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NEW INVESTIGATOR AWARD

Signal integration at the level of ion channel and exocytotic function in pancreatic β-cells

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INSULIN IS SECRETED IN A REGULATED MANNER from β-cells of the pancreatic islets of Langerhans in response to increased blood glucose following a meal. However, this statement belies the complexity of the processes regulating insulin release, which is also impacted by a plethora of neuronal, hormonal, autocrine, and nutrient signals. Many of the various factors stimulating or modulating insulin secretion have been well studied, such as glucose-dependent oxidative phosphorylation and metabolic signal generation (38) or glucagon-like peptide-1 (GLP-1) receptor-mediated cAMP production (40) among several others. It is therefore important to understand how these diverse signals regulate the machinery that controls insulin secretion and how the control of multiple effecter targets results in the precise regulation of secretion.

Why is it necessary for a signal, be it metabolic or receptor mediated, to target a plethora of effecters to achieve its intended result, in this case increased insulin secretion? The view, as it stands, is complex and fragmented. There are, for example, at least 10 (indeed, a bare minimum) identified effectors of GLP-1-induced insulin secretion (40). How do these effectors interact? Are they dependent on each other? One could ask whether the many separable targets affecting insulin secretion are in fact representative of a few key "effecter complexes" regulated by these signals. The present Perspective hypothesizes that at least some of the presumed metabolic and signaling targets at the plasma membrane are regulated together as a functional complex. Considered in this context is the "excitosome": the complex of ion channels and exocytotic proteins mediating downstream events in insulin secretion.

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The Secretory Mechanism

Some of the key mechanisms controlling insulin secretion are presented in Fig. 1*A* and have been reviewed extensively (13, 21, 22, 27, 42, 55, 62). The main points can be summarized as follows. A rise in plasma glucose leads to increased metabolism and an elevated cytoplasmic ATP/ADP ratio. This closes ATP-sensitive K^+ (K_{ATP}) channels, leading to β-cell depolarization, action potential firing, activation of voltage-dependent Ca^{2+} channels (VDCCs), and Ca^{2+} entry that triggers insulin granule exocytosis. β-Cell electrical activity is limited by the activation of voltage-dependent K^+ (Kv) channels, notably the Kv2.1 isoform, which mediate action potential repolarization. Superimposed on this, the triggering pathway, are one or more amplifying pathways that enhance secretion by promoting insulin granule recruitment to, and "priming" at, the plasma membrane. These include the receptor-mediated generation of cAMP and other signals and metabolic mediators such as NADPH (12, 21, 29, 38, 42). Candidates for *acute* regulatory points controlling secretion per se likely include *1*) key metabolic pathways within the mitochondria, *2*) the interaction of granules with, and activity of, the translocation machinery, and *3*) the molecular interactions of secretory machinery at the plasma membrane exocytotic sites that are the focus of the present perspective. Indeed, the plasma membrane is a key site of action of many signals that control insulin secretion.

What Defines the Exocytotic Machinery?

Following their biogenesis, insulin granules are trafficked to the cell periphery along microtubules (56). There, remodeling of the cortical actin network promotes translocation of granules to the plasma membrane (62). Once at the plasma membrane, insulin granules physically "dock" to the membrane through interaction of the vesicular and target-soluble NSF attachment protein receptor (v- and t-SNARE) proteins (Fig. 1*B*). SNARE proteins, together with a Ca^{2+} sensor, constitute the "minimum machinery" for the Ca^{2+}-dependent exocytosis of insulin (33). In the β-cell, these are represented by VAMP-2, syntaxin 1A/4, SNAP-25, and synaptotagmin VII/IX; this has been reviewed extensively (13, 17, 23, 30). Following a glucose-stimulated rise in intracellular Ca^{2+}, it is the SNARE proteins that mediate exocytosis by promoting the fusion of the opposing vesicular and plasma membranes.

