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Interaction of glipizide with human serum albumin

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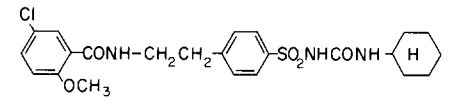
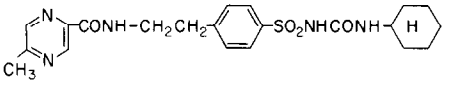
It is established that the hypoglycaemic sulphonylureas are extensively bound to human serum albumin at therapeutic plasma levels [1–3]. Binding of tolbutamide and chlorpropamide is apparently similar to that of many sulphonamides, while glyburide (I) (Fig. 1) interacts in a different manner [3]. In addition, glyburide is less susceptible to displacement by salts [4] or drug anions* than either tolbutamide or chlorpropamide.



I



II

Fig. 1. Chemical structures of glyburide (I) and glipizide (II).

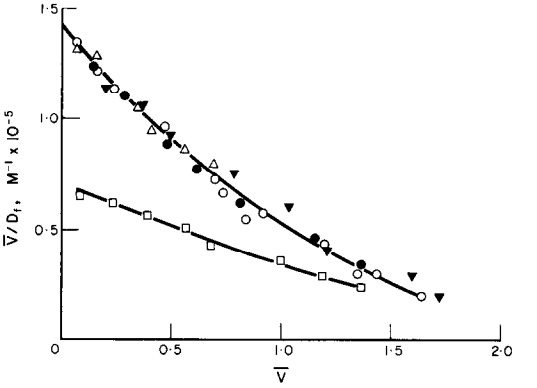


Fig. 2. Scatchard plot for interaction of glipizide with HSA at 37° in 0·067 M phosphate buffer: △, pH 6·4, 0·5% HSA; ○, pH 7·4, 0·5% HSA; ●, pH 7·4, 1% HSA; □, pH 7·4, 0·5% HSA + 0·154 M sodium chloride; and in 0·067 M tromethamine buffer: ▼, pH 7·4, 0·5% HSA.

A new sulphonylurea, glipizide (*N*-4-[*β*-(-methyl-pyrazine-2-carboxamide)-ethyl]-benzene sulphonyl-*N*′-cyclohexyl-urea) (II) (Fig. 1), has recently undergone clinical trial and is reportedly 200 times more potent than tolbutamide [5]. Preliminary dialysis studies of the drug (7 μg/ml) in pooled plasma from rat and dog indicated binding to the extent of 89 and 99 per cent respectively [6]. Dialysis of human plasma containing 0·4 μg/ml of glipizide indicated that 98 per cent was bound [7]. The relatively low volume of distribution estimated by these authors [7] was attributed in part to the high degree of plasma binding. In view of the fact that extensive binding may influence drug disposition, a more detailed investigation was made of the nature of the binding of glipizide to serum albumin.

^{14}C-Glipizide (radiochemical purity 98 per cent), labeled as described by Fuccella *et al.* [7], was diluted with unlabeled material and recrystallised prior to use. The final sp. act. was 0·22 μCi/mg, m.p. 204–5°. Solutions containing glipizide were estimated by liquid scintillation counting in Bray's solution for 50 min (counting range 270–20,000 dis/min above background). Human serum albumin (HSA) and the equilibrium dialysis procedure used to estimate binding have been described previously [3].

Data for the binding of glipizide are plotted in Fig. 2. Binding constants were calculated as described previously (Table 1) [3]. The interaction process is independent of protein concentration and pH within the range studied. It is of interest to note that glipizide interacts with only two sites on the HSA molecule. The value of the association constant for the first site is relatively low compared to glibenclamide and tolbutamide [3], but the secondary association constant is unusually high. This fact combined with the low therapeutic plasma concentrations during normal usage [7] results in a large percentage binding. The binding parameters determined at 37° (Table 1) predict that 98·4 per cent glipizide is bound at a plasma level of 0·4 μg/ml, assuming

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Table 1. Binding data for the interaction of glipizide with HSA in 0·067 M phosphate buffer, pH 7·4

