VOL. 42. NO. 2
NOVEMBER 1983

Research Communications in
Chemical Pathology and Pharmacology

STOICHIOMETRY OF CALCIUM BINDING BY HYPOGLYCEMIC SULFONYLUREAS

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Summary

In a two-phase bulk system for the study of Ca ionophoresis, certain hypoglycemic sulfonylureas, when added to the organic phase, were translocated into the aqueous medium. Nevertheless, the stoichiometry of Ca binding by these agents could be assessed by repeated mixing of the aqueous medium with organic phases. At saturation, the binding of Ca ranged from 0.5 to 1.0 mole/mole of sulfonylurea. A 1:1 molar ratio was observed with glibenclamide, and this was found to be compatible with the conformational analysis of the Ca-glibenclamide complex. There was a tight correlation between the stoichiometry of the Ca-sulfonylurea complex and the biological potency of each drug. This suggests that such a stoichiometry may be responsible, in part at least, for differences in the insulinotropic capacity of distinct hypoglycemic sulfonylureas.

Introduction

Distinct hypoglycemic sulfonylureas, when used at optimal concentration, increase to the same extent insulin

release from incubated pieces of rat pancreatic tissue (Malaisse, 1974). However, the concentration of sulfonylurea required to achieve a given secretory response is vastly different from one sulfonylurea to another. Thus, between the most and least potent agent, the ED_{50} for stimulation of insulin release spans a range of three orders of magnitude (Malaisse, 1974). We have recently identified a factor which may account, in part at least, for such differences in biological potency. Thus, the relative capacity of distinct hypoglycemic sulfonylureas to inhibit the specific binding of [^{3}H]glibenclamide or [^{3}H]gliquidone to multilamellar liposomes formed of egg yolk phosphatidylcholine was found to parallel their biological potency (Deleers and Malaisse, 1983). This situation, which is reminiscent of that found in intact islets (Sehlin, 1973; Hellman, 1974; Täljedal, 1974) led us to propose that the potency of distinct sulfonylureas, as insulinotropic agents, may depend on their ability to penetrate into the phospholipid domain of the cell membrane.

In the present work, we wish to propose that a second factor may account for the vastly different biological potencies of distinct hypoglycemic sulfonylureas. Our work is based on the view that the insulinotropic effect of these agents is somehow related to their ionophoretic capacity (Couturier and Malaisse, 1980a). Although the latter view was disputed by several investigators (Hellman, 1981; Henquin, 1982; Gylfe and Hellman, 1983), we have indicated elsewhere why we feel it premature to dismiss the ionopho-

retic hypothesis (Malaisse et al., 1983). The present work reveals that the stoichiometry of Ca binding by distinct hypoglycemic sulfonylureas correlates with their biological potency.

Materials and Methods

[^{3}H]glibenclamide (31 Ci/mole) and [^{3}H]gliquidone (5 Ci/mole) were kindly provided by H. Mertens (Hoechst, Belgium, Brussels, Belgium) and E. Rupprecht (Thomae, Biberach, FRG), respectively. Both sulfonylureas were tritiated in the cyclohexyl ring and their purity, as assessed by chromatography, greater than 96 %. Unlabelled sulfonylureas were all provided in the acid form, except gliquidone which was obtained as the Na salt.

To study Ca ionophoresis, an aqueous medium containing $^{45}CaCl_2$ was mixed for 1 min at room temperature with an organic phase formed of toluene and butanol (7/3, v/v) and containing a hypoglycemic sulfonylurea, as described in detail elsewhere (Malaisse et al., 1979). The precise composition of the aqueous medium is given in the text. In the absence of sulfonylurea, the translocation of Ca from an alkaline medium (0.2 ml; pH 10.5-11.0) containing $Ca(OH)_2$ 5 or 10 mM into the organic phase (0.6 ml) did not exceed 0.74 $\pm$ 0.09 nmol and 0.89 $\pm$ 0.16 nmol (n = 5 in each case), respectively. This represents, in terms of total Ca translocation (as illustrated in Fig. 3), less than 1 %. When the sulfonylurea-mediated Ca translocation was assessed by repeated

extractions, the reproducibility of the method, as judged from the difference between results collected in separate experiments, averaged 3.3 ± 1.6 % of the final result. The data illustrated in Figs. 2 and 3 are representative of two or more comparable experiments. In the experiments illustrated in Fig. 2, an exponential curve ($y = ae^{-bx}$) fitting the experimental data was calculated by aid of a desktop calculator, so that the integrated area under the curve (a/b) could be established.

