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Interaction of sulfonylurea with the pancreatic B-cell

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Key words: Pancreatic B-cell; interaction of sulfonylurea.

Introduction

Although hypoglycemic sulfonylureas may have several effects which are beneficial for the diabetic patient, there is no doubt that their ability to stimulate insulin release is an essential property[49, 64]. This insulinotropic capacity has previously been reviewed[32, 44, 56, 63] and is discussed in this article with emphasis on such mechanisms of action as are thought to be shared by various sulfonylurea derivatives with vastly different potencies. Attention is paid to how the insulin-releasing actions relate to the binding of sulfonylureas to B-cells and to the ensuing effects on metabolism and ion fluxes in these cells.

General aspects of the effects of sulfonylureas and related analogues

As covered in more detail by previous reviews, both first and second generation sulfonylureas can stimulate insulin release in the absence of glucose but are more effective as potentiators of glucose-initiated secretion. The detailed dynamics of the secretory response differs between various drugs, but rapidity of onset is a general characteristic. The immediate response of the B-cell to an acute challenge with sulfonylurea is significantly faster than the response to a sudden increase of glucose from basal to stimulatory concentrations. This difference is a reason for assuming that sulfonylureas act on a distal sequence of events in the physiological signal chain in the B-cell.
Cyclic AMP is in general an intracellular messenger effecting potentiation of insulin secretion in the presence of some initiator. The fact that sulfonylureas can raise the islet cyclic AMP level may therefore contribute to their potentiating action[21]. Whether the effect on cyclic AMP is due to the phosphodiesterase inhibiting properties of sulfonylureas is questionable in the light of data suggesting a poor ability of the drugs to enter into the B-cells (see below). There is an intricate inter-

relationship in the B-cell between Ca^{2+} and cyclic AMP[75]. As sulfonylureas influence Ca^{2+} as part of their action, the changes in cyclic AMP may be indirect consequences of more primary effects on the plasma membrane and ion fluxes.

The nature of the interaction of sulfonylureas with the B-cell plasma membrane to produce fast secretory responses is poorly understood. This matter will be discussed below in relation to experiments with cells and artificial membrane systems. Assuming that the in vivo hypoglycemic activity in rats reflects the insulin-releasing potencies of various sulfonylureas and related [(acylamino)alkyl] benzoic acids, Brown and Foubister[9] proposed a molecular model for the interaction of active drugs with the B-cell plasma membrane. The membrane site signalling secretion was suggested to be sensitive to the acidic SO_2NHCO group of sulfonylurea and to the similarly spaced COOH group of the non-sulfonylurea analogue HB 699. Related drugs with other substituents on the neighboring aromatic ring were ineffective, unless, as in the case of a methyl derivative, they could be metabolized to a carboxylic acid in vivo.

In an attempt to establish a unifying picture of stimulus-secretion coupling for glucose and sulfonylureas, it has previously been considered whether the drugs may interfere with membrane-located sulfhydryl groups of importance for the secretory signal chain[37,42]. According to the basic hypothesis[35,74], insulin release is favored by any mechanism that decreases the likelihood of two vicinal sulfurs forming a disulfide bridge in an ion-gating membrane protein. Nucleophilic interactions between membrane-located sulfur and the drug-presented carboxyl carbon or sulfonylurea sulfur are easily envisaged. However, in the experiments of Brown and Foubister[9] acetyl was an ineffective substitute for carboxyl or sulfonylurea groups. Nucleophilic interactions therefore seem unlikely, and the data favor the idea that the acidic nature of the substituent group is essential. The effect of introducing such a group in the vicinity of any receptor sulfur is difficult to assess. At a physiological pH, when the effective groups are partly dissociated, such an event can be expected to increase the electron density of the micro-environment, which might counteract oxidation of the membrane sulfurs and thus stimulate secretion. Perhaps the role of the aromatic ring carrying the acidic groups in all effective drugs of this type is to provide an anchor that helps to overcome the electrostatic repulsive forces inherent in such a local increase in electron density.

Sulfonylurea binding to the B-cells

The amounts of tolbutamide taken up by the pancreatic islets are small in comparison to the uptake by liver cells, which are permeable to the sulfonylureas[48]. It was early shown that the distribution volume for tolbutamide in isolated pancreatic islets only slightly exceeds that for extracellular space markers[39,67]. These and other observations indicate that sulfonylureas stimulate insulin release by binding to the surface of the B-cells. Although other studies[29,41,73] have established that some hypoglycemic sulfonylureas are distributed in apparent volumes exceeding the islet water space, there is indirect evidence that these compounds also stimulate insulin release by interacting with the plasma membranes.

