The Role of Ion Channels in Insulin Secretion

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Abstract Ion channels in beta cells regulate electrical and secretory activity in response to metabolic, pharmacologic, or neural signals by controlling the permeability to K^+ and Ca^{2+}. The ATP-sensitive K^+ channels act as a switch that responds to fuel secretagogues or sulfonylureas to initiate depolarization. This depolarization opens voltage-dependent calcium channels (VDCC) to increase the amplitude of free cytosolic Ca^{2+} levels ($[Ca^{2+}]i$), which triggers exocytosis. Acetyl choline and vasopressin (VP) both potentiate the acute effects of glucose on insulin secretion by generating inositol 1,4,5-trisphosphate to release intracellular Ca^{2+}; VP also potentiates sustained insulin secretion by effects on depolarization. In contrast, inhibitors of insulin secretion decrease $[Ca^{2+}]i$ by either hyperpolarizing the beta cell or by receptor-mediated, G-protein–coupled effects to decrease VDCC activity. Repolarization is initiated by voltage- and Ca^{2+}-activated K^+ channels. A human insulinoma voltage-dependent K^+ channel cDNA was recently cloned and two types of alpha1 subunits of the VDCC have been identified in insulin-secreting cell lines. Determining how ion channels regulate insulin secretion in normal and diabetic beta cells should provide pathophysiologic insight into the beta cell signal transduction defect characteristic of non-insulin dependent diabetes (NIDDM).

Key words: beta cells, ion channels, voltage-dependent calcium channels, sulfonylurea, ATP-sensitive K^+ channels, sulfonylurea receptor

Ion channels are pores in the membranes of the beta cell that regulate signal transduction pathways by controlling the permeability to K^+ and Ca^{2+}. The development of the patch clamp method, which can resolve current through individual ion channels, and the marriage of biophysical and biochemical studies has rapidly advanced the understanding of electrically excitable cells. The resting beta cell membrane is maintained at very negative potentials, approximately -70 mV, by selective ion permeability [reviewed in Rajan et al., 1990]. Two forces, ionic concentration gradients and electrical potential differences across the plasma membrane, regulate ion flux. As the ambient glucose concentration increases above a threshold level the beta cell becomes progressively depolararized, increasing electrical activity characterized by slow waves with superimposed oscillatory spikes. These electrical slow waves and spikes are reflected by an increase in the amplitude of $[Ca^{2+}]i$ pulses and pulsatile insulin release [Longo et al., 1991]. In contrast, the net loss of positive charge from the beta cell (hyperpolarization) decreases electrical activity and insulin secretion. A consensus has emerged that the $[Ca^{2+}]i$ is the major "second messenger" that regulates beta cell insulin secretion. Determining how ion channels control $[Ca^{2+}]i$ is central to understanding how the beta cell regulates insulin secretion.

NIDDM affects over 12,000,000 Americans. Early in the disease insulin release in response to glucose is delayed [reviewed in Boyd et al., 1990] and the normal pulsatile insulin secretory pattern is lost [O'Reilley et al., 1988]. Initially, this defect is limited to glucose alone, and other insulin secretagogues like sulfonylureas elicit robust secretory responses. Sulfonylureas were discovered during World War II when certain antibiotics of the sulfa class were found to produce hypoglycemia. These drugs are potent insulin secretagogues and are now given to restore glucose homeostasis in millions of patients with NIDDM [reviewed in Boyd et al., 1990]. The sulfonylurea high affinity binding site on the beta cell may contain an ion channel and by first understanding the sulfonylurea/glucose signal transduction pathway it may then be possible to unravel the defects that are a hallmark of NIDDM. In this Prospect I will review recent studies on how ion channels control insulin secretion, stressing areas where I think new hy-

potheses and experimental data should resolve unanswered questions. The style is like that of an editorial and the references are highly selected and not inclusive.

