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Binding of hypoglycaemic sulphonylureas to an artificial phospholipid bilayer

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Summary. Hypoglycaemic sulphonylureas bind to multilamellar liposomes formed of egg yolk phosphatidylcholine. In this artificial model, both *specific* and *non-specific* components of the binding phenomenon can be characterized by the same criteria as those used in studies performed with natural membranes. The relative ability of distinct sulphonylureas to inhibit the binding of ^{3}H-glibenclamide or ^{3}H-gliquidone to the liposomes parallels their relative potency as insulin secretagogues. It is proposed that the insertion of hypoglycaemic sulphonylureas into the phospholipid domain of the B cell membrane could represent a primary event in the mechanism by which these agents stimulate insulin release.

Key words: Liposomes, phospholipid, hypoglycaemic sulphonylurea, glibenclamide, gliquidone, gliclazide, tolbutamide, chlorpropamide.

Hypoglycaemic sulphonylureas stimulate insulin release, apparently by facilitating the inflow of Ca^{2+} into the pancreatic B cell [1, 2]. Most authors favour the view that the stimulation of Ca^{2+} inflow is secondary to a decrease in K^+ conductance, leading to depolarization of the plasma membrane and gating of voltage-sensitive calcium channels [3–6]. The molecular mechanism responsible for the change in K^+ permeability is not known [7]. Radioisotopic studies of sulphonylurea uptake by isolated islets suggest that these hypoglycaemic agents are bound to the plasma membrane of pancreatic B cells [8–10], which could be equipped with sulphonylurea receptors [11]. The cell boundary [12] could therefore represent the primary site of action of hypoglycaemic sulphonylureas.

The aim of the present study was to investigate how far the binding of sulphonylureas to natural membranes could be simulated in an artificial model of phospholipid bilayer.

Materials and methods

Egg yolk phosphatidylcholine was purchased from Sigma (St. Louis, Missouri, USA). ^{3}H-glibenclamide (31.1 Ci/mmol) was kindly provided by M.H. Mertens (Hoechst Belgium, Brussels, Belgium), and ^{3}H-gliquidone (4.8 Ci/mmol) by Dr N. Kaubisch (Boehringer, Ingelheim, FRG) and Dr Rupprecht (Thomae, Biberach, FRG). Both sulphonylureas were tritiated in the cyclohexyl ring and their purity assessed by chromatographic analysis to be >96%. Unlabelled glibenclamide and tolbutamide were obtained from Hoechst, gliquidone from Thomae, gliclazide from Servier Benelux, Brussels, Belgium and chlorpropamide from Pfizer, Brussels, Belgium.

Multilamellar liposomes of egg yolk phosphatidylcholine were formed as described elsewhere [13] in a Tris-HCl buffer (50 mmol/l, pH 7.30) yielding a final concentration of 2 mg/ml. The sulphonylureas were added to this buffer from stock solutions prepared in a mixture of ethanol and 0.1 N NaOH (1/1, v/v), the final concentration of these solvents not exceeding 0.2% (v/v). Aliquots (0.25 ml) of the liposomal suspension were placed in polyethylene microcentrifuge tubes (Beckman, Palo Alto, California, USA), incubated for 5–60 min at 37 °C, and centrifuged for 30 min at 4 °C and 3,000 *g*. The supernatant solution was then removed and the bottom of the tube containing the pellet of liposomes cut, placed in a counting vial containing 6 ml of scintillation fluid (Aquasol-2; New England Nuclear, Boston, Massachusetts, USA), and eventually examined for its radioactive content.

The specific radioactivity of ^{3}H-glibenclamide or ^{3}H-gliquidone was kept unchanged in all experiments, except for measurement of the non-specific binding in which case unlabelled sulphonylurea was added at a final concentration of 0.1 mmol/l. Such a non-specific binding was measured for each condition under study. Results are expressed as mean ± SEM values together with the numbers of individual determinations (n).

Results

At a 20 nmol/l concentration of ^{3}H-gliquidone, the apparent binding of the sulphonylurea to the liposomes reached an equilibrium value within 15 min of exposure to the drug (Figure 1). At the same concentration of the radioactive compound but in the presence of a much

higher concentration of unlabelled gliquidone (0.1 mmol/l), the radioactivity associated with the liposomes also rapidly reached equilibrium. Further experiments were performed over 30 min incubation.

