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SYNERGISTIC EFFECTS OF HYPOGLYCAEMIC SULPHONYLUREAS AND ANTIBIOTIC IONOPHORES UPON CALCIUM TRANSLOCATION

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1 Hypoglycaemic sulphonylureas, such as tolbutamide and gliclazide, provoke the translocation of calcium from an aqueous medium into or across a hydrophobic region. The combined effect of sulphonylureas and antibiotic ionophores upon such a process was investigated.

2 The magnitude of the sulphonylurea-induced translocation of calcium was more marked in the presence than in the absence of A23187. Gliclazide and tolbutamide also enhanced, although less markedly, X537A-mediated calcium translocation. The effect of the sulphonylureas was even less marked in the presence of both ionophores, which acted synergistically in causing calcium translocation.

3 A non-hypoglycaemic sulphonylurea and diazoxide failed to affect ionophore-mediated calcium translocation. Gliclazide failed to enhance X537A-mediated sodium translocation.

4 It is proposed that the primary site of action of hypoglycaemic sulphonylureas upon calcium-dependent physiological processes may correspond to a drug-induced facilitation of calcium transport across the plasma membrane, as mediated by native ionophores.

Introduction

According to Hellman and his colleagues, hypoglycaemic sulphonylureas do not penetrate the pancreatic β-cell beyond its plasma membrane (Hellman, Sehlin & Täljedal, 1971; 1973b; 1976; Hellman, Lernmark, Sehlin & Täljedal, 1973a; Hellman & Täljedal, 1975). This finding suggests that the primary site of action of these drugs may be located at the level of the cell boundary, as defined elsewhere (Orci, Ravazzola, Amherdt & Malaisse-Lagae, 1974). Recent biochemical observations in isolated pancreatic islets led us to postulate that the insulinotropic action of tolbutamide was primarily due to interference with the transport of ions across the cell membrane (Kawazu, Sener, Couturier & Malaisse, 1980). This view was further considered in the light of investigations indicating that hypoglycaemic sulphonylureas are able to translocate calcium into or across a hydrophobic region (Couturier & Malaisse, 1980b; Malaisse, Couturier & Valverde, 1980).

In the present paper, we have examined the extent to which hypoglycaemic and hyperglycaemic sulphonamides may affect the process of calcium translocation, as mediated by the antibiotic ionophores, A23187 and X537A. Some of these observations have been briefly described elsewhere (Malaisse, Sener, Herchuelz, Hutton, Devis, Somers Blondel, Malaisse-Lagae & Orci, 1977).

Methods

A small volume (0.2 to 0.3 ml) of a HEPES-buffered solution (25 mM; pH 7.0) containing, unless stated otherwise, Na^+ 122, Cl^- 120 and K^+ 5 mEq/l, and $^{45}CaCl_2$ (or $^{22}NaCl$) was vigorously mixed by mechanical means (Vortex-Genie, Bohemia, N.Y., U.S.A.) at room temperature for 1 min with an equal volume of an organic mixture (toluene: butanol, 7:3, v:v) containing, as required, the drug or association of drugs under study. An aliquot (0.1 ml) of the supernatant organic phase was then examined for its radioactive content (ct/min in 0.1 ml), from which its apparent calcium (or sodium) concentration (M) was calculated (M = specific activity of the initial aqueous phase $\times$ ct min^{-1} l^{-1}), as explained in greater detail elsewhere (Malaisse, Valverde, Devis, Somers & Couturier, 1979).