GLUCOSE TRANSPORT AND METABOLISM

Glucose is the major physiologic insulin secretagogue. Its site of action has been heavily investigated. This sugar is rapidly taken up into the beta cell by specific, high K_m glucose transporters. These transporters reside primarily in an intracellular compartment in unstimulated cells and are rapidly shuttled to the plasma membrane by insulin. Five related glucose transporters have been cloned from various tissues. The major beta cell transporter is called GLUT-2 [reviewed by Bell et al., 1990]. Following its uptake glucose is phosphorylated by glucokinase to glucose-6-phosphate which enters glycolysis. In the fed state this enzyme may be the rate limiting step in glucose metabolism and function as the beta cell "glucose sensor" determining the magnitude of the insulin secretory response [Meglasson and Matschinsky, 1986]. The unique thing about glucose that separates it from most other extracellular signals is that it must be metabolized to initiate changes in electrical activity and insulin secretion. Inhibition of metabolism blocks both these processes.

ATP-SENSITIVE K⁺ CHANNELS: EFFECTS OF FUEL SECRETAGOGUES AND SULFONYLUREAS

The earliest ionic event associated with insulin secretion is a decrease in K^+ efflux [reviewed in Henquin, 1991]. Cook and Hales [1984] linked metabolism to ion flux through K^+ channels whose gating (open and closed states) is regulated by the products of metabolism, ATP or ADP. These studies were rapidly confirmed and extended by Ashcroft and coworkers and many others [reviewed in Ashcroft, 1988], and they showed that ATP appears to bind directly to the cytoplasmic surface of the ATP-sensitive K^+ channel. Second messenger pathways do not seem to be involved. Corkey has hypothesized that oscillations in the glycolytic rate and the ATP/ADP ratio in the beta cell are entrained to oscillations in the activity of ATP-sensitive K^+ channels that in turn regulate membrane potential, $[Ca^{2+}]i$, and insulin release [see Longo et al., 1991]. A potential problem with this hypothesis has been underlined. In patch clamp studies half maximal closure of the ATP-sensitive K^+ channel occurs at 15 µM ATP levels. However, the

ATP levels in the islet are much higher, in the 3 to 6 millimolar range. Two explanations have been advanced to save the hypothesis and explain why these channels are not always closed by ATP. First, ADP might compete at equimolar concentrations with ATP for the same channel binding site and partially relieve the ATP-induced channel closure. The ATP-sensitive K^+ channels remain active when membrane patches are excised into solutions thought to be similar to those in vivo. Changing the ATP/ADP ratio from 5:1 to 1:5 increased K^+ channel activity twentyfold [Misler et al., 1986]. However, in the beta cells the free ADP levels are in the µM range much lower than the ATP levels. Thus, ADP probably has a separate binding site on the channel and may have independent effects to increase the opening frequency of the ATP-sensitive K^+ channel. Alternately, most ATP-sensitive K^+ channels in the beta cell might be closed under physiologic conditions and the closure of only a few more channels could then lead to depolarization [Cook et al., 1988]. In this model the resting beta cell would operate with minimal ion flux and energy expenditure.

Based on the Goldman equation a decrease in K^+ permeability alone would not be sufficient to depolarize the beta cell [Henquin, 1991]. Another current, not yet clearly identified, appears necessary. Using RIN cells, a rat insulinoma cell line that does not release insulin in response to glucose, Dunne suggested that changes in Na^+ permeability are essential for depolarization [Dunne et al., 1990]. These data contrast with other studies using normal mouse beta cells suggesting that depolarization can be initiated in the absence of extracellular Na^+ or in the presence of tetrodotoxin, a blocker of Na^+ channels. Henquin [1991] suggests that the specific permeability for Na^+, Cl^-, Ca^{2+}, or Mg^{2+} is not involved in the initial depolarization. He points out that since the cells remain depolarized at the threshold potential for these ions and that electrical activity can be demonstrated in the extracellular absence of these ions it is unlikely they are involved in the initial depolarization and Henquin further suggests, as does Cook [1988], that a non-specific leakage current moves the membrane current to a more positive level where a decrease in K^+ permeability can then depolarize the beta cell. Activators of ATP-sensitive K^+ channels like galanin [Sharp et al., 1989], diazoxide, or chromakalim and pinacidil lead to the loss of K^+ and positive charge from the cell,

hyperpolarization, and a decrease in insulin secretion (Fig. 1).