Figure 1 shows how different sulfonylureas are taken up in excess of the extracellular (sucrose) space in micro-dissected pancreatic islets. Evidently, both the first (upper panel) and second (lower panel) generation of sulfonylureas bind rapidly to the islets. There was no direct correlation between the potency of the individual drug and its ability to bind to the islets[43]. The potent second generation sulfonylurea glipizide was found to be incorporated to a similar extent as tolbutamide. Glibenclamide was exceptional among the sulfonylureas in not rapidly reaching uptake equilibrium but accumulating progressively in substantial amounts. The unusual binding characteristics of glibenclamide are also reflected in a protracted retention of the drug during washing of the islets in media lacking sulfonylureas. The amino reagent 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS), a strong inhibitor of the anion channels in the B-cell membrane, has been found to decrease the islet uptake of glibenclamide[35,38]. It is therefore possible that the progressive uptake of glibenclamide, after the initial binding, reflects permeation through anion channels. An intracellular appearance of glibenclamide might explain why prolonged exposure to this sulfonylurea has been found to result in functionally deficient B-cells[6,66].

The islet uptake of sulfonylureas is markedly suppressed in the presence of protein. The binding of glibenclamide, for example, was reduced by 65% when 0.5 mg/ml albumin was included in the incubation medium. In the presence of 5 mg/ml albumin the corresponding reduction of binding exceeded 90%. Various drugs, known to augment or prolong the insulin-releasing action of the sulfonylureas, increased the islet uptake at the expense of the binding to albumin[30]. Evidence for such a translocation of albumin-bound sulfonylurea to the islets is shown in figure 2. It can be seen that concentrations of phenylbutazone, lacking effects on the islet binding of glibenclamide in an albumin-free medium, significantly enhanced the amounts bound to the islets in medium containing 0.5 mg/ml albumin.

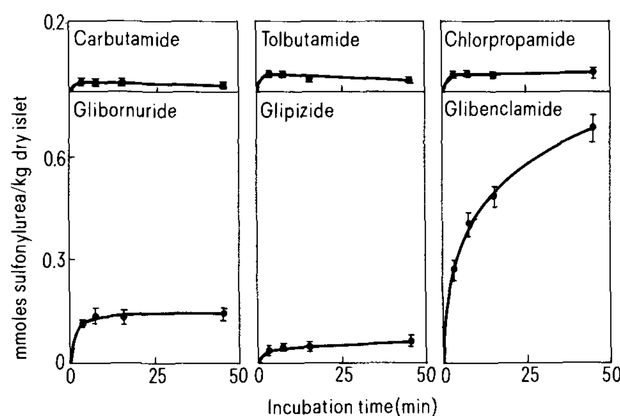

Figure 1. Islet uptake of sulfonylurea with time. The islets were exposed for various periods of time in an albumin-free medium to 20 µM radioactive sulfonylurea and 0.1 M sucrose. Glipizide was ^{14}C-labelled and the other sulfonylureas were in a tritiated form. The graphs indicate the islet content of sulfonylurea in excess of the sucrose space. Mean values ± SEM. Reproduced with permission from Hellman et al.[43].

With the demonstration of uptake characteristics for sulfonylureas indicating that the insulin-releasing action of these drugs is due to interaction with the periphery of the B-cells, it is important to consider the mechanisms for their binding to the plasma membrane. The complexity of the sulfonylurea molecule allows different types of binding[33]. In addition to ion-ion, ion-dipole, dipole-dipole, and van der Waals-London dispersion interactions with the acid sulfonamide group, there are also hydrophobic interactions with the non-polar moieties. The significance of the latter type of binding is apparent from the fact that large hydrophobic end groups can enhance the insulin-releasing potency. Current ideas of how the sulfonylureas bind to the B-cell are schematically illustrated in figure 3. In the proposed model attention is paid both to the fact that the sulfonylureas at physiological pH have a negative net charge and to the fact that there is an increased binding of such sulfonylureas which have large non-polar groups. The sulfonylurea binding to the B-cells can be assumed to result essentially from hydrophobic interactions counteracted by electrostatic repulsion from fixed negative charges at the cell surface. Shielding of the fixed negative charges should allow a stronger hydrophobic interaction, an idea compatible with the observation that cations increase the islet binding of sulfonylurea in a charge-dependent manner[42]. It is implicit from the model that sulfonylureas, if not permeating anion channels like possibly glibenclamide, are restricted to the outer half of the lipid bilayer.

Different sulfonylureas compete for binding sites in plasma membranes. These interactions have been analyzed systematically by using crude membrane fractions from rat brain and a B-cell tumor[50]. It was found that the binding of the sulfonylurea gliquidone involves a saturable component, an observation supposed to indicate the presence of a specific membrane receptor for sulfonylureas. However, it is noteworthy that the criteria adopted for specific binding are fulfilled also when sulfonylureas interact with phospholipid bilayers in an artificial system[19]. This observation raises serious doubts about the existence of specific sulfonylurea binding to the B-cell.

