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DISPLACEMENT OF TOLBUTAMIDE, GLIBENCLAMIDE AND CHLORPROPAMIDE FROM SERUM ALBUMIN BY ANIONIC DRUGS*

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Abstract The binding of tolbutamide, chlorpropamide and glibenclamide to human and bovine serum albumin has been estimated in the presence of a number of acidic drugs. It is shown that agreement between experimental data and that calculated using the competitive binding equation is very poor. The degree of displacement of tolbutamide and chlorpropamide is much greater than that calculated using the equation while displacement of glibenclamide is much less. These findings suggest that displacement is essentially non-competitive and that glibenclamide is less susceptible to displacement by acidic drugs than tolbutamide or chlorpropamide.

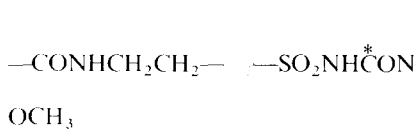
It is generally assumed that the degree of displacement of one drug by another from protein binding sites can be calculated using the well known competitive binding equation [1, 2]. Implicit in this is the further assumption that both drugs are bound to and compete for common discrete binding sites. This is particularly the case where binding constants are determined by measuring the displacement of a fluorescent [3, 4] or other probe.

The displacement of the sulphonylurea drugs from serum albumin has received considerable interest [6–9]. A variety of drugs can displace tolbutamide [6–10] and chlorpropamide [6–8, 10] from binding sites but little is known about the newer drug glibenclamide.

In this work displacement of the three sulphonylureas by several common acidic drugs has been estimated. The extent of displacement calculated using the competitive binding equation is compared with experimental data.

MATERIALS AND METHODS

Bovine serum albumin (BSA lot number 300-2060) and human serum albumin (HSA lot number 81c-13028) were both crystalline fraction V albumin obtained from Sigma Co. Tolbutamide and chlorpropamide have been described elsewhere [11]. [^{14}C]glibenclamide was labeled as follows:



The pKa was estimated as 6.5 ± 0.03 [11] and the sp. act. was 0.84 mCig^{-1}. Phenylbutazone and sulphaphenazole were donated by Ciba–Geigy and warfarin was donated by Endo Laboratories. All drugs including salicylic acid and paracetamol were recrystallised prior to use.

Dialysis procedure. Binding of tolbutamide and chlorpropamide was determined in the presence of displacing drugs using the dynamic dialysis technique [12]. The volume of the protein solution inside the dialysis bag was 7 ml and that of the external solution was 250 ml. This technique is unsuitable for use with glibenclamide because the drug is strongly bound to the membrane and has a very low rate constant for diffusion across the membrane [11]. Thus to study binding at the low therapeutically significant levels of glibenclamide, extended dialysis times plus a large correction for membrane binding would be necessary. An equilibrium dialysis technique was therefore used. Good agreement between the two dialysis techniques has been reported [13, 14]. Studies were carried out in sterile glass dialysis cells of 5-ml capacity. All dialysis studies were conducted at pH 7.4 in 0.067 M phosphate buffer. Binding parameters were calculated as described previously [14].

To estimate the effect of a co-solute drug on the binding of another, two approaches are possible. The more common is to study the binding in systems containing a fixed total concentration of co-solute. This has the substantial disadvantage that the binding profile of the drug under consideration is determined under conditions of varying co-solute activity since as binding of the drug varies binding of the co-solute also varies. The second approach is to determine the complete binding profile of the drug while maintaining a constant free concentration and thus a constant displacing effect of the co-solute. Only binding data obtained in this way is suitable for treatment according to the competitive binding equation. This equation states that for a particular class of identical and independent binding sites, the apparent association constant for a drug A, k'_A, in the presence of a competitor B is given by

$$k'_A = k_A(1 + k_B D_{fB}) \qquad 1.$$

where k_A and k_B are association constants for binding of A and B to protein and D_{fB} is the free concentration of competitor B.

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In dynamic dialysis procedures this is achieved by adding a suitable concentration of displacing drug to the albumin solution and the corresponding free concentration is maintained in the external compartment. When samples are removed from the external solution they are replaced with buffer containing the same co-solute concentration. As dialysis of the primary drug occurs, more co-solute will tend to bind to protein. Therefore, co-solute from the large external solution may enter the protein compartment. However, the same free concentration of co-solute should be maintained because of the reservoir effect of the large external compartment. For tolbutamide, chlorpropamide and the dynamic dialysis procedure, free concentrations of displacing drugs never varied by more than ± 2 per cent. Agreement of replicates for several systems indicated good reproducibility.

