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Evidence for binding of certain acidic drugs to α_1-acid glycoprotein

Human serum albumin (HSA) and α_1-acid glycoprotein (α_1-AGP) are the major circulating plasma proteins involved in drug binding. Acidic (anionic) drugs ionized at plasma pH, are assumed to be bound preferentially to HSA in plasma. The binding forces involve both hydrophobic and electrostatic interactions [1]. In contrast, α_1-AGP is the main plasma protein for the binding of basic (cationic) drugs [2]. In this case, the nature of the binding forces is not clearly established, though recent studies show that electrostatic interactions seem to be poorly involved: Lemaire and Tillement [3] showed that the different hydrophobic characteristics of some pindolol derivatives led to important differences in their binding. Then hydrophobic interactions between α_1-AGP and drugs are strongly to be expected. If this hypothesis is true, anionic drugs may also interact with α_1-AGP by hydrophobic bonds. So, the aim of this study is to investigate the qualitative and quantitative aspects of plasma binding of anionic drugs regarding their possible interactions with α_1-AGP. Two types of anionic drugs were used depending on the presence or absence of a carboxylic group. We attempted to find out whether acidic and basic drugs are bound to distinct or common sites on α_1-AGP.

Materials and methods. The binding of the drugs was measured by equilibrium dialysis at pH 7.4 in 0.066 M phosphate buffer ($\mu = 0.284$) at 37° for 4 hr using a Dianorm apparatus according to an experimental scheme previously described [4]. Drug solutions were prepared by isotopic dilution of a constant amount of [^{14}C]warfarin (49 Ci/mole, Amersham), [^{14}C]acenocoumarol (7 Ci/mole, Ciba-Geigy), [^{14}C]phenylbutazone (28 Ci/mole, Ciba-Geigy), [^{14}C]benoxaprofen (2.5 Ci/mole, Eli-Lilly), [^{14}C]indomethacin (13.8 Ci/mole, Merck Sharp & Dohme), [^{14}C]itanoxone (1.8 Ci/mole, Pierre Fabre), [^{14}C]salicylic acid (47 Ci/mole, Amersham), [^{14}C]clofibric acid (20 Ci/mole, CEA), [^{14}C]fenofibric acid (5.8 Ci/mole, Fournier) and [^{14}C]valproic acid (0.5 Ci/mole, CEA) with increasing amounts of unlabelled drugs. All labelled drugs had a chemical purity greater than 99%. α_1-AGP (Behringwerke) (99% pure) was dissolved in phosphate buffer at a concentration of 22.5 μM. Estimation of the binding parameters was performed by fitting the data to a theoretical relationship for drug–protein binding derived from the mass action law:

$$\frac{B}{R} = \frac{nKF}{1 + KF} \quad (1)$$

where B and F are the bound- and free-drug concentrations respectively, n and K the number of binding sites and the association constant, and R the total protein concentration. The data were analyzed by an iterative non-linear regression program using a Gauss–Newton algorithm [4]. Data obtained with an inhibitor were treated as follows: bound (B) vs free (F) ligand plots obtained without and with different amounts of inhibitor were analyzed altogether assuming either n or K values depended on the inhibitor concentration. The correct model was then chosen according to the best fit.

Some theoretical calculations were made to evaluate the respective concentrations of drug bound to HSA and α_1-AGP in plasma using therapeutic plasma levels. So the general equation used was:

$$B_i = K_i n_i RF \quad (2)$$

applied to each drug–protein binding of the ith class. Then

Table 1. Binding characteristics of some anionic drug–α_1-AGP interactions

Drug	% bound*	n	K (10^{-3} M^{-1})
Warfarin	88	1.09 ± 0.03	212 ± 38
Acenocoumarol	85	1.08 ± 0.02	201 ± 35
Phenylbutazone	26	0.71 ± 0.08	35 ± 9

*At a drug to α_1-AGP molar ratio of 0.04.

