Store-operated Ca²⁺ entry: a key component of the insulin secretion machinery

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Abstract

Normal plasma glucose level is ensured by the action of insulin, the major hypoglycemic hormone. Therefore, it is not surprising that insulin release from pancreatic β-cells of the islets of Langerhans is controlled by an array of balanced mechanisms in which glucose plays the leading role. Glucose triggers insulin secretion through the well-described pathway of ATP-driven closure of ATP-sensitive potassium channels (K_{ATP}), depolarization of the plasma membrane, and opening of the voltage-dependent Ca²⁺ channels (VDCC). The subsequent rapid rise in cytoplasmic free Ca²⁺ concentration triggers insulin exocytosis. However, despite more than 40 years of investigation, certain aspects of the intracellular Ca²⁺ responses to glucose and secretagogues remain unexplained, suggesting the involvement of additional Ca²⁺ channels. Here, we discuss the emerging role of store-operated Ca²⁺ channels carried by Orai1 and transient receptor potential canonical 1 (TRPC1) proteins and regulated by the stromal interaction molecule 1 (STIM1) in the control of glucose-induced insulin secretion. The role of other voltage-independent cation channels formed by other members of the TRP channels family is also addressed.

Introduction

Insulin secretion is driven by electrical activity and oscillations of intracellular Ca²⁺ concentration ($[Ca^{2+}]_i$) in pancreatic β-cells. The main driver of insulin secretion is plasma glucose; glucose metabolism produces ATP, which closes ATP-sensitive potassium channels (K_{ATP}) and cell depolarization, which, in turn, open voltage-dependent Ca²⁺ channels (VDCC). The sudden rise in intracellular Ca²⁺ levels causes exocytosis of insulin-containing vesicles. In addition to K_{ATP} channels and VDCC, a complex interplay between additional ion channels and intracellular Ca²⁺ stores produces unique patterns of oscillatory depolarization and $[Ca^{2+}]_i$ changes controlling insulin release (Gilon *et al.* 2014).

Since the concept of store-operated Ca²⁺ entry (SOCE) was first defined and characterized by Putney in the mid-1980s (Putney 1986), evidence has accumulated indicating that SOCE exists almost in all cell types. However, while SOCE has been shown to play a critical role in agonist-induced responses in many non-excitable cell types, direct evidence for its role in excitable cells is generally lacking.

This review focuses on recent advances in the field of SOCE in insulin-secreting pancreatic β-cells.

State of the art: current understanding of store-operated channels

The ubiquitous SOCE, first described by Putney in 1986 (Putney 1986), is the prominent mechanism of Ca^{2+} entry in nearly all non-excitable cells. The activation of a variety of phospholipase C (PLC)-coupled receptors leads to the formation of two second messengers, diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3). In turn, the IP_3 receptors mobilizes Ca^{2+} from the endoplasmic reticulum (ER), resulting in depletion of Ca^{2+} stores, which triggers extracellular Ca^{2+} influx named SOCE. In addition to rapidly refilling the depleted ER stores, which is essential to maintain proper ER functions, SOCE also triggers downstream signaling events involved in a wide variety of cell functions such as proliferation, growth, migration, inflammation and apoptosis. In addition, SOCE has been proposed to contribute to short-term cellular responses such as Ca^{2+} oscillations, exocytosis and muscle contractility. Despite the growing acknowledgment of the biological importance of SOCE into well-established models of Ca^{2+} homeostasis, the molecular mechanisms responsible for sensing ER Ca^{2+} depletion and triggering extracellular Ca^{2+} influx were the subject of intense debate since 1986, until the identification of stromal interaction molecule 1 (STIM1) in 2005. The STIM proteins are ER transmembrane proteins with a luminal C-terminal domain working as a Ca^{2+} sensor. Emptying of the ER Ca^{2+} stores sensed by this domain induces the rapid oligomerization of STIM1 dimers and translocation to ER/plasmalemmal junctional regions, where they physically interact with store-operated channels (SOCs) to induce SOCE. Despite high homology, the two isoforms, STIM1 and STIM2, have subtly distinct functions. STIM1 seems to be the main SOC-activating isoform, while STIM2, although its function is not completely elucidated, is a more sensitive sensor of ER luminal Ca^{2+} (reviewed in Prakriya 2013).