The method used for the conformational analysis of the Ca-glibenclamide complex is based on a strategy described in detail elsewhere (Ralston and DeCoen, 1974; Ralston et al., 1974; Brasseur et al., 1981, 1982; 1983; Deleers et al. 1983b). The conformational energy is empirically calculated as the sum of the Van der Waals interaction, the torsional potential and the electrostatic interaction, the latter being calculated for a dielectric constant of 3 (Deleers et al., 1982). The ten torsional angles were defined in sequential order from the cyclohexyl ring to the 5-chloro-2-methoxy-phenyl group (see below Fig. 4, from right to left). The Ca^{2+} ion was fixed at 2.65 Å (Smith and Duax, 1976) of the ionized N atom located between the carbonyl and sulfonyl functions (between angles α3 and α4). The N atom located between angles α8 and α9, next to the other carbonyl function, was also ionized. In a first systemic study, the seven torsional angles α3 to α9, inclusive, underwent successive increments of 60° each, whereas the angles α1, α2

and o10 were fixed at 180°. Our of the 6^7 or 279,936 conformers derived from this first study, the two configurations with a probability of existence of more than 8 % were then submitted to a minimization procedure (Nelder and Mead, 1965) bearing on all ten torsional angles, in order to further reduce the internal energy. The probability of existence of conformers was calculated from the internal energy according to a Boltzmann distribution (Deleers et al., 1983b). Stereoscopic views of the Ca-glibenclamide complex were established with aid of the PLUTO program (Motherwell and Clegg, 1978).

Results

1. Partition of sulfonylureas between aqueous and organic phases

Hypoglycemic sulfonylureas are able to translocate Ca from an aqueous medium into an organic phase (Couturier and Malaisse, 1980b). In order to establish the stoichiometry of Ca binding in such a two-phase bulk system, it is necessary that the ionophoretic agent remains located in the organic phase and that its Ca-binding capacity is close to saturation. The latter constrain requires that the experiments are conducted with a high concentration of Ca^{2+} and low concentration of H^+ in the initial aqueous medium (Couturier and Malaisse, 1980b). Fig. 1 indicates that, under these conditions, the hypoglycemic sulfonylurea may pass from the organic phase into the aqueous medium.

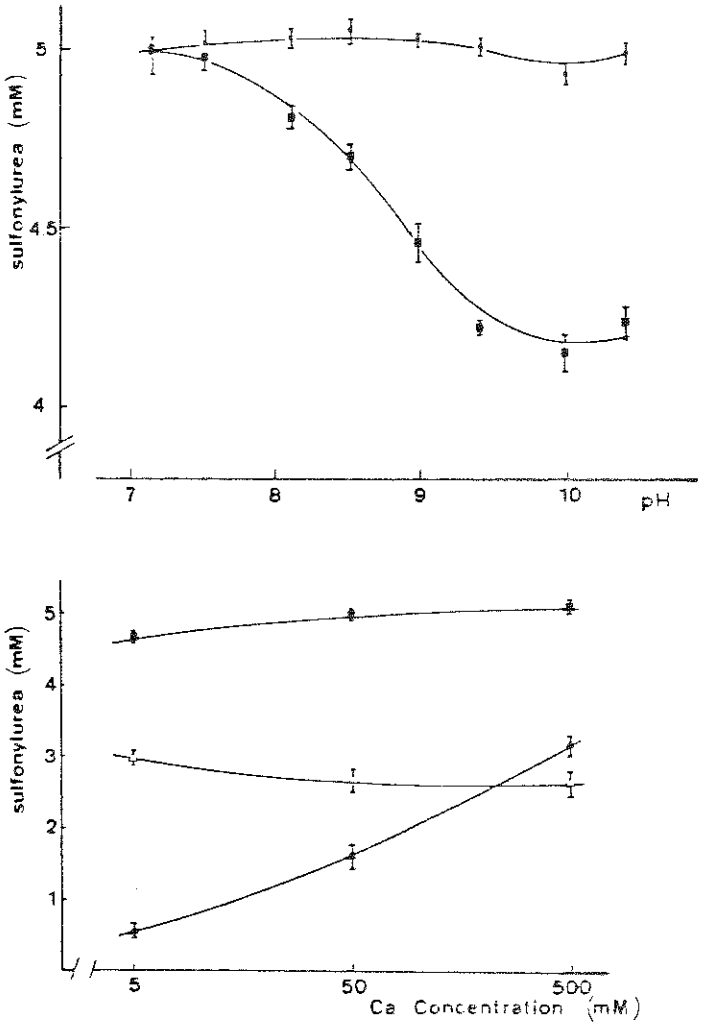

Fig. 1. Recovery of gliquidone (circles), glibenclamide (squares), gliclazide (open triangles) and glipizide (close triangles) in an organic phase after mixing with an aqueous medium containing either 5 mM $CaCl_2$ at variable pH (upper panel) or variable $CaCl_2$ concentrations at pH 10.5 (lower panel). The initial concentration of sulfonylurea in the organic phase amounted to 5 mM in all cases. The data were obtained by a radioisotopic (upper panel) or spectrophotometric procedure (lower panel). Mean values (± SEM) refer to 3-4 individual determinations.

