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SYNERGISTIC EFFECT OF HYPOGLYCEMIC SULFONYLUREAS AND NEGATIVE PHOSPHOLIPIDS ON CALCIUM TRANSPORT : IONIC AND CONFORMATIONAL ASPECTS

M. Deleers[+], P. Grognet[+], M. Mahy[+], R. Brasseur[‡] and W.J. Malaisse[+]

[+]Laboratory of Experimental Medicine and [‡] Laboratory of Macromolecules at Interfaces, Brussels University, Brussels, Belgium

SUMMARY

In a two-phase bulk system for the study of ionophoresis, the capacity of hypoglycemic sulfonylureas to translocate Ca^{2+} was enhanced in a synergistic manner by negatively charged phospholipids. High concentrations of Na^+ or K^+ had relatively little effect on sulfonylurea-mediated Ca^{2+} translocation. The acidity constant of hypoglycemic sulfonylureas ranged from 10^{-5} to 10^{-6}. The conformation analysis of Ca^{2+}-gliquidone complexes with a 1:1 or 1:2 stoichiometry and of a hybrid complex between Ca^{2+} and both gliquidone and phosphatidylserine revealed configurations suitable for Ca^{2+} transport across a hydrophobic domain. These findings raise the possibility that the cationic response of the pancreatic B-cell to hypoglycemic sulfonylureas may be due primarily to an alteration of both Ca^{2+} and H^+ transport.

INTRODUCTION

Hypoglycemic sulfonamides or sulfonylureas stimulate insulin release apparently by facilitating Ca^{2+} inflow and, hence,

by causing Ca^{2+} accumulation in the pancreatic B-cell (Malaisse et al., 1972; Lebrun et al., 1982). This may be due to a decrease in K^+ conductance leading to plasma membrane depolarization and the subsequent gating of voltage-sensitive Ca^{2+} channels (Meissner et al., 1980; Henquin, 1980; Henquin & Meissner, 1982). The molecular mechanism of these ionic changes is not known (Malaisse et al., 1983). Over recent years, consideration was given to the binding of the hypoglycemic drugs to specific membrane receptors (Kaubisch et al., 1982), their insertion in the phospholipid domain of membranes (Deleers & Malaisse, 1983), their action as permeant anion (Tarvin et al., 1981) and their ionophoretic capacity (Couturier & Malaisse, 1980a). The latter property was extensively investigated in artificial model membranes (Couturier & Malaisse, 1980b; Anjaneyulu et al., 1980; Deleers et al., 1980, 1981a, 1982, 1983a), but its relevance to the insulinotropic action of the hypoglycemic agents questioned by some other investigators (Gylfe & Hellman, 1983). The present report provides novel information on both the interaction between hypoglycemic sulfonylureas and distinct cations and the ionophoretic synergism between these agents and acid phospholipids.

MATERIALS AND METHODS

The ionophoretic translocation of Na^+, K^+ and Ca^{2+}, as judged from the fate of $^{22}Na^+$, $^{86}Rb^+$ and $^{45}Ca^{2+}$, respectively, was investigated in a two-phase bulk system, as described in detail elsewhere (Malaisse et al., 1979). The precise composition of both the initial organic phase, which contained a hypoglycemic

sulfonylurea and/or a phospholipid, and initial aqueous phase, which contained the radioactive cation, is given in the text.

The acidity constant (Ka) of hypoglycemic sulfonylureas was estimated from the measured pH of solutions of these drugs, at concentrations (C) ranging from 10 to 200 μM, in bidistilled water, according to the equation :

$$Ka = [H^+]^2 / \{[C] - [H^+]\} \quad (i)$$

The Ka values given in the text represent the mean of 4-6 determinations, each of which was made in duplicate at increasing concentrations of each drug.

The conformation analysis of Ca-gliquidone complexes is based on a strategy described elsewhere (Brasseur et al., 1981, 1982, 1983; Deleers et al., 1982, 1983b). Briefly, the total internal energy (i.e. the sum of Van der Waals interaction, torsional potential and electrostatic interaction) was calculated for each of the 6^6 conformations obtained when the six torsional angles (α_2, α_3, α_4, α_5, α_6 and α_8) in the Ca-gliquidone complex with a 1:1 stoichiometry (Fig. 1) were increased by 6 stepwise increments of 60° each. For the Ca-gliquidone complex with a 1:2 stoichiometry, the total internal energy was calculated in two successive systematic analysis, the first bearing on angles α_1, α_2, α'_2, α_8 and α'_8 and leading to the selection of a configuration with a 66.2 % probability and the second bearing on angles α_3, α'_3, α_4, α'_4, α_6 and α'_6 of the selected conformer. The hybrid gliquidone-Ca-phosphatidylserine complex was calculated using previous results of a systematic conformational analysis of diplamitoylphosphatidylserine (R. Brasseur, unpublished data), so that only one systematic analysis was performed.

