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OPPOSITE EFFECTS OF HYPOGLYCEMIC AND HYPERGLYCEMIC SULFONAMIDES UPON IONOPHORE-MEDIATED CALCIUM TRANSPORT

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Abstract—Hypoglycemic sulfonylureas (gliclazide, tolbutamide) were able to mediate Li–Ca or Na–Ca countertransport in an artificial system, and enhanced A23187-mediated Ca countertransport. Diazoxide failed to cause Ca countertransport and inhibited A23187-mediated countertransport. The inhibitory effect of diazoxide was abolished by gliclazide. Comparable results were obtained when an extract of pancreatic islets was used instead of A23187 as the reference ionophoretic material. The response to hypoglycemic and/or hyperglycemic sulfonamides in the present system was qualitatively identical to their effect upon ^{45}Ca net uptake and insulin release in intact islets. This analogy suggests that these drugs may act in the islet cells primarily by interfering with the transport of cations by native ionophores.

Hypoglycemic sulfonylureas (e.g. tolbutamide and gliclazide) stimulate insulin release [1], and are widely used in the treatment of non-insulin-dependent diabetic patients. The intimate mode of action of these drugs in the pancreatic B cell is poorly understood [2]. We have exposed elsewhere our reasons for believing that the insulinotropic action of hypoglycemic sulfonylureas cannot be attributed to a primary effect on either the metabolism of nutrients or the synthesis and breakdown of cyclic AMP in islet cells [3]. As an alternative explanation, it was proposed that the primary effect of hypoglycemic sulfonylureas consists in a stimulation of Na and Ca transport across the B cell plasma membrane [3, 4]. This view is based, *inter alia*, on the findings that hypoglycemic sulfonylureas are able to translocate Na and Ca into or across a hydrophobic domain, and that the latter process is suppressed by organic calcium antagonists (e.g. suloctidil) which are potent inhibitors of sulfonylurea-stimulated ^{45}Ca net uptake and insulin release in isolated pancreatic islets [3–8].

We now wish to report on the effect of gliclazide and diazoxide upon the uphill transport of calcium as mediated by either the antibiotic ionophore A23187 or native ionophoretic material extracted from pancreatic islets. In such a system, we were able to disclose for the first time opposite effects of hypoglycemic and hyperglycemic sulfonamides, respectively, upon the ionophore-mediated transport of calcium.

MATERIAL AND METHODS

In all experiments, 0.15 ml of an organic mixture (toluene : butanol, 7/3, v/v) was transferred back and forth between two tubes each containing 0.30 ml of aqueous medium and 0.05 ml of the same organic mixture. The two aqueous media consisted of a triethanolamine–HCl buffer (20 mM, pH 7) with initially the same concentration of $CaCl_2$ (^{40}Ca and ^{45}Ca) but different concentrations (2 and 200 mM, respectively) of either LiCl or NaCl. The organic phase contained, as required, gliclazide, tolbutamide, diazoxide, A23187 and an extract of pancreatic islets prepared as described elsewhere [9]. After each transfer, the aqueous medium and organic mixture were vigorously mixed for 1 min to ensure equilibrium partition of cations between these two phases [10]. After each two or three back-and-forth transfers of the organic mixture, paired samples (10 μl) were removed from the aqueous media with a microsyringe, and examined for their radioactive content. The present system for the study of ionophore-mediated cation transport was selected [11] instead of the Pressman cell because, in the latter model, several months would be required to complete a single experiment at the low concentration of ionophoretic material here used. In control experiments performed in the absence of any drug, no translocation of ^{45}Ca could be detected whether in the absence or presence of a Na^+ or Li^+ gradient (data not shown). The results are presented as the difference in ^{45}Ca content (Δ) of paired samples and expressed as a percentage of the total radioactivity found in the first two samples.

