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REVIEW

New Mechanisms for Sulfonylurea Control of Insulin Secretion

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Oral antidiabetic sulfonylureas like tolbutamide and glyburide have been used to treat patients with noninsulin dependent diabetes mellitus. These agents lower blood glucose by stimulating insulin secretion from the pancreatic islets of Langerhans. A major component of this stimulation is sulfonylurea-mediated closure of the ATP-inhibited potassium channels (K_{ATP} channels) of islet β-cells. Closure of these channels leads to cell depolarization, calcium uptake, and insulin exocytosis. Progress leading up to the recent cloning of the high-affinity sulfonylurea receptor and reconstitution of the K_{ATP} channel is reviewed in this article together with new data showing that sulfonylureas may control secretion by activating a novel chloride ion channel, inhibiting an islet Na/K/ATPase or via distal stimulation of granule exocytosis by a kinase C dependent mechanism.

Key Words: K_{ATP} channels; sulfonylureas; insulin secretion; potassium channels; chloride channels; sulfonylurea receptor.

Introduction

The islets of Langerhans of patients with noninsulin dependent diabetes mellitus (NIDDM) contain insulin-secreting β-cells but these cells secrete abnormally (Porte, 1991). Oral antidiabetic sulfonylureas are used in the treatment of NIDDM, as these drugs lower plasma glucose levels (Pfeifer et al., 1984). Tolbutamide is the prototype "first generation" sulfonylurea, whereas the more lipophilic glyburide is the best known "second generation" sulfonylurea. Although it has been known for years that sulfonylureas increase insulin secretion (Henquin, 1990), the cellular and molecular mechanisms involved have only recently been identified and warrant a brief review of progress made in this area. In addition to the cloning of the high-affinity sulfonylurea receptor (SUR) (Aguilar-Bryan et al., 1995) and reconstitution of the K_{ATP} channel (Inagaki et al., 1995b), major breakthroughs in cellular diabetes research, and additional sites of sulfonylurea action have been identified. We have identified a novel sulfonylurea-regulated chloride conductance in insulin secreting cells, whereas others have found effects of sulfonylureas on other cellular processes that might augment secretion.

Readers interested in comprehensive reviews on sulfonylureas and related topics are directed elsewhere (sulfonylurea therapy: Gerich, 1989; sulfonylurea receptors and their signaling pathways: Boyd et al., 1990; Boyd et al., 1991; Gylfe et al., 1984; Panten et al., 1992; K_{ATP} channels: Ashcroft and Rorsman, 1989, 1995; Dunne and Petersen, 1991; β-cell electrophysiology: Ashcroft and Rorsman, 1995; Ashcroft and Rorsman, 1989; Satin and Smolen, 1994).

Inhibition of ATP-Sensitive Potassium Channels (K_{ATP} Channels) by Glucose Metabolism Triggers Membrane Electrical Activity and Calcium Influx in Pancreatic Islet B-Cells

The membrane potential of pancreatic islet β-cells is controlled by an ATP-sensitive potassium channel (K_{ATP} channel), which couples islet membrane electrical activity to plasma glucose concentration (Ashcroft and Rorsman, 1989, 1995). K_{ATP} is a 50–70 pS, potassium-selective channel that is directly inhibited by intracellular ATP (Cook and Hales, 1984; Rorsman and Trube, 1985; for reviews *see* Ashcroft and Rorsman, 1989, 1995; Dunne and Petersen, 1991).

Cook and Hales (1984) first described the K_{ATP} channels of pancreatic islet β-cells and proposed that they coupled cellular ATP production to membrane depolarization. K_{ATP} channel activity in cell-attached patches was found to be inhibited by glucose metabolism (rat: Ashcroft et al., 1984; Misler et al., 1986; mouse: Rorsman and Trube, 1985; human: Misler et al., 1989), which supported the hypothesis that elevated plasma glucose and concomitantly increased cellular fuel metabolism controlled islet electrical activity via increased cytosolic [ATP]. K_{ATP} channel closure has been found to depolarize the β-cell to

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a threshold voltage near −50 mV, where β-cell Ca channel opening and electrical activity is triggered (Cook et al., 1988; Ashcroft and Rorsman, 1989; Satin and Smolen, 1994). Electrical activity, in turn, causes increased Ca influx, elevated cellular [Ca^{2+}], and increased Ca-dependent exocytosis of insulin granules (Arkhammer et al., 1984; Ashcroft and Rorsman, 1989, 1995; Gillis and Misler, 1992). This "consensus model" is now well-accepted by many workers in the field (*see* Ashcroft and Rorsman, 1995). Importantly, human islets have been shown to also contain K_{ATP} and many other ion channels present in rodent, suggesting that many of the same signaling pathways are probably utilized by human pancreatic islets (Ashcroft et al., 1989; Misler et al., 1989, 1992).