Table 1

Slope (μM of Ca^{2+}/mM of sulfonylurea) of the regression lines characterizing the increment in Ca^{2+} translocation at increasing concentrations (up to 6.4 mM or more) of hypoglycemic sulfonylureas (HS) in the absence or presence of a fixed concentration (50 μg/ml) of phosphatidic acid (PA) or phosphatidylserine (PS)

	HS alone	HS + PA	HS + PS
Tolbutamide	0.019	0.067	0.206
Glipizide	0.022	0.069	0.039
Glibenclamide	0.061	0.203	0.179
Gliclazide	0.086	0.328	0.394
Gliquidone	0.564	3.976	1.148

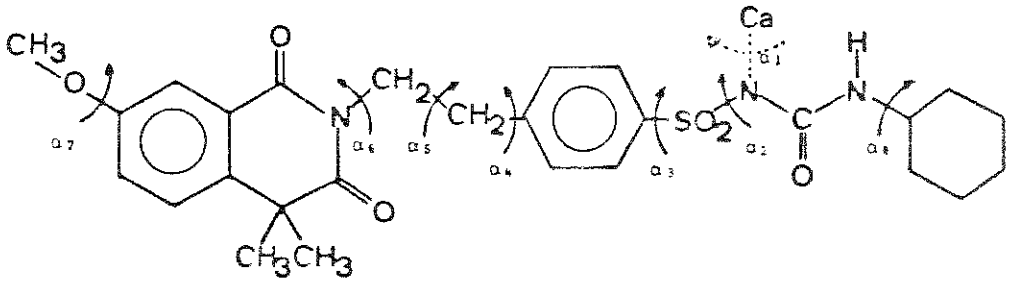

Fig. 1. Molecular structure of gliquidone with indication of the torsional angles. This view refers to part of a Ca^{2+}-gliquidone complex with a 1:2 stoichiometry.

on angles α_1, α_2 and α_3 in the gliquidone molecule and the first three angles of serine (-Ca-O-CO-,-CO-CH-, and -CH-CH_2-). In all cases, the Ca^{2+} atom was first fixed at 2.30 Å of a first ionized site in the gliquidone molecule, at the level of the N atom in the -SO_2-NH-CO- sequence (Brasseur et al., 1981, 1982, 1983; Deleers et al., 1982, 1983b). The most probable conformer selected for each type of complex was then submitted to a further analysis using a simplex minimization procedure (Nelder & Mead, 1965). The latter analysis involved all torsional angles. The two Ca-gliquidone complexes with a 1:1 and 1:2 stoichiometry, respectively, were calculated in a medium with a dielectric constant of 3, whereas the hybrid complex was oriented at a simulated lipid-water interface, using dielectric constants of 3 and 30 (Brasseur et al., 1982; Deleers et al., 1983b).

RESULTS

1. Synergism with phospholipids

Hypoglycemic sulfonylureas and negatively charged phospholipids, when located in an organic phase, were able to translocate Ca^{2+} from an aqueous medium (0.2 ml) consisting of a Tris-HCl buffer (20 mM, pH 7.4) and containing 120 mM NaCl, 0.2 mM Ca^{2+} and a tracer amount of $^{45}Ca^{2+}$ (37 KBq/ml) into the organic phase (0.2 ml), which consisted of a mixture of toluene-butanol (7:3, v:v). At a fixed concentration of acid phospholipid (50 µg/ml), the increment in Ca translocation due to the hypoglycemic sulfonylureas (1.6 to 9.6 mM) was more marked than in the absence of phospholipid (Figs. 2 and 3). Table 1 indicates that the

