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Competitive Binding of Long-Chain Free Fatty Acids, Octanoate, and Chlorophenoxyisobutyrate to Albumin

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SUMMARY

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The mechanism of binding of certain fatty acids and the drug chlorophenoxyisobutyrate (CPIB) to defatted bovine serum albumin has been studied by analysis of the mutual displacement of these ligands. The displacement of one ligand by another was measured at pH 7.45 by equilibrium partitioning between hexane and an aqueous phase, and by ultrafiltration. The ligand-inhibitor pairs were [^{14}C]palmitate vs. CPIB, octanoate, or stearate, [^{14}C]CPIB vs. octanoate or palmitate, and [^{14}C]octanoate vs. CPIB. Association constants (K_a) were measured for ligands in separate experiments, and inhibition constants (K_i) for competitors were estimated from linear double-reciprocal plots of the low ligand concentration region. In all cases a competitive type of displacement of ligand from a high-affinity site(s) by the inhibitor was observed. When K_a inhibitor was equal to or greater than K_a ligand, the inhibition constant (K_i) equaled K_a inhibitor, indicative of classical competitive inhibition. An example of this type is [^{14}C]CPIB ($K_a = 2 \times 10^5$ M^{-1}) displacement by palmitate ($K_i = K_a = 5 \times 10^6$ M^{-1}). However, when K_a inhibitor was less than K_a ligand, as in the case of [^{14}C]palmitate displacement by CPIB, the calculated K_i was less than K_a inhibitor. Furthermore, K_i decreased as the inhibitor concentration rose. Although data can be interpreted by a model in which common primary binding sites exist on albumin for medium- or long-chain free fatty acids and CPIB, a more complex interpretation than simple competitive inhibition may be required to explain the unequal K_i and K_a values.

INTRODUCTION

The recognition that serum albumin has a large number of anionic binding sites (1) has prompted many investigations into the nature of the ligand-albumin interaction. An apparently competitive displacement of one ligand from albumin by another was noted in 1950 by Karush (2), using dodecyl sulfate and *p*-(2-hydroxy-5-methylphenylazo)benzoic acid. The current view is that competition in the classical sense occurs only if both ligands have approximately the same association constant (K_a), in which case the inhibition constant (K_i) will equal K_a. If the respective K_a values for ligands X and Y differ by a factor of 10 or more, the primary binding sites on albumin are presumed to be different. For example, Goodman (3) found that nearly 2 moles of long-chain

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FFA[1] per mole of albumin were required before the binding of methyl orange to albumin was affected, suggesting that the primary binding sites are not similar. The difference between the high-affinity K_a values of FFA and methyl orange of 10^7 M^{-1} and 1.3×10^5 M^{-1}, respectively, supports this model. Recently de Miranda *et al.* (4) have interpreted the similarity of K_a and K_i constants for the fatty acids hexanoate, heptanoate, and octanoate as evidence of a common first binding site. Since the octanoate-butyrate pair did not possess equal K_i and K_a constants, however, it was concluded that the first binding sites for these fatty acids are not identical. The data of Rudman *et al.* (5), showing that, *in vivo*, long-chain FFA do not displace drugs such as salicylate, sulfadiazine, and diphenylhydantoin from albumin until a FFA to albumin ratio of 3 is reached, are compatible with the concept of dissimilar primary binding sites. In fact, these experiments have been taken to indicate that physiological variations in circulating FFA do not affect drug-albumin association significantly. Spector *et al.* (6–8) concluded that long-chain FFA can compete with drugs only at secondary binding sites; at primary FFA sites, any interaction between FFA and drug is allosteric.

We have recently shown that under certain conditions a weakly bound ligand (chlorophenoxyisobutyrate) can displace long-chain FFA from albumin (9). The condition is that a hydrophobic phase be present to trap the nonpolar unbound fatty acid, but not the more hydrophilic drug. In this manner the partitioning of the hydrophobic ligand reduces the aqueous unbound concentration, allowing more fatty acid to dissociate. The present experiments utilize this technique to define more quantitatively the displacement of fatty acid by a weaker ligand and the mechanism of drug-FFA-albumin interaction. By comparing the association and inhibition constants of ligand-FFA pairs, the question whether separate groups of binding sites exist for weak and strong ligands has also been examined.

[1] The abbreviations used are: FFA, free fatty acid(s); CPIB, chlorophenoxyisobutyrate; BSA, bovine serum albumin.