RESULTS

When the two aqueous media contained initially the same concentration of Ca and when gliclazide (5 mg/ml) was added to the organic mixture, Ca was progressively transported (Fig. 1) from the medium containing little Li^+ (2 mM) to that containing Li^+ in high concentration (200 mM). The magnitude of this phenomenon of uphill Ca translocation was

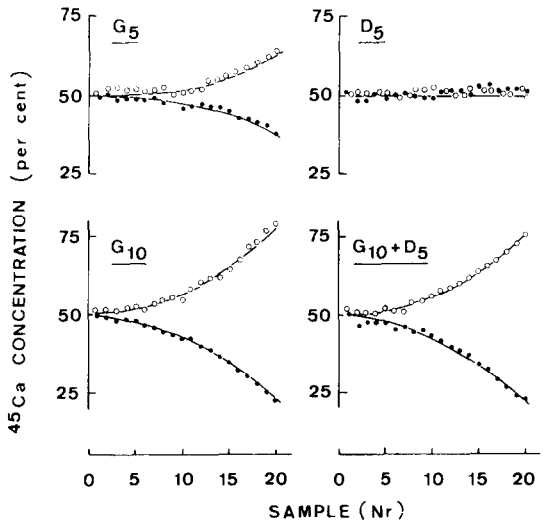

Fig. 1. The translocation of ^{45}Ca was mediated by an organic phase containing, as required, gliclazide (G) and diazoxide (D) at the stated concentration (mg/ml), and occurred between aqueous media intially containing $^{45}CaCl_2$ (0.2 mM; 1 μCi/ml) and either 2 (● closed circles) or 200 (○ open circles) mM LiCl. The ^{45}Ca content of the aqueous media was measured in paired samples removed after each two back-and-forth transfers of the organic phase, and is expressed as percentage of the total radioactive content of the first samples.

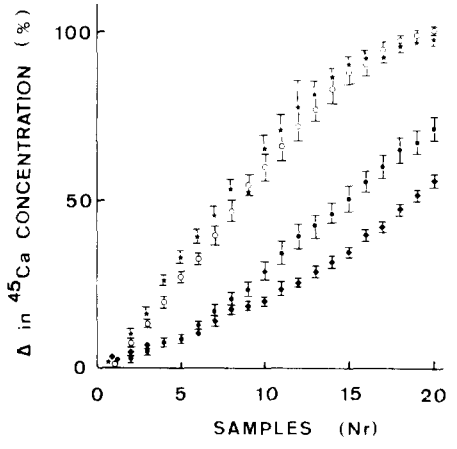

Fig. 2. The translocation of ^{45}Ca was mediated by an organic phase containing A23187 (0.1 mM) either alone (● closed circles; middle curve) or in combination with diazoxide (5 mg/ml, ♦ diamonds, lower curve), gliclazide (5 mg/ml; ○, open circles) or both sulfonamides (★ stars). The difference in ^{45}Ca content of paired samples (mean ± S.E.M.; N = 3 in each case) is expressed as in Fig. 1. Other experimental conditions were the same as those given in the legend to Fig. 1.

related to the concentration of gliclazide (Fig. 1, Table 1). Diazoxide (5 or 10 mg/ml) failed both to cause Ca translocation and to affect significantly gliclazide- or tolbutamide-mediated Ca countertransport (Table 1). When used at the same concentration (10 mg/ml), tolbutamide was less active than gliclazide.

In the second series of experiments, the ionophore A23187 was added to the organic mixture. A23187 alone caused Li–Ca countertransport (Fig. 2), confirming a recent report [11]. Gliclazide facilitated and diazoxide inhibited the phenomenon of A23187-mediated Ca countertransport ($P < 0.005$). In the concomitant presence of A23187, gliclazide and diazoxide, the results were not significantly different from those found with A23187 in the sole presence of gliclazide. Thus, in the presence of A23187, the slope of the regression line characterizing the increasing difference in ^{45}Ca concentration (Δ) as a function of the sample number (samples 1–15 inclusive) ranged as follows: diazoxide (2.28 ± 0.06) < control (3.65 ± 0.14) < gliclazide ($6.30 \pm \pm 0.15$) ~ gliclazide + diazoxide (6.41 ± 0.15), each mean value (± sample S. E.) being derived from 3 separate experiments.