Properties of K_{ATP} Channels and Nucleotide Sensitivity of Channel Gating

β-cell K_{ATP} channels open in clusters or bursts separated by longer closed periods lasting hundreds of milliseconds (Ashcroft and Rorsman, 1989). Channel amplitude exhibited weak inward rectification owing to Mg^{2+} blockade of open channels at positive potentials (Cook and Hales, 1984; Rorsman and Trube, 1985; Findlay et al., 1985).

Nucleotide regulation of K_{ATP} is complex and incompletely understood. The most direct and best understood of the nucleotide effects on K_{ATP} is the inhibitory action of adenosine triphosphate (ATP), adenosine diphosphate (ADP), or adenosine monophosphate (AMP) applied to the cytoplasmic side of inside/out membrane patches (Cook and Hales, 1984). These nucleotides were found to directly inhibit K channel activity in the order ATP>ADP>AMP and did not require nucleotide hydrolysis or complexation with Mg^{2+} since nonhydrolyzable analogs like adenylylimidodiphosphate (AMP-PNP) and Mg^{2+}-free species were effective inhibitors (Ashcroft and Rorsman, 1989, 1995; Dunne and Petersen, 1991).

When complexed with Mg^{2+}, these nucleotides activated K_{ATP}. Thus, Mg-ADP or Mg-GDP increased K_{ATP} channel activity in the presence or absence of ATP (Dunne and Petersen, 1991; Ashcroft and Rorsman, 1995). Stimulation by Mg-ADP occurred at lower doses than ADP-mediated inhibition. Nucleotide-mediated stimulation required the presence of Mg and, unlike direct blockade, was not mimicked by nonhydrolyzable ADP analogs, suggesting a modulatory site distinct from that mediating inhibition (Hopkins et al., 1992).

Besides direct inhibitory, as well as modulatory, nucleotide actions, it is likely that phosphorylation of K_{ATP} occurs, since the number of openable K_{ATP} channels resident in a patch has been found to decrease over tens of seconds following patch excision (Cook and Hales, 1984; Ashcroft and Rorsman, 1989). This "rundown" of activity was at least partially reversed by conditions favoring phosphorylation (reviewed in Ashcroft and Rorsman, 1995; Dunne and Petersen, 1991). Brief application of MgATP or ADP, for instance, often restored K_{ATP} activity following rundown (*see* Dunne and Petersen, 1991; Ashcroft and Rorsman, 1995). It may be that consensus A- and C-kinase phosphorylation sites on the sulfonylurea receptor are involved in the tonic phosphorylation of K_{ATP} (Aguilar-Bryan et al., 1995).

K_{ATP} Channels Are Inhibited by Sulfonylureas

Sulfonylureas inhibited K_{ATP} channels when applied directly to inside/out membrane patches (Sturgess et al., 1984; Trube et al., 1986; Dunne et al., 1987; Gillis et al., 1989). That this inhibition could be observed in "cell free" patches suggested that the K_{ATP} channel either contained a high-affinity sulfonylurea binding site itself or was in close association with it (Sturgess et al., 1984; Zunkler et al., 1988). The exact nature of this relationship was unclear, however, as many studies attempting to addess this issue were inconclusive or conflicting. Thus, it was found that continual passaging of hamster insulinoma tumor (HIT) (Aguilar-Bryan et al., 1992), but not CRG-1 (Khan et al., 1993), insulinoma cells down-regulated the number of high-affinity sulfonylurea receptors and inhibited K_{ATP} channel activity in parallel. The results obtained with HIT cells suggested that K_{ATP} was SUR or tightly associated with SUR. Proks and Ashcroft (1993) found that exposure of K_{ATP} channels to trypsin reduced channel sensitivity to sulfonylureas and ADP, which suggested that a site necessary for SUR regulation of K_{ATP} activity was disrupted by the enzyme. Functional coupling of SUR and K_{ATP} activity was demonstrated by the observation that sulfonylurea binding, inhibition of K_{ATP} channel activity, and suppression of Rb tracer efflux from islets, as well as insulin secretion, all had similar sensitivities to glyburide or tolbutamide and displayed similar rank order of potency among different sulfonylureas (e.g., Schmid-Antomarchi, 1987; Boyd et al., 1991).

