Hypothesis

Insulinotropic Effects of Hypoglycaemic and Hyperglycaemic Sulphonamides: The Ionophoretic Hypothesis

E. Couturier and W. J. Malaisse

Laboratory of Experimental Medicine, Brussels Free University, Brussels, Belgium

Summary. Hypoglycaemic sulphonamides stimulate net uptake of $^{45}\text{Ca}^{++}$ and insulin release in isolated pancreatic islets. These effects are antagonized by organic calcium-antagonists (e.g. suloctidil). In an artificial system, hypoglycaemic sulphonamides, such as gliclazide, stimulate the translocation of calcium into or across a hydrophobic immiscible domain, a process enhanced by the antibiotic ionophore A 23187 and antagonized by suloctidil. In this artificial system, the A 23187-mediated process of calcium countertransport is stimulated by gliclazide and inhibited by diazoxide. It is postulated that the insulinotropic action of hypoglycaemic and hyperglycaemic sulphonamides is primarily attributable to the ionophoretic action of these drugs.

Key words: Hypoglycaemic sulphonylureas, diazoxide, calcium, insulin release, ionophores, organic calcium antagonists.

Hypoglycaemic sulphonylureas stimulate insulin release from the pancreatic B-cell. The mechanism of such an insulinotropic effect is poorly understood [1]. Within the framework of current concepts concerning the cytophysiology of insulin release [2], three primary sites of action could be proposed to account for the insulinotropic action of sulphonylureas: a facilitation of nutrient metabolism, an interference with cyclic AMP synthesis or breakdown, or a remodelling of ionic fluxes in the B-cell.

Biochemical studies, reviewed elsewhere [1, 3], do not support the first of these 3 hypotheses.

Although hypoglycaemic sulphonylureas were occasionally claimed to activate adenylate cyclase [4] and repeatedly found to inhibit phosphodiesterase

[5–7] in islet homogenates, the relevance of the latter finding may be questioned since sulphonylureas apparently do not penetrate the B-cell beyond the plasma membrane [1]. A drug-induced increase in the cyclic AMP content of intact islets could be secondary to a cytosolic accumulation of Ca^{2+} [8].

The third hypothesis, namely that of a primary action of hypoglycaemic sulphonylureas upon ionic movements, may involve two distinct mechanisms. First, sulphonylureas may cause a decrease in K^{+} conductance [9, 10] leading to subsequent cell depolarization and gating of voltage-dependent Ca^{2+} channels; however, under suitable conditions, tolbutamide is able to stimulate Ca^{2+} influx and insulin release without decreasing K^{+} conductance [11]. Second, sulphonylureas may directly interfere with an ionophoretic modality of Ca transport across the B-cell plasma membrane [12–15]. This hypothesis has recently been scrutinized in great detail [16–18].

The present report documents some selected experimental findings in support of the ionophoretic hypothesis.

Methodological Considerations

Insulin release was measured in groups of 8 pancreatic islets, removed from fed female albino rats and incubated for 60 min at 37°C in a bicarbonate-buffered solution [19]. The net uptake of $^{45}\text{Ca}^{2+}$ by the islets was measured over 90 min incubation, followed by repeated washes of the islets in order to remove extracellular radioactivity [20].

Two methods were used to characterize the ionophoretic capacity of sulphonylureas and other drugs. In the first method, a small volume (0.2 ml) of a Hepes-NaOH buffer (25 mmol/l; pH 7.0) containing Na^{+} 125, K^{+} 5 and Cl^{-} 120 mmol/l and ^{45}Ca

Fig. 1. Effect of gliclazide (62 μmol/l) and suloctidil (5 μmol/l) upon insulin release (upper panel; *n*: 13) and ⁴⁵Ca net uptake (middle panel; *n*: 39–57) by isolated islets incubated in the presence of 5.6 mmol/l glucose. The translocation of calcium from an aqueous Hepes buffer (25 mmol/l; pH 7.0) containing Na⁺ 125, K⁺ 5 and Cl⁻ 120 mmol/l and ⁴⁵Ca (1.4 μmol/l) into an organic phase (toluene/butanol, 7/3, v/v) containing, as required, gliclazide alone (3.0 mmol/l) or in combination with suloctidil (0.3 mmol/l) is expressed as the concentration of calcium of the organic phase (lower panel; *n* = 3). The stimulant action of gliclazide and the inhibitory effect of suloctidil upon gliclazide-stimulated processes were highly significant (*P* < 0.001) in all cases. Note that the molar ratio of gliclazide/suloctidil (10–12) was comparable in the experiments performed with pancreatic islets and the artificial system, respectively