METHODS

Equilibrium partitioning between long-chain FFA and CPIB was carried out as previously described (9, 10). Generally 1.0 ml of 0.2 mM BSA in a salts buffer (0.1 M NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, and 25 mM phosphate, pH 7.45) was equilibrated with 1.0 ml of hexane that contained radioactive FFA. The reaction was carried out in 5-ml-capacity glass vials fitted with Teflon-lined screw caps, at 37° for 20–24 hr, with shaking. Scintillation counting of 0.2-ml aliquots of upper and lower phases was performed in glass vials of 5-ml capacity, using a 2:1 toluene–Triton solution and 0.4% 2,5-diphenyloxazole. Free aqueous (i.e., unbound) concentrations of fatty acids were calculated from partition ratios and the concentration in the hexane phase at equilibrium (9, 10).

Ultrafiltration of water-soluble ligands was done at 23–25° in centrifuge cones (Amicon, CF 25), with 0.1 mM BSA in 2.0 ml of the same physiological salts buffer, pH 7.45. Adherent water was removed from the sides by a brief preliminary centrifugation for 15 sec at 2000 rpm and discarded, and radioactivity in the ultrafiltrate resulting from a 60-sec centrifugation was determined.

To measure inhibition of [^{14}C]CPIB binding by long-chain FFA, a combination of equilibrium partitioning and ultrafiltration was used. This technique was necessary because of the limited solubility of palmitate and stearate in water. The aqueous phase contained 0.1 mM BSA in 2 ml of salts buffer, pH 7.45, plus drug. The FFA was added initially to 1 ml of the hexane phase. After equilibration for 20–24 hr at 37°, the hexane layer was removed by aspiration and the bottom, aqueous phase, containing bound plus free fatty acid and drug was ultrafiltered at 23° as described above. Free [^{14}C]CPIB was measured in the ultrafiltrate. In separate experiments, radioactive FFA was used to determine the concentration in the hexane and aqueous phases at equilibrium.

A Hewlett-Packard 9100 programmable

calculator was used to simulate the Scatchard binding equation (1) for a multisite model, assuming a maximum of 12 sites (11).

BSA, fraction V, 96–99% pure, was purchased from Sigma and defatted with charcoal (12) to remove endogenous FFA. All commercial radioactive FFA (New England Nuclear) were purified before use as described by Meisner (9).

Synthesis of [2-^{14}C]chlorophenoxyisobutyrate was accomplished according to Julia *et al.* (13), as modified by Dr. E. Ferdinandi, Ayerst Laboratories, Montreal:[2]

p-chlorophenol + [2-^{14}C]acetone + chloroform $\xrightarrow{\text{KOH}}$ [2-^{14}C]chlorophenoxyisobutyrate

Following preparative thin-layer chromatography and crystallization, the product was converted to the Na^+ salt; specific activity, 964 cpm/nmole; purity, 95%, by thin-layer chromatography in methylcyclohexane–acetone–acetic acid (70:30:1 by volume). The NMR spectrum of unlabeled CPIB synthesized as above was identical with that of the sample provided by Ayerst.

RESULTS

The effect of CPIB on palmitate binding to defatted BSA is shown in Fig. 1. In these experiments the use of a partitioning system provided a FFA concentration in the organic phase that was at least 10^3 times greater than the aqueous phase concentration. The "reservoir" of FFA assured that displacement by a competitive ligand did not greatly change the unbound FFA concentration in the aqueous phase. In the absence of drug the Scatchard plot of fatty acid binding is nonlinear (3, 14, 15), indicating the presence of nonidentical binding sites or negative interactions between sites. The solid line of Fig. 1 is a simulated line based upon the class model (1), and indicates the degree to which this analysis describes the experimental data. For palmitate the values are $n_1 = 4$, $k_1 = 4 \times 10^6$ M^{-1}; $n_2 = 2$, $k_2 = 8 \times 10^4$ M^{-1}, which are in reasonable agreement with previously reported analyses by other workers (3, 14, 15). Addition of CPIB to the aqueous phase at drug to albumin molar ratios of 2.5–25 displaces FFA from high-affinity sites as indicated by the lower (dashed) lines in Fig. 1. The Scatchard binding curves for palmitate in the presence of CPIB are still nonlinear, indicating that the nonequality of sites is maintained even in the presence of the drug.

Since the initial portions of the Scatchard plots were linear, we examined the mechanism of drug-albumin-FFA interaction on the first few high-affinity sites by converting the data to a double-reciprocal plot of (bound ligand)$^{-1}$ vs. (free ligand)$^{-1}$ (Fig. 2). Only the data for the first two or three high-affinity FFA sites are included, in order to simplify the analysis. In the absence of CPIB, the $K_{a\,app}$ (calculated from the intercept on the abscissa) for palmitate is 4.12×10^6 M^{-1}, in good agreement with the simulation of Fig. 1. The common $\bar{v}$ intercept indicates that CPIB displaces palmitate from high-affinity sites on albumin in an apparently competitive manner. Apparent K_a values for palmitate in the presence of CPIB are shown in Table 1.