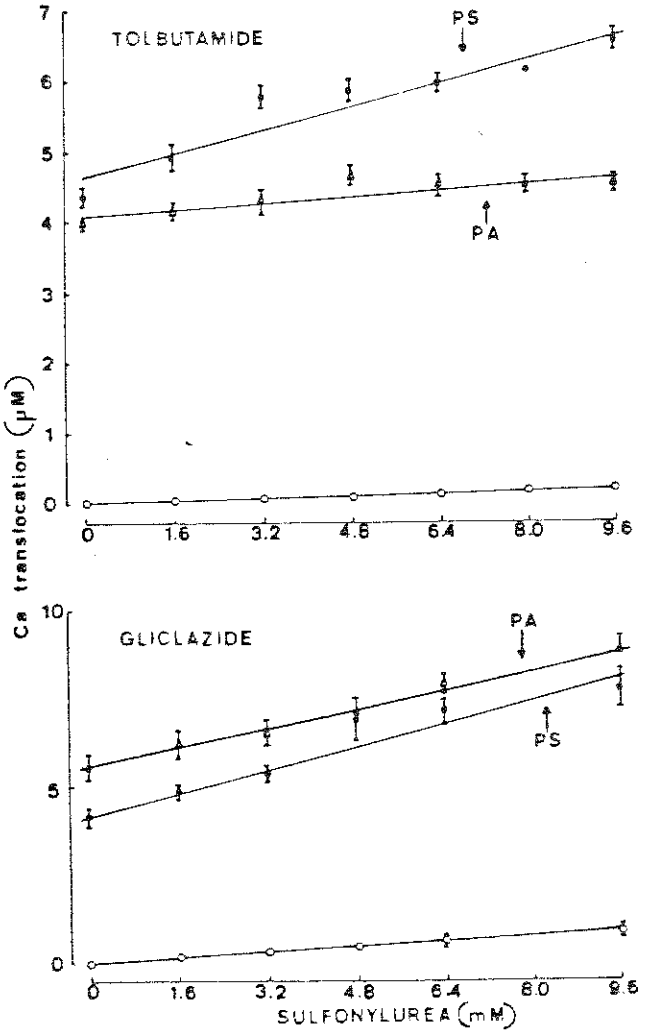

Fig. 2. Effect of increasing concentrations of tolbutamide (upper panel) or gliclazide (lower panel) upon Ca^{2+} translocation in the absence (open circles) or presence of phosphatidic acid (open triangles) or phosphatidylserine (closed circles). Mean values (± SEM) refer to 7-15 individual observations.

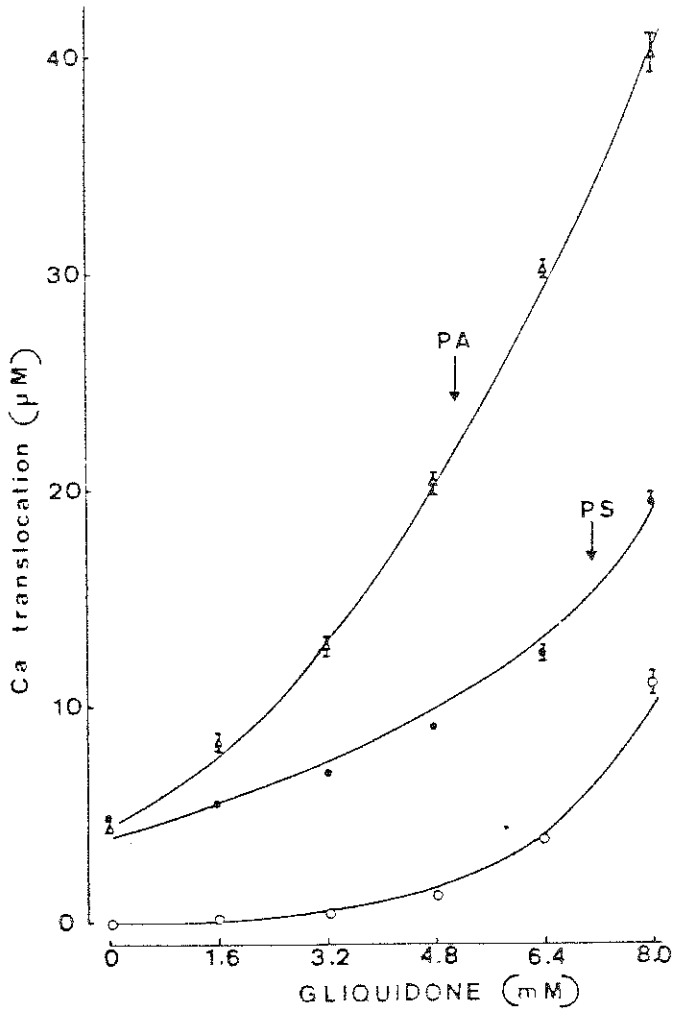

Fig. 3. Effect of increasing concentrations of gliquidone upon Ca^{2+} translocation in the absence (open circles) or presence of phosphatidic acid (open triangles) or phosphatidylserine (closed circles). Mean values (± SEM, if in excess of 0.2 μM) refer to 8 individual observations.