(1–10 μmol/l) was vigorously mixed for 1 min at room temperature with an equal volume of a mixture of toluene/butanol (7/3, v/v). An aliquot (0.1 ml) of the supernatant immiscible organic phase was then examined for its radioactive content by liquid scintillation [21]. In the second method [22], 0.15 ml of an organic mixture (toluene/butanol, 7/3, v/v) containing A 23187 (0.1 mmol/l) and, as required, gliclazide (16 mmol/l) or diazoxide (22 mmol/l) 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 mmol/l; pH 7.0) containing 0.2 mmol/l CaCl₂ (⁴⁰Ca and

Fig. 2. Effect of gliclazide and the antibiotic ionophore A23187 upon calcium translocation into an organic phase. The initial aqueous phase consisted of a Hepes buffer (25 mmol/l; pH 7.0) containing Na⁺ 125, K⁺ 5 and Cl⁻ 120 mmol/l and ⁴⁵Ca (10 μmol/l). The initial organic phase consisted of a toluene/butanol mixture (7/3, v/v). Gliclazide was added to the aqueous phase and A23187 to the organic phase, at the stated concentration. The Ca concentration of the final organic phase is shown as a function of the concentration of gliclazide. The slope of the regression line (*b*) was much higher (*P* < 0.001) in the presence of A23187 (upper line) than in its absence (lower line). Note the change in both elevation and relative scale of the 2 segments of the ordinate axis

⁴⁵Ca) and either 2 or 200 mmol/l LiCl. After each transfer, the aqueous medium and organic phase were vigorously mixed for 1 min. Samples were removed from the aqueous media after each two back-and-forth transfers of the organic phase, and examined for their radioactive content. In these experiments, a Li⁺ gradient was used because A 23187 is able to mediate Li-Ca but not Na-Ca countertransport [22].

All results are expressed as the mean (± SEM) together with the number of individual observations. The statistical significance of differences between mean values was estimated by use of Student's *t* test.

Stimulation by Hypoglycaemic Sulphonylureas of Ca Entry and Net Uptake in Isolated Islets

Figure 1 illustrates that gliclazide, like other hypoglycaemic sulphonylureas [3, 23], stimulates ^{45}Ca net uptake and insulin release from isolated islets. The time course [3, 23] and dose-action relationship [24] for the sulphonylurea-induced increase in ^{45}Ca net uptake, as well as its modulation by the ambient concentration of glucose, coincide with the time course and magnitude of the drug-induced increment in insulin release.

The capacity of hypoglycaemic sulphonylureas to stimulate Ca entry into the islet cells is documented by the observation that these agents provoke a dramatic and immediate increase in ^{45}Ca efflux from prelabelled islets and also by the abolition of this effect in the absence of extracellular Ca^{2+} [25], indicating that it corresponds to a process of Ca-Ca exchange between the influent ^{40}Ca and effluent ^{45}Ca [26]. The mechanism by which hypoglycaemic sulphonylureas stimulate Ca influx into the islet cells is the major theme of the present work.

Ionophoretic Effects of Hypoglycaemic Sulphonylureas

As illustrated in Figure 2, gliclazide is able to translocate Ca^{2+} from an aqueous solution into an organic phase chosen to mimic the hydrophobic domain of the plasma membrane. Further studies reported in detail elsewhere [16] allowed us to characterize the ionophoretic effect of hypoglycaemic sulphonylureas as follows. Over a much wider range of concentrations than that used in Figure 2, the amount of Ca^{2+} translocated into the organic phase was proportional to the square of the drug concentration, suggesting a 2/1 stoichiometry for the interaction between Ca^{2+} and the sulphonylureas. At increasing concentrations of Ca^{2+} in the initial aqueous phase, the phenomenon of Ca^{2+} translocation appeared as a saturable phenomenon. It was inhibited in a dose-related fashion by increasing concentrations of Na^+ or H^+ . Tolbutamide and gliclazide also provoked Na^+ translocation into the organic phase, the amount of Na^+ translocated being proportional to the drug concentration [16].