Table 1. Li–Ca countertransport in the presence of tolbutamide (T), gliclazide (G) and diazoxide (D)*

Drug (mg/ml)	$b \pm s_b$
T (10)	+ 1.651 ± 0.213†
T (10) + D (10)	+ 1.488 ± 0.114†
G (5)	+ 1.090 ± 0.151†
G (10)	+ 2.868 ± 0.189†
G (10) + D (5)	+ 2.701 ± 0.189†
D (5)	− 0.040 ± 0.034‡
D (10)	+ 0.043 ± 0.040‡

* All drugs were added to the organic phase at the stated concentration. The translocation of ^{45}Ca between media of low and high LiCl concentration (see Fig. 1) was calculated from the difference in radioactive content of paired samples (Δ) and expressed as a percentage of the total radioactivity of the first samples. The regression coefficients (Δ per sample) and its sample standard error ($b \pm s_b$) are shown together with the significance of the correlation coefficient.

† $P < 0.001$.

‡ Not significant.

In the last series of experiments, we investigated the effects of gliclazide and diazoxide upon Ca countertransport as mediated, in the presence of a Na^+ gradient, by ionophoretic material extracted from pancreatic islets (Fig. 3, Table 2). In the absence of the islet extract, gliclazide caused a modest uphill translocation of Ca, whereas diazoxide failed to do so and failed to significantly affect gliclazide-mediated Ca countertransport. The islet extract itself caused an obvious uphill translocation of Ca. This phenomenon was augmented by gliclazide and inhibited by diazoxide, both changes being significant. When both drugs were used in combination with the islet extract, the results were essentially the same as those found with the extract in the sole presence of gliclazide. Thus, no significant difference between the two latter experimental conditions could be detected, whether the translocation of ^{45}Ca was analysed by regression analysis or by comparison with the appropriate control values found when the

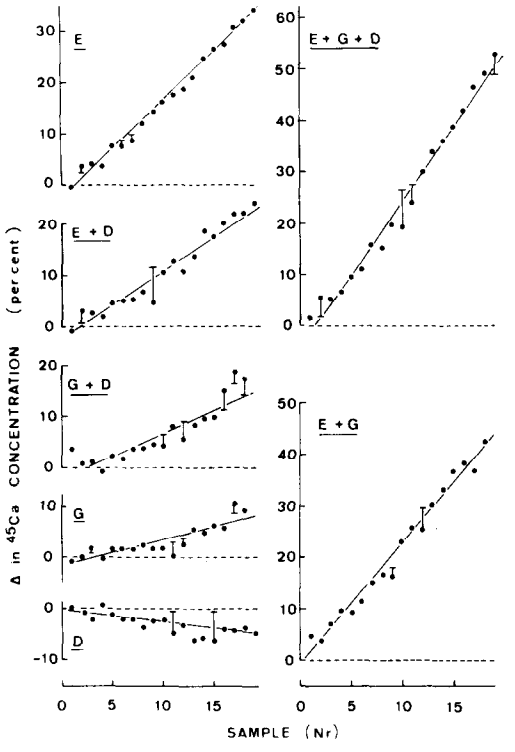

Fig. 3. The translocation of ^{45}Ca was mediated by an organic phase containing, as required, diazoxide (D; 4 mg/ml), gliclazide (G; 4 mg/ml) and an islet extract (E; each 0.4 ml of the organic phase contained material extracted from 125 islets), and occurred between aqueous media initially containing $^{45}CaCl_2$ (0.1 mM; 2 μCi/ml) and either 2 or 200 mM NaCl. The difference in ^{45}Ca content of the aqueous media (high minus low Na^+ concentration) was measured in paired samples obtained after each three back-and-forth transfers of the organic phase. Mean values are expressed as in Fig. 1 and refer to two to four individual experiments, the S.E.M. being shown only for the points most distant from the calculated regression line.

same islet extract had been used alone (Table 2, last column).

DISCUSSION

The present results clearly indicate that gliclazide and diazoxide exert opposite effects upon Ca countertransport in our artificial system. This finding should be considered in the light of the following observations.