In experiments performed in the presence of a 10 nmol/l concentration of ^{3}H-sulphonylurea and a 0.1 mmol/l concentration of the unlabelled drug, the total binding of glibenclamide and gliquidone averaged 13.3 ± 0.7 and 28.2 ± 0.9 nmol/mg of phospholipid, respectively ($n = 6$–10). This corresponds to approximately 1.00 ± 0.05 and 2.12 ± 0.07 mol sulphonylurea/100 mol phospholipid. If the binding of ^{3}H-sulphonylurea at this high concentration of the unlabelled agent is assumed to represent the *non-specific* binding, it should be substracted from other readings in order to characterize the *specific* binding of ^{3}H-sulphonylurea. In a range of concentrations from 0.2 to 10.0–15.0 nmol/l, such a specific binding was not quite proportional to the drug concentration (Fig. 2), the specific binding recorded at the highest concentration amounting to only 63% (glibenclamide) and 77% (gliquidone) of the theoretical values obtained by linear extrapolation of the data collected at the four lowest concentrations of sulphonylurea (i.e. between 0.2 and 2.0 nmol/l).

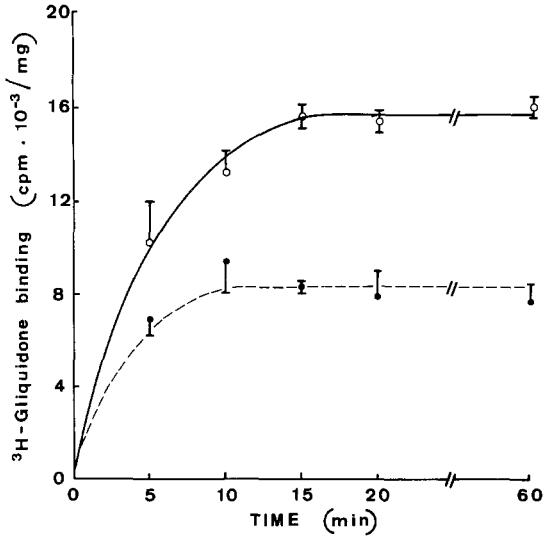

Fig. 1. Time course for the total (○——○) and non-specific (●----●) binding of ^{3}H-gliquidone (20 nmol/l) to liposomes as measured in the absence and presence of unlabelled gliquidone (0.1 mmol/l), respectively. Mean ± SEM values refer to four individual determinations and are expressed as cpm · 10^{-3}/mg phospholipid.

Figure 3 illustrates the effect of increasing concentrations of unlabelled gliquidone and gliclazide upon the specific binding of ^{3}H-glibenclamide (10.0 nmol/l). Within the range of concentrations under investigation (10.0 nmol/l to 0.1 mmol/l), the binding of ^{3}H-glibenclamide was much more severely inhibited by gliquidone than by gliclazide. Tolbutamide also slightly decreased ^{3}H-glibenclamide binding ($p < 0.01$), whereas chlorpropamide failed to affect ^{3}H-glibenclamide binding significantly ($p > 0.1$; data not shown).

The inhibition of ^{3}H-gliquidone-specific binding by unlabelled sulphonylureas is illustrated in Figure 4. The three drugs tested, namely gliclazide, tolbutamide, and chlorpropamide, all caused significant inhibition. The slopes of the regression lines for the inhibitory effect were not vastly different from one another. However, the concentrations of sulphonylurea required to cause 50% inhibition of ^{3}H-gliquidone-specific binding spanned a range of at least two orders of magnitude, averaging 0.6 μmol/l with gliclazide, 6.4 μmol/l with tolbutamide and 0.1 mmol/l with chlorpropamide.