All hypoglycaemic sulphonamides examined for such a purpose (i.e., tolbutamide, glycodiazin, gliclazide, glisoxepide, glipizide and glibenclamide) were able to translocate Ca^{2+} , whereas nonhypoglycaemic derivatives of either gliclazide or glipizide were unable to do so. Hypoglycaemic sulphonylureas were also able to provoke the translocation of ^{45}Ca from one aqueous medium into another across the

organic mixture [16]. The concentrations of sulphonylureas used in these experiments were not higher than those presumed to be found in the phospholipid domain of the B-cell plasma membrane when pancreatic islets are exposed to the same extracellular concentration of the drug as that used in most in vitro studies, this estimation taking into account the reported values for the uptake of hypoglycaemic sulphonylureas by isolated islets [1].

Combined Effect of Hypoglycaemic Sulphonylureas and Antibiotic Ionophores

The effect of hypoglycaemic sulphonylureas in causing Ca translocation into the organic phase is relatively modest at near-physiological Ca^{2+} concentration in the aqueous phase [16]. This is due to a relatively low affinity of hypoglycaemic sulphonylureas, so that saturation of their ionophoretic capacity (i.e. translocation of one Ca atom for each two molecules of drug present in the organic phase) requires much higher Ca^{2+} concentrations [16].

The gliclazide-induced increment in Ca translocation is dramatically enhanced when the drug is used in combination with the antibiotic ionophore A 23187 (Fig. 2). Such a cooperativity between distinct ionophoretic molecules, which is not an exceptional phenomenon [27], represents an important feature of the present hypothesis. Indeed, the sulphonylureas may be better able to affect Ca transport across membrane systems in the B-cell when acting in synergism with native ionophores [28]. Moreover, when hypoglycaemic sulphonylureas and other ionophoretic molecules (e.g., A 23187 or X 537 A) are used in combination, the transport of divalent cations is affected preferentially relative to that of monovalent cations. For instance, the combined use of X537A and gliclazide, which are both able to translocate Na and Ca into the organic phase when used separately, results in a synergistic effect upon the translocation of Ca, but not that of Na [17]. This dissociated behaviour is not surprising, since the translocation of Na, as distinct from that of Ca, corresponds to a 1/1 stoichiometry for the interaction between Na and either X537A or gliclazide [16, 29].

Inhibition by Organic Calcium-Antagonists of Gliclazide's Action

Organic calcium-antagonists such as verapamil, D600, nifedipine and suloctidil inhibit insulin release evoked by a variety of secretagogues such as glucose, α -ketoisocaproate, Ba^{2+} and extracellular Ca^{2+} in high concentrations [30–36]. The primary mode of action of the calcium-antagonists is to inhibit Ca^{2+}

entry into the islet cells [37]. The finding that verapamil also abolishes A23187-induced insulin release led us to postulate that the organic antagonist interacts with a Ca^{2+} -binding site common to both A23187 and the native ionophore, located at the B-cell membrane [38]. The latter concept was indeed validated, in the artificial system, using either A23187 [39] or native ionophoretic material extracted from isolated islets [28]. Further analogies between the artificial system and intact cells include a competitive protective effect of Ca^{2+} itself against the inhibitory action of verapamil [40] and the existence of a significant rank correlation between the relative efficiency of chemically-related drugs to inhibit Ca translocation into the organic phase and Ca^{2+} -dependent biological processes, respectively [41].

With this knowledge in mind, the fact that organic calcium-antagonists also inhibit ^{45}Ca net uptake and insulin release evoked by hypoglycaemic sulphonylureas in isolated islets [37, 42] is not surprising and, at first glance, does not throw much light on the mode of action of the hypoglycaemic agents. However, the finding illustrated in Figure 1 that suloctidil, in addition to inhibiting ^{45}Ca net uptake and insulin release in the islets, also inhibits gliclazide-mediated Ca translocation in the artificial system reveals a direct interference of the organic calcium-antagonist with the ionophoretic effect of gliclazide. Such a direct interference may explain why organic calcium-antagonists, at a given concentration, are better able to inhibit ^{45}Ca net uptake in islets exposed to hypoglycaemic sulphonylureas than in islets stimulated with glucose [37, 42].

Further experiments have indicated that the dose-action relationship for the inhibitory effect of suloctidil upon gliclazide- or tolbutamide-mediated Ca translocation displays the same features as those found with other ionophores [16], e.g., A23187. This similarity is important, since the dose-action relationship, when examined at various Ca^{2+} concentrations, allows the demonstration of competition between Ca^{2+} and its antagonist for a common site on the ionophoretic molecule [41].

