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STEREOSPECIFIC PLASMA BINDING OF GLIFLUMIDE, A NEW ANTIDIABETIC DRUG

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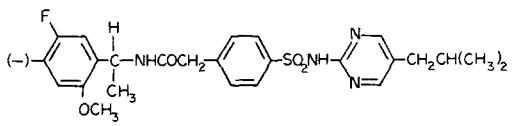
Abstract—Gliflumide is a new effective β-cytotropic agent with long-lasting activity. The compound is tightly bound to human plasma with a dissociation constant of 3.7×10^{-7} M. The optical enantiomer of gliflumide which is less potent in lowering blood glucose in rats has reduced affinity to plasma proteins indicating that binding is a stereospecific process. The comparison of dissociation constants of various antidiabetic drugs with the pattern of their biological activity suggests that the high affinity of gliflumide to plasma proteins may contribute to the delayed activity in animals and men.

Gliflumide ((S)-(−)-4-[*N*-(5-Isobutyl-2-pyrimidinyl)-sulfamoyl]-phenylacetic acid-1-(5-fluor-2-methoxy-phenyl)-ethyl amide), has been described as a new oral β-cytotropic antidiabetic drug of high potency and high efficacy [1]. The clinical properties are distinctly different of those of tolbutamide and glisoxepide. Oral or intravenous administration of the latter compounds results in an immediate stimulation of insulin secretion from the pancreas. The properties of gliflumide rather resemble those of the sulfonyl urea glibenclamide which is characterized by a slow onset and long-lasting release of insulin into the blood stream [2]. Until now, the mechanism of the protracted activity is not well understood. The purpose of this investigation was to determine whether protein binding might be one of the factors which contributes to the unusual activity pattern of gliflumide.



MATERIALS AND METHODS

Gliflumide and glisoxepide were kindly provided by Dr. C. Rufer, the radiolabeled compounds by Dr. P. E. Schulze, Schering AG, Berlin-Bergkamen. The specific activity of [^{3}H]gliflumide (generally labeled in the central phenyl ring) was 331 mCi/m-mole and 15.3 mCi/m-mole for [^{14}C]glisoxepide (labeled at C-2 of the hexamethylene-imine ring). Crystalline bovine serum albumin was obtained from Behring Werke, Marburg. Equilibrium dialysis was performed with an apparatus consisting of nine cells with a volume of one milliliter each, closely resembling the apparatus of Diachema AG, Switzerland. Cuprophan membranes were obtained from Technicon (Ireland) Ltd. The substances were dissolved in 0.05 M phosphate buffer with 10^{-3} M azide, pH 7.4, in concentrations as indicated in the figures and dialysed against diluted human plasma or bovine serum albumin for 24 hr at room temperature. Radioactivity was counted from aliquots of each cell in a liquid scintillation counter (Packard, Model 2450). Further experimental details and calculations are given elsewhere [3].

RESULTS

Using equilibrium dialysis both gliflumide and glisoxepide were bound to purified bovine serum albumin and diluted human plasma (Table 1). Under the experimental conditions gliflumide was almost exclusively bound to protein whereas binding of glisoxepide was less pronounced. This difference was particularly obvious on serial dilution of plasma (Fig. 1).

In order to determine binding parameters, radioactively labeled gliflumide was displaced from plasma with increasing concentrations of unlabeled compound (Fig. 2). Taking into account the changing specific activity, the data can be transformed into a saturation curve. From the concentration of free and bound ligand the dissociation constant and the binding capacity of plasma for gliflumide can be calculated according to the method of Scatchard [4]. The dissociation constant was determined to be 3.7×10^{-7} M. From the intercept with the abscissa one can estimate the binding capacity which was found to be 1 μmole of gliflumide per 1 ml of undiluted plasma (Fig. 3). The dissociation constant for glisoxepide determined with the same method was 1.5×10^{-5} M with a binding capacity of 2.5 μmoles/ml plasma.

