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SHORT COMMUNICATIONS

Thermodynamics of fatty acid anion displacement of warfarin and phenylbutazone from human albumin

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While it is recognized that the concentration of plasma free fatty acid (FFA) fluctuates considerably in response to changes in physical stress and food intake [1–4], these physiological changes in concentration generally have not been thought to have a significant effect on drug binding. However, in contrast to this view, recent work [5, 6] suggests that plasma FFA may have an important influence on the extent of drug albumin interactions. The manner in which FFA alters drug binding can be expected to vary with the mechanism(s) and site(s) of binding of a given drug and, in the cases of warfarin and phenylbutazone, is incompletely understood. This paper seeks to further examine certain published data concerning the human serum albumin binding of warfarin and phenylbutazone and their displacement of FFA in an effort to gain additional insight into the identity of their binding sites and the mechanism of their displacement.

The interactions of long-chain fatty acids with serum albumin was studied by Goodman [7] at a pH of 7·45 at 23°. Apparent association constants were determined by the method of Scatchard [8] and correspond to the binding of the fatty acid anions at this pH. The even-numbered saturated fatty acids from C_{12} to C_{18} were found to bind to three classes of sites (coordination numbers of $n_1 = 2$, $n_2 = 5 \pm 1$ and $n_3 \simeq 20$) and to have association constants which increased with increasing acid chain length. Solomon *et al.* [9, 10] have employed ultrafiltration to characterize the displacement of warfarin and phenylbutazone from human serum albumin by various drugs and fatty acids. Double-reciprocal plots showed apparent competitive displacement of both warfarin and phenylbutazone by lauric acid at acid–albumin molar ratios of 1·8 and 8·8 respectively. However, these authors [10] also found that stearic, myristic and lauric acids, at an acid–albumin molar ratio of 35, reduce the per cent bound of both drugs inversely with fatty acid chain length.

The latter evidence, together with the FFA–albumin binding data of Goodman [7], suggests in two ways that the predominant mechanism of drug displacement at the higher FFA levels is other than competition for binding sites. Competitive displacement would require that the fatty acid anions displace drug in rank order corresponding to their affinities for albumin; however, the inverse relationship is observed. Secondly, because the association constants of the fatty acid anions for albumin exceed those of warfarin and phenylbutazone by from one to three orders of magnitude, almost no binding of either drug would be expected to occur with simultaneous acid–albumin and acid drug molar ratios of 35 and 85 respectively.

The experimental observations can be explained if it is assumed that the displaced drug, warfarin or phenylbutazone, binds to n independent identical sites on serum albumin and that the effect of FFA of reducing the binding of drug results solely from a change in the binding affinity of the site. The apparent association constant, K, for drug binding to a given site is defined as

$$K = \frac{(DP)}{(D)(P)}$$

where (DP) and (P) are the concentrations of a given site occupied and unoccupied by drug, respectively, and (D) is the free drug concentration. The fraction of drug bound, F, is then given by the expression

$$F = \frac{n(DP)}{(D) + n(DP)}$$

It follows that, given F, the product $nK(P)$ can be calculated from the equation:

$$nK(P) = \frac{F}{1 - F}$$

If only the per cent drug bound is known or can be determined, an approximate apparent FFA-induced change in the free energy of binding can be calculated from the ratio, $K(P)/K'(P)'$, where the prime denotes values determined in the presence of FFA. Where values of (P) and $(P)'$ can be measured or calculated, the apparent FFA-induced free energy change in drug binding, $\Delta\Delta G$, can be determined from the relationship:

$$\Delta\Delta G = RT\ln(K/K')$$

This has been done for the data presented by Solomon, whose measurements were made in pH 7·4 phosphate buffer at 30°, and is shown in Table 1.

The close correspondence of $\Delta\Delta G$ between warfarin and phenylbutazone, pairwise with each of the three acids, suggests that these drugs are bound to the same primary site, as was also reported by Solomon *et al.* [9, 10], and that they are displaced from albumin by the same mechanism. This is of significance since, although there is general agreement [10–12] that phenylbutazone binds primarily to a single site on human albumin, various values have been reported for warfarin: a single primary site [9, 10], two primary sites [13, 14] and recently approximately 1·5 primary sites [15]. Similarities in binding are known wherein warfarin appears to bind to secondary sites [14, 15], as does phenylbutazone [12], and both drugs have been reported to bind to hydrophobic primary sites by other than ionic interactions [13].