Early studies supported that the transient receptor potential canonical (TRPC) channels family formed SOCs. The seven members of this family (TRPC1–C7) assemble as homo- or heterotetramers to form cation-permeable channels with modest Ca^{2+} selectivity. These channels are activated in response to receptor-mediated PLC activation via store-dependent and store-independent mechanisms. They typically generate relatively linear current–voltage relationships called ISOC with a reversal potential near 0 mV. However, the involvement of TRPC channels in the SOCs formation remains a highly contentious and unresolved issue (reviewed in Cheng *et al.* 2013).

In addition to TRPC proteins, the discovery in 2005 of the interaction between STIM1 and Orai1 proteins has created a whole new field of SOCE research. Orai1, which was first identified in mast and T cells via RNAi-mediated knockdown strategy, recapitulates the archetypical Ca^{2+} release-activated Ca^{2+} (CRAC) current (I_{CRAC}) (Prakriya 2013). This I_{CRAC} current is characterized by its responsiveness to store depletion, its small single-channel and whole-cell current densities, and its extreme selectivity for Ca^{2+} under physiological conditions (reversal potential near + 50 mV). There are three human Orai proteins (Orai1–3), but little is known about Orai2 and -3. The interaction between the cytoplasmic region of STIM1 (Orai-activating small fragment residues) and the C-terminal cytosolic segment of Orai1 is mandatory for the I_{CRAC} current (Prakriya 2013).

Several recent reports suggest that TRPC proteins, especially TRPC1 and -C4, interact with Orai1 to form functional SOCs activated by STIM1 in a variety of cell types. In that model, STIM1 may open TRPC channels by electrostatic interaction through its polybasic lysine-rich domains (Cheng *et al.* 2013). Conversely, STIM1/Orai1 and TRPC channels have been shown to bind and inhibit voltage-gated L-type Ca^{2+} channels in some models (Moreno & Vaca 2011, Sabourin *et al.* 2011).

Compared with the advances in many cell types, the physiological function of TRPC/Orai1-dependent SOCE is very unclear in β-cells.

SOCE in insulin secretion: from capacitive Ca^{2+} entry to the role of the STIM–Orai–TRPC complex

As mentioned in the introduction, pancreatic β-cells are excitable cells that depolarize in response to high glucose concentration. This electrical activity is characterized by typical bursting impulses, which in turn lead to $[Ca^{2+}]_i$ oscillations and subsequent insulin release oscillations. Thus, pancreatic β-cells rely largely on electrical activity and VDCC for extracellular Ca^{2+} influx. Nevertheless, it has been known for a long time that during glucose-induced depolarization, the increase in $[Ca^{2+}]_i$ is mediated not only by Ca^{2+} influx through VDCC, but also by Ca^{2+} released from the ER, and that ER Ca^{2+} stores are required to maintain the amplitude of glucose-induced $[Ca^{2+}]_i$ oscillations over time. ER Ca^{2+} release occurs both through passive leakage and active receptor-mediated release. Of note, in β-cells, active ER Ca^{2+} release is mainly due to IP_3-induced Ca^{2+} release. Ca^{2+}-induced Ca^{2+} release (CICR)

through RyRs may also occur upon large VDCC-mediated Ca²⁺ influx. However, this process is still controversial (reviewed in Gilon *et al.* 2014).