It is more difficult to maintain a particular free co-solute level in equilibrium dialysis because there is no reservoir effect. However, in the case of glibenclamide, it was present in such low concentrations compared to co-solutes, that little displacement of co-solutes occurred. It was possible to maintain free concentrations of phenylbutazone, warfarin and salicylate to within ± 5 per cent.

ANALYSIS

Tolbutamide, chlorpropamide and glibenclamide were estimated as described previously [11]. Paracetamol, sulphaphenazole, salicylate, phenylbutazone and warfarin were assayed spectrophotometrically at 245, 252, 299, 268 and 310 nm, respectively. Where necessary, small absorbance contributions due to tolbutamide, chlorpropamide and glibenclamide at these wavelengths were subtracted.

RESULTS

The effect of phenylbutazone, warfarin, salicylate, sulphaphenazole and paracetamol on binding constants of tolbutamide to BSA and HSA are shown in Table 1. Significant displacement of tolbutamide occurs in the first class of sites but there is little effect on the second class. It is interesting to note that the apparent association constant, k'_1, with BSA is very similar to that with HSA.

The binding constants for the various displacing compounds are listed in Table 2. Phenylbutazone, like tolbutamide, is bound to three primary sites on the albumin molecule. If these sites are common it might be expected that binding of tolbutamide in the presence of phenylbutazone could be described by the competitive binding equation. Values of the apparent primary association constants, k'_1, for tolbutamide were calculated using the respective binding parameters for tolbutamide and phenylbutazone and Eq. 1. (Table 1) These values are seven times greater than those observed experimentally.

Phenylbutazone caused greater displacement of chlorpropamide from BSA than warfarin at equivalent free concentrations (Table 3). Warfarin and chlor-

Table 1. Binding parameters for the interaction of tolbutamide with albumin at pH 7.4 and 37° in the presence of various drugs

Displacing agent	Mean free concentration ($M \times 10^4$)	Albumin	Binding parameters for tolbutamide n_1	k_1 ($M^{-1} \times 10^{-4}$)	n_2	k_2 ($M^{-1} \times 10^{-4}$)	Calculated k'_1 ($M^{-1} \times 10^{-4}$)
		BSA	2.98	24.82	8.12	3.39	
		HSA	2.27	21.86	8.21	1.71	
Phenylbutazone	0.08	BSA	2.86	1.02	8.08	3.21	7.63
	0.16	BSA	2.77	0.71	8.23	2.67	4.50
	0.16	HSA	2.22	0.63	8.07	1.64	4.37
Warfarin	0.04	BSA	2.93	2.38	8.06	3.24	
	0.16	BSA	3.12	1.05	8.15	3.19	
	0.16	HSA	2.25	1.66	8.17	1.55	
Salicylate	3.00	BSA	3.12	0.91	7.75	3.35	
Paracetamol	3.00	BSA	2.97	7.81	9.04	3.32	
	6.00	BSA	2.98	5.00	9.72	2.88	
Sulphaphenazole	0.32	BSA	2.82	14.70	8.47	2.40	
	0.64	BSA	3.13	6.58	8.06	1.75	

Table 2. Binding constants for drugs used to displace the sulphonylureas from serum albumin at 37° in 0.67 M phosphate buffer, pH 7.4

Drug	Albumin	n_1	k_1 ($M^{-1} \times 10^{-4}$)	n_2	k_2 ($M^{-1} \times 10^{-4}$)
Phenylbutazone	HSA	3.00	25.01	4.06	12.52
	BSA	3.18	27.80	4.21	22.86
Warfarin	HSA	1.84	14.71	5.01	15.20
	BSA	2.06	11.36	5.03	16.40
Salicylate	HSA	1.07	22.26	5.24	15.67
	BSA	1.18	21.03	5.03	16.72
Sulphaphenazole	BSA	1.02	9.20	5.30	11.31
Paracetamol	BSA	0.97	0.15	7.06	0.69

Table 3. Binding parameters for the interaction of chlorpropamide with BSA at pH 7.4 and 37° in the presence of warfarin and phenylbutazone