slope of the regression lines characterizing the translocation of Ca^{2+} at increasing concentrations of sulfonylureas was much higher in the presence than in the absence of phospholipid. Experiments performed with cardiolipin also revealed a comparable synergistic behaviour (data not shown).

2. Interference of monovalent cations

When the aqueous phase consisted of a Tris-HCl buffer (50 mM, pH 8.0) and contained $CaCl_2$ (5 mM), a tracer amount of $^{45}Ca^{2+}$ (74 KBq/ml) and variable amounts of NaCl or KCl, the translocation of Ca^{2+} mediated by distinct hypoglycemic sulfonylureas (5 mM) was inhibited, to a modest extent, at high concentrations of Na^+ or K^+ (Fig. 4). As a rule, the sulfonylurea-mediated translocation of Na^+ and K^+ was also modest at physiological pH, but increased at higher pH values (Fig. 5). In these experiments, the aqueous phase consisted of a Tris-HCl buffer (50 mM, variable pH) containing 100 mM NaCl or KCl and tracer amounts of $^{22}Na^+$ (296 KBq/ml) or $^{86}Rb^+$ (159 KBq/ml), whereas the organic phase contained the hypoglycemic sulfonylureas at 5.0 mM concentration.

The acidity constant (Ka) of sulfonylureas, as measured at concentrations ranging from 10 to 200 μM, averaged $10^{-5.0}$ for chlorpropamide, $10^{-5.3}$ for glibenclamide, $10^{-5.4}$ for glipizide, $10^{-5.5}$ for tolbutamide, $10^{-5.7}$ for gliclazide and $10^{-6.0}$ for glisoxepid. Thus, in all cases, the Ka was about 10^{10} to 10^{11} higher than that usually ascribed (Roberts & Caserio, 1967) to amides ($Ka \cong 10^{-16}$).