Glucose-induced Ca²⁺ influx through VDCC contributes to refilling ER Ca²⁺ stores. However, Ca²⁺ efflux also occurs and VDCC alone cannot ensure ER Ca²⁺ homeostasis at all times. This is especially true in the case of stimulation with IP₃-producing agents such as acetylcholine (ACh), which triggers massive ER Ca²⁺ export (reviewed in Gilon & Henquin 2001). Early studies performed in hyperpolarized β-cells clearly demonstrated the presence of SOCE stimulated by ER Ca²⁺ depletion in murine β-cells (Worley *et al.* 1994, Miura *et al.* 1996, Mears & Zimliki 2004). This capacitative Ca²⁺ entry is of much lower amplitude than that induced by glucose through VDCC. However, it is important to keep in mind that [Ca²⁺]ᵢ measurements in hyperpolarized cells mask a potential interplay with VDCC, thereby probably underestimating the influence of SOCs on Ca²⁺ influx.

Until recently, the identity of the channels mediating SOCE in β-cells remained unclear. Studies indicated that murine β-cells express several members of the TRPC protein family (reviewed in Jacobson & Philipson 2007). STIM1 and Orai1 are also present, and ER Ca²⁺ depletion stimulates STIM1 translocation to the plasma membrane to co-cluster with Orai1 in murine β-cells (Tamarina *et al.*

2008, Tian *et al.* 2012). Using pharmacological approach and dominant negative mutants of STIM1, Orai1 and TRPC1, we demonstrated that STIM1 is instrumental in the activation of both Orai1 and TRPC1 channels upon store depletion, either in response to chemical ER depletion using thapsigargin or in response to the glucose-stimulated insulin secretion (GSIS) secretagogue ACh. We further demonstrated that TRPC1, Orai1 and STIM1 form a ternary complex upon ER Ca²⁺ depletion (Sabourin *et al.* 2015). The inhibition of these channels severely impairs the second phase of insulin release and fully blocks the potentiating effect of ACh on insulin secretion. Interestingly, blockade of either Orai1 or TRPC1 reduces the amplitude of the [Ca²⁺]ᵢ oscillations in rat β-cells (Fig. 1), which supports a role of these proteins in the [Ca²⁺]ᵢ dynamics of GSIS. In addition to TRPC1, we and others observed that rodent β-cells express TRPC3, -C4, -C5 and -C6 (Jacobson & Philipson 2007, Sabourin *et al.* 2015). Our experiments showed that TRPC3 does not contribute to GSIS (Sabourin *et al.* 2015). However, it was recently suggested that TRPC3 may be involved in glucagon-like peptide 1 (GLP-1)-mediated Ca²⁺ influx in rat islets (Yamada *et al.* 2016). Further studies are required to determine the exact role of the different TRPC isoforms in β-cell function in response to glucose and secretagogues, as well as their potential interactions with VDCC.
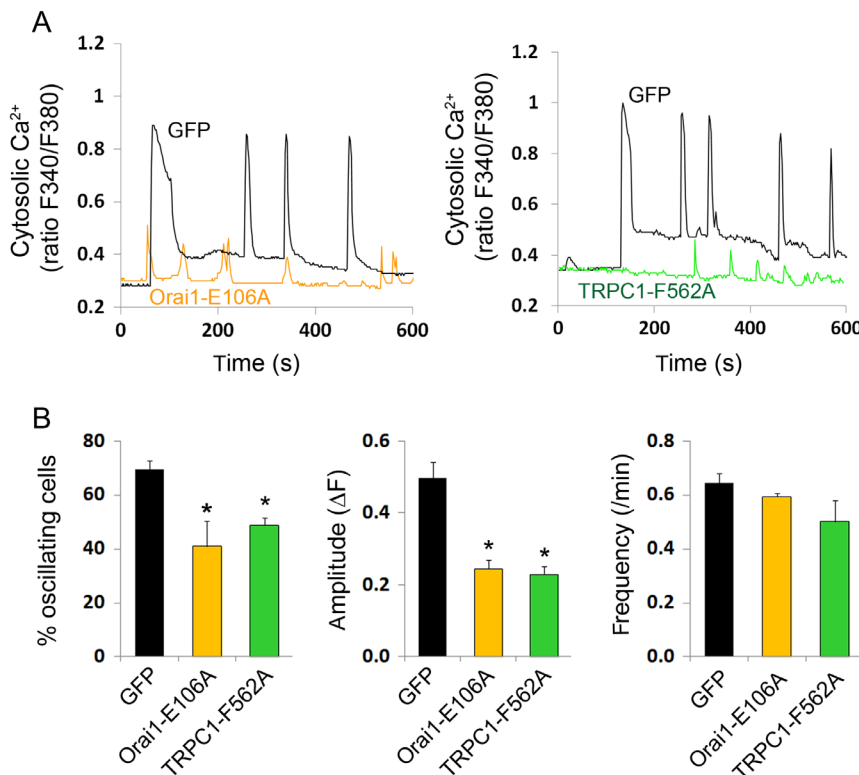