Displacing agent	Mean free concentration M × 10^4	Binding parameters for chlorpropamide				Calculated
		n_1	k_1 ($M^{-1} \times 10^{-4}$)	n_2	k_2 ($M^{-1} \times 10^{-2}$)	k_1' ($M^{-1} \times 10^{-4}$)
	—	1.94	4.67	8.93	4.14	
Warfarin	0.04	2.00	1.92	9.21	2.88	3.20
	0.16	1.94	1.09	9.14	1.86	1.64
Phenylbutazone	0.08	2.12	0.95	8.95	2.41	
	0.16	2.16	0.46	9.13	1.42	

Table 4. The effect of warfarin, phenylbutazone and salicylate on the interaction of glibenclamide with HSA at pH 7.4 and 37°

Displacing agent	Mean free concentration M × 10^4	Binding parameters for glibenclamide		Calculated
		n	k $M^{-1} \times 10^{-5}$	k' $M^{-1} \times 10^{-5}$
	—	1.82	7.64	
Warfarin	0.11	1.95	5.66	3.02
	0.26	1.76	5.47	1.57
Phenylbutazone	0.11	1.58	5.72	2.02
	0.24	1.53	4.52	1.11
	0.45	1.49	3.77	0.63
Salicylate	0.41	1.78	5.90	
	0.70	1.75	5.31	
	1.53	1.68	4.64	

propamide are both bound to two primary sites on the albumin molecule. However, the calculated values of k_1' for chlorpropamide in the presence of warfarin were again considerably greater than those observed experimentally (Table 3). A noteworthy point is that warfarin and phenylbutazone also appear to displace chlorpropamide from the secondary binding sites.

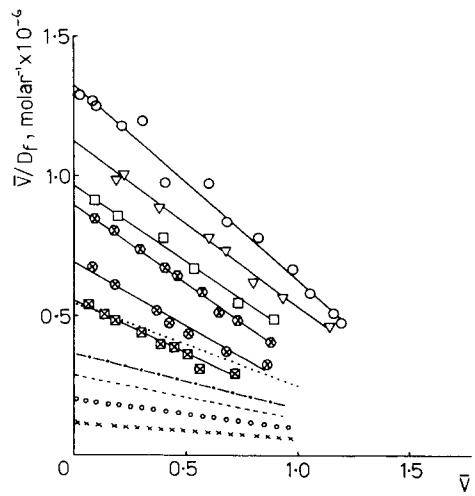

Fig. 1. The effect of phenylbutazone and warfarin on the binding of glibenclamide to 0.5% HSA at 37° in 0.067 M pH 7.4 phosphate buffer. ○ Glibenclamide alone. Warfarin free concentrations (M × 10^4): ▽ 0.11 (....)*; □ 0.26 (- - -)*. Phenylbutazone free concentration (M × 10^4): ⊗ 0.11 (——)*: ⊗ 0.24 (oooo)*; ⊠ 0.45 (xxxx)*.
* Binding curves predicted from k' values (Table 4) calculated from Eq. 1.

In contrast displacement of glibenclamide from HSA by phenylbutazone, warfarin and salicylate is far less than that predicted by the competitive binding equation (Table 4. Fig. 1). At the highest phenylbutazone free level (0.45 × 10^{-4} M) the association constant was reduced by a factor of only 2 compared to a 35-fold reduction in k_1 for tolbutamide in the presence of 0.16 × 10^{-4}M free phenylbutazone. Thus glibenclamide is far less susceptible to displacement by anionic drugs than tolbutamide and possibly chlorpropamide.

DISCUSSION

The standard competitive equation gives a very poor estimate of the degree of displacement for the systems considered here. In the case of tolbutamide and chlorpropamide displacement is far greater than that calculated. These drugs [11] and phenylbutazone [15] appear to bind to serum albumin by ionic forces. Calculations which account for electrostatic repulsion between the albumin molecule and sulphonylurea anions due to attachment of phenylbutazone anions produced little improvement in the fit of calculated data to that observed experimentally.

The possibility that materials may cause non-competitive displacement of drugs from plasma proteins has been recognized previously [15–17].

The environment around a binding site may facilitate hydrogen bond formation or van der Waal's attraction between drug and protein, contributing to the free energy of binding through either an enthalpic or entropic stabilisation [18]. If a competitor induced configurational changes in the albumin molecule thus

disturbing the favourable environment this could bring about displacement in addition to that caused by direct competition for common complexation sites [15]. The significance of these findings is that the degree of displacement cannot always be predicted directly by consideration of the respective association constants of the drug and displacing compound.