The general nature of the competition with palmitate as ligand was determined by using a long-chain FFA, stearate (Fig. 3), and a medium-chain FFA, octanoate (Fig. 4), as inhibitor. For the first four to six palmitate sites, as indicated by the ordinate intercept, a linear relationship was observed, analogous to the behavior of palmitate with CPIB. Both types of FFA behaved as apparent competitive inhibitors of palmitate.

To investigate the mechanism of FFA-albumin-drug interaction further, CPIB was used as the ligand, and FFA as the competitor. [2-^{14}C]CPIB was synthesized for these studies as outlined in METHODS, and the apparent association constants were obtained by examining the binding to defatted BSA (Fig. 5). The solid line is a computer simulation showing the "best fit" Scatchard model, where $n_1 = 1$, $k_1 = 2.2 \times 10^5$ M^{-1}; $n_2 = 11$, $k_2 = 0.8 \times 10^3$ M^{-1}.

[2] E. Ferdinandi, personal communication.

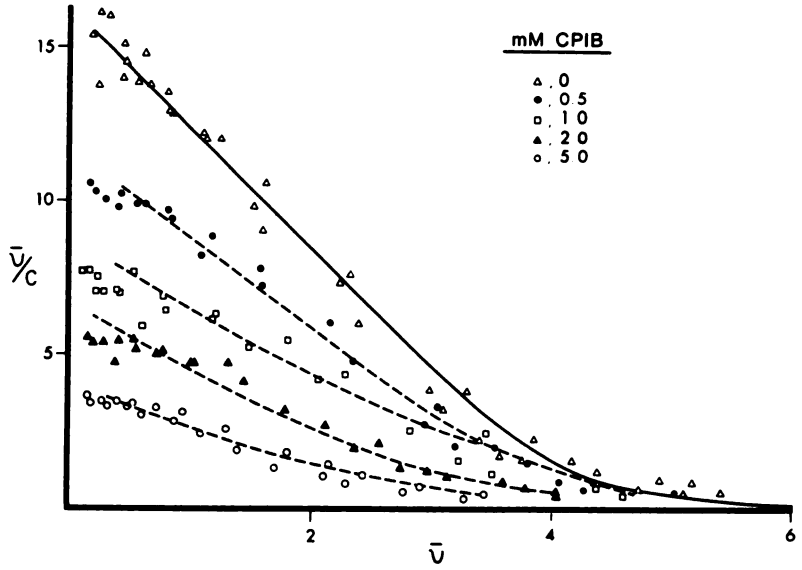

FIG. 1. *Scatchard plot of effect of chlorophenoxyisobutyrate on binding of [^{14}C]palmitate to BSA in a hexane/aqueous partitioning system*

The solid line represents a "best fit" computer simulation of the data, with n and k values as described in the text. Dashed lines are estimated fits for 0.5 mM (●), 1 mM (□), 2 mM (▲), and 5 mM (●) CPIB. $\bar{\nu}$ is moles of palmitate per mole of BSA; c is the micromolar concentration of free aqueous palmitate.

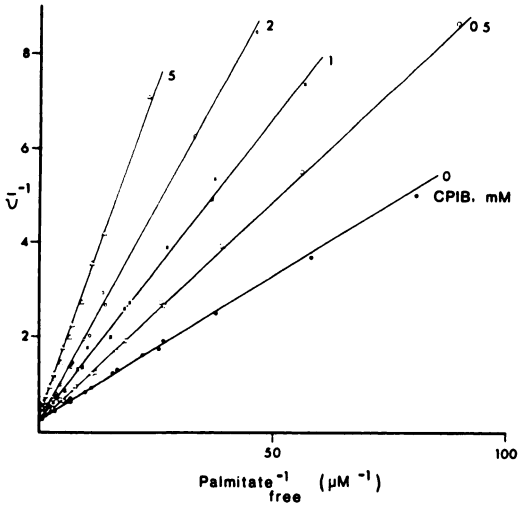

FIG. 2. *Double-reciprocal plot of effect of chlorophenoxyisobutyrate on [^{14}C]palmitate binding to high-affinity sites on BSA*

Data are from Fig. 1.

The constants are very similar to those obtained by Spector *et al.* (6), although the shape of the computed Scatchard plot is dissimilar, owing to the different number of low-affinity sites. For practical purposes, then, CPIB binds with moderately high affinity to only one site on albumin. An identical binding isotherm was obtained using defatted human serum albumin.