Discussion

In this study we have investigated the binding of hypoglycaemic sulphonylureas to liposomes formed of egg

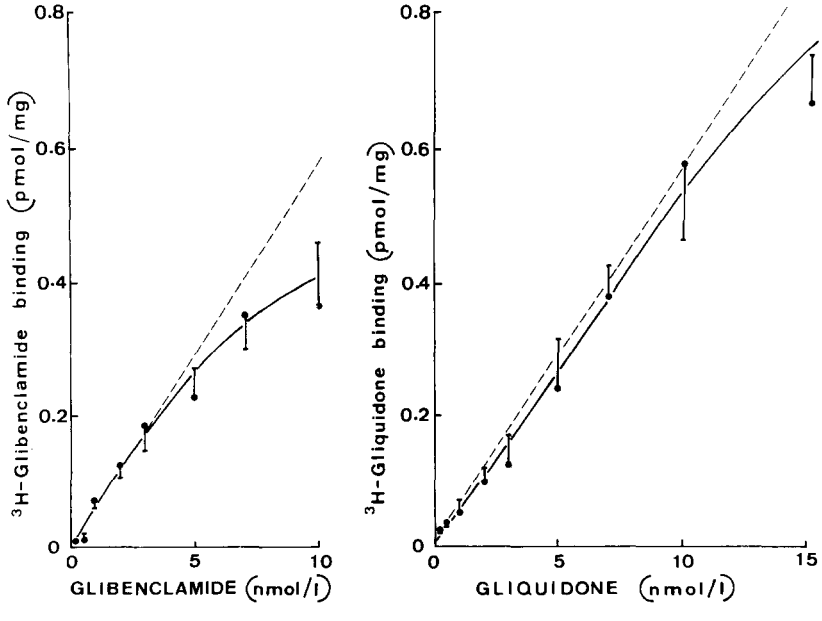

Fig. 2. Relationship between concentration and specific binding of ^{3}H-glibenclamide and ^{3}H-gliquidone to liposomes. Mean ± SEM values refer to six to ten individual determinations and are expressed as pmol sulphonylurea bound/mg phospholipid. The dotted lines illustrate relationships of proportionality with a slope derived from the data collected at the four lowest concentrations of sulphonylurea (0.2–2.0 nmol/l)

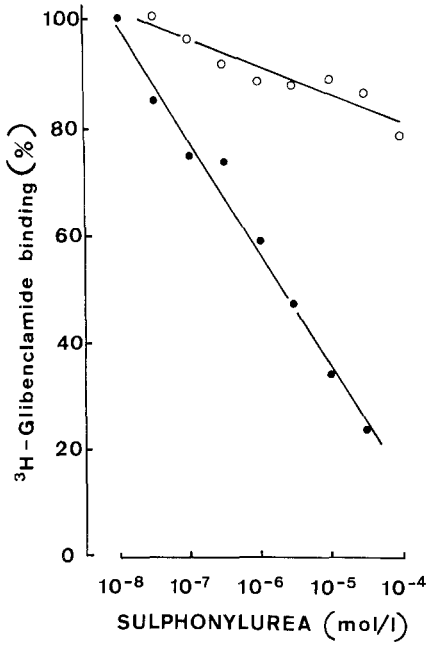

Fig. 3. Inhibition of the specific binding of ^{3}H-glibenclamide (10 nmol/l) to liposomes by increasing concentrations of unlabelled gliquidone (●——●) or gliclazide (○——○). Each value refers to the point-moving mean derived from quintuplate measurements performed at each concentration of sulphonylurea. The SEM for individual measurements averaged 9%.

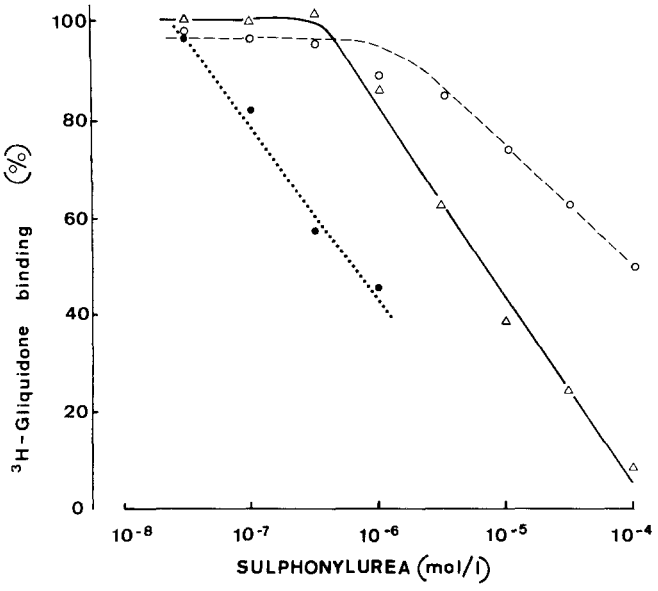

Fig. 4. Inhibition of the specific binding of ^{3}H-gliquidone (10 nmol/l) to liposomes by increasing concentrations of unlabelled gliclazide (●----●), tolbutamide (△——△) and chlorpropamide (○---○). Same presentation as in Fig. 3. The SEM for individual measurement averaged 17%.