While the SNARE machinery represents the minimum requirement for the Ca^{2+}-dependent exocytosis, the machinery impact-

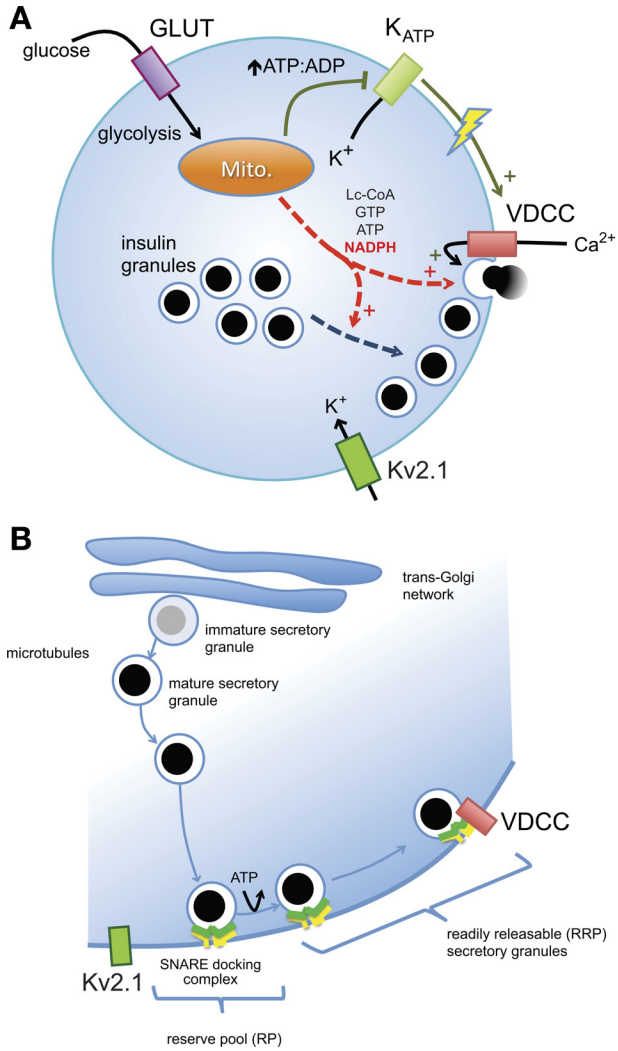

Fig. 1. Consensus model for glucose-stimulated insulin secretion from pancreatic β-cells. A: glucose entry through glucose transporters (GLUT) results in an increased intracellular ATP/ADP ratio. This results in inhibition of ATP-sensitive K^+ (K_{ATP}) channels, triggering electrical activity and opening of voltage-dependent Ca^{2+} channels (VDCC). Entry of Ca^{2+} triggers exocytosis of insulin granules at the plasma membrane. The voltage-dependent K^+ channel (Kv2.1) mediates a K^+ current that repolarizes β-cell action potentials, limiting Ca^{2+} entry and insulin secretion. Additional outputs from the mitochondria mediate a metabolic amplifying signal that further increases insulin secretion by facilitating insulin granule recruitment, docking, and/or exocytosis. B: once recruited to the plasma membrane, insulin granules dock through the association of vesicular and target-membrane SNARE proteins. Docked granules are "primed" for exocytosis in an ATP-dependent manner (whereby they become part of the readily releasable granule pool, able to undergo exocytosis in response to Ca^{2+}) and may physically associate with VDCCs and other channels (such as Kv2.1).

ing insulin secretion at the exocytotic site is much more vast. In reality, the exocytotic site consists of, and/or is influenced by, many interacting proteins. These include SNARE-interacting proteins like Munc18 (7), signaling proteins including the small G proteins (18, 62), cytoskeletal proteins such as actin filaments (62), and ion channels such as the VDCCs and Kv channels (13, 36). As such, this diverse range of proteins constitutes a regulatory complex, and control of the interaction between these players may prove to be an important determinant of insulin secretion.

Ion Channels

A diverse complement of ion channels is critical to pancreatic β-cell function (20, 54). More than a decade ago, it was recognized that SNARE proteins can interact with, and regulate, VDCCs (63). This was demonstrated to position β-cell secretory granules in close proximity to the sites of Ca^{2+} entry (3, 5, 64), allowing rapid local delivery of the Ca^{2+} signal for exocytosis (termed the "excitosome"). Other ion channels involved in insulin secretion also interact with and are regulated by SNARE proteins. For example, syntaxin 1A interacts with the nucleotide-binding domains of the K_{ATP} channel sulfonylurea receptor and modulates the regulatory effects of ADP (9, 32, 49). Syntaxin 1A also alters the gating and trafficking of Kv1.1 (16) and Kv2.1 (34, 35) by binding at the channels' COOH terminus. Similarly, VAMP2 (37) and SNAP-25 (45) inhibit the Kv2.1 channel by binding to its NH_2 terminus. While there are several caveats, (i.e., the use of overexpression and exogenous peptides of incompletely defined specificity), there is nonetheless ample evidence to suggest the regulation of ion channels by SNARE proteins. Future studies examining transgenic animal models and new approaches (FRET or single molecule imaging, for example) may be expected to provide further clarity regarding the specificity and physiological significance of SNARE-ion channel interactions. These findings have been interpreted in the context of SNARE-dependent tuning of excitation, whereby channel function and electrical activity are modulated depending on the status of SNARE interactions during vesicle docking and release (36).