A combination of ultrafiltration and equlibrium partitioning was employed to study the displacement of [^{14}C]CPIB by palmitate (Fig. 6). The advantage of this approach is that it provides a means of solubilizing palmitate in the organic phase over a wide concentration range, which, at equilibrium, allows a certain aqueous concentration, determined by partitioning. The effect of 0.1–0.5 mM palmitate on [^{14}C]CPIB binding to the high-affinity site for the drug on albumin is shown in Fig. 6. Palmitate acts as an apparently competitive inhibitor of CPIB for the primary binding site. Studies with [^{3}H]palmitate and [^{14}C]CPIB, not reported here, indicate that when the concentration of palmitate added to the organic phase is 0.1–0.5 mM, between 95% and 60%, respectively, is bound to BSA in the presence of 0.3 mM CPIB.

The competition between [^{14}C]CPIB and octanoate was examined by equilibrium dialysis (Fig. 7). The inhibitor reduced the

TABLE 1

Apparent association constants for ligands and inhibitors bound to bovine serum albumin

Ligand	Inhibitor	Inhibitor/albumin[a]	K_{app}[b]	K_i inhibitor[c]	K_a inhibitor[d]
			M^{-1}	M^{-1}	M^{-1}
Palmitate	Stearate	0	3.07×10^6		
		0.25	2.18×10^6	4.9×10^6	1.66×10^7
		0.5	1.19×10^6	6.0×10^6	
		1.0	0.64×10^6	4.3×10^6	
	Octanoate	0	6.5×10^6		
		2	4.0×10^6	1.12×10^4	7.2×10^5
		5	3.34×10^6	0.70×10^4	
		15	1.21×10^6	0.39×10^4	
	CPIB	0	4.12×10^6		
		0.5	2.72×10^6	4.9×10^3	
		1	1.97×10^6	4.3×10^3	$1.3\text{–}2.0 \times 10^5$
		2	1.39×10^6	3.1×10^3	
		5	0.88×10^6	1.8×10^3	
CPIB	Octanoate[e]	0	3.70×10^5		
		0.25	2.84×10^5	1.59×10^6	
		0.5	2.24×10^5	1.50×10^6	7.6×10^5
		1.0	1.15×10^5	1.61×10^6	
		2.0	0.33×10^5	2.08×10^6	
	Palmitate	0	2.04×10^5		
		1.0	1.47×10^5	1.2×10^7	$3\text{–}6 \times 10^6$
		2.5	0.77×10^5	1.5×10^7	
		5.0	0.24×10^5	2.0×10^7	
Octanoate	CPIB	0	7.58×10^5		
		0.5	3.94×10^5	1.56×10^5	
		1	1.97×10^5	1.72×10^5	$1.3\text{–}2.0 \times 10^5$
		2.5	1.32×10^5	0.75×10^5	
		5	1.00×10^5	0.37×10^5	

[a] Molar ratio of total inhibitor added to albumin. At equilibrium the inhibitor was present as $\text{unbound}_{\text{organic}}$ (when present), $\text{unbound}_{\text{aqueous}}$, and $\text{bound}_{\text{aqueous}}$.

[b] K_{app} = 1/slope ($\bar{V}_{max}$) from the double-reciprocal plots.

[c] K_i = apparent inhibitor constant, as described in the text.

[d] K_a inhibitor = value determined from double-reciprocal plots with only radioactive inhibitor present.

[e] All experiments were performed at 37°, except for [^{14}C]CPIB vs. octanoate, which was carried out at 23°.

apparent association constant for CPIB from 3.7×10^5 to 0.33×10^5 M^{-1} at an octanoate to BSA molar ratio of 2.

Analysis of the competition experiments described here is complex for several reasons. First, the concentration of free aqueous competitor is not simply equal to the total added, since a significant portion is bound to BSA and, in the case of the FFA, is also distributed into the hexane phase. Second, the concentrations of BSA, ligand, and competitor are all in the same range, and the concentration of free aqueous competitor will not be constant over a single curve, but will vary. Therefore, in order to analyze the binding data in terms of K_i values, the molar concentrations of free aqueous competitor were obtained from binding isotherms carried out in the presence of a concentration of ligand equal to approximately the average employed in each experiment. A typical ligand correction for ^{14}C-CPIB vs. palmitate (Fig. 6) is shown in Fig. 8, where the