Opposite Effects of Hypoglycaemic and Hyperglycaemic Sulphonamides upon Ionophore-mediated Calcium Transport

The data so far presented in this report refer to the ionophoretic capacity of hypoglycaemic sulphonylureas. The hyperglycaemic sulphonamide diazoxide, which inhibits ^{45}Ca net uptake and insulin release in isolated islets [20], exerts no major effect upon ^{45}Ca translocation in the two phase-system, whether in the presence or absence of antibiotic ionophores [16,

17]. The capacity of diazoxide to translocate Ca across the organic phase in the three phase-system is also negligible compared to that of gliclazide or tolbutamide [16, 18].

Opposite effects of hypoglycaemic and hyperglycaemic sulphonylureas, respectively, upon ionophore-mediated calcium transport were observed, when investigating the influence of these agents upon Na-Ca or Li-Ca countertransport. In this countertransport process, Ca^{2+} is transported by ionophores against its own chemical gradient and across the organic phase, provided that a suitable ion (Na^+ , Li^+) is distributed asymmetrically, so that the downhill flux of Na^+ or Li^+ is coupled with the uphill transport of Ca^{2+} in the opposite direction [22, 43]. Hypoglycaemic sulphonylureas are themselves able to mediate such a countertransport [18]. Diazoxide is unable to do so and does not affect sulphonylurea-mediated countertransport [18]. Gliclazide acts synergistically with A23187 in the process of countertransport, whereas diazoxide inhibits A23187-

Fig. 3. Effect of gliclazide and diazoxide upon A23187-mediated Li-Ca countertransport. At time zero, 0.3 ml of a triethanolamine-HCl buffer (20 mmol/l; pH 7.0) containing 0.2 mmol/l CaCl_2 (^{40}Ca and ^{45}Ca) and either 2 mmol/l (descending curves) or 200 mmol/l (ascending curves) LiCl was placed in each of two chambers. An organic immiscible phase (0.2 ml; toluene/butanol, 7/3, v/v) containing A23187 (0.1 mmol/l) and, as required gliclazide (16 mmol/l) or diazoxide (22 mmol/l) was transferred back-and-forth from one chamber to the other and thoroughly mixed for 1 min with the aqueous medium. Samples were removed from the aqueous media after each two back-and-forth transfers of the organic phase to establish their Ca^{2+} concentration. Mean values ($\pm$ SEM) refer to 3 separate experiments in each case. Note that gliclazide squares facilitated and diazoxide (open circles) inhibited Li-Ca countertransport, relative to the control values found with A23187 alone (closed circles)

mediated countertransport (Fig. 3). When both sulphonamides are used in combination with A23187, the results are not significantly different from those seen when only gliclazide is present together with A23187 [18]. Comparable results were obtained when native ionophores extracted from the islets were used instead of A23187 in this system [18].

These data add considerable support to the ionophoretic hypothesis here under consideration. They indeed reveal that the well known opposite effects of hyperglycaemic and hypoglycaemic sulphonylureas, respectively, upon ^{45}Ca net uptake [20, 23], ^{45}Ca efflux [25] and insulin release can be mimicked in the artificial system. Moreover, there are similarities with intact B-cells [44] where the inhibitory effect of diazoxide is suppressed and the stimulant action of gliclazide remains unaffected when both sulphonamides are used in combination.

Conclusion

The data presented in this report suggest that the ionophoretic capacity of hypoglycaemic and hyperglycaemic sulphonamides may play an important role in the expression of their insulinotropic action. Studies using more sophisticated models of artificial membranes are in progress in order to characterize further the influence of these drugs upon ion transport. Meanwhile, it is proposed that these drugs affect calcium movements in the B-cell, and possibly other organs (e.g. heart and platelets; see [45, 46]), by interference with the process of calcium transport as mediated by native ionophores.

Acknowledgements. We thank Mrs. M. Mahy and C. Demesmaecker for technical assistance and secretarial help. This work was supported by grants from the Belgian Foundation for Scientific Medical Research.