However, recent work from Ilana Lotan's group demonstrates that at least one of these interactions (specifically the syntaxin 1A-Kv2.1 interaction) facilitates exocytosis per se, independently of the actual ion conducting capacity of the channel (14, 57, 58). Additionally, Kv2.1 may not be the only channel to directly influence exocytosis (39), given recent reports that TRP-M channels modulate insulin secretion under conditions where they are unlikely to have any role in electrical or Ca^{2+} responses (6, 61). The mechanism by which ion channels may directly regulate exocytotic function is far from clear. The positive effect of Kv2.1 on exocytosis is suggested to involve the facilitation of secretory granule recruitment to the plasma membrane, possibly through the stabilization of t-SNARE complexes (15). A potential role for Kv2.1 in physically transducing membrane potential changes to the exocytotic machinery has also been proposed (48). Thus, it is pertinent to ask: is there a reciprocal relationship between β-cell ion channels and exocytosis such that these are regulated in concert, to the extent that the regulation of excitation and exocytosis becomes inseparable?

Do Signals Control the Ion Channel-SNARE Complex to Simultaneously Affect Ion Channel and Exocytotic Function?

The formation and status of the excitosome complex may be regulated by many signals that control insulin secretion (Fig. 2). For example the GLP-1 receptor, which binds and regulates Kv2.1 (46), increases β-cell electrical and exocytotic responses in concert (19). Arachidonic acid produced by the phospholipase A_2 (PLA_2) family of enzymes has a similar dual effect to both augment β-cell exocytosis (31) and inhibit Kv2.1 currents (28), consistent with a positive insulinotropic role for $iPLA_2\beta$

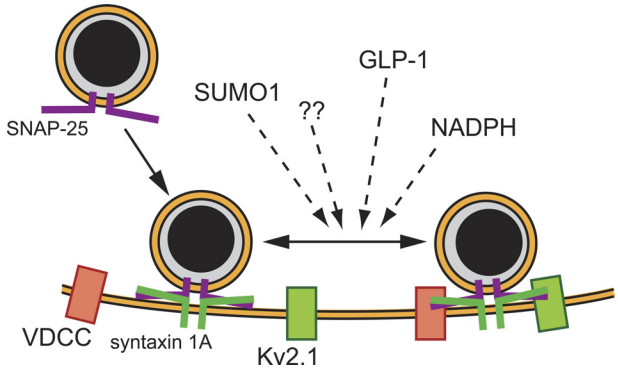

Fig. 2. Hypothetical model for the parallel regulation of exocytosis and ion channel function through the modulation of SNARE-channel complexes. The interaction of ion channels such as VDCCs and K^+ channels (Kv2.1) with exocytotic SNARE proteins (including SNAP-25 and syntaxin 1A) regulates channel function and conversely controls exocytotic capacity. Several signals known to affect insulin secretion (including GLP-1 receptor signaling, SUMOylation, and NADPH) have demonstrated effects on both exocytosis per se and ion channel activity. The hypothesis here is that these parallel effects result from modulation of the SNARE-channel complex (often termed the "excitosome").

shown in transgenic mice (2) and in human islets (50). It seems possible, then, that the function of any one protein, such as an ion channel, may be altered as a consequence of modulating the excitosome-exocytotic complex per se. Here, I provide evidence from work by our group and others on the regulation of Kv2.1 channel activity and exocytotic function in pancreatic β-cells to suggest that these are controlled in parallel. Indeed, this may suggest the control of a larger exocytotic-ion channel complex to affect secretion.

Metabolic regulation of ion channels and exocytotic efficacy. We (43) previously showed that increased NADPH, a candidate metabolic coupling factor, augments Kv2.1 inactivation and reduces the β-cell repolarizing K^+ current. This was proposed to enhance insulin secretion (47) and possibly be involved in the β-cell response to metabolic amplifying signals (24, 29, 38). However, inhibition of Kv2.1 seems an unlikely mechanism for metabolic amplification, since subplasma membrane Ca^{2+} is unaffected by the amplifying pathway (52). In any case, the metabolic amplification of insulin secretion occurs under conditions where Kv channel activity will have little effect on β-cell electrical responses (i.e., in the presence of high KCl and diazoxide, where the cells are held in a depolarized state). So why does NADPH regulate Kv2.1 channels in β-cells?

