Recent studies have identified a high-affinity receptor on the plasma membrane of the β-cell that is specific for all of the sulfonylureas. The most potent second-generation drugs, glyburide and glipizide, bind to the receptor and trigger insulin release at nanomolar concentrations. The affinity to the receptor-ligand interaction of all sulfonylureas correlates with their potency as insulin secretagogues, further implicating receptor occupancy with signal transduction. These drugs also inhibit the electrical activity of ATP-sensitive K⁺ channels and K⁺ efflux through these channels. The channels are also closed by the metabolism of the major insulin secretagogues, glucose and the amino acids, which signal insulin release by increasing the ATP level or the [ATP]-to-[ADP] ratio on the cytoplasmic side of the channel. Based on the channel number and the amount of K⁺ current they pass, it is possible to calculate that these channels control the resting membrane potential of the β-cell. Inactivation of the ATP-Inhibitable K⁺ channel results in a fall in the resting membrane potential, cell depolarization, and influx of extracellular Ca²⁺ through the voltage-dependent Ca²⁺ channel. The rise in intracellular free Ca²⁺ level triggers exocytosis. Thus, this is now possible to link either a stimulus from the metabolism of insulin secretagogues or the sulfonylureas to ionic and electrical events that elicit insulin release. These data also suggest that the sulfonylurea receptor or a closely associated protein is an ATP-sensitive K⁺ channel. Diabetes 37:847-50, 1988

CHARACTERIZATION OF SULFONYLUREA RECEPTOR
Studies by Schmid-Antomarchi et al. (1) in Nice, France, and my own laboratory (2) have shown remarkable agreement in the characteristics of the sulfonylurea receptor on the cell membranes of two insulin-secreting cell lines, an SV40 transformed hamster β-cell line (HIT cells) and a rat insulinoma (RIN cells). The binding of [³H]glyburide to the sulfonylurea receptor is of high affinity and comparable in both cell lines—with a Kᵦ of 0.24 nM to HIT cells in Houston and 0.3 nM to RIN cells in Nice. Scatchard analysis indicates a single class of binding sites. All of the other active sulfonylureas, e.g., glipizide and tolbutamide, displace the labeled glyburide from the receptor. The rank order of potency of sulfonylurea-receptor interaction is reflected by the biologic activity of the drugs on either stimulating insulin secretion from the HIT cells (2) or inhibiting K⁺ efflux (as measured by ⁸⁶Rb) from the RIN cells (1). The HIT cells contain about a six-fold-higher concentration of sulfonylurea receptors per milligram of β-cell membranes than the RIN cells. This again is reflected biologically, because these drugs are potent secretagogues when added to HIT cells, but they have much less impressive effects on insulin release from the RIN cells. These studies have clearly established that the sulfonylureas initiate their biologic effect by first interacting with specific high-affinity receptors on the β-cell membrane.

he molecular and cellular mechanisms by which sulfonylureas elicit insulin release have recently been clarified. I examine the evidence that the sulfonylurea receptor is an ATP-sensitive K⁺ channel and delineate how changes in the intracellular free-Ca²⁺ level ([Ca²⁺]) are connected to inactivation of this channel. The action of both glucose and sulfonylureas on the ATP-sensitive K⁺ channel allows speculation about the role of ion channels in signal transduction by sulfonylureas in non-β-cells. The literature cited is recent and not all inclusive.

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years that the major physiologic insulin secretagogues, i.e., glucose and amino acids, must be metabolized to elicit insulin release. Using \(^{86}\)Rb\(^+\) to monitor K\(^+\) flux, studies in islets have indicated that another early event in stimulus-secretion coupling is a decrease in K\(^+\) efflux from the \(\beta\)-cell, which is coupled to an increase in glycolysis (4). Recent patch-clamp studies have linked the metabolism of the insulin secretagogues with ionic events by identifying K\(^+\) channels, which are spontaneously active in the plasma membranes of dispersed \(\beta\)-cells or insulin-secreting cell lines and are gated by intracellular ATP levels or [ATP]/[ADP] (5–11; Fig. 1). The ATP does not have to be metabolized to close the channel. When open, these channels pass K\(^+\) out of the cell. The channels are present in high numbers, and calculations with the Nernst equation indicate that these channels control the resting membrane potential of the \(\beta\)-cell. If more of the channels open, the loss of K\(^+\) causes the cell to become more negatively charged and the membrane potential to become hyperpolarized. Conversely, closure of the channels decreases the membrane potential leading to depolarization.