Figure 1

Orai1 and TRPC1 blockade reduces the number of oscillating cells and the amplitude of glucose-induced intracellular Ca²⁺ oscillations in the rat insulinoma cell line INS-1E. Fura-2/AM imaging in INS-1E cells transfected with dominant negative Orai1-E106A or TRPC1-F562A mutants in KRBH supplemented with 20 mM glucose and 2 mM Ca²⁺. Transfected cells were loaded with 3 μM Fura-2/AM plus 10% (w/v) Pluronic F-127 (Life Technologies) for 45 min in KRBH supplemented with 2 mM glucose. Cells were acquired after Fura-2/AM excitation at 340/380 nm with a Visichrome holographic monochromator. Images were treated with MetaFluor to evaluate the ratio of fluorescence emitted at 340 and 380 nm and the fluorometric signal was measured as the ΔF (340/380 nm ratio). (A) Representative traces of INS-1E cells transfected with the control GFP, Orai1-E106A or TRPC1-F562A mutants. (B) Quantitative assessment of the percentage of transfected cells oscillating, of the Ca²⁺ oscillations amplitude (ΔF), and oscillations frequency per min. $n = 4$ experiments, $n = 15$ investigated cells/experiment minimum.

Early studies clearly demonstrated the existence of an inward current triggered by ER Ca^{2+} depletion in murine β-cells, which could depolarize the plasma membrane and activate VDCC (Worley *et al.* 1994, Dyachok & Gylfe 2001, Mears & Zimliki 2004). According to this hypothesis, the ER depletion and SOCs opening strongly amplify glucose-induced depolarization in murine β-cells (Miura *et al.* 1996, Roe *et al.* 1998). Of note, the TRPC3 and -C6 channels have been shown, in vascular smooth muscle cells, to trigger sufficient depolarization to open VDCC (Soboloff *et al.* 2005, Wang *et al.* 2008). Careful electrophysiology studies should be undertaken to determine the impact of the ternary complex Orai1/TRPC1/STIM1 in the electrical activity of β-cells.

In contrast, reports indicate that STIM1/Orai1 and TRPC channels may suppress VDCC via physical interaction in various cell types (Moreno & Vaca 2011, Sabourin *et al.* 2011). The direct or functional interactions between SOCs and VDCC in β-cells deserve further investigations to elucidate the effects of SOCE on VDCC and resulting regulation of GSIS.