There is strong but still indirect evidence that the ATP-sensitive K^+ channel or a closely-associated protein found on the plasma membranes of insulin-secreting cell lines and pancreatic beta cells, and brain or cardiac membranes, is the high affinity sulfonylurea receptor. Occupation of the sulfonylurea receptors inhibits the electrical activity of the ATP-sensitive K^+ channel and leads to depolarization. Following depolarization $[Ca^{2+}]i$ increases and triggers insulin secretion. The organic Ca^{2+} blockers interdict Ca^{2+} influx and establish clearly that the rise in $[Ca^{2+}]i$ is the trigger that initiates insulin secretion. There appears to be an interplay between glucose and the sulfonylureas on the ATP-sensitive K^+ channel with each lowering the effective concentration of the other secretagogue required to elicit insulin secretion.

Glucose also has unique effects to modulate insulin secretion which are not understood. The concentration dependent effects of the sulfonylureas on each step in beta cell signal transduction agree remarkably. The Kd of binding, the inhibition of 86Rb efflux and the ATP-sensitive K^+ channels, and the ED50 for both $[Ca^{2+}]i$ changes and insulin secretion all occur over the same concentration range of sulfonylureas. The
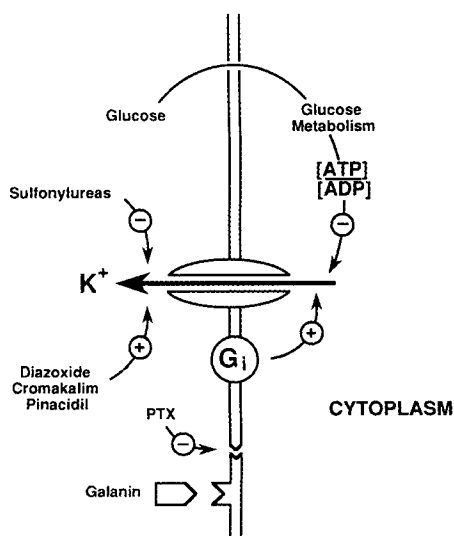

Fig. 1.   The ATP-sensitive channel in the beta cell. The channel is inactivated by ATP or the ATP-ADP ratio generated by metabolism or by high affinity sulfonyyureas binding. Channel openers include the vasodilators, chromakalim and pinacidil, as well as the inhibitor of insulin secretion, diazoxide. Galanin activates the channel through a pertussis toxin-sensitive G-protein which is probably in the Gi family.
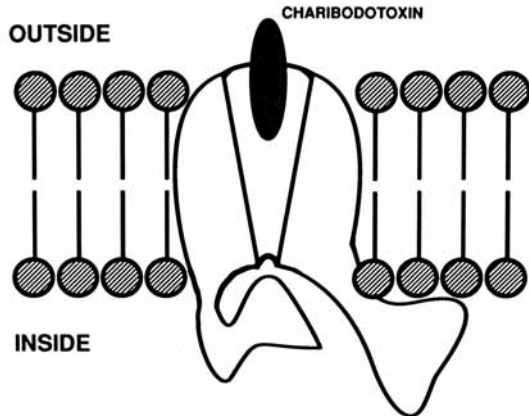
sulfonylurea receptor has been photoaffinity labeled and solubilized [Aguilar-Bryan et al., 1990] and in beta cell or brain membranes has a molecular weight of approximately 140 to 150 kDa [reviewed in Boyd et al., 1990]. The major challenge is to now determine the structure of this protein.

Do sulfonylureas have peripheral mechanisms of action that do not require an increase in endogenous insulin secretion? We think not. The studies reporting possible sulfonylurea receptors on other putative pharmacologic targets of sulfonylurea action, hepatocytes and adipocytes, have used low specific activity tracers and have not clearly demonstrated the high affinity receptor. Using a high specific activity sulfonylurea tracer we have not been able to confirm the existence of high affinity receptors of those tissues. Muscle requires further study. Low affinity binding can occur to proteins like albumin which can contaminate membrane preparations. These drugs don't have significant hypoglycemic effects when administered to patients with no beta cell function and insulin deficient diabetes (type I). When antidiabetic effects of sulfonylureas in NIDDM are clarified they will probably be mediated by increasing insulin secretion [reviewed in Boyd et al., 1990, and Boyd et al., 1991).