The K\(^+\) channels are closed by insulin secretagogues, glucose, mannose, glycerol, and l-leucine (10,11). Addition of metabolic inhibitors, which block utilization of a particular substrate, prevents the inhibitory effect on the K\(^+\) channel or reopens K\(^+\) channels already closed by that particular secretagogue (9). Although there are several different types of K\(^+\) channels, the patch-clamp studies can be performed under experimental conditions in which only K\(^+\) current through channels inhibited by ATP are being studied. The same ATP-sensitive K\(^+\) channels are inhibited by the sulfonylureas at drug concentrations similar to those that saturate the sulfonylurea receptor. The second-generation drugs, which are much more potent insulin secretagogues than the preceding generation, inhibit the ATP-sensitive channel, trigger insulin secretion, and decrease K\(^+\) efflux at low nanomolar concentrations (1,2). Second-generation drugs such as tolbutamide act at higher drug concentrations, in the micromolar range, to elicit the same biologic response. The correlation between the affinity of these drugs to bind to the receptor, inhibit \(^{86}\)Rb\(^+\) efflux, and trigger insulin secretion suggests that the sulfonylurea receptor or a closely associated protein is the ATP-sensitive K\(^+\) channel. This hypothesis was first advanced by Sturgess et al. (12).

Although the metabolizable insulin secretagogues and sulfonylureas both inhibit the ATP-sensitive K\(^+\) channel, they act at different sites. Outside-out excised patches of \(\beta\)-cell membranes or the binding of labeled glyburide to intact insulinoma cells reveals that the sulfonylureas elicit their effects by acting on receptors located on the outer surface of the plasma membrane. In contrast, ATP acts on the cytoplasmic surface of the membrane. The concept of a receptor as an ion channel is not novel, because the nicotinic acetylcholine receptor is an Na\(^+\) channel, and the dihydropyridine receptor is a Ca\(^{2+}\) channel (13,14). In both instances the specific ligand binds to the extracellular portion of the channel. The \(\gamma\)-aminobutyric acid–benzodiazepine receptor was recently isolated from bovine brain and shows homology with other ligand-gated receptor subunits (15). Thus there appears to be a superfamily of receptors that are ion channels.

There has been some reluctance in accepting the concept that ATP or ADP levels might act as intracellular signals that regulate a membrane channel directly rather than serving as a donor of phosphate in a phosphorylation reaction. The concentration of ATP in the \(\beta\)-cell is on the order of 3–5 mM, and patch-clamp studies indicate that the concentrations of ATP that inhibit the K\(^+\) channel are much lower, in the micromolar range (8–10). However, the patch-clamp technique may underestimate the concentration of ATP necessary to inhibit the channel by dialyzing away other intracellular factors that interact with ATP to regulate the channel. In support of this notion, the ATP sensitivity of channel activity is modified by addition of ADP. Because both nucleotides are present in \(\beta\)-cells, the reduction of the efficiency of ATP in inhibiting the sulfonylurea-blockable K\(^+\) channel may explain why the channel is still active at presumed intracellular ATP concentrations that should completely inactivate the channel (5,6,9,10).