In a first series of experiments, an aqueous medium (0.2 ml) consisting of a Tris-HCl buffer (50 mM; pH 7.1-10.4) and containing 5 mM $CaCl_2$ was mixed with an organic phase (0.2 ml) consisting of a mixture of toluene and butanol (7/3, v/v) and containing an unlabelled sulfonylurea (5 mM) together with a tracer amount of tritiated sulfonylurea. Under these conditions, virtually all the gliquidone remained located in the organic phase. However, about 16 % of the amount of glibenclamide initially added to the organic phase passed into the aqueous medium, as the initial pH of the latter medium was raised from 7.1 to 9.4 or more (Fig. 1, upper panel).

In a second series of experiments, the same organic phase (1.0 ml) containing an unlabelled sulfonylurea (5 mM) was mixed with an aqueous medium (1.0 ml) containing non-buffered Tris (20 mM, pH close to 10.5) and increasing concentrations of $CaCl_2$ (5 to 500 mM), the concentration of sulfonylurea in the final organic phase being monitored by spectrophotometry. In addition to glibenclamide, two other sulfonylureas (glipizide and gliclazide), which were not available in the radioactive form, were used in this second series of experiments. The spectrophotometric readings were performed at 305 nm (glibenclamide), 317 nm (glipizide) and 342 nm (gliclazide). Incidentally, tolbutamide, chlorpropamide and gliquidone could not be tested in this model, because they failed to display absorbance at the concentration and in the range of ultraviolet wave lengths under consideration. As shown in Fig. 1 (lower panel), a major frac-

tion of glipizide and gliclazide escaped from the initial organic phase. In the case of glipizide, the recovery of the drug in the organic phase increased when the initial $CaCl_2$ concentration of the aqueous medium was raised from 5 to 50 and 500 mM, as if the Ca-glipizide complex were to be more lipophilic than glipizide itself. The escape of glibenclamide from the organic phase was also somewhat more marked at low than at high Ca^{2+} concentration. This phenomenon was much less marked, however, in the case of glibenclamide than glipizide, the latter drug being therefore excluded from further experiments.

2. Ca translocation

In order to overcome the problems imposed by the escape of the sulfonylureas from the organic phase, the study of Ca translocation was performed by mixing a small volume (0.2 ml) of an aqueous medium containing only $Ca(OH)_2$ (2.5-15 mM; pH 10.5-11.0) and a tracer amount of $^{45}CaCl_2$ with a large volume (0.6 ml) of the usual organic phase containing a sulfonylurea (1.0 mM). After mixing, an aliquot (0.5 ml) of the organic phase was removed and replaced by an equal volume (0.5 ml) of the toluene/butanol mixture devoid of sulfonylurea. The aqueous medium and organic phases were again mixed together. This procedure was then repeated four times, so that most of the sulfonylurea initially added to the first organic phase would eventually be recovered during the 5 successive extractions. Each sample (0.5 ml) of the organic phase was examined for its radioactive

content. As illustrated in Fig. 2, the recovery of ^{45}Ca in the successive samples decreased in an exponential manner and, in most but not all cases, reached negligible values in the fifth sample. The total recovery of ^{45}Ca could be judged, therefore, either by summation of the readings collected in each sample or by integration of the logarithmic curve fitted on such readings. As shown in Fig. 3, the total amount of Ca translocated into the organic phases increased as the Ca^{2+} concentration of the initial aqueous medium was raised from 2.5 mM to higher values. Fairly stable recoveries were usually reached at the highest Ca^{2+} concentrations. These saturation values differed from one sulfonylurea to another, being close to 0.5-0.6 mole of Ca/mole of sulfonylurea in the case of chlorpropamide and tolbutamide and close to 1.0 mole of Ca/mole of sulfonylurea in the case of gliquidone and glibenclamide.

3. Relationship between stoichiometry, conformation and biological potency

The data illustrated in Fig. 3 indicate that, at saturation, the Ca-binding capacity of distinct sulfonylureas is characterized by distinct stoichiometries. The finding that certain sulfonylureas, such as gliquidone and glibenclamide, bind Ca^{2+} in a 1:1 molar ratio is compatible with the consideration that these agents display, in addition to a first ionization site common to all hypoglycemic sulfonylureas (Fig. 4), either a second ionization site or a potential binding site at the level of an electron pair not loca-