References

- Hellman B, Täljedal I-B (1975) Effects of sulphonylureas derivatives on pancreatic B-cells. In: Hasselblatt A, Bruchhausen Ev (eds) *Insulin II*. Springer, Berlin Heidelberg New York, p 175–194
- Malaisse WJ, Sener A, Herchuelz A, Hutton JC (1979) Insulin release: the fuel hypothesis. *Metabolism* 28: 373–386
- Kawazu S, Sener A, Couturier E, Malaisse WJ (in press) Metabolic, cationic and secretory effects of hypoglycaemic sulphonylureas in pancreatic islets. *Naunyn Schmiedebergs Arch Pharmacol*
- Levey GS, Schmidt WMI, Mintz DH (1972) Activation of adenylylase in a pancreatic islet cell adenoma by glucagon and tolbutamide. *Metabolism* 21: 93–98
- Ashcroft SJH, Randle PJ, Täljedal I-B (1972) Cyclic nucleotide phosphodiesterase activity in normal mouse pancreatic islets. *FEBS Lett* 20: 263–266
- Sams DJ, Montague W (1972) The role of adenosine 3':5'-cyclic monophosphate in the regulation of insulin release. Properties of islet-cell adenosine 3':5'-cyclic monophosphate phosphodiesterase. *Biochem J* 129: 945–952
- Goldfine ID, Perlman R, Roth J (1971) Inhibition of cyclic 3', 5'-AMP phosphodiesterase in islet cells and other tissues by tolbutamide. *Nature* 234: 295–297
- Valverde I, Vandermeers A, Anjaneyulu R, Malaisse WJ (1979) Calmodulin activation of adenylate cyclase in pancreatic islets. *Science* 206: 2225–2237
- Boschero AC, Malaisse WJ (1979) The stimulus-secretion coupling of glucose-induced insulin release. XXIX. Regulation of $^{86}\text{Rb}^+$ efflux from perfused islets. *Am J Physiol* 236: E139–E146
- Henquin JC (1980) Tolbutamide stimulation and inhibition of insulin release: studies of the underlying ionic mechanisms in isolated rat islets. *Diabetologia* 18: 151–160
- Malaisse WJ, Carpinelli AR, Herchuelz A (in press) Tolbutamide stimulates Ca^{2+} influx in islet cells without reducing K^+ conductance. *Diabetologia*
- Malaisse WJ, Sener A, Herchuelz A, Hutton JC, Devis G, Somers G, Blondel B, Malaisse-Lagae F, Orci L (1977) Sequential events in the process of glucose-induced insulin release. *Excerpta Medica Int Congr Series* 413: 95–102
- Malaisse WJ, Devis G, Hutton JC, Somers G (1977) Effect of insulinotropic agents on Ca^{2+} translocation in an artificial system. *Diabetes* 26: 407
- Hoek, JC, Corkey BE, Rich TL, Scarpa A, Williamson JR (1978) Sulphonylureas-induced changes in calcium permeability of biological membranes. *Diabetes* 27: 481
- Corkey BE (1978) Calcium transport properties of the hypoglycaemic sulphonylureas. *Fed Proc* 37: 1543
- Couturier E, Malaisse WJ (in press) Ionophoretic activity of hypoglycaemic sulphonylureas. *Arch Int Pharmacodyn*
- Couturier E, Malaisse WJ (in press) Synergistic effects of hypoglycaemic sulphonylureas and antibiotic ionophores upon calcium translocation. *Br J Pharmacol*
- Anjaneyulu R, Anjaneyulu K, Couturier E, Malaisse WJ (in press) Opposite effects of hypoglycaemic and hyperglycaemic sulphonylureas upon ionophore-mediated calcium transport. *Biochem Pharmacol*
- Malaisse WJ, Brisson G, Malaisse-Lagae F (1970) The stimulus-secretion coupling of glucose-induced insulin release. I. Interaction of epinephrine and alkaline earth cations. *J Lab Clin Med* 76: 895–902
- Malaisse-Lagae F, Malaisse WJ (1971) Stimulus-secretion coupling of glucose-induced insulin release. III. Uptake of ^{45}Ca by isolated islets of Langerhans. *Endocrinology* 88: 72–80
- Malaisse WJ, Valverde I, Devis G, Somers G, Couturier E (1979) Ionophore-mediated cation translocation in artificial systems. I. A23187-mediated calcium translocation. *Biochimie* 61: 1185–1192
- Malaisse WJ, Anjaneyulu K, Anjaneyulu R, Couturier E (1980) Ionophore-mediated Ca^{2+} countertransport: role of Na^+ , Li^+ or H^+ gradient. *Mol Cell Biochem* 30: 67–70
- Malaisse WJ, Mahy M, Brisson GR, Malaisse-Lagae F (1972) The stimulus-secretion coupling of glucose-induced insulin release. VIII. Combined effects of glucose and sulphonylureas. *Eur J Clin Invest* 2: 85–90
- Malaisse WJ, Sener A, Herchuelz A (1979) Effects of insulinotropic agents on cationic fluxes in islet cells. In: *Treatment of early diabetes*. Camerini-Davalos RA, Hanover G (eds) Plenum Press, New York, p 85–96
- Malaisse WJ, Pipeleers DG, Mahy M (1973) The stimulus-secretion coupling of glucose-induced insulin release. XII. Effects of diazoxide and gliclazide upon ^{45}Ca efflux from perfused islets. *Diabetologia* 9: 1–5