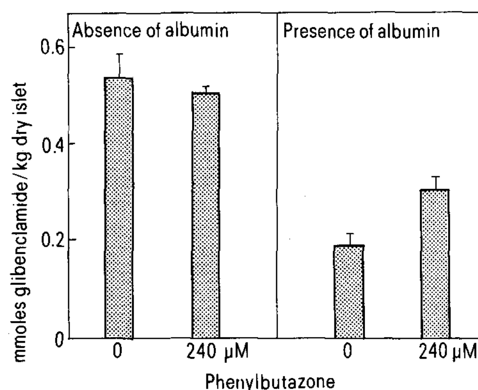

Figure 2. Islet uptake of glibenclamide in the presence and absence of albumin and phenylbutazone. The islets were incubated for 60 min with 20 µM ^{14}C-labelled glibenclamide and 0.1 M tritiated sucrose in the presence and absence of 240 µM phenylbutazone and 0.5 mg/ml albumin. The bars illustrate the incorporation of glibenclamide in excess of the sucrose space. Mean values ± SEM.

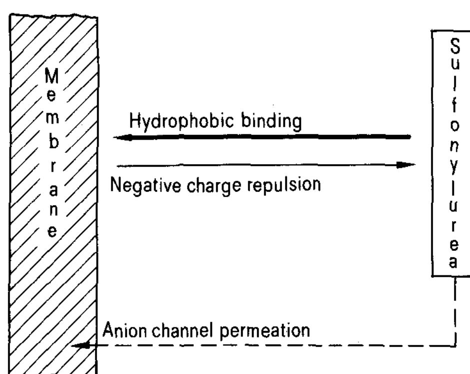

Figure 3. Model illustrating how hypoglycemic sulfonylureas bind to the β-cell membrane. In the case of glibenclamide there is possibly, in addition to superficial binding, a slow permeation through anion channels in the plasma membrane. Reproduced with permission from Hellman[32].

Sulfonylurea effects on nutrient metabolism

The possibility that hypoglycemic sulfonylureas stimulate insulin release by interacting with the nutrient metabolism of the B-cells has been considered in a number of studies. A problem in relating the insulinotropic effect to metabolic parameters is that the secretory response is very prompt, whereas substantial incubation periods are often required to detect metabolic changes. For example, it is difficult to decide whether sulfonylurea-induced oxygen consumption[72] is related to the initiation process or reflects the energy requirements of insulin discharge. Since the secretory response to sulfonylureas is more rapid than that to glucose[13,20], it is difficult to accept the proposal that secretion is stimulated by glycogen-derived intracellular glucose after release of amyloglucosidase from lysosomes labilized by sulfonylurea[55]. Furthermore, there is no convincing evidence that sulfonylureas increase the overall glucose metabolism in the pancreatic slets. Tolbutamide has been reported to stimulate[3], inhibit[51], or lack[4,51] effect on the rate of glucose oxidation and/or utilization. Glucose degradation has also been found to be unaffected by gliclazide and glibenclamide[4,25,51]. When other metabolic pathways were studied, tolbutamide had no effect on the oxidation of glutamine[56], palmitate or pyruvate[51], and glibenclamide did not influence the oxidation of leucine or alanine[40] or the concentrations of endogenous aspartic acid, γ-aminobutyric acid, glutamic acid, glycine, α-ketoglutarate, or leucine[15,25]. After pre-labelling with ^{14}C-glucose or ^{14}C-glutamine, the oxidation of endogenous substrates was not affected by glibenclamide[52].

The enzyme glutamate dehydrogenase has been envisaged as a site where regulatory molecules can influence B-cell metabolism and insulin release[23,28,71]. Among the sulfonylureas, carbutamide activates this enzyme[23,28], tolbutamide, glipizide and gliclazide lack effect[23,56], and glibenclamide is even an inhibitor[23]. However, it is not only the divergent actions that make glutamate dehydrogenase an unlikely mediator of sulfonylurea-stimu-

lated secretion but also the fact that the drugs do not seem to enter the B-cells when stimulating insulin release.
It is obviously difficult to find noteworthy metabolic effects related to sulfonylurea-induced initiation of insulin secretion. Nevertheless, one cannot entirely rule out the possibility that the drugs affect potential coupling factors such as NAD(P)H or ATP. Several reports indicate that sulfonylureas decrease, rather than increase, the B-cell content of ATP after incubation for 15 min or more[5,34,51]. However, no acute effects were observed in freeze-stop experiments after 30 sec[53]. Similarly, Panten[63] did not find any direct effects on the NAD(P)H fluorescence of intact islets. Kawazu et al.[51] even reported a decrease after 30 min, suggesting that metabolic changes may be a consequence, rather than a cause, of insulin release.