Since K_{ATP} inhibition by glucose metabolism may be incomplete in diabetics owing to abnormal metabolic signaling (McDonald, 1995), sulfonylureas might help control plasma glucose by directly blocking K_{ATP} channels and thus circumventing metabolism (Boyd et al., 1991). In support of this, high doses of glucose were found to be unable to completely inhibit K_{ATP} channel activity in streptozotocin-induced NIDDM rats, whereas tolbutamide remained effective (Tsuura et al., 1992). An inability to inhibit even the last 1% of the total K_{ATP} conductance in diabetic islets would interfere with stimulus-secretion coupling, since modeling predicted that over 99% of β-cell K_{ATP} channels must be closed for islets to depolarize (Cook et al., 1988). This seemingly subtle channel regulatory defect can thus account for the insufficient insulin secretion observed in response to glucose in diabetes.

Cloning of SUR, a High-Affinity Sulfonylurea Receptor

Previous studies carried out by several laboratories have characterized the high-affinity sulfonylurea receptor (SUR) as using biochemical techniques (Gaines et al., 1988; Kramer et al., 1988; Aguilar-Bryan et al., 1990, 1992; Nelson et al., 1992), which culminated in the characterization of a 140 kDa protein subsequently shown to be the high-affinity sulfonylurea receptor (Nelson et al., 1992).

Recently, Aguilar-Bryan et al. (1995) cloned rodent SUR. Initial peptide sequence information was obtained with photolabeling to an iodinated glyburide analog and the 140 kDa SUR was isolated from HIT cells. The amino terminal sequence was then determined from protease fragments of SUR. Degenerate primers constructed using the sequences of these fragments allowed PCR amplification of a fragment from an αTC cell glucagonoma cDNA library. An HIT cell cDNA library was then screened to obtained the larger fragment used to obtain full-length SUR cDNA. This sequence coded for a 177-kDa rat or hamster protein (140 kDa on SDS gels) which consisted of 1582 amino acids, with the two rodent sequences displaying 98% identity.

Northern analysis detected SUR mRNA in RIN, HIT, and αTC-6 cell lines and islets. Transient transfection of SUR into COS cells reconstituted specific high affinity sulfonylurea binding to the COS membranes. The K_i's obtained for rat and hamster SUR were 2 and 10 n*M*, respectively, in the range expected from binding studies (Gaines et al., 1988; Aguilar-Bryan et al., 1990, 1992; Nelson et al., 1992). In addition, the rank order of potency for displacement of iodinated glyburide was glyburide> iodoglyburide>tolbutamide, as expected. Hydrophobic analysis of SUR suggested its topology consisted of 13 transmembrane spanning domains and two Walker consensus sequences, the latter representing intracellular nucleotide binding folds (Aguilar-Bryan et al., 1995; Philipson and Steiner, 1995). The significance of these nucleotide binding folds to K_{ATP} function will be discussed later. SUR also contained 20 PKC and 3 PKA consensus phosphorylation sites (Aguilar-Bryan et al., 1995). Sequence homology with other cloned proteins showed SUR is a member of the ATP-binding cassette/traffic ATPase superfamily, a family of over 30 proteins including multiple drug resistance protein (MDR) and the cystic fibrosis transmembrane conductance regulator (CFTR; Hyde et al., 1990).