yolk phosphatidylcholine. Our results refer to a binding phenomenon rather than penetration of the drug into the vesicular lumen. Indeed, at the concentration of phospholipid used here (0.5 mg/250 µl), the intravesicular space of multilamellar liposomes does not exceed 1% of the volume of incubation medium [14], whereas the liposome-associated radioactivity represented 34%–64% of the total radioactivity present in each sample. Three major analogies can be found between the present results and those obtained with natural membranes.

First, in our system just as in brain membranes [11] or intact islets [15–17], the total binding of [^{3}H] sulphonylurea increased progressively, albeit not proportionally, as the concentration of sulphonylurea was raised in a range between 0.2 nmol/l and 0.1 mmol/l. At the latter concentration, the equilibrium value for total binding to intact islets averaged 0.15, 0.67 and 3.15 mmol/kg dry weight in the case of tolbutamide [15], glibornuride [16] and glibenclamide [17], respectively. If these values are representative of binding to the plasma membrane, as proposed by the Umeå group, they would correspond, in the case of glibenclamide, to a sulphonylurea/phospholipid molar ratio close to unity. This estimation is based on the knowledge that, in pancreatic islets, the plasma membrane/dry weight ratio is close to 1700 m^2/kg dry weight [18, 19] and that the content in phospholipids (averaged molecular weight taken as 750) of biological membranes is close to 1.4 mg/m^2 [20, 21]. However, if the hypoglycaemic sulphonylureas were located not only in the plasma membrane but also in the membrane of intracellular organelles – a view which cannot yet be ruled out – the glibenclamide/phospholipid molar ratio could fall from 1.0 to a value as low as 0.02, the total phospholipid content of islets averaging 230 mmol/kg protein [22]. In the present system and at the same initial concentration of sulphonylurea (0.1 mmol/l), the sulphonylurea/phospholipid ratio was close to 0.01–0.02. The latter values could be somewhat underestimated. Indeed, if allowance is made for the fact that we are dealing with multilamellar liposomes (approximately 5–15 layers) [23], the true binding to the outer bilayer of phospholipids could be higher than the value calculated by reference to the total amount of phospholipid present in each sample. There appears, therefore, to be a fair agreement between data collected in living cells and our artificial model.

A second analogy between the artificial and biological systems consists in the fact that it is possible, in both models, to isolate a component of the binding phenomenon which can be operationally defined as *specific binding*. The saturation of this specific binding appeared to be reached at much lower concentrations of sulphonylurea in brain membranes (1.0 nmol/l) than in liposomes. It could be disputed that our artificial membrane contains no specific receptors and hence the terms 'specific' and 'non-specific' binding were used here incorrectly. However, the aim of the present study was to test precisely whether artificial membranes devoid of specific receptor might behave phenomenologically in the same manner as natural membranes. Our experimental data demonstrate that, within limits, such is the case. A similar situation was recently observed for the binding of tumour-promoting phorbol esters to lipo-

Table 1. Relative biological potency of hypoglycaemic sulphonylureas (and a sulphonamide)

Sulphonylurea	Molecular weight (daltons)	A	B	C	D	E	F	G	H	K
Glibenclamide	493	0.25	0.31	0.40	0.25	0.78	0.25	0.50		
Glisoxepide	447	0.85	0.69	1.06	0.83	0.87		0.80		
Glipizide	445	1.30		0.94				1.00		
Gliquidone	528				4.17		4.64	3.00		
Glibornuride	354				5.00	5.10		5.00		
Gliclazide	323	27.65						32.00		
Tolazamide	311		53.85					40.00		
Carbutamide	271								199.04	
Tolbutamide	270		219.23	96.15	312.50	247.52	309.41	200.00	230.00	230.00
Glycodiazine[a]	309	235.15								
Chlorpropamide	276								450.00	526.80

[a] Sulphonamide.