3. Conformation analysis

Ca-gliquidone complexes may apparently display a 1:1 or 1:2

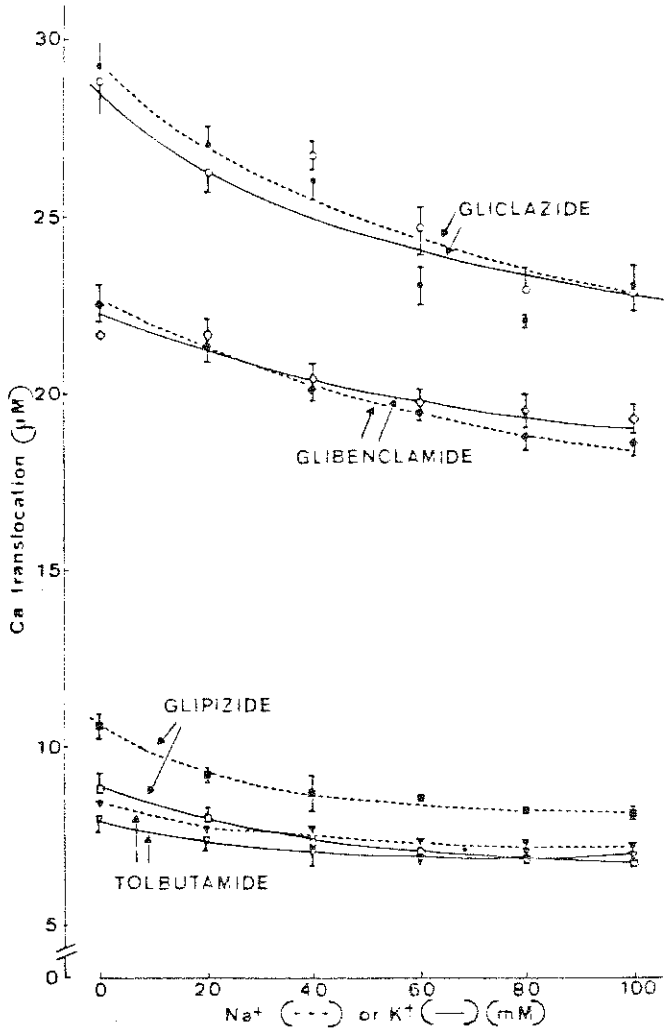

Fig. 4. Effect of increasing concentrations of NaCl or KCl upon Ca^{2+} translocation evoked by gliclazide (circles), glibenclamide (diamonds), glipizide (squares) or tolbutamide (triangles). Mean values (± SEM, if in excess of 0.1 μM) refer to 4 individual observations.

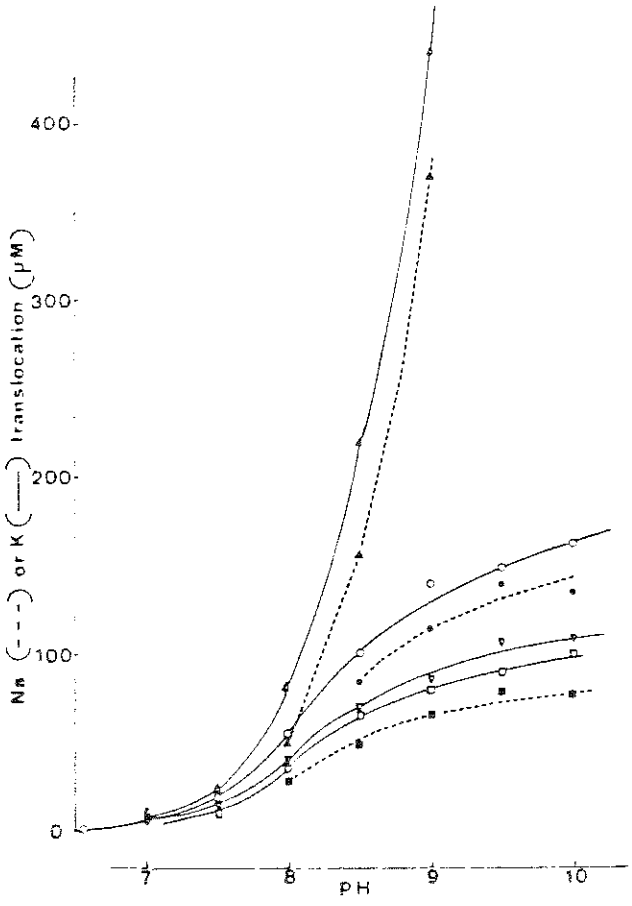

Fig. 5. Influence of pH upon the translocation of Na^+ (closed symbols, dotted line) or K^+ (open symbols, solid line) as mediated by glibenclamide (triangles pointing upward), gliclazide (circles), tolbutamide (triangles pointing downward) or glipizide (squares). Mean values refer to 4 individual determinations, the SEM usually not exceeding the size of the mean point.

stoichiometry (Deleers et al., 1983c). Moreover, the results illustrated in Fig. 3 suggest the existence of hybrid complexes between Ca^{2+}, gliquidone and phosphatidylserine. We conducted, therefore, a conformation analysis of these three types of complexes. In the most probable conformer (21 % of probability) of the 1:1 complex (Fig. 6), a distance of 4.67 Å separated the Ca^{2+} ion from the second binding site of gliquidone, namely the O atom situated on the isoquinolyl cycle in para of the dimethyl group. This conformer, like the most probable conformer (19.4%) of the 1:2 complex (Fig. 7), were characterized by a hydrophobic external surface. In the most probable conformer (25.2 %) of the hybrid phosphatidylserine-Ca^{2+}-gliquidone complex (Fig. 8), one of the two palmitoyl chains interacted with the gliquidone molecule, while the other hydrophobic chain remained perpendicular to the interface.

DISCUSSION

The present findings afford further information on the ionophoretic capacity of hypoglycemic sulfonylureas. First, they indicate, by comparison with previous findings (Couturier & Malaisse, 1980b), that the inhibitory effect of Na^+ and K^+ upon sulfonylurea-mediated Ca^{2+} translocation is tightly dependent on the Ca^{2+} concentration of the aqueous phase, the relative magnitude of such an inhibitory effect being inversely related to the Ca^{2+} concentration.