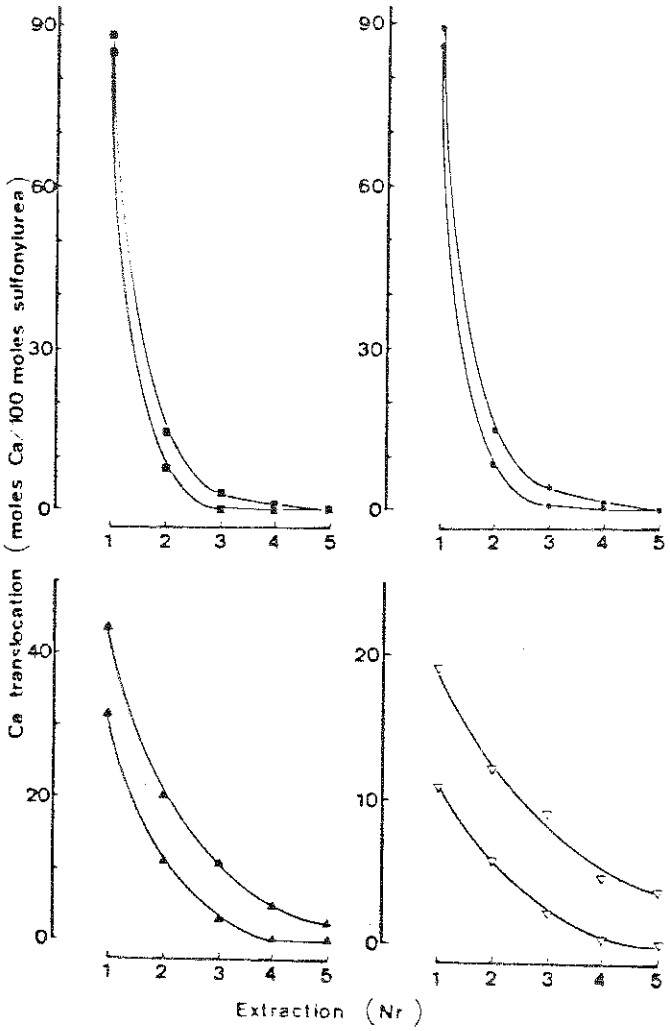

Fig. 2. Translocation of Ca after repeated mixing of an aqueous medium (0.2 ml; pH 10.5-11.0) initially containing either 5 mM (lower curves) or 10 mM (upper curves) $Ca(OH)_2$ and a tracer amount of $^{45}CaCl_2$ with an organic phase (0.6 ml) initially containing glibenclamide (upper left panel), gliquidone (upper right panel), gliclazide (lower left panel) or tolbutamide (lower right panel) at a 1.0 mM concentration. The amount of Ca recovered after each extraction is expressed relative to the initial concentration of sulfonylurea in the organic phase. Note the differences in scale of the ordinates axes.

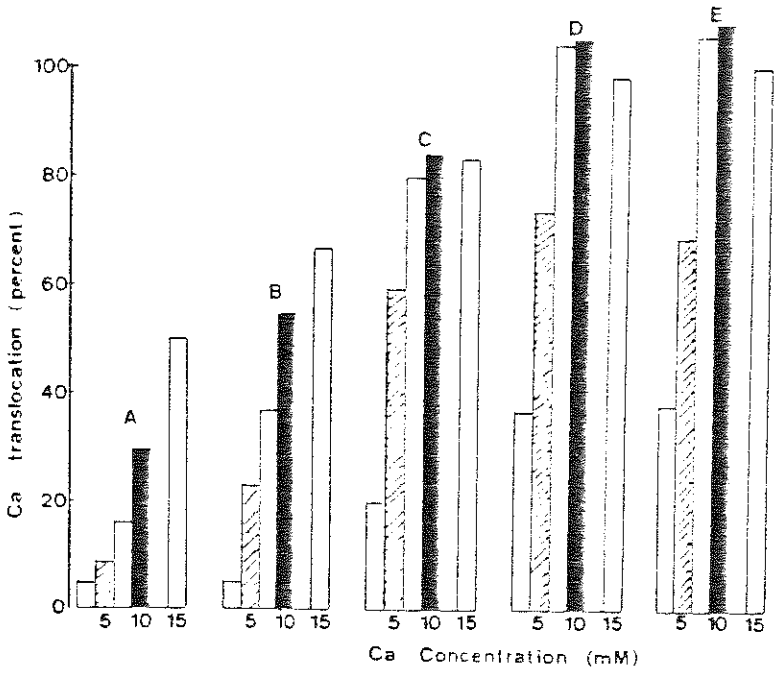

Fig. 3. Total translocation of Ca by chlorpropamide (A), tolbutamide (B), gliclazide (C), gliquidone (D) and glibenclamide (E) into an organic phase mixed with an aqueous medium containing $Ca(OH)_2$ at increasing concentrations (2.5 to 15 mM). The total translocation of Ca was calculated by exponential analysis of data such as those illustrated in Fig. 2, and expressed in percent of the Ca/sulfonylurea molar ratio.

ted in the vicinity of an ionization site and susceptible to provide a coordinative electron-donor link.