The concentration of the drug(s) given in the text refers to the initial concentration in the organic mixture. The final aqueous phase contained no detectable amount of ionophore (Malaisse *et al.*, 1979) or hypoglycaemic sulphonylurea (Couturier & Malaisse, 1980b). No significant trapping of radioactivity occurred at the interface between the aqueous and organic phases. Thus, when samples were removed from both the upper layer of the supernatant organic phase and bottom of the aqueous phase, the recovery of radioactivity averaged 100.3 $\pm$ 1.1, 99.2 $\pm$ 1.1 and

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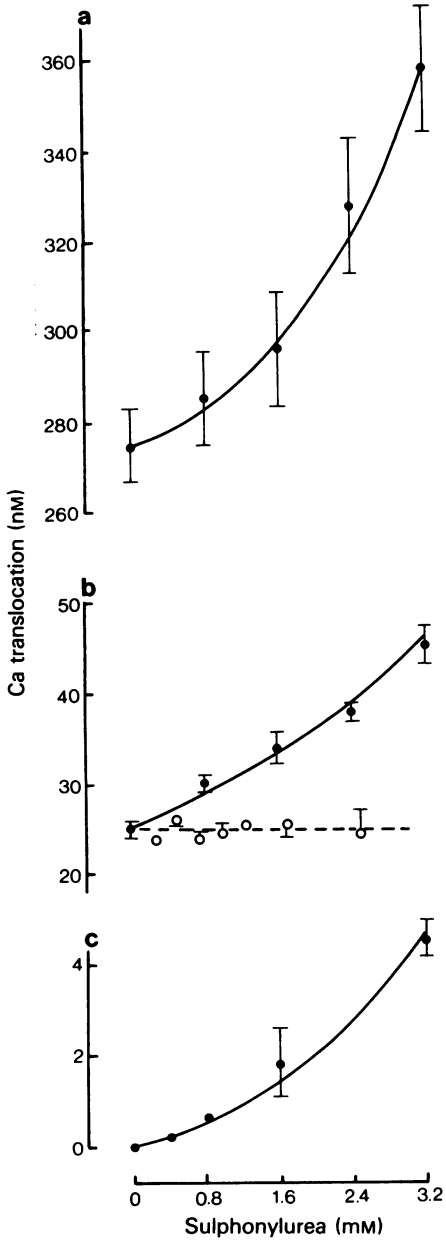
99.7 ± 0.6% (n = 3 in each case) of the amount added to each tube (100.0 ± 0.9%) in the presence of gliclazide (1.2 mM), A23187 (10 μM) or both, respectively. All results are expressed as the mean (±s.e. mean) together with the number of individual determinations (n).



Results

Effect of hypoglycaemic sulphonylureas upon A23187-mediated calcium translocation

Gliclazide enhanced A23187-mediated calcium translocation (Figure 1b). The sulphonylurea-induced increment in calcium translocation was about 4 times higher in the presence than in the absence of A23187 (Figure 1b and c), both series of experiments being performed at low concentrations of Ca^{2+} (0.8 to 1.3 μM) in the initial aqueous phase.

When the concentration of Ca^{2+} was raised to 0.2 mM, the sulphonylurea-induced increment in calcium translocation was further increased. However, relative to the control value found in the sole presence of A23187, such an increment appeared more marked at low than at high calcium concentrations (Figure 1a and b). The latter situation is reminiscent of that characterizing the dose-action relationship for A23187-mediated calcium translocation, in which case the relative increment in calcium translocation attributable to a given increase in ionophore concentration is also more marked at low than at high Ca^{2+} concentration (Malaisse *et al.*, 1979).

The gliclazide-induced increment in calcium translocation (Δ) was not proportional to the concentration of sulphonylurea (S). Instead, these variables appeared to be linked by the following equation

$$\Delta = aS^b \qquad \text{(i)}$$

in which a and b represent constants. Thus, a rectilinear dose-action relationship was obtained in logarithmic scales (Figure 2), according to the equation

$$\log\Delta = \log a + b \log S. \qquad \text{(ii)}$$

Tolbutamide affected A23187-mediated calcium translocation in a manner comparable to that seen with gliclazide (Figure 2). When plotted on logarithmic scales, the dose-action relationship for the sul-

Figure 1 Dose-reaction relationship for the ability of gliclazide to provoke calcium translocation into an organic immiscible phase. Mean values refer to 9 to 21 individual observations. The experiments were performed in the absence (c) or presence of A23187 10 μM (a) and 100 μM (b) at an initial Ca^{2+} concentration in the aqueous phase of 0.8 to 1.3 μM (b and c) or 0.2 mM (a). At the low Ca^{2+} concentrations, the control value (no gliclazide) for A23187-mediated calcium translocation corresponded to a mean of 35.8 ± 1.5 nM per μM of Ca^{2+} in the initial aqueous phase. The dotted line in the middle panel refers to data obtained with the non-hypoglycaemic derivative of gliclazide, S2574. No s.e. mean bar is shown when this was so small that it lay within the point.