Spector *et al.* [5] have proposed an allosteric mechanism to account for the effect of FFA on the albumin binding of chlorophenoxyisobutyrate ion, which has been shown to competitively displace warfarin [10]. The thermodynamic data derived in this paper also are consistent with an allosteric mechanism as a dominant mode of FFA displacement of drug from albumin for both warfarin and phenylbutazone. These data also suggest that the mechanism is the same for the three acids, since the calculated $\Delta\Delta G$ values do not show erratic changes with increases in fatty acid chain length. FFA-induced spatial rearrangements in human albumin sufficient to result in an allosteric influence on drug binding are not unexpected since bovine serum albumin is known to undergo significant conformational shifts in response to FFA [16]. The results obtained from the calculations in the present paper indicate that useful tests of the similarity of binding sites

Table 1. Thermodynamic data of warfarin and phenylbutazone displacement by FFA

Fatty acid	F	$nK(P)$	$(P)^*$ ($M \times 10^5$)	K ($M^{-1} \times 10^{-3}$)	ΔG (cal/mole)	$\Delta\Delta G$ (cal/mole)
			Warfarin			
None	0·829	4·85	6·70	72·3	−6740	0
Stearic	0·515	1·06	7·99	13·3	5720	1020
Myristic	0·306	0·44	8·85	4·98	−5129	1611
Lauric	0·160	0·19	9·44	2·02	−4584	2156
			Phenylbutazone			
None	0·913	10·49	6·36	165	−7237	0
Stearic	0·677	2·10	7·32	28·6	−6181	1056
Myristic	0·509	1·04	8·01	12·9	−5703	1534
Lauric	0·286	0·40	8·93	4·49	−5065	2172

* Values of (P) were calculated from the reported [10] values of F and the total concentrations of drug and albumin assuming $n = 1$.

and mechanisms for pairs of drugs, such as warfarin and phenylbutazone, are possible via molecular probes such as fatty acids.

Department of Pharmaceutics, EDWARD G. RIPPIE
College of Pharmacy,
University of Minnesota,
Minneapolis, Minn. 55455, U.S.A.

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Elevation of central γ-aminobutyric acid levels by isoniazid in mice and convulsant thresholds

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The accumulating evidence for a role of γ-aminobutyric acid (GABA) as a central inhibitory transmitter [1, 2] has prompted several studies about a relationship between the central levels or the metabolism of this amino acid and the sensitivity to convulsions. The results of these studies have been contradictory: some authors did not find any such relationship [3–5], whereas the results of others seem to indicate a role of GABA for central excitability [6] or seizure activity [7–9]. Interpretation of the results was made difficult by the use of drugs with powerful pharmacodynamic properties, as for example thiosemicarbazide or aminooxyacetic acid, as tools for alterations in the central GABA metabolism. Recently, however, Perry and Hansen [10] reported that isoniazid administered in the food over several days in nontoxic doses was able to elevate central GABA levels considerably. Wood, Peesker and Urton [11] and Wood and Peesker [12] showed that treatment with isoniazid indeed protected chicks against seizures elicited by exposure to hyperbaric oxygen or by injection of picrotoxin or pentetrazole, but was ineffective in rodents against hyperbaric oxygen seizures.

Since a daily uptake of 100 mg/kg isoniazid in the drinking water was tolerated by mice without behavioral changes, and since this treatment neither seemed to affect the central metabolism of monoamines, the effect of which on convulsant thresholds had been studied previously [13], we thought it worthwhile to look for a correlation between central GABA concentration and the electro- and chemoconvulsant thresholds in this species.

MATERIAL AND METHODS

The study was done in mice of NMRI-strain (Mollegaard Hansens Avlslaboratorier A/S, Fjby, DK-4632 Fl.