The relative biological potency of each drug was judged from (A) the dose-action relationship for insulin release by pieces of rat pancreatic tissue [26, 27]; (B) the dose-action relationship for the maximal fall in blood glucose concentration after oral administration to normal subjects [28]; (C) the dose-action relationship for the maximal decrease in blood glucose concentration after intravenous administration to normal conscious dogs [29]; (D) the doses causing a 30% decrease in blood glucose concentration after intravenous administration to normal healthy volunteers [30, 31]; (E) the doses yielding comparable integrated glycaemic profiles after intravenous administration to normal subjects [32]; (F) the doses provoking comparable glycaemic decreases after oral or intravenous administration to normal subjects [33, 34]; (G) the minimal therapeutic doses [11, 35], chlorpropamide being excluded from this series because of its unusually long half-life; (H) the increments in glucose fractional removal rate (K value) observed after intravenous administration of a low dose (5 mg/kg body weight) of each drug to anaesthetized dogs [36, 37]; and (K) the decrease in blood glucose concentration observed 2 h after oral administration of a low dose (10 mg/kg body weight) of sulphonylurea to monkeys [38]. In each column, the data are expressed relative to one another, according to the reference(s) cited. The absolute values in different columns were adjusted to provide comparable data for the same drug(s). Some agents, which are not cited elsewhere in this report, are listed in the Table because they were used to establish the correspondence between the different columns. All comparisons refer to the concentration or dosage required to achieve a given secretory or glycaemic response, results being expressed in weight units rather than molar units

somes and biological membranes, respectively [24]. It should be emphasized that the distinction between specific and non-specific binding in the present work neither implies nor rules out the existence of distinct modalities of interaction between sulphonylureas and phospholipids. The present data merely reflect a lack of proportionality between total binding and drug concentration. Further work is required both to characterize the modality or modalities of interaction (e.g. hydrophobic *versus* hydrophilic) between hypoglycaemic sulphonylureas and phospholipids and to distinguish, in biological membranes, between the insertion of these agents in the phospholipid domain and their possible binding to membrane-associated proteins.

A third and striking analogy was found in the ability of distinct sulphonylureas to inhibit the binding of a given drug. In the liposomes, the K_i for inhibition of ^{3}H-glibenclamide by gliquidone was close to 2.1 μmol/l, whereas biologically less potent sulphonylureas (gliclazide, tolbutamide, chlorpropamide) tested at concentrations up to 0.1 mmol/l caused only modest to marginal decreases in ^{3}H-glibenclamide-specific binding. The relative potency of gliclazide, tolbutamide and chlorpropamide in inhibiting ^{3}H-gliquidone binding to liposomes paralleled their respective biological potency as insulin secretagogues (Table 1). The K_i was close to 6.4 μmol/l with tolbutamide, which compares favourably with the values of 2.7 μmol/l reported by Kaubisch et al. in brain membranes [11]. Likewise, in intact islets from obese-hyperglycaemic mice, the total binding of ^{3}H-glibenclamide is unaffected by tolbutamide [25], that of ^{3}H-glibornuride reduced by glibenclamide (−21%) but not by tolbutamide [16], and that of ^{3}H-tolbutamide inhibited (−34%) by glibenclamide [15], all experiments being carried out at a 20 μmol/l concentration of ^{3}H-sulphonylurea and a 0.1 mmol/l concentration of the unlabelled potential inhibitor. Thus, whether in intact islets, brain membranes or liposomes, there was a close parallel between the relative biological potency of distinct sulphonylureas as insulin secretagogues and their relative ability to cause reciprocal inhibition of binding.

These analogies raise the idea that the insertion of hypoglycaemic sulphonylureas in the phospholipid domain of the B cell membrane may represent the first step in the sequence of cytophysiological events leading to stimulation of insulin release by these agents. Hence, the ability of distinct sulphonylureas to penetrate into the phospholipid domain of cell membrane(s) could well represent one of the main factors conditioning their biological potency.

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