β-cell K_{ATP} Is an Inward Rectifier K Channel

No potassium channel activity was observed when the SUR gene was expressed alone, which suggested that the sulfonylurea receptor was either separate from the K_{ATP} channel or required additional subunits for functional channel activity (Aguilar-Bryan et al., 1995). A number of inward rectifiers have been shown to exist in islet cells and insulinomas although their role in islet physiology and their relation to native K_{ATP} channels is unclear (Bond et al., 1995a; Ferrer et al., 1995; Inagaki et al., 1995a; Sakura et al., 1995). The resolution of this issue came with the report that co-expression of SUR with a particular islet inward rectifier K channel subunit, Kir 6.2 (or β-cell inward rectifier [BIR]) in COS cells reconstituted K_{ATP} function (Inagaki et al., 1995b). Kir 6.2 was cloned by homology using sequences from another islet inward rectifier (uK_{ATP}-1; Inagaki et al., 1995a) to screen human islet cDNA libraries. The longest clone coded for a 390 amino acid protein with the prototypical structure and sequence homology expected of the inward rectifier family (e.g., ROMK1, IRK1, GIRK1, CIR; Chandy and Gutman, 1993). Both human and mouse Kir 6.2 were isolated and had 96% amino acid identity. Unlike uKATP-1, Kir 6.2 was present in insulin-releasing cells where it colocalized with SUR. Kir 6.2 was found to have a wider tissue distribution than SUR (J. Bryan, personal communication). Fluorescence localization studies showed that the SUR and Kir 6.2 genes were located on adjacent loci on human chromosome 11 (at p15.1), which suggested that the genes encoding these two intimately related proteins may have evolved from a larger ancestral gene. Inagaki et al. (1995b) suggested that the two gene products, Kir 6.2 and SUR, were the two subunits, named KATP-a and KATP-β, respectively, of a heteromultimeric K_{ATP} channel.

Expression of mSUR (mouse) and hKir 6.2 (human) in COS cells revealed a 76 pS, weakly-inward rectifying K channel (with Mg^{2+}_i present) that was blocked by ATP. The K_i for ATP of 10 μ*M* was similar to the 15-μ*M* value originally reported by Cook and Hales (1984) for native ß-cell channels. Furthermore, the channel was inhibited by nonhydrolyzable ATP analogs, 1 m*M* ADP or 0.1 μ*M* glyburide, and activated by diazoxide. Increased efflux of radiolabeled Rb following metabolic poisoning was dose-dependently inhibited by glyburide (K_i = 1.8 n*M*) or tolbutamide (K_i = 32 μ*M*). In the absence of inhibitors, diazoxide stimulated efflux with a K_a of 60 μ*M*, with all of these values being compatible with those from native K_{ATP} channels (Inagaki et al., 1995b). Thus, it was concluded that the β-cell K_{ATP} channel was comprised of both an inward rectifying (α-KATP) subunit Kir 6.2 and a sulfonylurea receptor subunit (β-KATP), with both required for full reconstitution of K_{ATP}. Expression of Kir 6.2 alone produced no channel activity (Inagaki et al., 1995b; Ammala et al., 1996). β-KATP was presumed to confer both adenine nucleotide and sulfonylurea sensitivity to the α/β complex.

Inagaki et al. (1995b) found SUR selectively coupled Kir 6.2. However, coexpression studies by Ammala et al. (1996), using the same Kir 6.2 and SUR transcripts that demonstrated functional KATP channels (Sakura et al., 1995), also found that SUR may "promiscuously" couple to Kir 1.1a/ROMK1 (Ho et al., 1993) or KIR 6.1/u-KATP-1 (Inagaki et al., 1995a) inward rectifier subunits. The basis for this discrepancy is not known, although some of this

"promiscuous coupling" could be owing to endogenous K_{ATP} channels present in untransfected HEK cells rather than heterologously expressed rectifiers (Krapavinsky et al., 1995b; Sui et al., 1996). Ammala et al. (1996) also found that alterations in pipet [ATP] did not alter the endogenous inward rectifier currents of HEK cells and suggested by analogy that SUR may not confer nucleotide sensitivity to K_{ATP} either. While Ammala et al. pointed out that this may not hold for β-cell K_{ATP} channels, manipulating cellular [ATP] by altering pipet [ATP] may not be a stringent test for ATP-sensitivity since efficient dialysis and control of cellular [ATP] may be thwarted by endogenous membrane ion pump activity, residual metabolic machinery present in broken cells, or inadequate diffusion and exchange of ATP through pipet tips.