VOLTAGE- AND CALCIUM-ACTIVATED POTASSIUM CHANNELS

Voltage-activated K^+ channels, and Ca^{2+}-activated K^+ channels also called the "maxi K^+ channel" because of their high conductance, are also found on beta cell plasma membranes. These latter channels respond to both changes in voltage or intracellular Ca^{2+}. Potassium channels are blocked by tetraethylmethonium ions (TEA) (Fig. 2) or carybdotoxin, a peptide found in scorpion venom. Studies with TEA suggest voltage- or Ca^{2+}-activated K^+ channels are involved in repolarization, but probably do not play a role in depolarization elicited by glucose [Henquin, 1990] as originally suggested by Ribalet [Ribalet et al., 1988] in his studies of the RIN cell.

Mutations in *Drosophila* at a locus called Shaker led to the cloning of voltage-activated K^+ channels. Based on sequence similarities a family of voltage-sensitive K^+ channel genes was then cloned from insect and mammalian sources including a human insulinoma [Phillipson et al., 1991]. This channel was also found in normal human islets and has a deduced sequence of 613

Fig. 2. The putative voltage-sensitive K⁺ channel in the beta cell. The channel pore probably is shaped like an hourglass. At the inner surface there appear to be two ball and chain domains that can inactivate the channel by plugging the pore. TEA can block the channel at the external mouth and at another site near the cytoplasmic inner area of the pore, whereas caribdotoxin blocks at the external side of the pore [adapted from Ruppersberg et al., 1991].

amino acids. A 4 kb transcript was found in the RNA from the hamster and rat insulin-secreting cell lines, but not other human tissues, indicating either that the channel is not widely expressed or its abundance is lower in other tissues. When the synthetic RNA from this channel was expressed in *Xenopus* oocytes, the channel had characteristics of a delayed rectifier, a K⁺ channel that permits a greater flux of K⁺ ions at hyperpolarized membrane potentials rather than during depolarized membrane potentials. Phillipson et al. [1991] have also suggested that this Shaker-related K⁺ channel could restore the resting membrane potential after depolarization and contribute to the regulation of insulin secretion. They also speculated that if this channel was overexpressed in some human insulinomas or in fetal islets it might stabilize the resting membrane potential below that level required to depolarize the cell. This hypothesis would fit with the clinical data showing reduced insulin responsiveness to glucose in some patients with insulinomas and the observation that fetal islets also release insulin poorly to this secretagogue.

Another recent advance in channel biology came from *Drosophila* genetics when Ca²⁺-activated K⁺ channels were cloned from a locus termed slowpoke (*slo*) where mutations were known to alter this type of current. This channel contains a predicted polypeptide domain that is somewhat similar to the voltage-activated K⁺ channels, but the entire protein is about twice as large as those channels and contains two other potential membrane spanning regions at the carboxyl terminus [Atkinson et al., 1991].

REGULATION OF VOLTAGE-DEPENDENT CALCIUM CHANNELS

VDCCs are regulated by [Ca²⁺]i and other second messengers (cAMP and possibly protein kinase C) and could also be activated directly by changes in the ambient glucose concentration [Smith et al., 1989]. Influx of Ca²⁺ through these channels restricts further Ca²⁺ entry in several excitable cell types [see Rajan et al., 1990, for review]. Santin and Cook found both fast Ca²⁺ inactivated and slow voltage-inactivated Ca²⁺ channels in HIT cells [Santin et al., 1989]. Keizer and Smolen have used mathematical modeling to investigate the role of ion channels in insulin secretion [Keizer et al., 1991]. Using a model with only ATP-sensitive K⁺ channels and delayed rectifier K⁺ channels it was not possible to produce a normal pulsatile or bursting pattern. However, by modifying the parameters to increase the Ca²⁺ channel open times they were able to model robust bursting patterns similar to those seen in normal islets. They suggest that both ATP-sensitive K⁺ channels and Ca²⁺ channels serve as a glucose sensors.