26. Herchuelz A, Couturier E, Malaisse WJ (1980) Regulation of calcium fluxes in pancreatic islets: glucose-induced calcium-calcium exchange. *Am J Physiol* 238: E96–E103
27. Couturier E, Deleers M, Malaisse WJ (in press) Synergism between distinct ionophores. *Pharmacol Res Commun*
28. Valverde I, Malaisse WJ (1979) Ionophoretic activity in pancreatic islets. *Biochem Biophys Res Commun* 89: 386–395
29. Couturier E, Malaisse WJ (1980) Ionophore-mediated cation translocation in artificial systems. II. X537A-mediated calcium and sodium translocation. *Biochimie* 62: 177–180
30. Devis G, Somers G, Van Obberghen E, Malaisse WJ (1975) Calcium-antagonists and islet function. I. Inhibition of insulin release by verapamil. *Diabetes* 24: 547–551
31. Malaisse WJ, Devis G, Pipeleers DG, Somers G (1976) Calcium-antagonists and islet function. IV. Effect of D600. *Diabetologia* 12: 77–81
32. Somers G, Devis G, Van Obberghen E, Malaisse WJ (1976) Calcium-antagonists and islet function. VI. Effect of barium. *Pflügers Arch* 365: 21–28
33. Malaisse WJ (1977) Calcium-antagonists and islet function. X. Effect of suloctidil. *Arch Int Pharmacodyn* 228: 339–344
34. Malaisse WJ, Boschero AC (1977) Calcium-antagonists and islet function. XI. Effect of nifedipine. *Horm Res* 8: 203–209
35. Devis G, Somers G, Malaisse WJ (1975) Stimulation of insulin release by calcium. *Biochem Biophys Res Commun* 67: 525–529
36. Devis G, Somers G, Malaisse WJ (1977) Dynamics of calcium-induced insulin release. *Diabetologia* 13: 531–536
37. Malaisse WJ, Herchuelz A, Levy J, Sener A (1977) Calcium-antagonists and islet function. III. The possible site of action of verapamil. *Biochem Pharmacol* 26: 735–740
38. Somers G, Devis G, Malaisse WJ (1976) Analogy between native and exogenous ionophores in the pancreatic B-cell. *FEBS Lett* 66: 20–22
39. Malaisse WJ, Devis G, Somers G (1977) Inhibition by verapamil of ionophore-mediated calcium translocation. *Experientia* 33: 1035–1036
40. Malaisse WJ (1979) Protective effect of calcium against the verapamil-induced inhibition of ionophore-mediated calcium translocation. *Experientia* 35: 1578–1579
41. Malaisse WJ, Somers G, Valverde I, Couturier E (in press) Organic calcium-antagonists and calcium-ionophores. *Arzneimittelforsch*
42. Malaisse WJ, Sener A, Devis G, Somers G (1976) Calcium-antagonists and islet function. V. Effect of R33711. *Horm Metab Res* 8: 434–438
43. Malaisse WJ, Couturier E (1978) Ionophoretic model for Na-Ca countertransport. *Nature* 275: 664–665
44. Brisson GR, Malaisse WJ (1971) Insulinotropic effect and possible mode of action of a new potent sulfonylurea (BS-4231). *Can J Physiol Pharmacol* 49: 536–544
45. Linden J, Brooker G (1978) The positive insulinotropic action of sulfonylureas. A mechanism independent of cyclic adenosine 3', 5'-monophosphate. *Diabetes* 27: 694–698
46. Verry M, Bryon PA, Dechavanne M, Lagarde M, Vainer H (1975) Etude des phénomènes biochimiques du métabolisme plaquettaire. Le gliclazide, agent régulateur de la physiologie plaquettaire. *J Pharmacol Clin* 2: 199–208

Received: May 19, 1980

W. J. Malaisse
Laboratory of Experimental Medicine
Brussels Free University
115 Bvd. de Waterloo
B-1000 Brussels
Belgium