Previous attempts to demonstrate changes in cytosolic [ATP]/[ADP] in islets have been equivocal. Recently, Meglsson et al. (16) presented information showing that glucose rapidly increased [ATP]/[ADP] in islets. Studies by Cook and...
ikeuchi (17) showed that glucose and tolbutamide share effects on ATP-inhibitable K+ channels, and Misler et al. (18) showed that the ATP channel is also present on human islet cells and is inhibited by glibenclamide or tolbutamide. These data provide further support for the hypothesis that the energy state of the β-cell is a coupling factor in the cytosol, linking increased fuel metabolism, ionic and electrical events, and insulin secretion and that sulfonylureas act at the same site in the secretory pathway.

Recent studies with fruit flies (Drosophila) have led to the cloning of a gene locus called the Shaker locus, which is thought to code for a K+ channel (19). The name derives from observations that flies lacking this portion of DNA develop shaking movements of the legs when anesthetized. Because no K+ channels have been chemically isolated, the cloning of the first K+ channel should prove to be the initial step in unraveling the diversity of K+ channels that exist. In these insects, multiple K+ channel components appear to arise from a single gene by alternative splicing of the messenger RNA (20). The predicted gene products from the Shaker locus show sequences similar to the voltage-sensitive Na+ channel and the rabbit muscle dihydropyridine receptor (a Ca2+ channel), again indicating that this class of K+ channels will also be similar in structure to other receptors or ion channels.

SULFONYLUREA RECEPTORS ON NON-β-CELLS

It is logical to think that sulfonylurea receptors exist on non-β-cells where they have pharmacologic effects, but this hypothesis remains unproved. One of the difficulties in demonstrating receptors on hepatocyte, adipocyte, and muscle cell lines or liver or fat cells is that the nonspecific binding of the labeled drug is much higher to these cell membranes than to HIT or RIN cells or brain membranes. The receptor cannot yet be demonstrated with confidence. However, because the receptor has been found on brain membranes, it may be possible to demonstrate sulfonylurea receptors on putative non-β-cell targets of these drugs with improved ligands having higher specific activity. The number of sulfonylurea receptors on the brain membranes is only ~10% of those found on the HIT cells, and the function of the receptor in the central nervous system is unclear. Preliminary patch-clamp studies from several laboratories suggest that the ATP-sensitive K+ channel is not ubiquitous and will have a selective distribution.

Two other approaches to evaluating the peripheral effects of the sulfonylureas on non-β-cells are in their infancy. In preliminary studies, we have not yet been able to demonstrate changes in [Ca2+]i in cell lines that are model systems of the major peripheral target tissue of the sulfonylureas, i.e., muscle, fat, and liver. With fura 2 to measure [Ca2+]i in cultured liver cells (HepG2), differentiated fat cells (3T3-L1), and a skeletal muscle line (BC3H1), sulfonylureas and/or insulin did not alter [Ca2+]i (21). Previous studies from several laboratories have established that the sulfonylureas increase glucose uptake into each of these cell lines. These data raise the possibility that there is an alternative system by which sulfonylureas signal their effects on peripheral targets. It is also possible that the sulfonylureas have their antidiabetogenic effects on non-β-cells indirectly by either increasing insulin secretion or potentiating the action of insulin on muscle, fat, or liver tissue. The first of the latter two hypotheses would not require that specific receptors will be found on these peripheral tissues. The data are not clear on this point. However, Draznin et al. (22) have successfully demonstrated an increase in [Ca2+]i when sulfonylureas or insulin were added to isolated rat adipocytes. Furthermore, patch-clamp studies have shown that Ca2+ channels exist in 3T3 fibroblasts, the origin of the 3T3-L1 fat cells, although the function of voltage-dependent Ca2+ channels in these nonexcitable cells is unclear (23).