Initial Attempts to Clone the K_{ATP} Channel

Earlier attempts to clone K_{ATP} were unsuccessful but are briefly summarized here for completeness. Ashford et al. (1994) cloned a putative inward rectifer K_{ATP} channel, rc-KATP from rat heart cDNA using PCR (Ashford et al., 1994). rc-KATP-1 encoded a protein of 417 amino acids with homology to other inward rectifiers. Northern analysis suggested rc-KATP-1 was present in heart and nervous structures but absent from islet cell lines (Ashford et al., 1994). Expression of rc-KATP-1 in HEK or BHK cells was associated with K_{ATP}-like channel activity although rc-KATP-1 was not inhibited by sulfonylureas. A second putative K_{ATP} channel was cloned from insulinoma cDNA and termed KATP-2 because of its strong homology (71% identity) to rc-KATP (KATP-1; Tsaur et al., 1992). KATP-2 was present in brain and HIT cells but not heart (Tsaur et al., 1995) and was found to localize to the human chromosome locus 21q22.1. Tsaur et al. (1995) and Zhang et al. (1995) tested for linkage between KATP-2 and NIDDM, MODY, or GDM forms of diabetes but obtained only negative results.

Krapivinsky et al. (1995) subsequently showed that rc-KATP-1 or KATP-1 was not a K_{ATP} channel but a subunit of the acetylcholine-gated K channel of heart ($I_{K(ACh)}$; Krapivinsky et al., 1995a). Earlier studies identified GIRK1 as the clone encoding the K(ACh) channel but GIRK1 did not completely reconstitute all of the characteristics expected of K(ACh) (Krapivinsky et al., 1995a). Krapivinsky et al. (1995a) found that GIRK-1 was closely associated with a 45-kDa protein they named CIR (cardiac inward rectifier), which was nearly identical (only differing by two amino acids) to rc-KATP. Coexpression of CIR and GIRK1 completely reconstituted K(ACh) activity, whereas CIR expressed alone encoded brief channel-like events that did not resemble any native ion channel (Krapivinsky et al., 1995a). Krapivinsky et al. (1995) concluded that CIR was not a native channel or related to K_{ATP} channels. Instead, they suggested that CIR and GIRK1 were two subunits of a heteromultimeric complex mediating $I_{K(ACh)}$. Further work showed that CIR was not a component of cardiac K_{ATP} (Krapivinsky et al., 1995b). In addition, K_{ATP} channel activity was found even in untransfected control HEK cells, suggesting that the K_{ATP} activity ascribed to rc-KATP-1 by Ashford et al. (1994) might have been mediated by endogenous K_{ATP} channels (Krapivinsky et al., 1995b). Ashford et al. (1995) subsequently retracted their paper based on their inability to reproduce their initial findings (Ashford et al., 1996).

Analysis of SUR Mutations in PHHI Provides Evidence that SUR Is the K_{ATP} Nucleotide Sensor

The identification of two nucleotide binding domains within SUR suggested that this subunit might mediate the nucleotide sensitivity of K_{ATP} channels. Nucleotides binding to SUR would thus regulate K_{ATP} channel activity via interactions between SUR and Kir 6.2.

In support of this hypothesis, Thomas et al. (1995) found that SUR localized to human chromosome 11p15.1, a locus previously shown to be the gene for persistent hyperinsulinemic hypoglycemia of infancy (or PHHI), a rare autosomal recessive disorder characterized by abnormally high and unregulated insulin secretion despite hypoglycemia. Analysis of SUR nucleotide sequences from PHHI patients disclosed mutations in the second nucleotide binding fold (or NBF2), which caused premature truncation and disruption of NBF2, in essence eliminating NBF2 (*see* Aguilar-Bryan and Bryan, 1996). No SUR mRNA was detected in pancreatic tissue from these affected children. Thomas et al. (1995) suggested that loss of function mutations in NBF2 were the cause of familial PHHI as they resulted in little or no SUR expression. If SUR loss resulted in loss of functional K_{ATP} channel activity, PHHI islets would be persistently depolarized. This would account for their excessive, and unregulated, insulin secretion (Thomas et al., 1995). Indeed, when Kir 6.2 was coexpressed with SUR mutated in vitro to resemble the mutant SUR of PHHI patients, no K_{ATP} channel activity or metabolically-dependent Rb efflux was observed (Clement et al., unpublished; cited in Aguilar-Bryan and Bryan, 1996).