Sulfonylurea effects on B-cell membrane potential and transport of monovalent ions

Matthews and Dean[59] reported that tolbutamide depolarizes the B-cells. Later work provided a more complete picture in showing that glibenclamide[60] and tolbutamide[47,61] evoke electrical depolarization as well as spike activity in 3 mM glucose. The activity induced by tolbutamide is suppressed by diazoxide, which also hyperpolarizes the B-cells[47]. In a glucose-free medium, concentrations of tolbutamide below 74 μM slightly depolarized the B-cells without giving rise to spike activity[47].
Studies on ion fluxes in isolated islets have been performed to elucidate the electric and secretory effects of sulfonylureas. Tolbutamide had no effect on $^{22}Na^+$ uptake in brief incubations[51]. It is therefore doubtful whether a change in Na^+ permeability is involved in the drug action. It is generally thought that the glucose-induced depolarization of the B-cells involves modulation of the K^+ permeability[7,45,69,70]. Tolbutamide at high concentrations (0.4–0.7 mM) diminished the efflux of $^{86}Rb^+$ (K^+ analogue) in a monophasic manner at 3 mM glucose[8,46,47]. In 6 mM glucose, however, 74 μM tolbutamide transiently enhanced the $^{86}Rb^+$ efflux, while 0.4 mM of the drug induced a more sustained increase[47]. Glibenclamide (1 μM) temporarily diminished the $^{86}Rb^+$ efflux in glucose-free medium (Norlund, Lindström and Sehlin, unpublished work). A secondary rise of $^{86}Rb^+$ efflux in the presence of glibenclamide, as well as the increased efflux with tolbutamide in 6 mM glucose, may be due to Ca^{2+} uptake and activation of Ca^{2+}-dependent K^+ channels[46].
The uptake of $^{86}Rb^+$ by islets is also inhibited by tolbutamide or glibenclamide. In particular, the initial uptake of $^{86}Rb^+$, representing the rate of K^+ influx, was reduced by 0.07–0.7 mM tolbutamide[47,51] or by 10 nM–0.2 mM glibenclamide (Norlund, Lindström and Sehlin, unpublished work). Effects on net accumulation after longer periods of incubation are conflicting. Thus, in one study 0.7 mM tolbutamide had no effect[51], but in another study 0.07 or 0.4 mM tolbutamide was moderately inhibitory[47]. $^{86}Rb^+$ accumulation was not affected by 0.01–10 μM glibenclamide but decreased at the very high concentration of 200 μM (Norlund, Lindström and Sehlin, unpublished work). Analysis of the interaction between glibenclamide and ouabain showed that the whole effect of 0.1 μM glibenclamide on $^{86}Rb^+$ influx in *ob/ob* mouse islets was on the ouabain-resistant (1 mM ouabain) part, whereas no effect was found on the ouabain-sensitive part reflecting the Na^+/K^+ pump (Norlund, Lindström and Sehlin, unpublished work). It has been suggested that tolbutamide-induced depolarization of the B-cells is due to a decrease in K^+ permeability[46,61] that triggers voltage-dependent Ca^{2+} influx and insulin release[31,46,51]. The data on glibenclamide seem to be in accord with a similar chain of events. Glibenclamide increases the rate of $^{36}Cl^-$ influx in mouse islets[68]. This effect may be relevant for the observed secretory interactions between glibenclamide and SITS, a blocker of anion transport[38].
New evidence for direct effects of glibenclamide on the B-cell membrane has recently been obtained. Microscopic measurements of isolated, living B-cells in suspension showed that glibenclamide increases the osmotic resistance of the B-cells, i.e. the drug counteracted the swelling induced by a hypo-osmolar medium[62]. This effect may be due to increased membrane permeability (see below) and/or to a more specific action of glibenclamide in enhancing a mechanism for volume regulation based on K^+ extrusion (Norlund, Lindström and Sehlin, unpublished work). It is so far not clear how the B-cell volume regulation is related to electrogenic K^+ fluxes and insulin release.

Sulfonylurea action on Ca^{2+} fluxes in intact cells

The requirement for extracellular Ca^{2+} in sulfonylurea-induced insulin release is well established[14]. The drugs promote Ca^{2+} influx into the B-cells, as suggested by depolarization and the appearance of action potential-like spikes[16,61] and verified by an increased ^{45}Ca uptake[36,57]. The rapidly initiated sulfonylurea-stimulated ^{45}Ca uptake, which decays with time, fulfills the criteria of Ca^{2+} influx through voltage-dependent channels subject to progressive inactivation[46]. Only sulfonylureas with insulin-releasing effects promote the entry of Ca^{2+} (Hellman[31]). Moreover, the stimulation of Ca^{2+} uptake appears to have some specificity for the pancreatic B-cells. Concentrations of tolbutamide which significantly enhance ^{45}Ca uptake into isolated pancreatic islets have no effect on the posterior pituitary or adrenal medulla[26]. Like other insulin secretagogues with stimulatory effects on Ca^{2+} entry into B-cells, sulfonylureas increase the efflux of radioactivity from islets preloaded with ^{45}Ca[31,54,58]. This effect resembles that on insulin release in being prompt and dependent on extracellular Ca^{2+} but is not simply the result of exocytosis of calcium in the B-cell granules. In analogy with the action of other depolarizing agents, the stimulation of ^{45}Ca efflux by sulfonylureas can be expected to result from increased entry of non-radioactive Ca^{2+} exchanging with the ^{45}Ca in intracellular stores[31]. The sulfonylurea action on ^{45}Ca efflux from isolated islets differs from that of glucose in lacking the inhibitory component that is unmasked by lowering the extracellular Ca^{2+} concentration. In a Ca^{2+}-deficient medium sulfonylurea neither inhibits (like glu-

cose) nor stimulates (like carboxylic Ca^{2+} ionophores) the efflux of ^{45}Ca (fig. 4). It is therefore likely that the sulfonylurea-induced increase of cytosolic Ca^{2+} initiating insulin release reflects an enhanced membrane permeability to the ion without much involvement of intracellular Ca^{2+} stores.