An increase in cAMP potentiates glucose-stimulated insulin secretion by increasing the influx of Ca²⁺ through VDCC. This is probably mediated by phosphorylation of the channel, but this has not been demonstrated by direct studies. In contrast, certain G-proteins are important negative modulators of ion channels in beta cells [see Rajan, 1990, for review]. Our studies have shown that in depolarized HIT cells somatostatin or alpha2 receptor occupation activates G-proteins that interact directly with the VDCC to inhibit Ca²⁺ current, increases in [Ca²⁺]i, and insulin secretion. This process is blocked by pertussis toxin pretreatment [Hsu et al., 1991a, 1991b; Keahey et al., 1989a]. In addition, these inhibitors of insulin release have been shown to have additional effects more distal in the secretory process and at much higher concentrations can alter cAMP generation by effects on adenylate cyclase.

Acetyl choline occupies muscarinic receptors on the beta cell to activate phospholipase C releasing diacylglycerol and inositol 1,4,5-trisphosphate, Ins (1,4,5) P3. This signal molecule binds to a specific receptor on the endoplas-

mic reticulum in the beta cell that if similar to those recently cloned from brain will be part of an intracellular Ca^{2+} release channel. In the absence of glucose cholinergic stimuli are poor insulin secretagogues, but they potentiate the glucose effects on secretion. The posterior pituitary peptide, vasopressin, is found in the islet and may regulate insulin release. In the presence of glucose, vasopressin potentiates the acute release of insulin by activating phosphoinositide turnover, Ins (1,4,5) P3 generation, and intracellular Ca^{2+} release [Gao et al., 1990]. In addition, vasopressin further depolarizes the beta cell, and Ca^{2+} flows into the cell through VDCC to sustain insulin release. The exact messenger that activates beta cell depolarization by vasopressin is still unclear, but Ins (1,4,5) P3 itself or inositol 1,3,4,5 tetrakisphosphate, an Ins (1,4,5) P3 metabolite, or both, acting in concert, are candidates for this latter effect.

VOLTAGE-DEPENDENT CALCIUM CHANNELS

In different tissues 4 types of Ca^{2+} channels have been described called L, T, N, and P channels. L-type channels have long-lasting activity and are sensitive to the Ca^{2+} channel blockers, the dihydropyridines (DHPs). Nimodopine, a DHP, blocks depolarization induced Ca^{2+} currents, changes in $[Ca^{2+}]i$, and insulin secretion from HIT cells over the same concentration range [Keahey et al., 1989] suggesting that the L-type Ca^{2+} channels control the plateau and spike electrical activity activated by various insulin secretagogues. Other types of Ca^{2+} channels may also exist in beta cells, but it remains to be clearly demonstrated that they play a significant role in signal transduction.

The development of agonists that bind to specific sites on the VDCCs led to the isolation of DHP-binding proteins from skeletal muscle T-tubules [reviewed in Rajan et al., 1990]. The consensus view is that the Ca^{2+} channel consists of 5 distinct subunits: alpha1, alpha2, beta, gamma and delta [see Rajan, 1990, for references]. The putative structure of the alpha 1 subunit is shown in Figure 3. It contains 4 homologous hydrophobic repeat domains (I–IV) that each contain at least 6 alpha helical membrane spanning regions (S). Four of these domains appear to form a pore in Ca^{2+} and Na^+ channels. The known K^+ channels are smaller and contain only one domain. It is thought that four K^+ channels associate to form a pore. The S4 region of each voltage-sensitive ion channel type con-
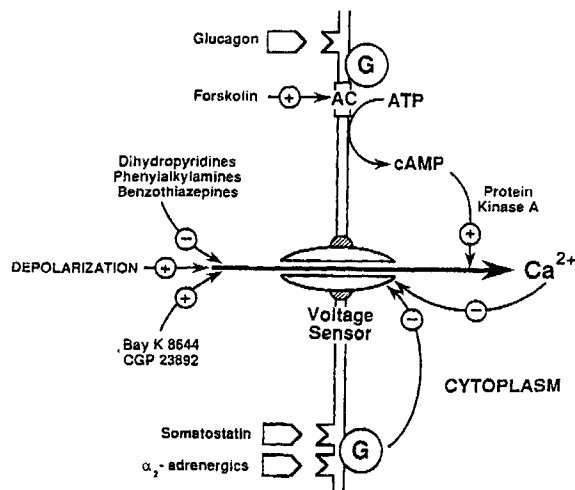