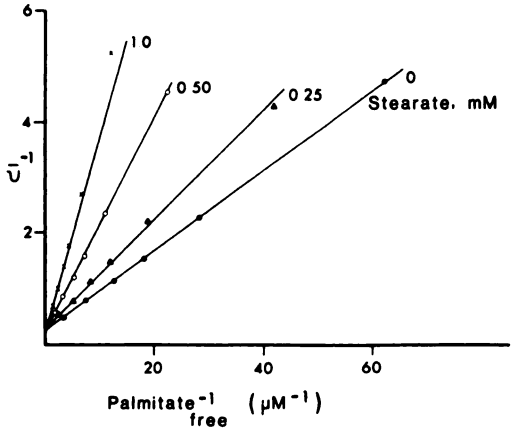

FIG. 3. *Double-reciprocal plot of effect of stearate on [^{14}C]palmitate binding to BSA*

Both FFA were added to the hexane phase of the partitioning system and allowed to equilibrate for 20 hr at 37° with shaking. The initial concentration of stearate in the hexane phase is shown. The abscissa shows the micromolar concentration of free palmitate (reciprocal).

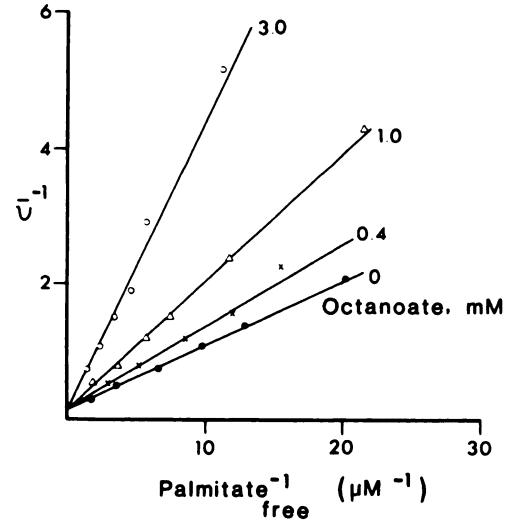

FIG. 4. *Double-reciprocal plot of effect of octanoate on [^{3}H]palmitate binding to BSA*

Octanoate, which did not partition in hexane, was added in the concentrations shown to the aqueous phase of a partitioning system.

binding of [^{14}C]palmitate to 0.2 mM BSA in the presence of 0.5 mM CPIB was determined. This concentration was chosen because it represents an intermediate [^{14}C]CPIB level used in Fig. 6. The binding is decreased 30% at a palmitate to BSA ratio of 1:5 (the ratio used in Fig. 6), and by progressively smaller amounts at higher FFA to BSA molar ratios. The concentration of free palmitate thus obtained was used to estimate the K_i of palmitate for [^{14}C]CPIB binding. Similarly, in the case of [^{14}C]palmitate vs. stearate, the free competitor (stearate) concentration was found to be increased about 50% by palmitate, but even here the straight-line nature of the $1/\bar{v}$ vs. 1/free plot (Fig. 3) argues in favor of its validity.

The free inhibitor concentration was then used to obtain an apparent association constant (K_i) for the inhibitor according to Edsall and Wyman (16):

$$K_i = \frac{1}{[I]}\left(\frac{K_a}{K_a'} - 1\right)$$

where K_a and K_a' are the association constants of ligand measured without (K_a) and with (K_a') inhibitor, and $[I]$ is the molar concentration of free competitor in the aqueous phase.

Table 1 summarizes these data. For simple competitive inhibition, the K_i should be independent of competitor concentration and should equal the K_a determined by direct binding. For a competitive pair of ligands this consideration should hold true for *each* ligand. It is striking that with octanoate and CPIB vs. palmitate as ligand the values of K_i differ appreciably from the respective K_a values. For example, the K_i of CPIB for palmitate is 4×10^3 M^{-1}, which is $1/50$ the K_a of 2.2×10^5 M^{-1}. On the other hand, the K_i of stearate for [^{14}C]palmitate binding is 5×10^6 M^{-1}, nearly equivalent to the K_a for stearate of 1.7×10^7 M^{-1}. The K_i values for octanoate and palmitate vs. CPIB are also very similar to the K_a values. It is instructive to compare the palmitate-CPIB pair: although the K_i of CPIB is only 2% of the K_a of CPIB (approximately 10^3 vs. 10^5), the K_i of palmitate for [^{14}C]CPIB of $1.2\text{–}2.0 \times 10^7$ M^{-1} is slightly greater than the palmitate K_a of 5×10^6 M^{-1}. In other words, although both CPIB and palmitate are apparent competitive inhibitors of the binding of one another, only when palmitate is the inhibitor does the system behave as class-