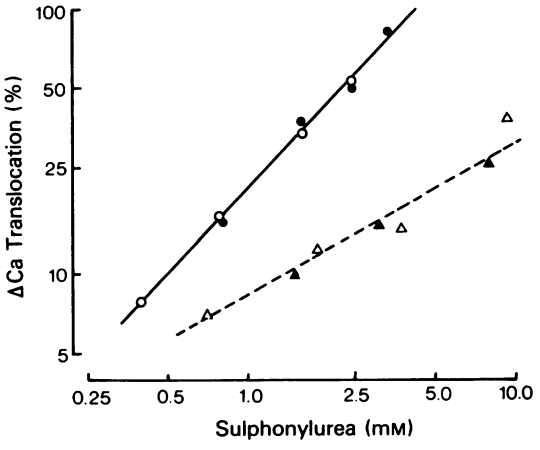


Figure 2 Dose-action relationship for the effect of gliclazide (closed symbols) and tolbutamide (open symbols) upon calcium translocation evoked by either A23187 (circles) or X537A (triangles). The experimental conditions are defined in the legends of Figure 1b and Figure 3. The sulphonylurea-induced increment in calcium translocation (Δ) is expressed as a % of the control value found in the absence of sulphonylurea, and is plotted as a function of the drug concentration (logarithmic scales).

phonylurea-induced increment in calcium translocation, relative to control value, yielded a slope (±sample standard deviation of the regression coefficient) of 1.12 ± 0.09 and 1.06 ± 0.01 with gliclazide and tolbutamide, respectively (Figure 2). Glipizide also augmented A23187-mediated calcium translocation. However, over the same range of sulphonylurea concentration (0.8 to 3.2 mM), the enhancing action of glipizide represented less than half of that seen with either gliclazide or tolbutamide (data not shown).

The non-insulinotropic derivative of gliclazide, S2574 (*N*-(4-carboxy-benzene-sulphonyl)-*N'*-[3-aza-3-bicyclo(3,3,0)-octyl]-urea), failed to affect A23187-mediated calcium translocation (Figure 1). S2574 also fails to cause calcium translocation in the absence of ionophore (Couturier & Malaisse, 1980b).

Effect of hypoglycaemic sulphonylureas upon X537A-mediated calcium translocation

Both tolbutamide and gliclazide enhanced X537A-mediated calcium translocation (Figure 3). However, the relative magnitude of such an increase was less marked than in the presence of A23187 (Figure 2). As judged from the slope of the dose-action relationship, the sulphonylurea-induced increment in calcium translocation, relative to the control value and as a function of the drug concentration (logarithmic scales), averaged 1.09 ± 0.03 in the presence of A23187 as distinct from only 0.62 ± 0.07 in the presence of X537A ($P < 0.001$). Incidentally, the lesser sensitivity towards sulphonylureas of X537A as distinct from A23187 cannot be attributed to the observed difference in calcium translocation evoked by each of these ionophores when used in the absence of sulphonylurea. At the low Ca^{2+} concentration used in the present experiments, X537A 1.4 mM translocates 3.43 ± 0.08 nM per μM of Ca^{2+}, whereas as little as 0.1 mM A23187 translocates 35.8 ± 1.5 nM per μM of Ca^{2+}. For reasons already mentioned, such a difference should, everything else being equal, allow sulphonylureas to cause a greater relative increase in calcium translocation with X537A than with A23187.