[Ca2+]i: SIGNAL FOR SULFONYLUREAS

The voltage-dependent Ca2+ channel is intimately involved in the stimulus-secretion coupling by sulfonylureas. After depolarization, this channel transmits the Ca2+ into the cell that is the second messenger that signals exocytosis. Our studies measuring [Ca2+]i, in HIT cells have shown that the sulfonylureas increase [Ca2+]i in a dose-dependent manner, and the source of the Ca2+ is an extracellular pool. Chelation of Ca2+ in the medium completely inhibits insulin secretion and sulfonylurea-induced changes in [Ca2+]i (24). All three classes of Ca2+-channel antagonists—phenylalkylamines, dihydropyridines, and benzodiazepines—inhibit glibenclamide- or tolbutamide-stimulated insulin secretion and the rise in [Ca2+]i in a parallel and dose-dependent manner (25).

There is no evidence for a direct effect of the sulfonylureas on the voltage-dependent Ca2+ channel. Drugs that bind specifically to these channels do not displace the labeled glibenclamide from the membranes, and conversely, in patch-clamp studies, a series of compounds that block Ca2+ channels do not affect the sulfonylurea and ATP-sensitive K+ channels (1). The effect of sulfonylureas can be demonstrated in outside-out patches, suggesting that the effect of the sulfonylureas on the K+ channel is a direct one and does not involve a second messenger. Glyburide binding to intact RIN cells is fully dissociable, agreeing with the electrophysiologic data that the drugs act on a site on the plasma membrane (1).

The biochemical mechanisms by which an increase in [Ca2+]i is coupled to the distal steps of the secretory process are still the least understood facet of stimulus-secretion coupling. As more free Ca2+ becomes available it would bind to the ubiquitous Ca2+-binding protein calmodulin, changing the configuration of the molecule to the active state (26) and allowing it to interact with various calmodulin-binding proteins, many of which are enzymes. In smooth muscle, the initial generation of tension correlates with phosphorylation of the myosin light chain (27). Thus, a reasonable hypothesis is that a similar pathway is found in the β-cell, because all of the enzymes to bring about this process are present in islet tissue. One enzyme, the myosin light-chain kinase, co-purifies with secretory granule membranes isolated from the pituitary (28), placing the enzyme at a strategic site to control exocytosis. We have recently shown that the rise in [Ca2+]i triggered by HIT cell depolarization is associated with the rapid phosphorylation of three proteins in association with insulin release (29). Phosphorylation, the rise in [Ca2+]i, and insulin secretion can all be inhibited by the prior addition of the Ca2+-channel blocker verapamil. Although one of these proteins has the correct molecular weight and isoelectric point to be the myosin light chain, the exact identity of the
Ca$^{2+}$-dependent phosphoproteins remains to be established.

Earlier studies suggested that sulfonylureas signal insulin release by effects of the adenylate cyclase-cAMP system (30,31). However, we found no alteration in the cAMP content of HIT cells treated with either glyburide or tolbutamide. The concentrations of these drugs that alter either phosphodiesterase or cAMP content of islets are much higher than that required to bind to the sulfonylurea receptor and stimulate insulin secretion. Furthermore, it is not necessary to evoke an action on cAMP to explain either the patch-clamp findings or the direct measurements of [Ca$^{2+}$] in the signaling pathway in sulfonylurea-stimulated insulin secretion.

Although there is excellent agreement on the concentration of sulfonylureas that saturates the receptor and elicits insulin release, the dose-response curve of the increase in [Ca$^{2+}$] elicited by sulfonylureas in HIT cells is shifted to the right. These data suggest that, although sulfonylureas signal the release of insulin by a change in [Ca$^{2+}$], there are additional potentiating intracellular signals. The most likely pathway that could potentiate the receptor-ligand interaction is the activation of protein kinase C. There is preliminary information that sulfonylureas also increase the turnover of phosphoinositides and the diacylglycerol released from the plasma membrane that could activate protein kinase C (32).

In summary, sulfonylureas elicit insulin release by binding to a high-affinity receptor on the plasma membrane. The tight coupling between receptor affinity, decreasing K$^+$ efflux, inhibition of ATP-modulated K$^+$ channels, and insulin secretion suggests the sulfonylurea receptor may be an ion channel—possibly ATP-inhibitable K$^+$ channel.

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REFERENCES