In the exploration of the Ca^{2+} movements induced by sulfonylureas advantage has been taken of a clonal cell line (RINm5F) established from a transplantable rat islet tumor. The relevance of this preparation for studying sulfonylurea effects on Ca^{2+} fluxes became evident with the demonstration that these cells respond with stimulated ^{45}Ca efflux during perifusion[1]. With access to large amounts of RINm5F cells it has been possible to measure net fluxes of calcium by monitoring the concentration in a suspension medium containing micromolar concentrations of the ion. At such low concentrations the net uptake of Ca^{2+} was unaffected by opening of the potential-dependent channels following from depolarization of the RINm5F cells[24]. In figure 5 the effect of tolbutamide on the net uptake of Ca^{2+} has been evaluated by dual wavelength recordings of the metallochromic indicator arsenazo III. In support for the absence of a primary action of sulfonylureas on the intracellular distribution of Ca^{2+}, tolbutamide did not influence the net uptake of Ca^{2+} when this process was stimulated by glucose, and the carboxylic ionophore A-23187 mobilized Ca^{2+}.

In view of the depolarizing effects of sulfonylureas it is not surprising that these compounds favor the entry of Ca^{2+} through voltage-dependent channels. However, it has also been suggested that the increased inflow of

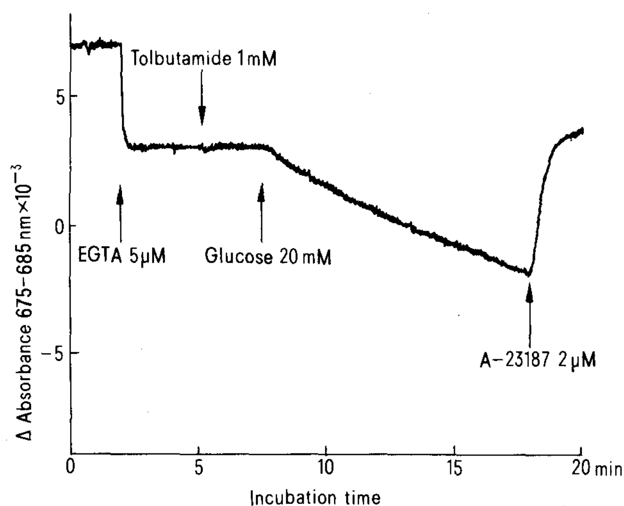

Figure 5. Effects of tolbutamide, glucose and A-23187 on the net fluxes of Ca^{2+}. The RINm5F cells were suspended in 1 ml medium at a concentration of 6.4 mg protein/ml. The medium, which was buffered at pH 7.4 with 25 mM Hepes, contained 20 µM arsenazo III, 20 µM phenol red and at the beginning of the experiment 22 µM Ca^{2+} as determined by EGTA titrations. Whereas the absorbance difference 499–525 nm of phenol red was used to clamp pH at 7.4 by additions of NaOH, the wavelength pair 675–685 nm was utilized to monitor continuously the Ca^{2+} activity of the medium with the aid of arsenazo III.