Table 2. Simulated distributions of bound acidic drugs in serum

Drug	Per cent bound to HSA (600 μM)	Per cent bound to α_1-AGP (22.5 μM)
Warfarin	94	5
Acenocoumarol	90	9
Phenylbutazone	99.3	0.3

HSA and α_1-AGP concentrations used are in the normal range. The sum of the two computed bound fractions is close to the value observed in human plasma [4, 5]. The following drug HSA binding parameters were used: warfarin: $n_1 = 2$, $K_1 = 90{,}000\ M^{-1}$ and $n_2 = 7.8$, $K_2 = 1300\ M^{-1}$; acenocoumarol: $n_1 = 3.35$, $K_1 = 27{,}000\ M^{-1}$ and $n_2K_2 = 4100\ M^{-1}$; phenylbutazone: $n_1 = 1.4$, $K_1 = 230{,}000\ M^{-1}$ and $n_2 = 3.7$, $K_2 = 5600\ M^{-1}$.

the total bound ligand concentration (B) is:

$$B = \Sigma B_i \tag{3}$$

Then:

$$\frac{B}{T} = \frac{(\Sigma K_i n_i)R}{1 + (\Sigma K_i n_i)R} \tag{4}$$

with T ($= F + B$) being the total drug concentration.

Results. Clofibric, fenofibric, salicylic and valproic acids do not bind to α_1-AGP whereas benoxaprofen, indomethacin and itanoxone at a molar drug to α_1-AGP ratio of 0.04 bind very poorly with fractions bound about 10%. In contrast, the percentages of bound warfarin, acenocoumarol or phenylbutazone are noticeably higher, between 90 and 26%. Table 1 summarizes the binding parameters obtained for these three drugs. The results demonstrate that there is one site for these drugs. Warfarin and acenocoumarol

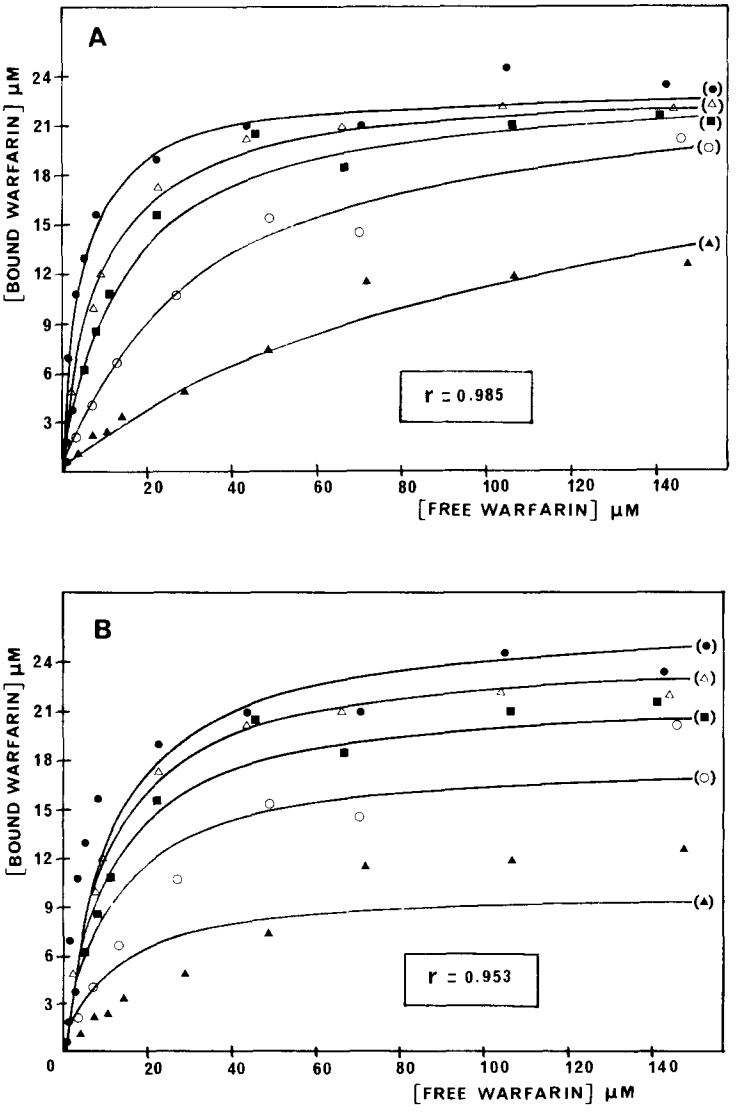

Fig. 1. Binding of warfarin to α_1-AGP (22.5 μM), alone (●), with 25 (△), 50 (■), 100 (○) and 200 (▲) μM propranolol. In panel A binding data are fitted assuming that only K values vary (competitive inhibition), in panel B only n values are assumed to vary (non-competitive inhibition) r is the correlation coefficient for each type of analysis.

bind to α_1-AGP with a high affinity, whereas phenylbutazone affinity is noticeably lower.