Aberrant SOCE is observed in a growing number of diseases, such as cardiovascular, pulmonary, skin diseases and inflammation. Also, mutations in TRPC proteins have been linked to several channelopathies, and single-nucleotide polymorphisms in the *TRPC1* gene are associated with type 2 diabetes mellitus (T2DM) (reviewed in Nilius & Szallasi 2014). Mutations in *STIM1* and *ORAI1* have also been linked to devastating immunodeficiency diseases termed CRAC channelopathies. Additionally, STIM1/Orai1 dysregulations are involved in a broad range of diseases including cancer, neurodegenerative and cardiovascular diseases (Prakriya 2013). In our recent study, we demonstrated that SOCE function is altered in a rat β-cell line after prolonged exposure to high concentration of glucose (Sabourin *et al.* 2015), suggesting a potential dysfunction of SOCE in diabetes. So far, no link was found between Orai1, STIM1 or TRPCs and β-cell defects. Unfortunately, no data are available yet reporting the role of these proteins in insulin secretion *in vivo*. The study of mouse invalidated for Orai1 and STIM1 has demonstrated the importance of these proteins in development and growth (reviewed in Feske 2009), but their crippling phenotypes do not allow for proper study of β-cell function (*Orai1⁻/⁻* mice on a mixed genetic background are smaller, immunodeficient and present alopecia; *Stim1⁻/⁻* mice generated in various ways perish *in utero* or *perinatali* of respiratory failure). The limited current knowledge for this potentially key mechanism in insulin release calls for the generation of tissue-specific knockout mice to evaluate the importance of SOCE *in vivo* in normal and pathological situations leading to diabetes.

Other TRP channels in β-cells

Other reports point out to additional channels of the TRP family as key players in β-cell function. Rodent and human primary β-cells and/or insulin-secreting cell lines express a variety of TRP channels belonging to the vanilloid (TRPV2 and -4), ankyrin (TRPA1) and melastatin (TRPM2–5) subfamilies (reviewed in Colsoul *et al.* 2013).

TRPM2 forms functional Ca^{2+}-permeable channels in β-cells, which contribute to insulin release in response to glucose and exendin-4 (a GLP-1 receptor agonist) and *Trpm2⁻/⁻* mice show hypoinsulinemia and resultant hyperglycemia. However, TRPM2 seems to regulate insulin secretion independently of its role as a Ca^{2+} channel and no correlation was detected between TRPM2 variants and the risk for developing T2DM. GSIS is also significantly impaired in pancreatic islets from *Trpm5⁻/⁻* mice, resulting in impaired glucose tolerance. This appears to be due to a lack of glucose-induced fast $[Ca^{2+}]_i$ oscillations in those mice. In their study, Colsoul and coworkers hypothesized that these oscillations are lost due to an impairment of the Ca^{2+}-activated Na^+/K^+ current, which could be driven by the monocation-permeable TRPM5 channels. New reports further suggest a role of TRPM5 in the potentiating effect of GLP-1 in rat islets (Krishnan *et al.* 2014) and the GLP-1 effects are negligible in *Trpm4⁻/⁻* or *Trpm5⁻/⁻* islets (Shigeto *et al.* 2015). Of note, an association was recently found between TRPM5 variants and prediabetic phenotypes in individuals who are at risk for developing T2DM. The Ca^{2+}-activated TRPM3 channels are also present in rodent and human islets. However, despite some findings indicating that these channels might form functional channels in β-cells, it is unlikely that they play a major role in physiologic insulin release. Finally, TRPA1 activation by mustard oil and 4-hydroxy-2-nonenal stimulates insulin release and the TRPA1 antagonist HC-030031 inhibits GSIS, suggesting a role for TRPA1 in β-cell function (Colsoul *et al.* 2013).

Conclusion

The model suggesting that GSIS relies only on VDCC has shown its limits and accumulating evidence now supports a growing importance for voltage-independent cation channels in β-cell function. These channels offer a new paradigm of $[Ca^{2+}]_i$ dynamics and pancreatic β-cell

excitability. Our findings especially stress the critical role of the ternary TRPC1/Orai1/STIM1 complex in Ca^{2+} influx, the amplitude of Ca^{2+} oscillations, and correlated GSIS. The existence of Orai2 and -3, as well as STIM2, and other TRP proteins potentially interacting with the Orai1/TRPC1/STIM1 complex, calls for further studies to characterize the regulation of this new class of channels in β-cells.

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