In order to test the specificity of binding, displacement of labeled gliflumide by a structurally closely related compound was performed. Previous studies

Table 1. Binding of gliflumide and glisoxepide to bovine serum albumin and diluted human plasma after dialysis for 22 hr at room temperature*

	Percentage of bound ligand	
	Bovine serum albumin 10 mg protein/ml	Diluted human plasma 8.5 mg protein/ml
Gliflumide	92 ± 0.5	94 ± 0.2
Glisoxepide	44 ± 1.4	48 ± 0.9

* Concentration of ligand was 1.6×10^{-5} M ($\bar{x} \pm S_{\bar{x}}$; $n = 3$).

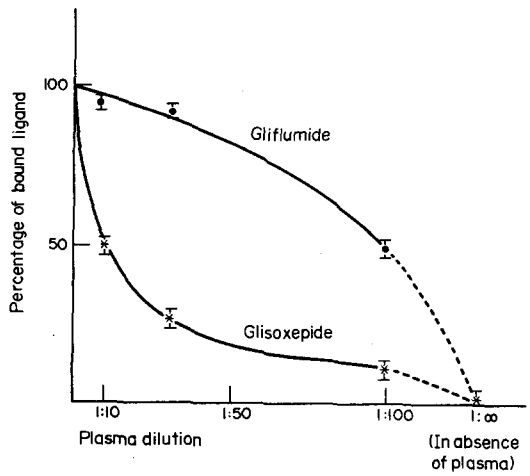


Fig. 1. Dependency of binding from plasma dilution. Dialysis was performed for 24 hr at room temperature. Concentration of ligand 1.6×10^{-5} M. The points represent the means of three independent experiments.

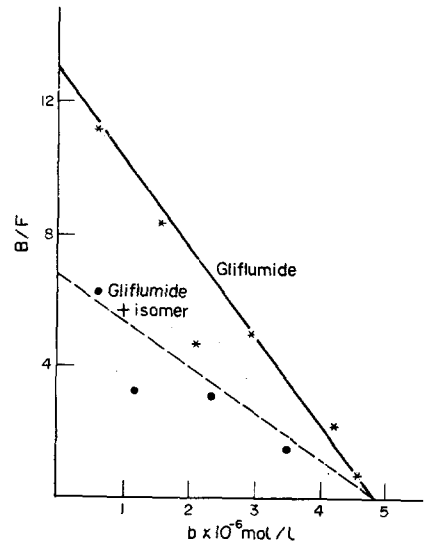


Fig. 3. Determination of binding parameters of gliflumide and its R-enantiomer according to Scatchard [4]. Plasma dilution 1:200. The concentration of gliflumide varied between 7.5×10^{-7} and 1.6×10^{-5} M. The concentration of the enantiomer in the assay was 4×10^{-6} M ($r = 0.94$ for gliflumide and 0.92 for the R-enantiomer; the points represent the means of two independent experiments).

have shown that the introduction of the asymmetric carbon atom into the molecule yields two optical isomers which differ in their relative potency by a factor of twenty as measured in the blood sugar lowering effect in rats [2], the more active compound being the S-isomer gliflumide. The biologically less active enantiomer (R-isomer) was added in a fixed concentration (4×10^{-6} M) to increasing amounts of gliflumide. From Scatchard plot analysis it is possible to calculate the dissociation constant for the R-enantiomer [5, 6] according to the equation

$$K_d = (K_s \cdot c_x / K'_s - K_s)'$$

where K_s = dissociation constant of gliflumide; K'_s = apparent dissociation constant of gliflumide in presence of 4×10^{-6} M R-enantiomer, and c_x = concentration of R-enantiomer in the assay (4×10^{-6} M). The dissociation constant of the R-enantiomer was found to be 4.4×10^{-6} M and hence is about one tenth of that of gliflumide (Fig. 3). The reduction of binding by a structurally closely related compound suggests that binding to plasma proteins is a highly specific process.