First, the present system represents a useful model for the study of such processes as Na–Ca countertransport [12] and Ca–Ca exchange [13] and the influence thereupon of pharmacological agents such as organic calcium antagonists [13–15]. We had observed previously that hypoglycemic sulfonylureas are able to translocate Ca into [5] or across [16] the organic mixture in the absence of any gradient of monovalent cations. Diazoxide exerts no obvious effect upon calcium transport in the absence of a gradient of monovalent cations [16]. In the present study, the effect of hypoglycemic sulfonylureas and diazoxide alone or in combination with either the antibiotic ionophore A23187 or native ionophores extracted from pancreatic islets was tested in the presence of a Li^+ or Na^+ gradient because such a situation made it possible to reveal the opposite effects of gliclazide (or tolbutamide) and diazoxide, respectively.

Second, in pancreatic islet cells, like in other tissues [17], a process of Na–Ca countertransport is thought to participate in the uphill extrusion of Ca from the cell against the prevalent electrochemical gradient [18]. It could be argued, therefore, that the stimulation by gliclazide or such a process cannot account for the stimulatory effect of gliclazide upon ^{45}Ca net uptake by islet cells. However, other aspects of the ionophoretic effects of gliclazide should be taken into account. On one hand, the modulatory effect of this drug upon Ca transport may also concern the downhill influx of Ca into the islet cells; and indeed, hypoglycemic sulfonylureas stimulate both the downhill transport of Ca in the present system

Table 2. Na–Ca countertransport in the presence of diazoxide (D), gliclazide (G) and an islet extract (E)*

Line	Agent(s)	$b \pm s_b$	N	P	Δ (% of control)
1	D	−0.241 ± 0.123‡	37		
2	G	+0.522 ± 0.087†	37		
3	G + D	+0.889 ± 0.108†	37	vs 2: NS	
4	E + D	+1.324 ± 0.113†	48	vs 5: < 0.01	74.1 ± 3.8† (10)
5	E	+1.907 ± 0.083†	75		100.0 (20)
6	E + G	+2.351 ± 0.121†	48	vs 5: < 0.03	141.6 ± 5.4† (15)
7	E + G + D	+2.882 ± 0.127†	38	vs 6: NS	133.8 ± 7.5† (10)

* The regression coefficients and their sample standard errors ($b \pm s_b$), as derived from the data illustrated in Fig. 3, are given together with the significance of the correlation coefficient and the number of individual measurements (N). Also shown is the significance (P) of differences between mean slopes, taking into account the number of individual experiments in each group. In the last column, the Δ in ^{45}Ca content of the aqueous media (samples 12–16 inclusive) are expressed as percentage of the paired control value found when the same islet extract was used alone; mean values (± S.E.M.) are shown together with the significance of differences between experimental and control values and the number of individual measurements (in parentheses).

† $P < 0.001$.

‡ Not significant.

[16] and the entry of Ca into the islet cells [3]. On the other hand, a stimulation of Na influx may lead to an increase in the cytosolic concentration of Na and, by doing so, cause both mobilization of Ca from intracellular stores [19] and secondary inhibition of Ca outwards transport; and indeed, hypoglycemic sulfonylureas stimulate Na translocation in the organic phase [16] and cause both increased net uptake of ^{22}Na [4] and inhibition of Ca outward transport [20] in the islet cells.

Third, the concentration of tolbutamide or gliclazide added to the organic mixture was not higher than that presumed to be found in the hydrophobic domain of the plasma membrane, taking into account the estimated volume for such a domain (0.6 per cent of the cell volume) and the published values for uptake of sulfonylureas by isolated islets [21, 22]. In these calculations, it was assumed that islet cells are spherical structures with a diameter of 10 μm and a plasma membrane thickness of 10 nm, and that sulfonylureas do not penetrate islet cells beyond the plasma membrane [2]. If these assumptions are correct, the biological material extracted from the islets, although being considerably diluted in the organic mixture, was exposed to a concentration of sulfonylurea of the same order of magnitude as that present in the plasma membrane of intact cells when the latter are exposed to incubation media containing the drug at the same concentration as that used in most *in vitro* studies [7, 8, 21, 22].