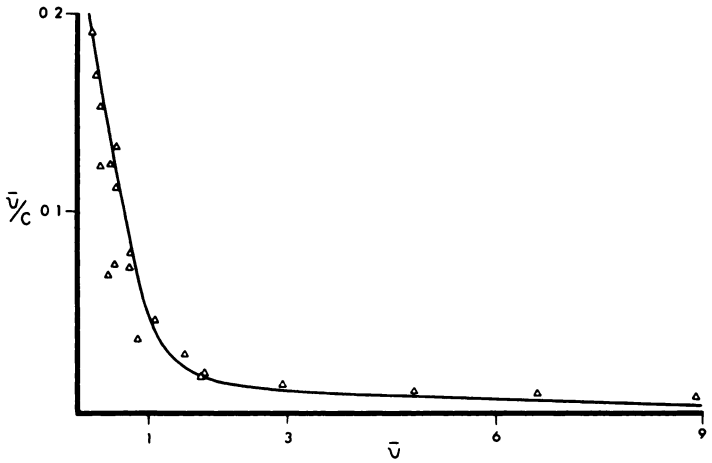

FIG. 5. *Scatchard plot of [2-^{14}C]chlorophenoxyisobutyrate binding to BSA*

The solid line represents a "best fit" computer simulation, where $n_1 = 1$, $k_1 = 2.2 \times 10^5$; $n_2 = 11$, $k_2 = 0.8 \times 10^3$. The simulated values are slightly low for $\bar{v} > 2$ because of the use of a 12-site model yielding an abbreviated x intercept. The [^{14}C]CPIB was equilibrated at 37° for 16 hr in 0.1 mM BSA, pH 7.45, before ultrafiltration.

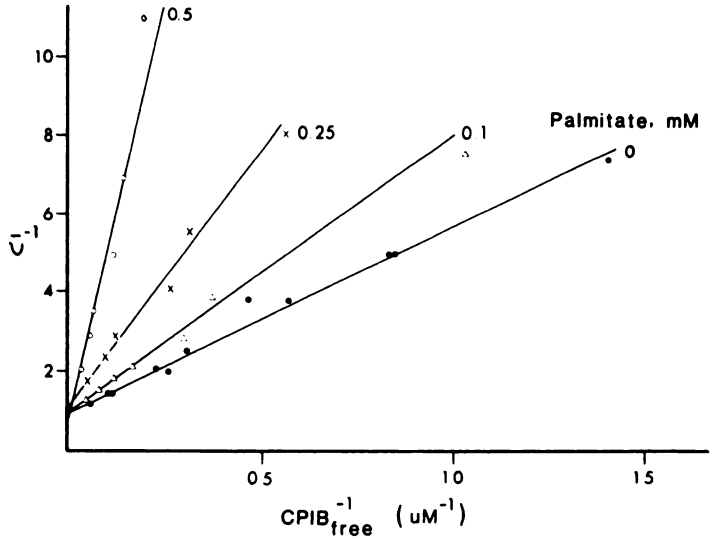

FIG. 6. *Double-reciprocal plot of effect of palmitate on [^{14}C]CPIB binding to BSA*

Palmitate was added to the hexane phase at the concentrations shown, and [^{14}C]CPIB was added to the aqueous phase. After equilibration for 16 hr at 37°, the aqueous phase was ultrafiltered.

ical simple competition. Finally, the data show that the K_i values of CPIB and octanoate for [^{14}C]palmitate binding and the K_i CPIB for [^{14}C]octanoate binding decrease as the inhibitor concentration is increased. For the other inhibitor-ligand pairs, however, the inhibition constants either do not change appreciably or even increase slightly.

DISCUSSION

Our data do not fully agree with the assessment that different groups of high-affinity binding sites exist on albumin for FFA and drugs (4, 8, 17). Rather, many of the data are consistent with the interpretation that ligands containing COOH groups bind in a mutually exclusive sense

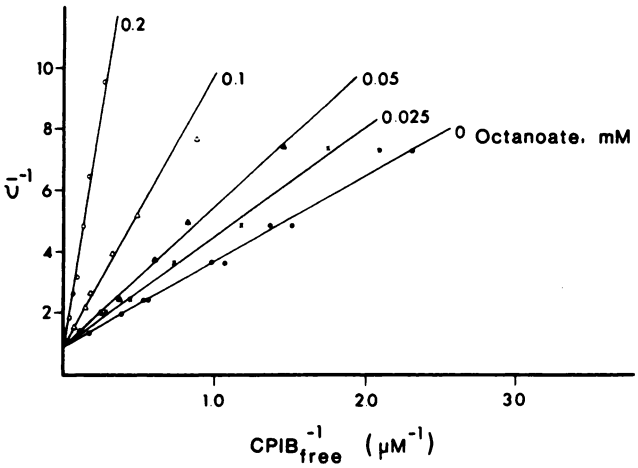

FIG. 7. *Double-reciprocal plot of effect of octanoate on [^{14}C]CPIB binding to BSA*
Ligand and inhibitor were equilibrated for 30 min at 23° in salts–0.1 mM BSA, pH 7.45, before ultrafiltration. Octanoate was added in the concentrations shown.