Conformation analysis of the Ca-glibenclamide complex provided two configurations with a probability of existence exceeding 8 % (Table 1). In these two conformers, the distance between the Ca^{2+} ion and the second ionization site in the glibenclamide molecule amounted to no more than 4.45

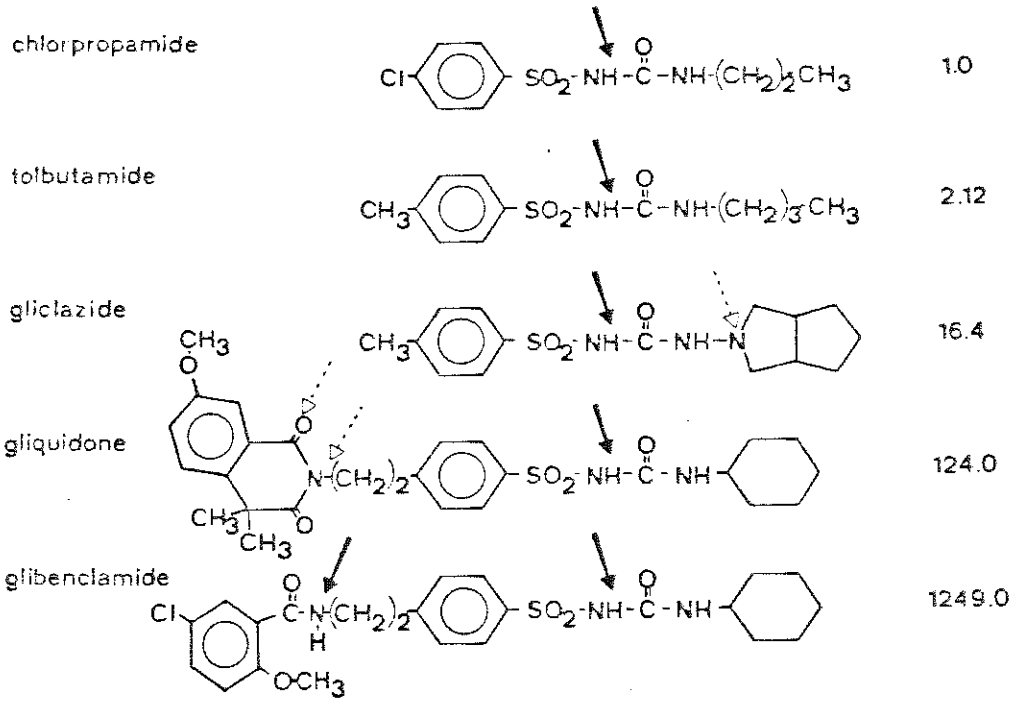

Fig. 4. Chemical structure and biological potency of five hypoglycemic sulfonylureas. The solid arrows indicate sites of ionization. The dotted arrows refer to potential binding sites, as judged from the presence of one or two electron pair(s) not located in the vicinity of an ionization site. The biological potency of the five drugs, in terms of their insulinotropic capacity, is expressed relative to that of chlorpropamide, according to data reported elsewhere (Deleers and Malaisse, 1983).

to 4.52 Å. The conformation analysis further indicated that the molecule of glibenclamide is indeed susceptible to affect a suitable configuration for the insertion of a Ca^{2+} ion in a cryptic hydrophilic cavity (Fig. 5). In this configuration, the external surface of the globular complex is hydrophobic. This arrangement would be quite favourable for

Table 1

Most probable conformers of the Ca-glibenclamide complex. The probability of existence and the values for torsional angles (°) are given for each conformer

Probability	64.5 %	16.1 %
Angle α1	153.1	155.2
α2	96.8	107.8
α3	77.3	33.8
α4	188.7	175.8
α5	236.0	273.8
α6	58.7	267.6
α7	13.2	0.6
α8	181.3	197.8
α9	133.8	21.7
α10	190.3	244.6

the transport of Ca^{2+} by glibenclamide across the phospholipid domain of cell membranes.

Fig. 6 illustrates the tight correlation ($r = 0.974$; $P < 0.01$) found between the Ca-binding capacity of five disting hypoglycemic sulfonylureas and their respective biological potency (logarithmic scale).