	First class of sites					Second class of sites				
Temp.	n_1	k_1 ($M^{-1} \times 10^{-4}$)	ΔG° (kcal/mole)	ΔH° (kcal/mole)	ΔS° (e.u.)	n_2	k_2 ($M^{-1} \times 10^{-4}$)	ΔG° (kcal/mole)	ΔH° (kcal/mole)	ΔS° (e.u.)
11·5°	0·98	18·89	−6·87	−3·66	11·28	1·06	4·08	−6·00	−3·11	10·16
25·0°	1·00	13·10	−6·99	−3·66	11·17	1·01	3·07	−6·12	−3·11	10·09
36·5°	0·99	11·38	−7·16	−3·66	11·31	1·03	2·62	−6·26	−3·11	10·16
46·0°	0·95	9·05	−7·24	−3·66	11·21	1·03	2·21	−6·34	−3·11	10·13

a serum albumin concentration of 3 per cent. This agrees well with the previous estimate of 98 per cent bound to pooled human serum [7].

Binding of glipizide to both sites increases with decreasing temperature. The thermodynamic parameters, calculated by standard methods, are given in Table 1. The substantial temperature dependence suggests that ionic forces are not involved in the interaction unlike the binding of sulphonamides [8, 9], tolbutamide and chlorpropamide [3]. The free energy of binding is the result of almost equal contributions by a negative enthalpy change and a positive entropy change. This is consistent with binding by van der Waals forces [10] and is supported by the pH independent binding (Fig. 2). At pH 6·4 where the drug is 76 per cent ionised (pKa = 5·9), binding is almost identical to that at pH 7·4 where 97 per cent is ionised. Thus, albumin possesses equal affinity for both non-ionised and ionised glipizide which further suggests that ionic forces are not involved.

The interaction of glipizide with HSA thus resembles that of glyburide rather than tolbutamide [3]. This is significant in that glyburide is only weakly displaced by drug anions such as phenylbutazone, warfarin and salicylate, whereas tolbutamide is very strongly displaced.* For this reason, it is possible that glipizide may be a suitable sulphonylurea for use in conjunction with anionic drugs to reduce displacement hazards.

Previously, it was shown that tromethamine buffer and chloride greatly reduce the binding to HSA of tolbutamide but not glyburide [4]. From Fig. 2 it can be seen that the interaction of glipizide with HSA is not reduced significantly by tromethamine. Furthermore, the presence of 0·154 M sodium chloride reduces glipizide association constants k_1 and k_2 by only $\frac{1}{2}$ and $\frac{1}{3}$, respectively, compared to a 30-fold reduction in k_1 for tolbutamide [4]. Therefore, in this respect also, binding of glipizide resembles that of glyburide rather than tolbutamide or sulphamethizole [9]. This suggests that, because of the non-ionic nature of the interaction, the choice of buffer for binding studies *in vitro* of glipizide is not as critical as for drugs bound solely as anions.

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Tissue uptake of δ-aminolaevulinic acid

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Acute intermittent porphyria is a genetically inherited disease in which there is an increased hepatic biosynthesis and excessive urinary excretion of prophyrins and their precursors δ-aminolaevulinic acid (ALA) and porphobilinogen. ALA is also present in abnormally high plasma concentrations. The normal plasma ALA level is usually taken to be zero; however, concentrations as high as 24 μg/ml have been reported in acute porphyric attack [1].

It is known that porphobilinogen and the porphyrins are pharmacologically inactive and it is, therefore, unlikely that they play any direct role in the development of the signs and symptoms of acute porphyria [2]. Recently however, there has been increasing evidence that ALA may be an aetiological factor in the production of the clinical manifestations of the disease.

In vitro studies have shown that ALA is capable of inhibiting red cell and brain ATPase, and membrane sodium transport [3, 4]. Such actions of ALA *in vivo* could affect tissue function. To exert such actions however, it would be necessary for ALA to penetrate into these tissues. It is already known that ALA can cross the blood-brain barrier and thereby enter the brain [5].

The present experiment was designed to see if ALA was taken up into tissues other than the brain and if so, how long it remained unchanged in certain tissues.

Since it was necessary to evaluate tissue ALA levels which had been in equilibrium with a blood ALA concentration similar to that found in acute porphyria, ALA was injected intraperitoneally in rats and the resulting blood concentrations were measured. Blood samples were removed from a cannulated carotid artery in the anaesthetized rat and the ALA content was estimated at times after an intra-