Binding of warfarin to α_1-AGP is represented in Fig. 1. Displacement of the hyperbola occurs when propranolol is added. The data are analyzed using either a competitive model of inhibition (K values vary with an unchanged n value), or a non-competitive model (n values vary with an unchanged K value). When propranolol is assumed to produce competitive inhibition, the data are adequately fitted (Fig. 1A) whereas the non-competitive pattern of inhibition does not appear to fit the data conveniently (Fig. 1B). The binding parameters of the warfarin, acenocoumarol and phenylbutazone interactions with α_1-AGP plus those generally reported for their interactions with HSA [4, 5] were used to simulate binding percentages in serum over a therapeutic range of each drug. Table 2 summarizes the distributions of bound drugs between HSA and α_1-AGP in plasma.

Discussion. Numerous studies have demonstrated the high-affinity binding of many anionic drugs to HSA [6]. Since this binding to 600 μM HSA can account for almost all anionic drug binding in plasma, the possibility that other proteins could also bind anionic drugs was poorly investigated.

Most of the anionic drugs studied in this communication are not bound to α_1-AGP, or the degree of binding is so low that negligible α_1-AGP binding in plasma is then expected. Nevertheless, warfarin and acenocoumarol were found to bind to α_1-AGP with a high affinity, even greater than their association constant to HSA. Phenylbutazone is also bound to α_1-AGP, but with a lower, intermediate affinity.

Propranolol inhibits the warfarin–α_1-AGP binding according to a competitive mechanism. Thus it can be assumed that some acidic and basic drugs share one common site on α_1-AGP. As the nature of the binding forces involved has been often questioned, this feature shows that electrostatic interactions are not likely to occur in the high-affinity interactions between these drugs and α_1-AGP.

Since we checked the α_1-AGP binding of a limited number of acidic drugs, the present work is not intended to ascertain the rules by which anionic drugs are capable of binding to α_1-AGP. However, some features are emphasized by this study. Anionic drugs which exhibit a high or intermediate α_1-AGP binding affinity do not exhibit any carboxylic moiety, and share a common specific binding site on HSA, called site I by Sudlow *et al.* [7] or the warfarin site by Fehske *et al.* [8] and Sjöholm *et al.* [9]. In contrast, all the drugs poorly or not bound to α_1-AGP exhibit carboxylic groups and specifically bind to the other HSA binding site, called site II or the diazepam site respectively by the same authors.

Finally, the simulation of the distribution of bound drugs between α_1-AGP and HSA in plasma shows that α_1-AGP binding, though relatively low, is not negligible for acenocoumarol and warfarin. Since α_1-AGP shows large fluctuations due both to physiological and pathological conditions, the concentration of the drug–α_1-AGP combination is likely to vary; the extent of binding in plasma may also change [2].

In summary, the binding of some acidic drugs to α_1-AGP was studied by equilibrium dialysis at 37°, pH 7.4. Certain acidic drugs bound to α_1-AGP at one binding site with a high affinity. Though the α_1-AGP plasma concentration is far lower than the HSA concentration, the association constants of some acidic drugs with α_1-AGP are high enough to suggest that binding to α_1-AGP will contribute significantly to the total plasma binding of these drugs.

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Evidence that 3-aminopicolinate stimulates glutamine metabolism by rat renal cortical tissue *in vitro*

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Man *et al.* [1] have recently shown that 3-aminopicolinate, a hyperglycemic agent [2, 3] that stimulates the activity of phosphoenolpyruvate carboxykinase (EC 4.1.1.32) isolated from rat liver [4] and renal cortex [1] accelerates the extraction of glutamine by the rat kidney *in vivo*. However, these authors failed to demonstrate that 3-aminopicolinate affects glutamine removal when rat renal cortical slices are used [1].

The present report shows that suitable concentrations of 3-aminopicolinate exert a stimulatory effect on glutamine