Fig. 3. Dihydropyridine-sensitive voltage-dependent Ca^{2+} channel in beta cells. This channel is activated by cell depolarization. The Ca^{2+} channel agonists, Bay K 8644 and CGP 23892, and elevated cAMP levels promote Ca^{2+} influx. Three classes of organic Ca^{2+} channel blockers (dihydropyridines, phenylalkylamines, or benzothiazipines) and elevated $[Ca^{2+}]i$ restrict Ca^{2+} influx into the cell. Inhibitors of insulin secretion, somatostatin, and alpha2 adrenergic agonists inhibit this channel through a pertussis toxin-sensitive G-protein. AC, adenylate cyclase.

tains a string of positively charged amino acids thought to be the voltage sensor. A change in electrostatic force could cause a conformational change in the channel protein. (Fig. 4).

When transvected into L-cells the alpha 1 subunits form stable cell lines that express DHP-sensitive Ca^{2+} currents [Perez-Reyes et al., 1989]. However, the kinetics of these transvected channels were much slower than expected for skeletal muscle Ca^{2+} currents. The recent coexpression of the alpha1 subunit with the beta subunit in L-cells has generated cell lines with Ca^{2+} currents with normal kinetics [Lacerda et al., 1991; Varadi et al., 1991]. In contrast to the alpha1 subunit, the alpha2 subunit does not contain binding sites for Ca^{2+} antagonists or phosphorylation sites and, when coexpressed in Xenopus oocytes with the alpha1 subunit, only increase Ca^{2+} current about twofold. Its role in channel function is unclear. Tissue specific isoforms of the alpha1 subunit have been cloned from skeletal, cardiac, and smooth muscle as well as lung and brain.

The full sequence of the alpha1 subunit of any secretory cell has not been published, but based on work in progress appears close at hand. Using PCR and oligonucleotide primers synthesized from the conserved regions in the skeletal
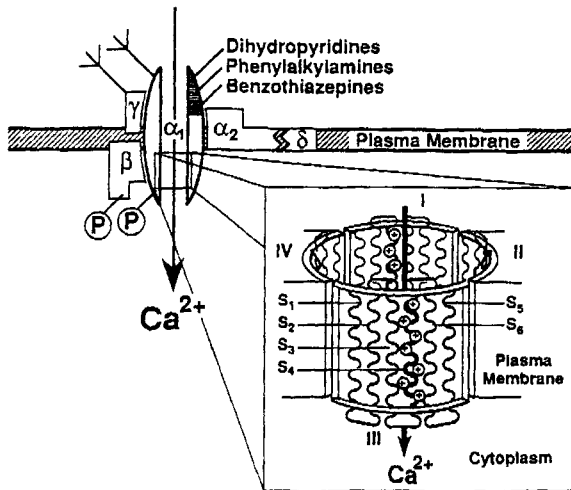
Fig. 4. Primary structure of the Ca^{2+} channel. The putative structure of the dihydropyridine-sensitive Ca^{2+} channel of skeletal muscle based on the work of Catterall and Campbell (see Rajan et al., 1990, for complete references). This channel contains 5 proteins thought to be subunits. The alpha1 is the primary functional unit and when expressed in mouse L-cells it generates Ca^{2+} currents with activation kinetics about 100-fold slower than expected for a muscle Ca^{2+} channel. It contains binding sites for all three classes of the organic Ca^{2+} channel blockers and is subject to phosphorylation. The beta subunit can also be phosphorylated and when coexpressed with the alpha1 subunit in mouse L-cells generates Ca^{2+} currents with normal activation kinetics. The magnified view shows that the channel has four homologous repeat domains (I–IV) each containing 6 transmembrane segments (S1–S6). The S4 segment contains an array of positively charged amino acids which is highly conserved in all voltage-sensitive ion channels and may serve as the voltage sensor.