Renström and colleagues (25, 53) have demonstrated the direct facilitation of insulin exocytosis by NADPH. An effect of NADPH on the very distal events in insulin secretion such as granule docking and/or priming is consistent with the view that metabolic amplification occurs downstream of Ca^{2+} entry (52). One can hypothesize that NADPH enhances exocytosis in part by altering the interaction of SNARE proteins with ion channels, including Kv2.1. This seems plausible, given that Kv channels, including those in human and rodent islets, bind NADPH (1, 8) and that NADPH and syntaxin 1A overlap in their mechanism for the inhibition of Kv2.1. Both cause a leftward shift in the voltage dependence of channel inactivation (34, 35, 43). Thus, while NADPH inhibition of Kv2.1 channels is unlikely in itself to mediate the amplifying effects of glucose on insulin secretion, this observation is not necessarily unrelated to metabolic amplification. Indeed, although there is not yet any direct experimental evidence, this may be indicative of the control of a larger complex to effect the facilitation of insulin exocytosis.

Similarly, work by Kakei's group (65, 66) has demonstrated the regulation of Kv2.1 currents by the metabolic generation of ATP. This is suggested to either up- or downregulate channel activity, depending on the membrane potential examined (66). This work, interpreted in a purely excitatory sense, suggests that metabolic regulation of Kv2.1 contributes to the physiological regulation of β-cell electrical and Ca^{2+} responses to glucose. However, it could be asked whether the effects of glucose on Kv2.1 current are perhaps secondary to the modulation of an exocytotic channel-SNARE complex, particularly since the priming of secretory granules for release is also dependent on the metabolic generation of ATP (42, 55).

SUMOylation and the regulation of ion channels/exocytosis. Kv2.1 in human β-cells can be inhibited by SUMO1 (10). This has recently been shown to occur through a direct SUMOylation of Lys^{470} in the channel COOH terminus (51). We assumed that inhibition of Kv2.1 by SUMOylation would increase insulin secretion on the basis of known effects of Kv2.1 inhibition to enhance electrical activity, Ca^{2+} responses, and insulin secretion (26, 41, 44, 60). Instead, SUMOylation inhibits glucose-stimulated insulin secretion from mouse and human islets and has no effect on islet Ca^{2+} responses to glucose (11). This results from a direct inhibition of Ca^{2+}-dependent insulin exocytosis. While we have attributed the exocytotic effect in part to SUMOylation of the exocytotic Ca^{2+} sensor synaptotagmin VII (11), it is relevant to ask: are the effects of SUMOylation on Kv2.1 activity and exocytotic function mutually exclusive?

Indeed, we find that several SUMO1-associated proteins are pulled down with Kv2.1, including syntaxin 1A (unpublished observations). Furthermore, the Kv2.1 SUMOylation site (51) is located within the C1a domain of the channel deemed critical for syntaxin 1A binding (58) and which our recent work demonstrates is required for Ca^{2+}-dependent insulin exocytosis from human β-cells (Dai et al., unpublished observation). It will be interesting to determine whether the SUMOylation status of the channel alters its interaction with syntaxin 1A. Again, while direct evidence is lacking, we can hypothesize that this contributes to the regulation of exocytosis per se by SUMO1 and that effects on Kv2.1 activity occur in parallel to modulation of the excitosome complex.

Concluding Remarks

The interactions controlling insulin exocytosis at the plasma membrane and their regulation are complicated. Beyond the SNARE machinery, many interacting adaptor and signaling molecules regulate exocytosis. This complex would comprise not only SNAREs and ion channels (i.e., the excitosome) but also receptors and signal integrators [such as snapin (59)]. But is such a complex consistent with the spatial constraints of the exocytotic site? How dynamic are the interactions expected to be [syntaxin itself is remarkably dynamic in its association with the exocytotic site (4)]? Future work should examine these questions and whether the various excitosome complex interactions (such as Kv2.1-syntaxin 1A) contribute to the

regulation of insulin secretion by various metabolic and receptor mediated signals.

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DISCLOSURES

No conflicts of interest are reported by the author.

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