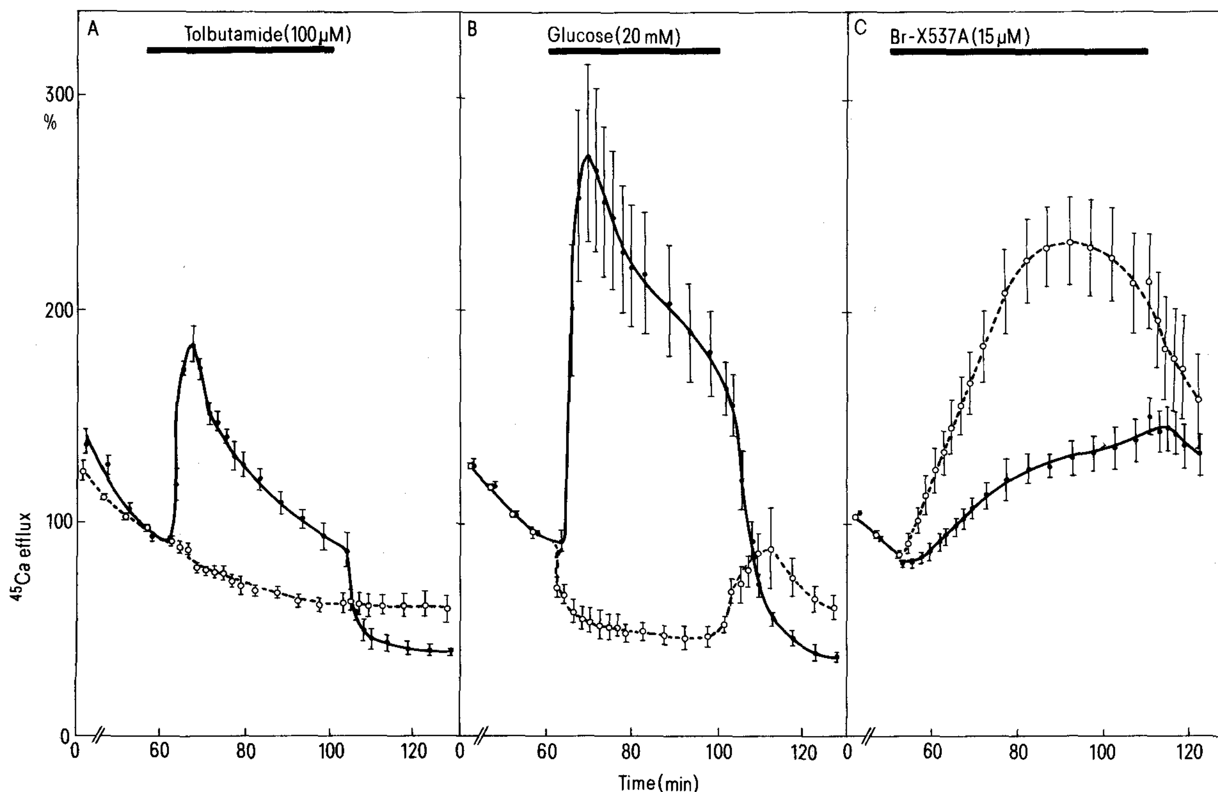

Figure 4. Effects of tolbutamide, glucose and the Ca^{2+} ionophore bromolasalocide on ^{45}Ca efflux from isolated islets. The islets were perifused in medium containing 1.28 mM Ca^{2+} (●) or deficient in Ca^{2+} and supplemented with 0.5 mM EGTA (○). During the period indicated by the horizontal black bar 100 µM tolbutamide (A), 20 mM glucose (B) or 40 µM bromolasalocide (C) was introduced into the perifusion medium. The efflux of ^{45}Ca is shown as the percentage of that recorded in the individual experiment during the 10-min-period preceeding the modification of the medium composition. Mean values ± SEM.

Ca^{2+} into the B-cells reflects the ability of the drugs to mediate exchange diffusion similar to that induced by carboxylic Ca^{2+} ionophores[10,11,17]. The studies of how the hypoglycemic sulfonylureas affect the Ca^{2+} fluxes in intact cells provide no support for an ionophoretic action of the compounds. The observation that gliclazide slightly potentiates the effect of the ionophore A-23187 in mediating the outflow of ^{45}Ca from islets in a Ca^{2+}-deficient medium[56] might be a phenomenon related to unspecific labilization of the plasma membrane (see below). Major arguments against the ionophore hypothesis are that the sulfonylurea-stimulated ^{45}Ca efflux depends not only on extracellular Ca^{2+} but also on K^+ (Hellman[33]) and that even very high concentrations of sulfonylurea do not stimulate the process more than other depolarizing agents[31].

Sulfonylurea action on Ca^{2+} fluxes in artificial systems

The ionophore hypothesis for the mechanism of action of hypoglycemic sulfonylureas has been tested in various model systems. One of these models involves translocation of ^{45}Ca from an aqueous phase into an immiscible organic phase of toluene/butanol during vigorous shaking[10]. In a modification of the technique, ^{45}Ca translocation from one aqueous phase to another was studied by shaking with a common organic phase. In these systems tolbutamide, gliclazide and glipizide were reported to mediate exchange diffusion in a manner similar to that of A-23187[11]; the potency of the sulfonylureas increased in the presence of the antibiotic ionophore[12]. Sulfonylureas also potentiated the ability of an islet extract to facilitate exchange diffusion[2].

Some criticism can be directed against the above-mentioned techniques as valid models of the situation in B-cells. For example, it is unclear whether similar results would have been obtained if the sulfonylureas had been added to one of the aqueous phases of a Pressman cell conventionally used for defining ionophores[65]. The observations were made after addition of the sulfonylureas to the organic phase at concentrations much higher than those required for maximum stimulation of insulin release. It might be argued that there is an accumulation of the drugs in the plasma membrane of the B-cells[43], justifying the use of such high concentrations. However, the possibility that the conformational state of the sulfonylureas differ between a toluene/butanol phase and a highly structured phospholipid bilayer must not be overlooked. As discussed above, most sulfonylureas seem to be restricted to the outer water/lipid interface of the B-cell plasma membrane and never enter the interior of the membrane. The ionophore-like properties in toluene/butanol will therefore probably be absent in a lipid bilayer except, possibly, when the drugs are mixed with the lipid before the formation of the bilayer. A serious objection against the ionophore hypothesis is the lack of correlation between the ionophoretic capacity of the sulfonylureas in toluene/butanol and their insulinotropic potency[11].