muscle and cardiac Ca^{2+} channel alpha1 subunits, [Perez-Reyes et al., 1990] have probed the diversity of L-type channels in RNA isolated from two secretory cell types, HIT cells and ovarian tissue, as well as that from many other non-endocrine tissues. The PCR amplified products, which spanned a region from the loop that joins domain III and IV through most of domain IV, were sequenced. A gene product was found in beta cells, ovary, and brain that had sequence similarities to partial published sequences of brain Ca^{2+} channels. This was felt to be a third type of Ca^{2+} channel called neuroendocrine because of its similarity to brain channels and its distribution in endocrine cells. The PCR work shows that the HIT cell RNA contains at least two types of calcium channels, the neuroendocrine channel and a "cardiac-like" channel. The full-length clone of a rat brain alpha1 subunit has recently been reported. This channel is shorter at the carboxy terminus than skeletal

muscle or cardiac alpha1 subunits [Hui et al., 1991]. Alternate splicing of a primary transcript encodes variants at two regions of the channel, in the putative cytoplasmic loop between domains I and II and at the IVS3 region where splice variants were reported by Perez-Reyes et al. [1989]. In the region amplified by PCR by Perez-Reyes this rat brain channel appears to be quite similar to one of the HIT cell neuroendocrine alpha1 subunits. Two other recently cloned rat brain alpha1 subunits are related to cardiac channels sharing 95% identity to the DHP-sensitive cardiac channel [Snutch et al., 1991]. One of these channels appears similar to the HIT cell cardiac-like channel, called Ca Ch 2a by Perez-Reyes et al. [1990].

CONCLUSIONS

One can now conclude with some certainty that 5 major ion channels—ATP-sensitive K^+ channels, voltage-activated K^+ channels, calcium-activated K^+ channels, and two L-type Ca^{2+} channels—play a pivotal role in regulating insulin secretion (Fig. 5). The Ca^{2+} channels appear related to brain channels and the human voltage-dependent K^+ channel is a Shaker channel homolog. The rapid advances in determining both the molecular structure and factors that regulate the function of these channels promise to add to the understanding of beta cell signal transduction.

PROSPECTS FOR FUTURE RESEARCH

The recent cloning of the first Ca^{2+}-activated K^+ channel from Drosophila and progress in several laboratories in pursuit of the sulfonylurea receptor should ultimately lead to the determination of the structure of these two other classes of beta cell K^+ channels. Full-length cDNA clones of beta cell VDCC alpha1 subunits should soon be available. It will be first necessary to know how many alpha1 Ca^{2+} channels exist in the beta cell and how many different splice variants are utilized, and to determine how these channels relate to those found in other endocrine cells. Determining if all 5 proteins isolated together from skeletal muscle as a Ca^{2+} channel complex are found as subunits in other excitable cell types and are necessary for the Ca^{2+} channels to function is a challenge for all investigators. Several studies suggest this may not be the case. The expression studies in mouse L-cells [Varadi et al., 1991] suggest that the alpha1 and beta proteins are the most impor-
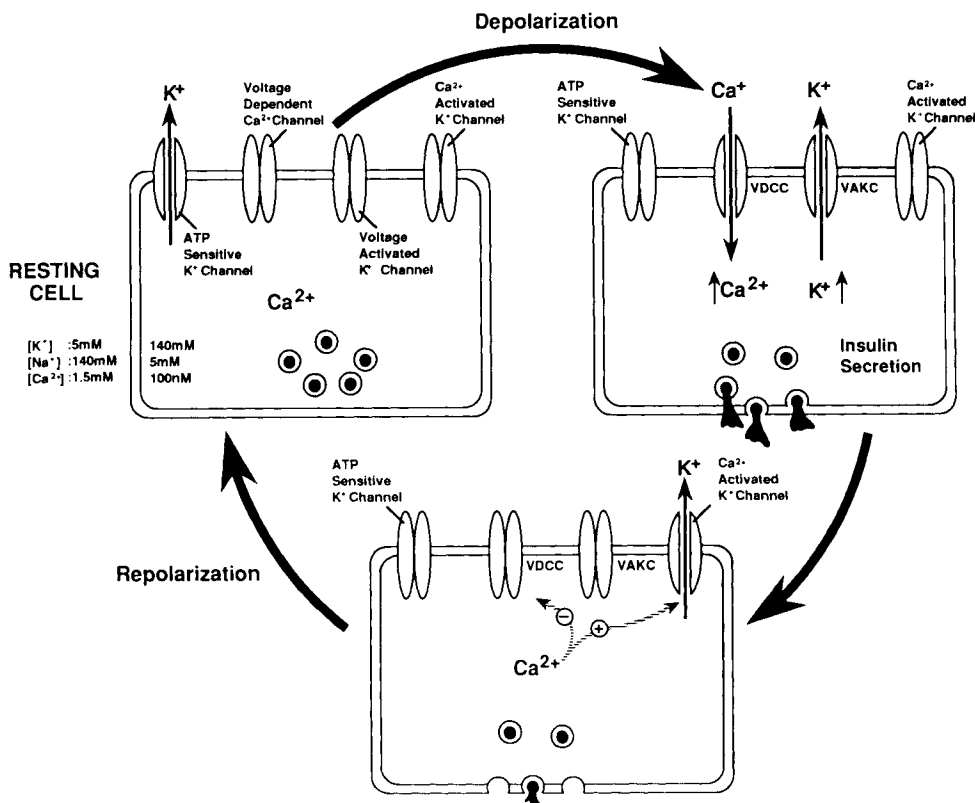
Fig. 5. Ion channel modulation of insulin secretion. The resting membrane potential in pancreatic beta cells is set by the K$^+$ conductance through ATP-sensitive K$^+$ channels and a probable leak current (not clearly identified). Closure of the ATP-sensitive K$^+$ channel leads to depolarization and Ca^{2+} influx through voltage-dependent Ca^{2+} channels. The rise in Ca^{2+} triggers insulin secretion, inactivates the Ca^{2+} channels, and, in concert with the change in membrane potential, the rise in [Ca^{2+}]i activates Ca^{2+}-activated K$^+$ channels and voltage-activated K$^+$ channels to enhance K$^+$ efflux. This results in repolarization of the cell and reversion to the resting state.