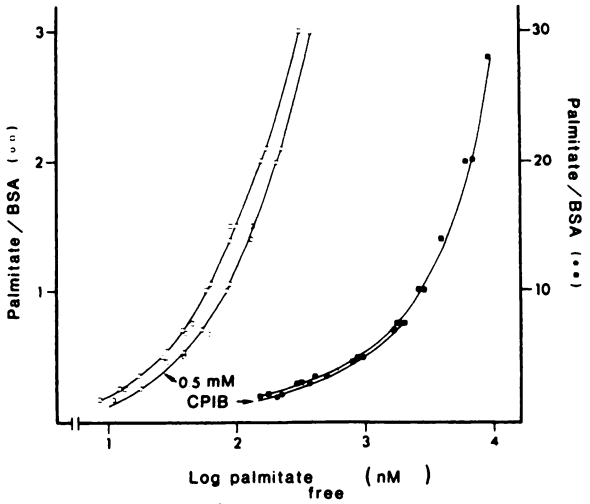

FIG. 8. *Unbound palmitate concentration vs. molar ratio of palmitate to BSA*
[^{14}C]Palmitate was added to the hexane phase and equilibrated in a partitioning system for 16 hr. Unbound palmitate was computed from the molar fraction remaining in the hexane phase and the partition ratio of palmitate. ○ and ●, 0.5 mM total CPIB in the aqueous phase. Palmitate/BSA is the molar ratio of the total palmitate and BSA present in the system.

to the same set of sites, independent of the affinity of the ligand toward albumin. The primary evidence for this concept is that although the reciprocal binding plots for high-affinity sites are indicative of competitive binding, i.e., complete displacement of competitor at saturating ligand concentrations, with two of the competitors ([^{14}C]palmitate vs. octanoate or CPIB) the K_i is not equal to the K_a, but rather is 10–100-fold less. In the case of [^{14}C]octanoate vs. CPIB, the $K_i = K_a$ of CPIB initially, but decreases as the concentration of CPIB is raised. Furthermore, with four competitors ([^{14}C]palmitate vs. stearate, [^{14}C]octanoate vs. CPIB, [^{14}C]CPIB vs. octanoate, and [^{14}C]CPIB vs. palmitate) the K_i is essentially equal to the K_a determined directly. Whether it is correct to compare K_i with K_a in this situation is unclear, since deviations from linearity in the double-reciprocal plot

would be expected to occur as a result of variable free inhibitor concentrations. Computer simulations have verified that nonlinear double-reciprocal plots are obtained, bending downward at high ligand concentration in the presence of competitor.[3] Empirically, however, since the experimental points for the double-reciprocal plots remain linear and apparently competitive, we feel that it is appropriate to compare the constants. In either event, these experiments are not completely consistent with simple competition and require a more complex interpretation.