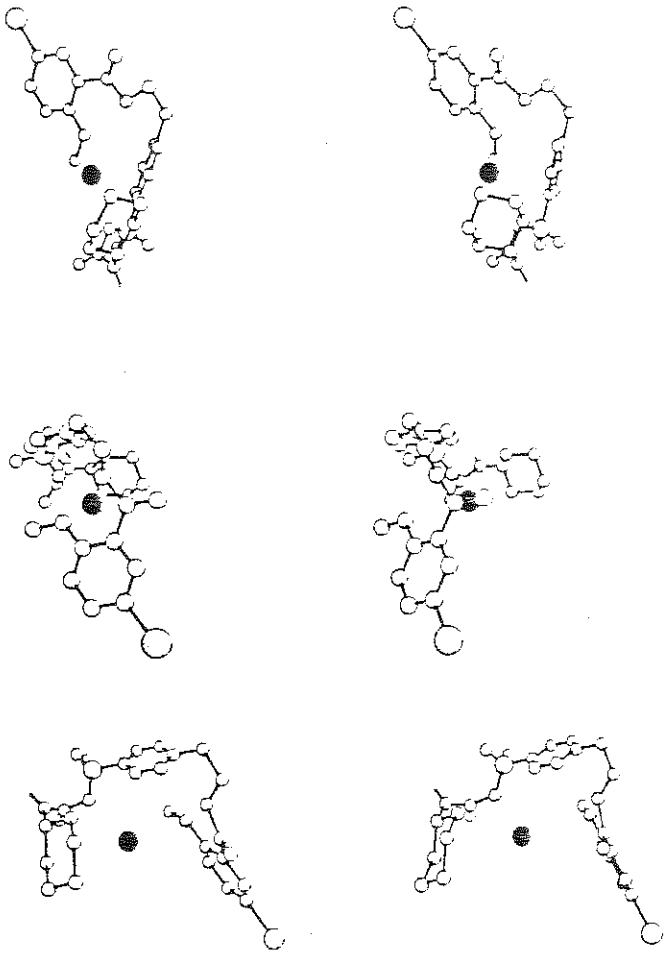

Fig. 5. Stereoscopic views of the most probable conformer for the Ca-glibenclamide complex. The views were taken, from top to bottom, along 3 perpendicular axes. The black circles refer to the Ca^{2+} ion.

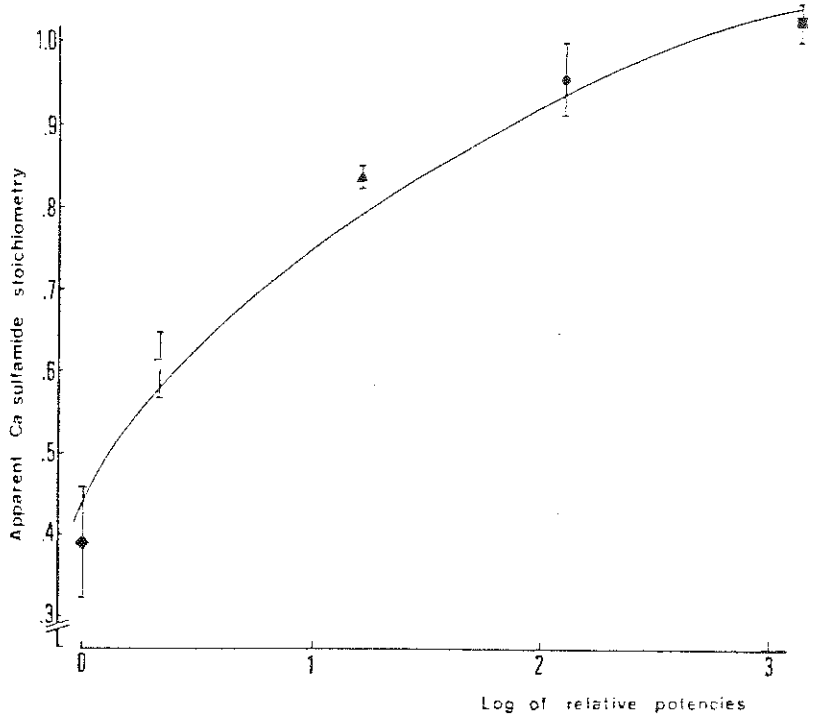

Fig. 6. Relationship between the apparent stoichiometry for Ca binding by distinct hypoglycemic sulfonylureas (diamond : chlorpropamide; open triangle : tolbutamide; closed triangle : gliclazide; circle : gliquidone; square : glibenclamide) and their biological potency (logarithmic scale; see Fig. 4). Mean values (± SEM) for binding capacity refer to the measurements performed at high Ca^{2+} concentrations (10 and 15 mM; see Fig. 3) and were calculated by both summation of the primary data (see Fig. 2) and their exponential extrapolation.

Discussion

The present study illustrates some of the difficulties encountered in investigating the stoichiometry of Ca binding by ionophores in the two-phase bulk system. In previous studies, we had proposed that each Ca^{2+} ion is bound by two

molecules of sulfonylurea. This view was based on the following observations. First, at non-saturating Ca^{2+} concentrations, the translocation of Ca represents a power function of the concentration of such sulfonylureas as tolbutamide and gliclazide (Couturier and Malaisse, 1980b). This behaviour is similar to that found with the ionophores A23187 or X537A, which indeed form a Ca-ionophore complex with a 1:2 stoichiometry (Malaisse et al., 1979; Couturier and Malaisse, 1980c). Second, the process of sulfonylurea-mediated Ca translocation is characterized by a 2:1 molar ratio for the competition between H^+ and Ca^{2+}, respectively (Deleers et al., 1981). Last, the conformational analysis of a Ca-gliclazide complex with a 1:2 stoichiometry provides results in agreement with those derived from the study of the Ca-gliclazide complex by nuclear magnetic resonance (Deleers et al., 1982).

The present work reveals that certains hypoglycemic sulfonylureas (e.g. gliquidone and glibenclamide) may bind Ca in a 1:1 molar ratio. This finding was found compatible with the chemical structure of these sulfonylureas. Moreover, the conformational analysis of the Ca-glibenclamide complex with such a 1:1 stoichiometry provided a configuration well suited for the transport of Ca^{2+} across the hydrophobic domain.

