stereoselectivity at higher total drug binding [18, 19]. The presence of two binding sites on α_1AGP for propranolol may reflect dif-

Table 1. Dissociation constants (k_d) and number of binding sites (n) for lidocaine, verapamil, propafenone and propranolol binding to α_1-acid glycoprotein

	Class 1		Class 2	
	n_1	k_{d_1} (M^{-1})	n_2	k_{d_2} (M^{-1})
Lidocaine	0.403	5.79×10^{-6}		
Verapamil	0.615	3.43×10^{-6}		
Propafenone	0.792	7.62×10^{-6}	0.202	600×10^{-8}
Propranolol	0.580	2.56×10^{-6}	1.13	1.97×10^{-4}

Table 2. K_d, pK_a, log P and log Q values for lidocaine, verapamil, propafenone and propanolol

	K_d (M^{-1})	pK_a	Log P	Log Q
Lidocaine	5.79×10^{-6}	7.9*	2.19	2.3
Verapamil	3.43×10^{-6}	9.4†	3.3¶‡	1.3
Propafenone	7.0×10^{-6}	9.0§	4.0‖	2.4
Propranolol	2.56×10^{-6}	9.3*	3.1	1.2

* pK_a and log P values for lidocaine and propranolol are from Courtney [20].

† pK_a for verapamil is from Knoll Pharmaceutical.

‡ Log P for verapamil was determined in our laboratory.

§ pK_a for propafenone is from Kohlhardt and Seifert [21].

‖ Log P for propafenone (determined by the Hansch fragmentation method) is from Ken Courtney (unpublished data).

ferences in the binding characteristics of the stereoisomers.

Since physical chemical properties have been shown to influence the binding of drugs to serum proteins, we also examined the relationship between the binding affinity of the four drugs studied with their distribution coefficient in octanol-H_2O (log Q) (Table 2). The correlation between the dissociation constants and log Q ($r = 0.96$) was significant (Fig. 3). There was no significant correlation between dissociation constants and octanol-H_2O partition coefficients (log P) which is the distribution coefficient corrected for ionization of the compounds. Using phenothiazine derivatives, Hulshoff and Perrin [22] have demonstrated a similar relationship between binding to albumin and octanol-H_2O partition coefficients. They, however, noted a more satisfactory correlation when the distribution coefficient was corrected for the degree of ionization.

The presence of impurities in α_1AGP may explain differences in the binding characteristics of some basic drugs which have been noted by different investigators. We initially conducted a series of binding experiments using the α_1AGP as obtained without further purification. We noted that the binding of verapamil and propafenone was less than expected and that the results were variable and not reproducible. Because of these results we purified the glycoprotein as outlined in Materials and Methods. The first stage of the purification was designed to remove heavy metal ions which may have contaminated α_1AGP during the isolation process. Kerkay and Westphal [23] have shown that heavy metal ions inhibit the binding of progesterone to α_1AGP in a noncompetitive process. The second stage involved recrystallization and removal of lipophilic contaminants. Ganguly *et al.* [12] previously have shown that lipophilic contaminants of α_1AGP also may inhibit drug binding to α_1AGP.

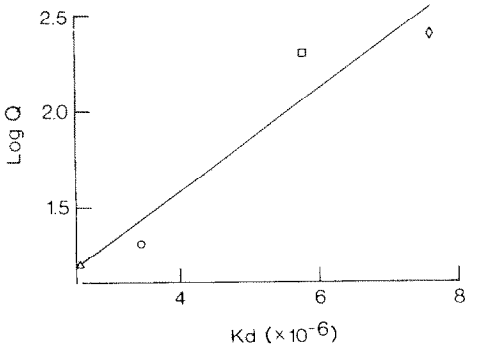

Fig. 3. Relationship between the distribution coefficient in octanol-H_2O (log Q) and dissociation constants (M^{-1}) of lidocaine (□), verapamil (○), propafenone (◇), and propranolol (△).

In summary, we have evaluated the binding of four antiarrhythmic drugs to purified α_1AGP. They all appear to bind to a similar high-affinity site, and two of the drugs studied, propafenone and propranolol, have at least one other site to which they bind.

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