Second, the present data indicate that the acidity constant of hypoglycemic sulfonylureas is in the 10^{-5} to 10^{-6} range. Since protonophores may be more efficient in transporting H^+ at

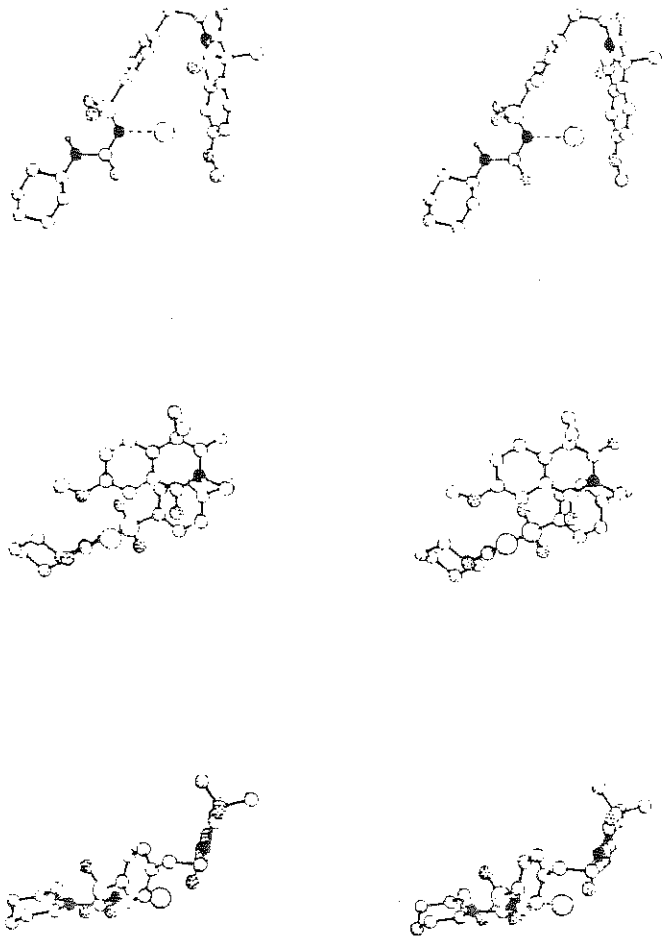

Fig. 6. Stereoscopic views along 3 perpendicular axes of the most probable conformer of the Ca-gliquidone complex with a 1:1 stoichiometry. H, C and S (or Ca) atoms are shown as open circles of increasing size. N and O atoms are shown as black and dotted symbols, respectively.

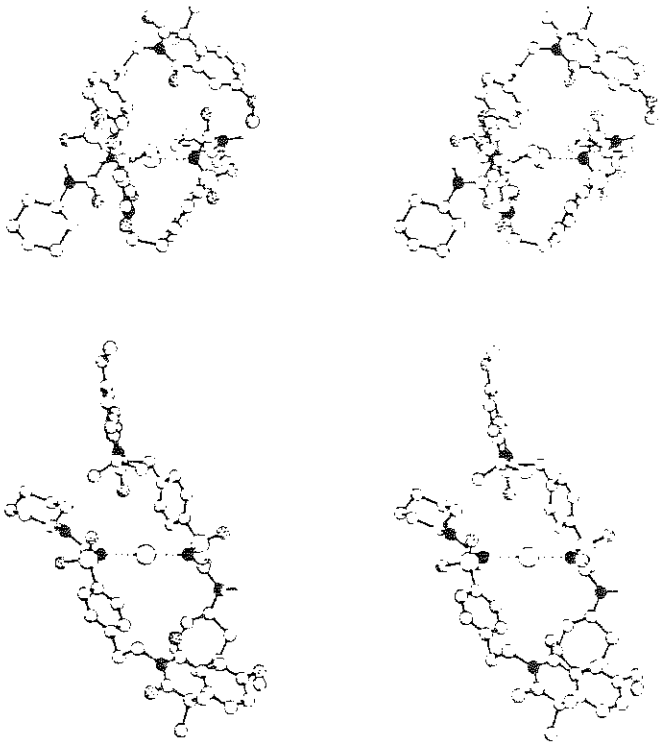

Fig. 7. Stereoscopic view along 2 perpendicular axes of the most probable conformer of the Ca-gliquidone complex with a 1:2 stoichiometry. Same presentation as in Fig. 6.