Last, there was a striking analogy between the present results and the effects of drugs upon ^{45}Ca uptake and insulin release in isolated islets, these processes being stimulated by gliclazide and inhibited by diazoxide [20, 23]. Moreover, when the two sulfonamides were used in combination, the effect of diazoxide was masked by that of gliclazide. Likewise, hypoglycemic sulfonylureas abolish the inhibitory effect of diazoxide upon glucose-stimulated insulin release [24]. In view of these analogies and the abovementioned considerations, it is tempting to speculate that the interference of hypoglycemic and hyperglycemic sulfonylureas with the transport of cations, as mediated by native ionophores, represents a primary effect of these drugs in the islet cells and, hence, accounts for their insulinotropic action.

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REFERENCES

1. A. Loubatières, *Acta diabet. lat.* **6**, (suppl. 1), 20 (1969).
2. B. Hellman and I. B. Täljedal, in *Insulin* (Part 2) (Eds. A. Hasselblatt and F. V. Bruchhausen), p. 175–194. Springer, Berlin (1976).
3. E. Couturier and W. J. Malaisse, *Diabetes*, submitted for publication.
4. S. Kawazu, A. Sener, E. Couturier and W. J. Malaisse, *Naunyn Schmiedeberg's Archs pharmac.* in press.
5. W. J. Malaisse, A. Sener, A. Herchuelz, J. C. Hutton, G. Devis, G. Somers, B. Blondel, F. Malaisse-Lagae and L. Orci, *Excerpta med. I. C. S.* **413**, 95 (1977).
6. W. J. Malaisse, E. Couturier and I. Valverde, *Proc. R. Soc. Med.*, in press (1980).
7. W. J. Malaisse, A. Herchuelz, J. Levy and A. Sener, *Biochem. Pharmac.* **26**, 735 (1977).
8. W. J. Malaisse, A. Sener, G. Devis and G. Somers, *Horm. Metab. Res.* **8**, 434 (1976).
9. I. Valverde and W. J. Malaisse, *Biochem. biophys. Res. Commun.* **89**, 386 (1979).
10. W. J. Malaisse, I. Valverde, G. Devis, G. Somers and E. Couturier, *Biochimie,* **61**, 1185 (1979).
11. W. J. Malaisse, K. Anjaneyulu, R. Anjaneyulu and E. Couturier, *Molec. cell. Biochem.*, in press (1980).
12. W. J. Malaisse and E. Couturier, *Nature, Lond.* **275**, 664 (1978).
13. E. Couturier and W. J. Malaisse, *J. inorg. Biochem.* **12**, 57 (1980).
14. W. J. Malaisse, G. Devis and G. Somers, *Experientia* **33**, 1035 (1977).
15. W. J. Malaisse, *Experientia* **35**, 1578 (1979).
16. E. Couturier and W. J. Malaisse, *Arch. int. Pharmacodyn. Thér.*, in press.
17. M. P. Blaustein, *Rev. Physiol. Biochem. Pharmac.* **70**, 33 (1974).
18. R. D. G. Milner and C. N. Hales, in *The Structure and Metabolism of the Pancreatic Islets* (Eds. S. Falkmer, B. Hellman and I. B. Täljedal), pp. 489–493. Pergamon Press, Oxford (1970).
19. A. Herchuelz and W. J. Malaisse, *J. Physiol., Lond.* **302**, in press (1980).
20. W. J. Malaisse, D. G. Pipeleers and M. Mahy, *Diabetologia* **9**, 1 (1973).
21. B. Hellman, J. Sehlin and I. -B. Täljedal, *Biochem. biophys. Res. Commun.* **45**, 1384 (1971).
22. B. Hellman, Å. Lernmark, J. Sehlin and I. -B. Täljedal, *Fedn. Eur. biochem. Soc. Lett.* **34**, 347 (1973).
23. F. Malaisse-Lagae and W. J. Malaisse, *Endocrinology* **88**, 72 (1971).
24. G. R. Brisson and W. J. Malaisse, *Can. J. Physiol. Pharmac.* **49**, 536 (1971).