Nichols et al. (1996) also identified a point mutation, G1479R, in SUR from PHHI patients, which was distinct from the PHHI truncation mutants studied by Thomas et al. (1995) but which also resided in NBF2. The effect of this mutation on K_{ATP} function was determined by in vitro coexpression of hamster SUR containing G1479R with Kir 6.2 in COS cells. Wild-type K_{ATP} channels activated in response to diazoxide or metabolic poisoning while G1479R mutants showed activity in response to diazoxide but not in response to metabolic poisoning. Diazoxide-activated PHHI K_{ATP} channels had normal pore properties and ATP sensitivity but, unlike wild-type K_{ATP}, MgADP failed to antagonize ATP in these mutant channels. In contrast,

mutations engineered in NBF1 had normal MgADP sensitivity (Nichols et al., 1996).

Dunne et al. (1995) obtained islet β-cells from four neonates undergoing pancreatectomy as treatment for PHHI. Despite hypoglycemic conditions, no K_{ATP} channel activity was observed in cell-attached patches. Significantly, the cells displayed spontaneous electrical activity, elevated resting $[Ca^{2+}]_i$ and calcium oscillations even under basal conditions. In excised patches, abnormal islet K channels were observed that had a conductance of 20 picosiemens (pS) and were activated by ATP, ADP, somatostatin, and diazoxide. Philipson et al. (1996) reported that PHHI islets displayed voltage-sensitive currents but no K_{ATP} currents and were persistently depolarized.

These data provide direct and compelling evidence suggesting that SUR couples changes in cytoplasmic nucleotide concentration to K_{ATP} channel activity, as originally proposed by Aguilar-Bryan et al. (1995), and that mutations in the second nucleotide binding fold of SUR underlie PHHI by disrupting nucleotide sensing. In addition, cytoplasmic MgADP rather than ATP appears to be a crucial metabolic coupling factor (Nichols et al., 1996). In summary, the successful reconstitution of K_{ATP} in vitro, the correlation between loss of K_{ATP} activity and either naturally occurring SUR mutations in PHHI or engineered SUR mutations mimicking PHHI strongly supports the view that K_{ATP} is a heteromultimer of two subunits, Kir 6.2 and SUR, with SUR functioning as the sulfonylurea receptor and nucleotide sensor of the channel complex (Inagaki et al., 1995; Aguilar-Bryan and Bryan, 1996).

Sulfonylureas Also Activate an Islet Cell Cl Channel, $I_{Cl,islet}$

Islet β-cells have a Cl^- channel ($I_{Cl,islet}$) that is modulated by glyburide (Kinard and Satin, 1995). As for Cl^- channels of other systems, $I_{Cl,islet}$ was also activated by cell swelling (Kinard and Satin, 1995; Best et al., 1996) or cAMP (Kinard and Satin, 1995). $I_{Cl,islet}$ showed strong outward rectification, showed selectivity among anions, and was blocked by the Cl^- channel blockers DIDS (Kinard and Satin, 1995), niflumic acid and NPPB (Kinard and Satin, 1996). Interestingly, 1 μ*M* glyburide activated $I_{Cl,islet}$ in HIT cells (Kinard and Satin, 1995) and rat β-cells (L. Best, personal communication), whereas higher doses (100 μ*M*) inhibited $I_{Cl,islet}$ (Kinard and Satin, 1995). Glyburide activation would be expected to depolarize islets since, at negative membrane potentials, $I_{Cl,islet}$ mediated an inward current (Satin and Kinard, 1995; Best et al., 1996). Activation of $I_{Cl,islet}$ may contribute to islet depolarization (Britsch et al., 1994) and increased insulin secretion observed in response to hypotonic solutions (Blackard et al., 1975). Preactivation of $I_{Cl,islet}$ by 1 μ*M* glyburide might also account for the improved "osmotic resistance" observed in response to hypotonic conditions (Norlund and Sehlin, 1984).

The selectivity sequence (Br>Cl>I), cAMP activation, and glyburide block of $I_{Cl,islet}$ are all properties shared with CFTR, a Cl channel with significant homology to SUR (Aguilar-Bryan et al., 1995; Aguilar-Bryan and Bryan, 1996). However, unlike $I_{Cl,islet}$, CFTR showed little outward rectification and was insensitive to DIDS or cell swelling, suggesting CFTR was unlikely to underlie most of $I_{Cl,islet}$. The sulfonylurea sensitivity of $I_{Cl,islet}$ suggested the channel may be coupled to a sulfonylurea receptor. This may be another example of promiscuity on the part of SUR (Ammala et al., 1996), although the higher concentrations needed for $I_{Cl,islet}$ activation suggest a sulfonylurea receptor with lower affinity (Aguilar-Bryan et al., 1992). The K_{ATP} channels of heart cells, for example, have been shown to be less sensitive to sulfonylureas as compared to β-cell K_{ATP} channels (Ripoll et al., 1992), an indication that there may be a family of SURs with different sulfonylurea affinities in nature, although this remains to be established.