The displacement of tolbutamide from its primary sites by warfarin, salicylate, paracetamol and sulphaphenazole deserves consideration. These four drugs are bound to only one or two primary sites (Table 2) but they cause displacement in the three primary tolbutamide binding sites. Therefore it is difficult to explain the displacement in terms of competition for common sites. It could be argued that competition occurs due to overlap of binding sites of the two drugs.

Warfarin, for example, would then compete for only two of the three tolbutamide binding sites. However, the three primary sites for tolbutamide are apparently still equivalent (Table 1) and the concept of overlapping sites does not seem to apply.

It has been suggested previously that the tendency of one drug to displace another depends on its ability to distort the albumin molecule [19]. The mechanism of displacement may therefore be essentially non-competitive.

The displacement of glibenclamide from albumin by phenylbutazone, warfarin or salicylate is far less than that calculated by the competitive binding equation, (Eq. 1), assuming competition for both binding sites. This may be related to its mechanism of binding. Glibenclamide is bound by non-ionic forces whereas tolbutamide and chlorpropamide appear to be bound chiefly by ionic forces [11]. In addition anions would not displace glibenclamide by increasing the charge on the albumin molecule. The displacement that does occur may be due to an effect of the drugs on tertiary albumin structure. If this is the case, the effect is small possibly because the sites of attachment are remote from those of glibenclamide.

Although the binding parameters cannot be used quantitatively to calculate displacement, there does appear to be a rank order correlation between the binding constants of displacing drugs and the extent to which they reduce binding of a particular sulphonylurea. For example, phenylbutazone is the most strongly bound compound in Table 2 and appears to be the most effective at a given free concentration. Warfarin is more weakly bound and is less effective. The degree of displacement is therefore still a function of the number of drug molecules bound even though the mechanism of displacement may be essentially non-competitive.

The binding of glibenclamide is relatively unaffected by therapeutic levels of these displacing drugs. At most the free glibenclamide level would be doubled. Hence glibenclamide may be a safer drug to use when there is likelihood of concurrent administration of other drugs. It should be emphasized that the displacing drugs used in this work were all anions. It is possible that drugs bound by non-ionic forces, similar to glibenclamide may cause more substantial displacement.

REFERENCES

1. J. Steinhardt and J. A. Reynolds, in *Multiple Equilibria in Proteins* pp. 12–16. Academic Press, New York (1969).
2. J. T. Edsall and J. Wyman, *Biophysical Chemistry* pp. 591–662. Academic Press, New York (1958).
3. H. W. Jun, R. T. Mayer, C. M. Himel and L. A. Luzzi, *J. pharm. Sci.* **60,** 1821 (1971).
4. P. Hsu, J. K. H. Ma and L. A. Luzzi, *J. pharm. Sci.* **63,** 570 (1974).
5. H. Buttner and F. Portwich, *Klin. Wschr.* **45,** 225 (1967).
6. H. Wishinsky, E. J. Glasser and S. Perkal, *Diabetes, Suppl.* **11,** 18 (1962).
7. J. Judis, *J. pharm. Sci.* **61,** 89 (1972).
8. J. Judis, *J. pharm. Sci.* **62,** 232 (1973).
9. L. K. Christensen, J. M. Hansen and M. Kristensen, *Lancet* **2,** 1298 (1963).
10. B. Nillson and A. Agren, *Acta pharm. suecica* **11,** 391 (1974).
11. M. J. Crooks and K. F. Brown, *J. Pharm. Pharmac.* **26,** 304 (1974).
12. M. C. Meyer and D. E. Guttman, *J. pharm. Sci.* **57,** 1627 (1968).
13. M. C. Meyer and D. E. Guttman, *J. pharm. Sci.* **59,** 39 (1970).
14. M. J. Crooks and K. F. Brown, *J. pharm. Sci.* **62,** 1904 (1973).
15. A. Hasselblatt, *Proceedings of the European Society for the Study of Drug Toxicity, Vol. XIII.* Toxicological Problems of Drug Combinations, 89 (1972).
16. E. M. Sellers and J. Koch-Weser, *Clin. Pharmac. Ther.* **11,** 524 (1970).
17. D. Rudman, T. J. Bixler and A. F. Del Rio, *J. Pharmac. exp. Ther.* **176,** 261 (1971).
18. E. Karush, *J. Am. chem. Soc.* **72,** 2705 (1950).
19. P. M. Keen, *Br. J. Pharmac.* **26,** 704 (1966).