Table 1 Combined effects of X537A, A23187 and gliclazide upon calcium translocation

X537A (mM)	*A23187* (mM)	*Gliclazide* (mM)	*Ca translocation* (nM)	Δ *gliclazide* (%)
1.4	—	—	4.6 ± 0.1 (9)	—
1.4	—	1.6	5.4 ± 0.1 (3)	+15.6 ± 2.2[b]
—	0.1	—	49.7 ± 0.8 (9)	—
—	0.1	1.6	82.1 ± 5.8 (6)	+65.2 ± 11.8[c]
1.4	0.1	—	79.1 ± 0.3 (5)	—
1.4	0.1	1.6	79.3 ± 1.1 (9)	+0.9 ± 1.3[NS]
1.4	0.1	3.2	89.2 ± 3.3 (15)	+12.8 ± 5.7[a]

The experiments were performed at an initial Ca^{2+} concentration of 1.4 μM in the aqueous phase. The last column indicates the increment (Δ) in calcium translocation attributable to gliclazide as a percentage of the appropriate mean control value found in the absence of sulphonylurea, together with its statistical significance (NS: not significant; [a]$P < 0.05$; [b]$P < 0.02$; [c]$P < 0.005$).

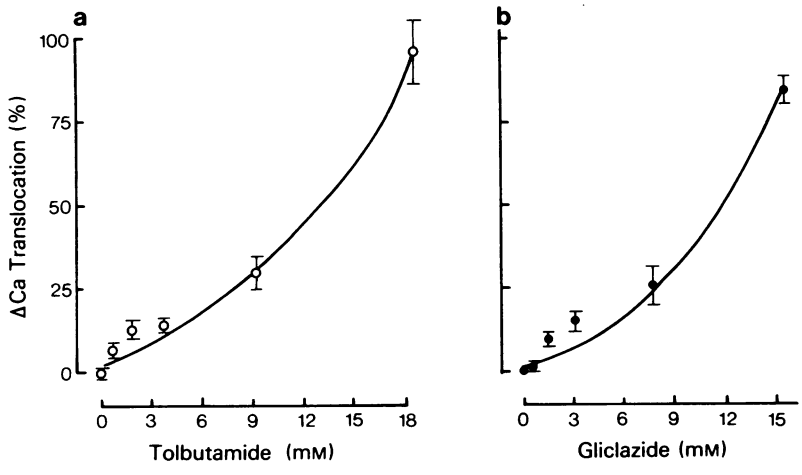


Figure 3 Dose-action relationship for the effect of tolbutamide (a) and gliclazide (b) in enhancing X537A-mediated Ca translocation. The sulphonylurea-induced increment in Ca translocation (△) is expressed as a % of the control value found in the absence of sulphonylurea. Each point refers to 3 to 31 individual measurements. The experiments were performed in the presence of X537A 1.4 mM and with initial concentrations of Ca^{2+} in the aqueous phase ranging from 1.3 to 11.2 μM. The control value for X537A-mediated calcium translocation averaged 3.43 ± 0.08 nM (n = 25) per μM of Ca^{2+} in the initial aqueous phase.

Combined effects of A23187, X537A and gliclazide

When used in combination, the ionophores A23187 and X537A caused a translocation of calcium much higher than that expected from the sum of their individual effects (Table 1). The enhancing action of gliclazide upon ionophore-mediated calcium translocation was considerably reduced in the presence of both ionophores. Thus, no significant effect of gliclazide 1.6 mM was observed, and the concentration of sulphonylurea had to be raised to 3.2 mM to detect a barely significant increment in ionophore-mediated calcium translocation.

Effect of diazoxide upon ionophore-mediated calcium translocation

Diazoxide failed to affect significantly A23187-mediated calcium translocation (Table 2). Similarly diazoxide (1.1 to 2.2 mM) failed to affect X537A-mediated calcium translocation, the values found in the presence of diazoxide averaging 101.2 ± 2.5% (n = 6) of the mean control value obtained in the absence of diazoxide.

Effect of hypoglycaemic sulphonylureas upon X537A-mediated sodium translocation

X537A (1.4 mM) stimulated sodium translocation, with a mean value close to 10 μM of sodium translocated per mM of Na^+ in the initial aqueous phase. Both at low (0.3 μM) and high (121 mM) Na concentrations, gliclazide (up to 3.2 mM) failed to cause any detectable increase in X537A-mediated sodium translocation.