The lipid bilayer in the form of liposomes is another model which has been utilized in the testing of the ionophore hypothesis of sulfonylurea action. With this model it was shown that liposomes prepared from lipid containing gliclazide released ^{45}Ca more rapidly than liposomes lacking the sulfonylurea[17]. Liposomal internalization of Pr^{3+} was also shown after addition of 0.6–2 mM glibenclamide or gliclazide to a liposomal suspension[18]. Such concentrations of glibenclamide are much higher than those required for maximum stimulation of insulin release and are not even soluble at physiological pH. It is therefore noteworthy that the pH was as high as 9 during the addition of the sulfonylureas. The observed translocation of cations is not necessarily due to ionophoresis but may rather reflect instability of the liposomal preparation. Considerably lower concentrations of glibenclamide have been shown to labilize membranes, as indicated by the release of the 'stability marker' 5,6-carboxyfluorescein from liposomes[27] and of B-glucuronidase and acid phosphatase from a lysosomal fraction of pancreatic islets[22]. As a matter of fact, measurements of the net release of Ca^{2+} from liposomes containing 100 mM $CaCl_2$ provided direct evidence against the ionophore hypothesis[27]. Although it was possible to detect exchange diffusion by A-23187 with the utmost sensitivity, even very high concentrations of tolbutamide (fig. 6) or glibenclamide lacked ionophoretic action.

Exchange diffusion has also been studied in a chromaffin granule preparation[26]. Being essentially impermeable to H^+ and Ca^{2+} at appropriate experimental conditions, these granules are ideal for studies of ionophoretic actions. The acid interior of the granules can be utilized to

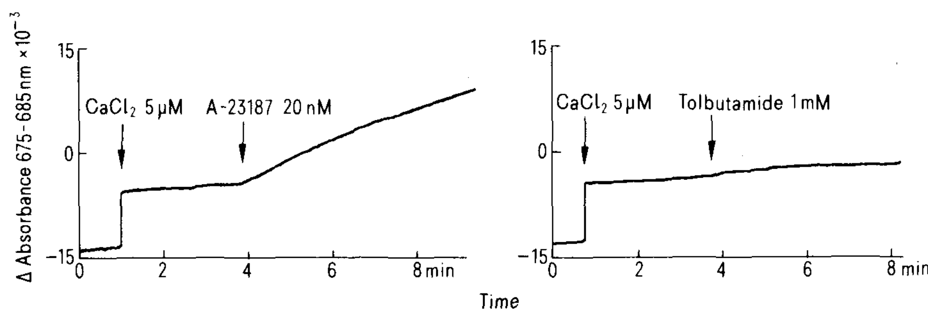

Figure 6. Effect of tolbutamide on Ca^{2+} efflux from liposomes. The cuvette, which was thermostated at 35 °C, contained 975 μl of 255 mM sucrose, 30 mM Tris-maleate (pH 7.0), 20 μM arsenazo III and 25 μl liposomes loaded with 100 mM $CaCl_2$ and 0.1 mM Tris-maleate (pH 7.0). The absorbance difference 675–685 nm of arsenazo III was used to indicate variations in the Ca^{2+} concentration of the medium. For calibration purposes the experiments were started by the addition of Ca^{2+}. The effect of tolbutamide was evaluated in relation to that of the Ca^{2+} ionophore A-23187. Reproduced with permission from Gylfe et al.[27].

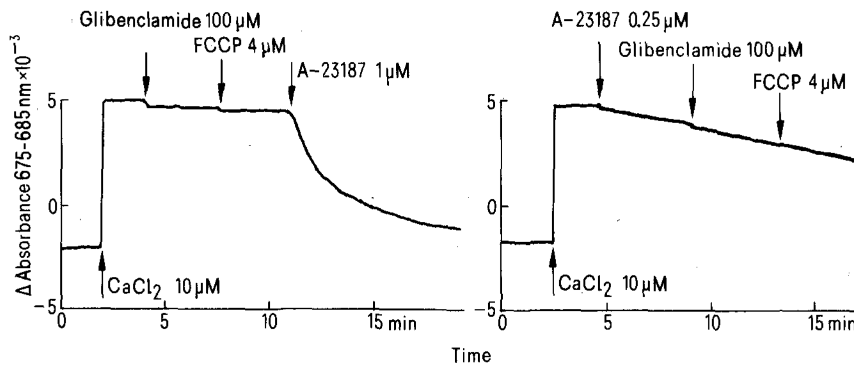

Figure 7. Effect of glibenclamide on net Ca^{2+} fluxes in chromaffin granules. The cuvette contained 250 μl of 270 mM sucrose, 30 mM Tris-maleate (pH 7.0), 20 μM arsenazo III, and chromaffin granules (protein 7.1 mg/ml) The absorbance difference at 675–685 nm of arsenazo III was used to indicate variations in the Ca^{2+} concentration of the medium. The left panel shows the concentration of Ca^{2+} in the medium after the addition of glibenclamide alone or in combination with FCCP. The rigth panel indicates the corresponding data in medium supplemented with A-23187. Reproduced with permission from Gylfe and Hellman[26].