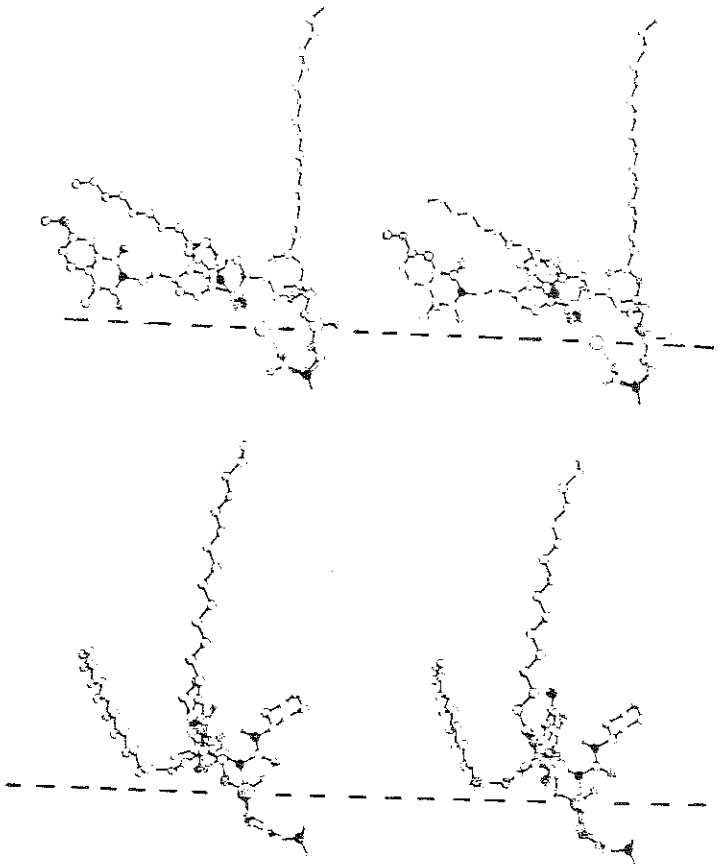

Fig. 8. Stereoscopic views of the most probable conformer of the hybrid complex between Ca, phosphatidylserine and gliquidone. The two views are taken at 90° of one another. In both cases, the complex is shown perpendicular to the simulated lipid-water interface. Such an interface was assumed to pass through the Ca^{2+} ion. Atomic symbols as in Fig. 6.

a pH close to their pKa (Dilger & Mc Laughlin, 1979; Mc Laughlin & Dilger, 1980), it is conceivable that hypoglycemic sulfonylureas transport H^+ from acidic to less acidic sites in the B-cell, e.g. from secretory granules into the cytoplasm or from the cytoplasm into the extracellular fluid. The latter movement could be coupled to the entry of Ca^{2+} into the B-cell.

Last, our ionophoretic and conformational data indicate that hypoglycemic sulfonylureas, e.g. gliquidone, and negative phospholipids, e.g. phosphatidylserine, may act cooperatively in mediating Ca^{2+} transport and may form a hybrid Ca-complex. The existence of hybrid complexes between distinct ionophoretic molecules, first demonstrated in the case of the antibiotic ionophores A23187 and X537A (Couturier et al., 1980; Deleers et al., 1981), was previously also documented in the case of gliclazide and A23187 (Deleers et al., 1982). The present findings lend support to the view that hypoglycemic sulfonylureas may affect Ca^{2+} transport by a synergistic behaviour with native ionophores in the islet cells (Couturier & Malaisse, 1980a). The postulated hybrid complex and the homologous Ca-gliquidone complexes display configurations suitable for the transport of Ca^{2+} across the phospholipid domain of cell membranes. In the homologous complexes, the highly hydrophilic sites tended to be masked by the hydrophobic periphery of the complex. In the hybrid complex, the area occupied by the hydrophobic relative to hydrophilic part of the complex was higher than in phosphatidylserine molecules. Such a situation could favour the formation of inverted micelles and, by a tunneling process, the delivery of Ca^{2+} at the opposite side of a phospholipid bilayer (De Kruijff & Cullis,

1980; Verkleijet al., 1980; Mandersloot et al., 1981).

In conclusion, the present work draws attention to the possible significance of ionophoretic events as determinants of the cationic response in islet cells exposed to hypoglycemic sulfonylureas, with emphasis on the synergistic behaviour between these insulin secretagogues and endogenous phospholipids.

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