The nature of the binding protein for gliflumide in plasma remains still unclear. The number of binding sites determined according to the Scatchard equation was rather high and suggested that albumin is the main binding protein. This idea was further supported by the fact that bovine serum albumin exhibited substantial binding under the conditions applied in the present experiments (Table 1). In addition, pre-

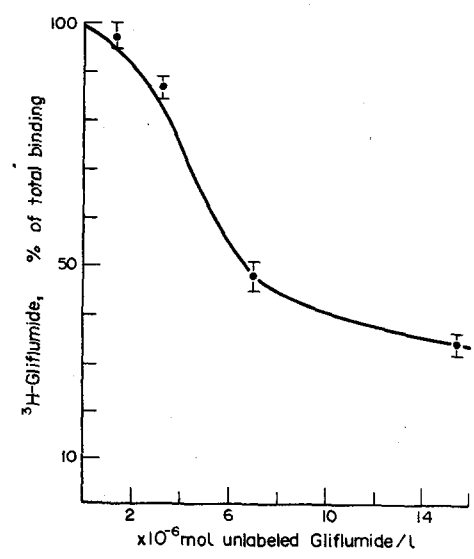


Fig. 2. Displacement of 7.5×10^{-7} M [^{3}H]gliflumide by increasing concentrations of unlabeled compound. Plasma dilution 1:200 ($\bar{x} \pm S_{\bar{x}}$; n = 3).

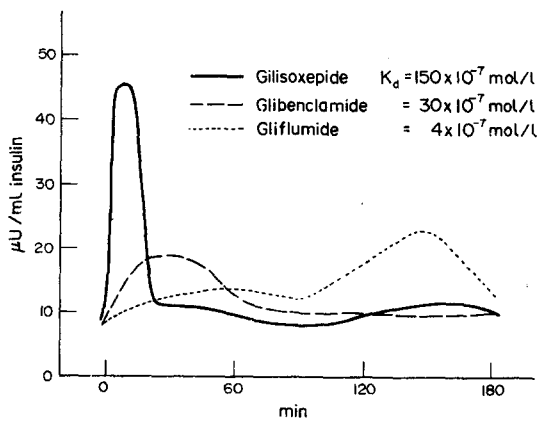


Fig. 4. Dissociation constants of antidiabetic drugs, and plasma insulin in healthy volunteers after i.v. injection (data adapted from [2, 6, 7, 11]).

liminary steady-state electrophoretic studies revealed that gliflumide is indeed attached to albumin.

DISCUSSION

Using equilibrium dialysis it could be demonstrated that gliflumide is strongly bound to human plasma proteins. With a dissociation constant of 3.7×10^{-7} M the affinity is about ten times higher than that described for glibenclamide with 3×10^{-6} M [6, 7]. Binding of glisoxepide (1.5×10^{-5} M) is in the range of tolbutamide which was found to be approximately 3×10^{-5} M [6–9]. The pharmacologically active plasma concentrations of both glisoxepide and gliflumide are in the order of 0.15 ng/ml [2]. The differences in plasma binding between glibenclamide and tolbutamide have been ascribed to the fact that glibenclamide is bound by a nonionic mechanism while binding of tolbutamide involves primarily ionic forces [10]. The finding that the R-enantiomer of gliflumide binds less strongly indicates that antidiabetic drugs are bound to plasma by a stereospecific process.

It is very unlikely that the dissociation constants of antidiabetic drugs to human serum proteins are correlated with the intrinsic activity of these compounds at the β-cell and thus with their blood sugar-lowering potency. This is most strikingly apparent when tolbutamide and glibenclamide are compared, which differ in their biological activity by a factor of 1000. However, it is conceivable that the dissociation of the β-cytotropic compound from plasma may be a rate-limiting step for the availability of the drug at the target cell. A high affinity to plasma proteins may therefore result in a slow and long-lasting biological effect as measured, e.g. in insulin release and blood glucose depression. This possibility is shown in Fig. 4 in which the dissociation constants for glisoxepide, glibenclamide and gliflumide were related to their maximal biological effect. Insulin release after i.v. injection of glisoxepide occurred already after 3 min and decreased to the initial level within 20–30 min. Insulin output after glibenclamide was delayed and lasted for about 60 min whereas maximal insulin release after gliflumide was only reached after 150 min. A similar pattern was observed for the blood glucose levels [2, 11].

The clinical value of compounds with a biological activity of such long duration has still to be carefully assessed. However, it is important to note that plasma binding may considerably contribute to the activity profile of a drug *in vivo*.

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