The existence of two different stoichiometries for Ca-sulfonylurea interaction (1:1 and 1:2 molar ratio) could account for a number of prior observations. First, it implies

that the finding of a 2:1 molar ratio for H^+:Ca^{2+} competition in the process of sulfonylurea-mediated Ca translocation is not sufficient to postulate a 1:2 molar ratio for the Ca-sulfonylurea complex. Second, our data do not exclude that certain sulfonylureas may form two types of complexes with, respectively, a 1:2 and 1:1 molar ratio for Ca-binding ty the drug. A mixed population of Ca-sulfonylurea complexes could account for the existence of intermediate values, between the extremes of 0.5 and 1.0, for the Ca/sulfonylurea molar ratio (Fig. 3). Third, the stoichiometry of the Ca-sulfonylurea complex may affect the pattern of the dose-action relationship for Ca translocation or transport at increasing concentrations of the drug. Such a relationship would be expected to be ruled by a law of proportionality when the Ca/sulfonylurea molar ratio is close to unity. However, when the Ca/sulfonylurea ratio is close to 0.5, the translocation of Ca at non-saturating Ca^{2+} concentrations would be expected to represent a power function of the sulfonylurea concentration (Malaisse et al., 1979). The two types of relationship were indeed observed in a recent study of praseodymium transport by hypoglycemic sulfonylureas across liposomal membranes (Deleers et al., 1983a). Last, the existence of a second ionized site in the glibenclamide molecule could conceivably account for the fact that the derivative of glibenclamide HB699, or 4-[2-(5-chloro-2-methoxy-benzamido)-ethyl]-benzoic acid, which is devoid of the sulfonylurea group, is nevertheless able to

stimulate Ca uptake and insulin release in isolated rat islets (Füssganger and Wojcikowski, 1977; Puech et al., 1983).

It could be argued that our ionophoretic data were obtained at high Ca^{2+} concentrations and high pH and, hence, may not be relevant to the situation found in the immediate vicinity of cell membranes. However, the data illustrated in Fig. 3 indicate that the differences in Ca-binding capacity of distinct sulfonylureas were even more marked, in relative terms, at low (2.5 mM) than high (10-15 mM) Ca^{2+} concentrations. It is conceivable, therefore, that differences in Ca binding by distinct agents also occur under physiological conditions.

It is well established that the efficiency of ionophore-mediated Ca transport across artificial membranes depends, inter alia, on both the concentration of ionophore in the phospholipid bilayer (Deleers and Malaisse, 1980) and the stoichiometry of the Ca-ionophore complex (Deleers et al., 1981; Deleers and Malaisse, 1982). When each Ca^{2+} ion is bound to two molecules of ionophore, the formation of the complex is affected by the lateral mobility of the ionophore in the plane of the bilayer, whereas such is not the case for complexes characterized by a 1:1 stoichiometry. Therefore, the present findings, taken in conjunction with our recent data on the binding of sulfonylureas to artificial bilayers (Deleers and Malaisse, 1983), point to both the insertion of these drugs into the phospholipid domain of the B-cell membrane and the stoichiometry for Ca binding as two

possible determinants of the relative insulinotropic potency of distinct hypoglycemic sulfonylureas.

Acknowledgements

This work was supported by a grant from the Belgian Ministry of Scientific Policy. We thank M. Mahy for technical assistance and C. Demesmaeker for secretarial help.

References

Brasseur, R., Goormaghtigh, E., and Ruysschaert, J.M. (1981). Theoretical conformational analysis of phospholipid bilayers. *Biochem. biophys. Res. Commun.* 103, 301.

Brasseur, R., Deleers, M., Malaisse, W.J., and Ruysschaert, J.M. (1982). Conformational analysis of the calcium-A23187 complex at a lipid-water interface. *Proc. Natl. Acad. Sci. USA* 79, 2895.

Brasseur, R., Deleers, M., and Malaisse, W.J. (1983). Conformational analysis of the calcium transport antagonist gallopamil. *Biochem. Pharmacol.* 32, 437.

Couturier, E., and Malaisse, W.J. (1980a). Insulinotropic effects of hypoglycemic and hyperglycemic sulphonamides : the ionophoretic hypothesis. *Diabetologia* 19, 335.

Couturier, E., and Malaisse, W.J. (1980b). Ionophoretic activity of hypoglycemic sulfonylureas. *Arch. Int. Pharmacodyn. Therap.* 245, 323.

Couturier, E., and Malaisse, W.J. (1980c). Ionophore-mediated cation translocation in artificial systems. II. X537A-mediated calcium and sodium translocation. *Biochimie* 62, 177.

Deleers, M., and Malaisse, W.J. (1980). Ionophore-mediated calcium exchange diffusion in liposomes. *Biochem. Biophys. Res. Commun.* 95, 650.

Deleers, M., Couturier, E., Mahy, M., Gelbcke, M., and Malaisse, W.J. (1981a). Ionophoretic property of hypoglycemic sulfonylureas. Competition between H^+ and Ca^{2+}. *Ann. Endocrinol.* 42, 297.