tant and Lacerda and his colleagues [1991] in the laboratories of Lutz Birnbaumer and Arthur Brown have questioned if the other proteins are true subunits and necessary for Ca^{2+} channel function. Comparison of the full-length clones of the DHP-sensitive Ca^{2+} channels in endocrine secretory cells, and the effect of mutations on function should help define what determines ion selectivity, activation, and inactivation characteristics and define important regulatory regions that allow the channel to respond to second messengers, G-proteins, and other intracellular regulators. Alpha and beta subunits contain phosphorylation sites that are probably involved in the regulation of the Ca^{2+} channels, but this hypothesis requires direct proof. The structural differences in the tissue specific isoforms of Ca^{2+} channels appear to explain the lack of cross reactivity of antibodies raised against skeletal or cardiac muscle Ca^{2+} channels to beta cell channel proteins. It may be possible to take advantage of these structural differences

to generate antibodies specific for the beta cell channels and determine at the protein level how these isoforms are distributed and expressed. Antibodies against specific regions of these molecules will also be important in further defining structure function relationships.

How might this basic information relate to NIDDM, a disease that clearly has a genetic etiology? The cloned beta cell channel proteins will certainly be used as candidate genes to try to more clearly focus in on the regions of the genome that are hiding the diabetes genes. This is a heterogeneous disease and many different abnormalities can lead to this common disease. One has to explain defects in beta cell signal transduction that may not become manifest for 40 or more years after birth. Stresses that challenge the beta cell secretory capacity like pregnancy, obesity, or other endocrinopathies can temporarily impair glucose homeostasis and result in the first clinical manifestations heralded by hyperglycemia. This suggests that patients

inherit beta cell abnormalities that, when challenged by a second stress leading to hyperglycemia, may cause beta cell dysfunction. We know, at the clinical level, that lowering the secretory demands on the beta cell with dietary modification or an exercise program often restores glucose homeostasis. Sulfonylureas can also bring about improved glucose tolerance probably by improving signal transduction in the abnormal beta cell. Thus, the initial defects leading to NIDDM in many patients will probably result from defects in the signals that regulate the metabolically responsive ion channels.

ACKNOWLEDGMENTS

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