Several possibilities are conceivable to explain the above inconsistencies. (a) The suggestion has been made that drugs and long-chain FFA do not share the same primary sites on albumin (6–8). If the weaker binding sites for CPIB ($K_a = 10^3$ M^{-1}) were responsible for displacing the FFA from its tight binding sites ($K_a = 10^7$ M^{-1}), the K_i of 10^3 M^{-1} would be explicable. However, this would leave the high-affinity CPIB site to correspond to either a low-affinity FFA site or a site that does not bind FFA. Neither of these seems plausible, since the reciprocal experiment ([^{14}C]CPIB vs. palmitate) shows that displacement of CPIB from its high-affinity site occurs by simple competition with the high-affinity site of palmitate, as judged by the similar K_i and K_a of palmitate. In a simple "crossover" model, the K_i for palmitate should be *lower* than K_a, analogous to the drug data. Thus it is not reasonable to conclude that the high-affinity drug binding competes for the low-affinity FFA site on albumin, and vice versa. Deviations from the expected equality between K_i and K_a are due to some other cause. (b) The binding of FFA studied in Figs. 2–4 represents the first two or three high-affinity sites. It is known (3) that weaker binding sites also exist and become important at higher FFA concentrations. As FFA is displaced from tightly bound sites by CPIB, unbound FFA may reach aqueous concentrations such that binding occurs to lower-affinity FFA sites. Thus added CPIB would not actually displace the expected amount of FFA but rather would cause a certain amount of "spillage" from high-affinity to lower-affinity sites. The more weakly bound drug, having only a single high-affinity site, would be displaced competitively into the aqueous phase by FFA. In principle, this concept is capable of explaining qualitatively the observed difference between K_i and K_a. It may also be able to account for the decreasing K_i values with increasing competitor concentration found in these same situations. However, preliminary computer simulations using the Scatchard (1) multiple binding site equation, assuming competitive behavior of the inhibitor, have not yet been successful in accounting for the unequal K_i and K_a data.[4] (c) One type of interaction could occur such that CPIB or octanoate would bind to a different site from FFA, but in a competitive "allosteric" manner. In this case the K_i would equal K_a for both ligands only if displacement occurred with a single conformational change of albumin. On the other hand, if both CPIB and FFA bound allosterically and produced a different conformation state, the K_i calculated above would not be the correct measure of binding to albumin. (d) Schrier *et al.* (18) have presented evidence for aqueous phase dimerization of carboxylic acids by the formation of pairwise hydrophobic bonds, which may provide an explanation for the low K_i for CPIB and octanoate. Since only the monomeric form is presumed to bind to albumin, the decrease in K_i values shown in Table 1 would be due to the concentration dependence of dimerization. Initial attempts to substantiate this have been unsuccessful. (e) Interaction between binding sites through ligand-induced conformational changes of albumin, leading to negative cooperativity of binding, would also account for much of the data. Such negative cooperativity has not been excluded as an explanation for the multitude of FFA binding sites on albumin. If the drug binds to the same sites as FFA but causes a different conformational change, and hence a different affinity for succeed-

[3] H. Meisner and K. Neet, unpublished observations.

[4] K. Neet and H. Meisner, unpublished observations.

ing drug or FFA molecules, the observed data on ligand-inhibitor pairs could be obtained. In fact, this model possesses such flexibility that nearly any type of behavior could be explained. Such a complex model cannot be tested by binding curves alone and should not be adopted until simpler models have been considered and discarded.

In conclusion, we have demonstrated that binding of the long-chain FFA palmitate and stearate, on the one hand, and binding of the drug CPIB and the medium-chain FFA octanoate, on the other, completely displace each other from high-affinity sites. Such observations, while consistent with the assumption that similar sites exist on albumin for drugs and long-chain FFA, may be more complex than simple competitive inhibition, since the K_i for CPIB and octanoate is not equal to the respective K_a values. Experiments are in progress to examine the role of competitive allosterism [see (c)] as an explanation for the nonclassical competition, and possibly of the decreasing K_i values as well.

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REFERENCES

1. Scatchard, G. (1949) *Ann. N. Y. Acad. Sci.*, **51**, 660-672.
2. Karush, F. (1950) *J. Am. Chem. Soc.*, **72**, 2705-2713.
3. Goodman, D. (1958) *J. Am. Chem. Soc.*, **80**, 3892-3898.
4. de Miranda, R., Eikelboom, T. & van Os, G. (1976) *Mol. Pharmacol.*, **12**, 454-462.
5. Rudman, D., Bixler, T. & Del Rio, A. (1971) *J. Pharmacol. Exp. Ther.*, **176**, 261-272.
6. Spector, A., Santos, E., Ashbrook, J. & Fletcher, J. (1973) *Ann. N. Y. Acad. Sci.*, **226**, 247-258.
7. Santos, E. & Spector, A. (1974) *Mol. Pharmacol.*, **10**, 519-528.
8. Spector, A. (1975) *J. Lipid Res.*, **16**, 165-179.
9. Meisner, H. (1977) *Mol. Pharmacol.*, **13**, 224-232.
10. Goodman, D. (1958) *J. Am. Chem. Soc.*, **80**, 3887-3892.
11. Fletcher, J., Spector, A. & Ashbrook, J. (1970) *Biochemistry*, **9**, 4580-4587.
12. Chen, R. (1967) *J. Biol. Chem.*, **242**, 173-181.
14. Spector, A., John, K. & Fletcher, J. (1969) *J. Lipid Res.*, **10**, 56-67.
15. Ashbrook, J., Spector, A., Santos, E. & Fletcher, J. (1975) *J. Biol. Chem.*, **250**, 2333-2338.
16. Edsall, J. & Wyman, F. (1958) *Biophysical Chemistry*, Vol. 1, p. 652, Academic Press, New York.
17. Sudlow, G., Birkett, D. & Wade, D. (1975) *Mol. Pharmacol.*, **11**, 824-832.
18. Schrier, E., Pottle, M. & Scheraga, H. (1964) *J. Am. Chem. Soc.*, **86**, 3444-3449.