drive Ca^{2+} uptake by Ca^{2+}/H^+ exchange diffusion mediated by A-23187. In this system neither tolbutamide nor glibenclamide (fig. 7) had any effect on the net fluxes of Ca^{2+} whether the sulfonylurea was tested alone or in combination with the protonophore FCCP. Moreover, the sulfonylureas did not affect the ionophoretic properties of A-23187. In order to test the possibility that sulfonylureas are neutral ionophores or channel-forming quasi-ionophores the conductance of a 'black' lipid membrane was measured in medium containing Na^+, K^+, Mg^{2+}, Ca^{2+}, and Cl^- (Gylfe et al.[27]). Despite the fact that the sensitivity of this system allowed the detection of single ion channels, there was no measurable change in conductivity upon addition of high concentrations of tolbutamide or glibenclamide.

Conclusions

Sulfonylureas have a variety of effects on pancreatic B-cells. In the present review an attempt has been made to identify those that appear fundamental from a mechanistic point of view and in that sense common to all hypoglycemic drugs tested. On several points the available experimental information is limited. With this reservation in mind, the following general hypothesis is presented for the insulin-releasing action of this class of drugs. Hypoglycemic sulfonylureas and related [(acylamino)alkyl]benzoic acids bind to the B-cell plasma membrane, a step in which hydrophobic anchoring is essential. Dissociated acidic COOH or SO_2NHCO groups in the drugs are thus presented to an ion-gating protein in the plasma membrane, possibly in the vicinity of a pair of sulfur atoms. The reduced state of these sulfurs is promoted, preventing the formation of a disulfide bridge. K^+ permeability is thereby decreased, favoring depolarization of the B-cell and Ca^{2+} influx through voltage-dependent channels. Finally, Ca^{2+} triggers the physiological apparatus for discharge of the insulin secretory granules. The effect of this insulin-releasing signal chain is amplified by cyclic AMP which increases in the B-cell as a consequence of depolarization and Ca^{2+} influx. This hypothesis does not attribute an ionophoretic role to the sulfonylureas per se, because various experiments with cells and artificial membrane systems render such an idea apparently less tenable.

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Stimulus-secretion coupling in the pancreatic B-cell: concluding remarks

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Key words. Pancreatic B-cell; stimulus-secretion coupling.

The pancreatic B-cell may be viewed as a fuel-sensor organ. Thus, its secretory activity is mainly but not exclusively regulated by the level of circulating nutrients, and its main secretory product, insulin, regulates the uptake or release of nutrients in extrapancreatic tissues. The influence of non-nutrient secretagogues, e.g. catecholamines, cholinergic agents or gastrointestinal hormones, upon insulin release in vivo does not detract from such a schematic view. Indeed, the immediate effect of hormones and neurotransmitters upon the pancreatic B-cell allows modulation of nutrient-regulated insulin release at times when the supply or consumption of nutrients are dramatically modified, e.g. during muscular exercise or after food intake.

The functional organization of the B-cell can also be conceived of within the framework of this fuel concept. Thus, changes in the concentration of circulating nutrients are sensed by the B-cell through changes in the rate of nutrients oxidation. Increasing attention should be paid, therefore, to the regulation of metabolic events in islet cells exposed to the heterogenous constellation of circulating nutrients at their physiological concentration[1].

Several coupling factors may be generated by the metabolism of nutrients and affect distal events in the secretory sequence. For instance, changes in redox state, intracellular pH and ATP availability may influence the movements of ions in the islet cells or other cellular events involved in the stimulation of insulin release[2].

It is obvious that glucose and other insulin secretagogues dramatically affect ionic fluxes in the islet cells, this being associated with induction of bioelectrical activity. The precise determinism of the changes in membrane potential and their relevance to the exocytosis of secretory granules remain, however, to be fully elucidated[3].

The use of the fluorescent calcium-indicator quin-2 has recently allowed to validate the concept, already advanced almost 20 years ago, that the stimulation of insulin release usually coincides with in increase in cytosolic Ca^{2+} activity. The regulation of cytosolic Ca^{2+} concentration depends not solely on the net balance between Ca^{2+} influx and efflux across the plasma membrane but also on the sequestration or release of Ca^{2+} by such organelles as the endoplasmic reticulum and mitochondria[4].

The response to a rise in cytosolic Ca^{2+} concentration may be mediated, in part at least, by the Ca^{2+}-binding regulatory protein, calmodulin. Calmodulin as well as calmodulin-binding proteins are present in islet cells and Ca-calmodulin affects the activity of a number of enzymes in islet homogenates or subcellular fractions. However, further studies are required to define the precise role played by calmodulin in the secretory sequence[5].