Deleers, M., Couturier, E., and Malaisse, W.J. (1981b). Ionomycin mediated calcium transport in rigid and fluid liposomes. *Cell Calcium* 2, 159.

Deleers, M., Brasseur, R., Gelbcke, M., and Malaisse, W.J. (1982). Homologous and hybrid calcium complexes of A23187 and hypoglycemic sulfonylureas : nuclear magnetic resonance and conformational analysis. *J. Inorg. Biochem.* 16, 215.

Deleers, M., and Malaisse, W.J. (1982). Influence of membrane viscosity on the lateral and transverse mobility of carboxylic ionophores. *Chem. Phys. Lipids* 31, 227.

Deleers, M., and Malaisse, W.J. (1983). Binding of hypoglycaemic sulphonylureas to an artificial phospholipid bilayer. *Diabetologia* in press.

Deleers, M., Gelbcke, M., and Malaisse, W.J. (1983a). Transport of Pr^{3+} by hypoglycemic sulfonylureas across liposomal membranes. *FEBS Letters* 151, 269.

Deleers, M., Brasseur, R., Ruysschaert, J.M., and Malaisse, W.J. (1983b). Conformational analysis of phorbol esters at a simulated membrane/water interface. *Biophys. Chem.* 17, 313.

Fussgänger, R.D., and Wojcikowski, C. (1977). Stimulation of insulin secretion of the isolated perfused rat pancreas by a new hypoglycemic agent, an acyl-amino-alcyl benzoic acid derivative. *Diabetologia* 13, 394.

Gylfe, E., and Hellman, B. (1983). Lack of Ca^{2+} ionophoretic activity of hypoglycemic sulphonylureas in excitable cells and isolated secretory granules. *Molec. Pharmacol.* 22, 715.

Hellman, B. (1974). Factors affecting the uptake of glibenclamide in microdissected pancreatic islets rich in ß-cells. *Pharmacology* 11, 257.

Hellman, B. (1981). Tolbutamide-stimulation of $^{45}Ca^{2+}$ fluxes in microdissected pancreatic islets rich in B-cells. *Molec. Pharmacol.* 20, 83.

Henquin, J.C., and Meissner, H.P. (1982). Opposite effects of tolbutamide and diazoxide on ^{86}Rb fluxes and membrane potentials in pancreatic B-cells. *Biochem. Pharmacol.* 31, 1407.

Malaisse, W.J. (1974). Insulinotropic action of different sulfonylureas in vitro : a comparative study. In B. Glasson and A. Benakis (eds.). *Cyclic AMP, New anti-diabetic drugs, Parasitosis Chemotherapy.* Médecine et Hygiène, Genève. Pp. 67-79.

Malaisse, W.J., Valverde, I., Devis, G., Somers, G., and Couturier, E. (1979). Ionophore-mediated cation translocation in artificial systems. I. A23187-mediated calcium translocation. *Biochimie* 61, 1185.

Malaisse, W.J., Hubinont, C., Lebrun, P., Herchuelz, A., Couturier, E., Deleers, M., Malaisse-Lagae, F., and Sener, A. (1983). Mode of action of hypoglycaemic sulfonylureas in the pancreatic β-cell : coinciding and conflicting views. In M. Serrano Rios and L.P. Krall (eds.). *Clinical and Pharmacological Activities of Sulfonylurea Drugs*. Excerpta Medica, Amsterdam. Pp. 24-39.

Motherwell, S., and Clegg, W. (1978). PLUTO program. University of Cambridge, England.

Nelder, J.A., and Mead, R. (1965). A simplex method for function minimization. *Computer J.* 7, 308.

Puech, R., Manteghetti, M., Ribes, G., Wollheim, C.B., and Loubatières-Mariani, M.-M. (1983). Enhancement of insulin release and islet cell calcium content by an acyl-aminoacyl benzoic acid derivative, HB699. *Horm. Metab. Res.* in press.

Ralston, E., and DeCoen, J.L. (1974). Folding of polypeptide chains induced by the amino-acid side chains. *J. Mol. Biol.* 83, 393.

Ralston, E., DeCoen, J.L., and Walter, R. (1974). Tertiary structure of H-Pro-Leu-Gly-NH_2, the factor that inhibits release of melanocyte stimulating hormone, derived by conformational energy calculations. *Proc. Natl. Acad. Sci. USA* 71, 1142.

Sehlin, J. (1973). Evidence for specific binding of tolbutamide to the plasma membrane of the pancreatic B-cells. *Acta diabetol. lat.* 5, 1052.

Smith, G.D., and Duax, W.L. (1976). Crystal and molecular structure of the calcium ion complex of A23187. *J. Am. Chem. Soc.* 98, 1578.

Täljedal, I.B. (1974). Uptake of glibornuride by microdissected pancreatic islets. *Horm. Res.* 5, 211.