Table 2 Effect of diazoxide upon A23187-mediated calcium translocation

Diazoxide (mM)	*Calcium translocation (% of control)*
Nil	100.0 ± 1.9 (30)
0.14	101.4 ± 4.4 (21)
0.72	98.2 ± 2.0 (18)
1.08	102.3 ± 3.3 (21)
1.44	106.6 ± 4.4 (18)
2.17	105.5 ± 5.3 (21)

The results are expressed as a percentage of the mean control value found in the absence of diazoxide. The experiments were performed at a low initial concentration of Ca^{2+} in the aqueous phase (ca. 1.0 μM) and in the presence of A23187 0.1 mM. The concentrations of monovalent anions in the initial aqueous phase varied from 0 to 120 mEq/l for Cl^- and 7 to 127 mEq/l for (K^+ + Na^+). Hence, the control value for calcium translocation varied between the extreme values of 56.7 ± 3.0 nM (Cl^- 120, K^+ 5 and Na^+ 122 mEq/l) and 121.1 ± 6.4 nM (no Cl^-, no K^+ and Na^+ 7 mEq/l).

Discussion

The present findings demonstrate that hypoglycaemic sulphonylureas, such as tolbutamide and gliclazide facilitate the translocation of calcium into a hydrophobic region as mediated by the antibiotic ionophores, A23187 or X537A. A non-insulinotropic analogue of gliclazide and the hyperglycaemic sulphonamide, diazoxide, failed to affect the ionophore-mediated process of calcium translocation. No facilitatory effect of gliclazide upon X537A-mediated sodium translocation could be detected.

The results obtained with sulphonylurea in the presence of A23187 (or X537A) are comparable to those obtained when the concentration of the ionophore itself is increased. Indeed both the dose-action relationship for the increment in calcium translocation and the influence thereupon of the initial Ca^{2+} concentration of the aqueous phase are identical at increasing concentrations of A23187 or at a fixed concentration of A23187 and increasing concentrations of sulphonylureas. We have previously shown that the magnitude of calcium translocation as a function of the ionophore and Ca^{2+} concentrations is compatible with a 1/2 stoichiometry for calcium-ionophore interaction (Couturier & Malaisse, 1980a; Malaisse *et al.*, 1979). The same stoichiometry may apply to the calcium-sulphonylurea interaction (Couturier & Malaisse, 1980b).

Our results confirm that distinct ionophores may act in a cooperative manner upon the process of calcium translocation (Couturier, Deleers & Malaisse, 1980). Moreover, the experimental data indicate that the capacity of sulphonylureas to enhance calcium translocation is not identical with different ionophores or combination of ionophores. The existence of such a variability suggests but does not demonstrate, that calcium may form a hybrid complex with sulphonylureas and certain ionophores.

From the biological standpoint, the specificity of the ionophoretic response towards hypoglycaemic as distinct from non-hypoglycaemic or hyperglycaemic sulphonamides and its specificity towards calcium as distinct from sodium are compatible with the view that the interaction of hypoglycaemic sulphonylureas with native ionophores, such as those recently characterized in isolated pancreatic islets (Valverde & Malaisse, 1979), represents a fundamental event in the mechanism by which these drugs modify calcium handling in the pancreatic β-cell and, possibly, in other target organs affected by hypoglycaemic sulphonylureas, e.g. heart and platelets (Roth, Prout, Goldfine, Wolfe, Muenzer, Grauer & Marcus, 1971; Verry, Bryon, Dechavanne, Lagarde & Vainer, 1975; Linden & Brooker, 1978).

In conclusion, although the ionophoretic capacity of the hypoglycaemic sulphonylureas themselves is rather modest (as a result of low affinity constant for calcium; see Couturier & Malaisse, 1980b), these drugs may be much better able to translocate calcium across biological membranes when acting in synergism with native ionophoretic systems.

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