Sulfonylureas Inhibit the Electrogenic Na/K/ATPase of HIT Insulinoma Cells, a Putative Mechanism for Depolarizing Islets and Stimulating Insulin Release

Sulfonylureas also appear to affect active ion fluxes in β-cells. Ribalet et al. (1996) recently described an electrogenic Na pump current in HIT cells, which was blocked by glyburide or tolbutamide at doses THAT bind some low-affinity membrane proteins (Ribalet et al., 1996). The IC_{50} for glyburide inhibition of this pump current was 68 n*M*. The authors proposed that glyburide binding to a high-affinity receptor (presumably SUR) closed K_{ATP} channels, while low affinity binding inhibited both K_{ATP} channels and Na pump activity. Secretion would presumably be stimulated by the blockade of tonically hyperpolarizing Na pump current and/or by intracellular $[Na^+]$ accumulation, although the Na^+_i-sensitivity of the pump would limit this mechanism to a transient effect as intracellular $[Na^+]$ rose. Some evidence suggesting a possible interaction between the K_{ATP} channel and the Na/K/ATPase was also obtained.

Ribalet et al. (1996) argued that, since insulin secretion and Na pump current were affected by similar doses of glyburide (10–40 n*M*) compared to the higher sensitivity of K_{ATP} channels (low n*M*), inhibition of the Na pump by glyburide most likely mediated the insulinotropic action of the sulfonylureas. However, good agreement between the ED_{50}'s for K_{ATP} inhibition and secretion have been obtained previously after correcting for glyburide binding to serum albumen (Panten et al., 1992; Boyd et al., 1993). It may be that low doses of glyburide stimulate secretion by closing K_{ATP} channels, whereas other mechanisms like pump inhibition come into play at higher doses.

Enhancement of Insulin Exocytosis by Sulfonylureas Owing to a Distal, PKC-Dependent Mechanism

All of the mechanisms discussed so far involve the alteration of ionic fluxes at the β-cell membrane following sulfonylurea binding to its receptor. These changes in turn translate into depolarization of the β-cell membrane owing to decreased passive net K efflux through K_{ATP}, increased net Cl efflux through $I_{CP,\ islet}$ or decreased active outward flux mediated by a Na/K/ATPase. Depolarization ultimately leads to the increased intracellular [Ca^{2+}], which stimulates the release of insulin granules (Ashcroft and Rorsman, 1995).

However, Eliasson et al. (1996) recently presented evidence for a far different sulfonylurea mechanism in which secretion is stimulated by a direct interaction between the secretory apparatus (or a closely related protein) and the sulfonylurea receptor. Importantly, this pathway is Ca-independent. Eliasson et al. (1996) measured insulin secretion in single mouse β-cells by monitoring changes in their electrical capacitance. Since granule exocytosis increases membrane area and thus membrane capacitance, tracking the latter during an experiment reflects changes owing to the fusion or retrieval of exocytotic vesicles. Phase analysis allows the resistive and capacitative components of membrane impedance owing to ion conductance or membrane capacitance, respectively, to be separated (Gillis and Misler, 1992). Eliasson et al. (1996) stimulated Ca^{2+} influx through voltage-dependent Ca^{2+} channels with a depolarizing voltage pulse and then rapidly monitored C_m using phase-tracking. In addition, in some experiments, FURA-2 was used to monitor changes in intracellular [Ca^{2+}] associated with Ca channel activation.

Pulse depolarizations from –65 mV to 0 mV triggered inward Ca^{2+} currents associated with increased cell capacitance on the order of 25 fF. Ca-dependent increments in cell capacitance were significantly enhanced (≈ 3 ×) by 100 m*M* tolbutamide or 0.1 μ*M* glyburide. Sulfonylurea action was independent of Ca influx since the drugs did not increase Ca^{2+} current amplitude and did not involve the drug-induced islet cell depolarization (since the voltage-clamp kept membrane potential fixed). Thus the effects noted were unrelated to the depolarizing actions of sulfonylureas via K_{ATP} inhibition.

Sulfonylurea action was also not a result of decreased calcium buffering (and thus a rise in free [Ca^{2+}]) since the intracellular [Ca^{2+}] transients associated with Ca current activation were not increased by sulfonylureas. Ca-evoked secretion was increased by activation of cAMP-dependent protein kinase with forskolin but the percent potentiation owing to tolbutamide was unaffected by forskolin or by a blocker of cAMP-dependent protein kinase, R_p-cAMP. In contrast, enhancement of exocytosis following treatment with the PKC stimulator PMA or application of the PKC inhibitor bisindolylmaleimide abolished potentiation owing to either tolbutamide or glyburide. This suggested that sulfonylureas enhanced secretion through activation of PKC (Ashcroft et al., 1994; Elisson et al., 1996). The ED_{50} for tolbutamide potentiation of exocytosis was found to be 32 μ*M*, with a Hill coefficent of 2.5.

Eliasson et al. (1996) concluded that the potentiation owing to sulfonylureas may be clinically relevant. They estimate that as much as 75% of sulfonylurea effects on secretion in depolarized cells may be a result of effects on exocytosis and suggest that sulfonylureas may interact with receptors in secretory granule membranes. An SUR subtype may thus be part of a complex of molecules regulating secretion. Phosphorylation of consensus PKC sites on granule SUR might thus modulate the interaction between the receptor and as yet unidentified granular protein, modifying secretion. Eliasson et al. (1996) cite several papers pointing to significant sulfonlyurea binding to intracellular membranes in support of this hypothesis (Carpentier et al., 1986), although how sulfonylurea binding to SUR alters PKC activity remains obscure.

Quantifying the contribution of this mechanism to the net insulinotropic action of sulfonylureas may be difficult, however, since it requires determining whether the initial depolarizing action of the sulfonylureas owing to low nanomolar suppression of K_{ATP} channels under unclamped conditions is more or less important than positive modulation of secretion produced by higher doses (tens of n*M*) when exocytosis is evoked by patch clamp depolarizations and Ca influx. Of course, without initial depolarization to open the Ca channels, no secretion is triggered to modulate (Cook et al., 1988; Ashcroft and Rorsman, 1989). This situation is reminiscent of glucose itself, which (besides closing K_{ATP} channels and thereby depolarizing islets and provoking Ca-dependent secretion) modulates secretion via a parallel, distal pathway, independently of K_{ATP} (Gembal et al., 1992). Gembel et al. (1992) discriminated between the triggering role of glucose and a parallel modulatory role, with both possibly involved in stimulus-secretion coupling. Again, without K_{ATP} inhibition by glucose metabolism and, thus, depolarization, no insulin secretion occurs via this parallel pathway. It thus seems likely that K_{ATP} channel closure is a crucial initial step in the consensus pathway linking sulfonylurea binding to insulin release, as for glucose-mediated insulin release. If so, then it seems hard to accept the hypothesis that most SURs are intracellular as suggested by Eliasson et al. (1996). Instead, it would seem likely that SUR-Kir 6.2 complexes at the cell surface occur in sufficient density to mediate the "consensus pathway" while a fraction of the cell's SURs may be intracellular and thus mediate distal effects of the drugs.

K_{ATP} Channels, Sulfonylurea Receptors, and Future Prospects

A number of developments in the sulfonylurea receptor field bode well for the future of cellular diabetes research. The cloning of K_{ATP} will permit detailed studies of its structure-function relationship and facilitate screening for possible K_{ATP} mutations in patients with diabetes mellitus or other diseases. Site-directed mutagenesis will likely be carried out to identify the K_{ATP} sequences associated with nucleotide sensing (begun by Nichols et al., 1996), Mg^{2+} blockade, sulfonylureas binding and ATP gating, among other properties. It will also be important to more fully understand the nature of the molecular interaction between the a and b subunits (Inagaki et al., 1995b).

Of the novel actions of sulfonylureas reviewed above, which are actually involved in stimulating insulin release pharmacologically at clinically relevant doses?; how do they compare quantitatively with K_{ATP} inhibition? For an important homeostatic hormone like insulin, several independent or parallel pathways are likely to control its synthesis and secretion. With multiple control points and pathways, there may turn out to be multiple targets for the sulfonylureas or future antidiabetic drugs to act upon.

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