IP_3Rs and nSOCE—Tied Together at Two Ends

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Abstract

All living organisms need to respond appropriately to changes in the extracellular milieu. Cellular mechanisms that enable such responses evolved in parallel with organismal complexity and intracellular Ca^{2+} signaling is one such mechanism where extracellular signals received at the cell membrane communicate with endoplasmic reticular stores of Ca^{2+}, to stimulate appropriate Ca^{2+}-mediated changes in cellular physiology. The amplitude and dynamics of endoplasmic reticulum (ER)-Ca^{2+} release in response to extracellular signals determines the nature of the cellular response. An understanding of how ER-Ca^{2+} channels might regulate cellular Ca^{2+} signaling in different cell types is lacking. In a recent paper, this question has been addressed in the context of neurons (Chakraborty et al., 2023) and the implications of these new findings are discussed here.

Keywords

Ca^{2+}, calcium release-activated channel, endoplasmic reticulum (ER), IP_3 receptors, Orai, signaling, store-operated calcium entry (SOCE), stromal-interaction molecule (STIM)

Two classes of intracellular Ca^{2+} channels that receive inputs from the cell surface are the endoplasmic reticulum (ER)-localized inositol 1,4,5 trisphosphate receptor (IP_3R) and the ryanodine receptor (RyR). Of these, the IP_3R releases ER-Ca^{2+} upon generation of IP_3 in response to activation of either phospholipase Cβ (PLCβ) or γ by membrane-bound G-protein coupled receptors or receptor tyrosine kinases, respectively, whereas Ca^{2+} release from the RyR occurs primarily in response to changes in cytoplasmic Ca^{2+} or membrane depolarization, upon entry of extracellular Ca^{2+} through plasma membrane (PM) localized channels (Woll and Petegem, 2022). Thus, a range of extracellular agonists that include hormones, peptides, antigens, and neurotransmitters stimulate IP_3-mediated Ca^{2+} release, whereas voltage-gated or ligand-gated-channels on excitable cells stimulate Ca^{2+}-induced Ca^{2+} release from the RyR (Woll and van Petegem, 2022).

A consequence of ER-Ca^{2+} release is depletion of ER-store Ca^{2+}, replenishment of which is essential for normal chaperone function in the ER as well as Ca^{2+} transfer to mitochondria. Replenishment of ER-store Ca^{2+} occurs through store-operated Ca^{2+} entry (SOCE), where ER-localized stromal-interaction molecule (STIM) proteins sense the drop in ER-Ca^{2+} and communicate with the membrane-localized Ca^{2+} channel Orai, leading to channel opening, entry of extracellular Ca^{2+} and refilling of ER-store Ca^{2+} (Prakriya and Lewis, 2015). It has long been thought that non-excitable and excitable cells might have different means of restoring ER-Ca^{2+} stores. In excitable cells, frequent stimulation of voltage-gated and ligand-gated membrane channels allows for multiple modes of Ca^{2+} entry possibly rendering STIM/Orai SOCE redundant. However, evidence of SOCE in excitable cells has mounted over the years (Bouron, 2023). Indeed, SOCE was identified in excitable cells well before the molecular mechanism of SOCE through STIM/Orai was elucidated in non-excitable cells (Emptage et al., 2001; Baba et al., 2003). Genetic studies also identified a requirement for the IP_3R and the SOCE components STIM and Orai in regulating *Drosophila* flight (Venkiteswaran and Hasan, 2009; Sharma and Hasan, 2020). Investigation of cellular Ca^{2+} changes in primary cultures of *Drosophila* neurons first identified attenuated SOCE in IP_3R mutant neurons, despite pharmacological depletion of ER-store Ca^{2+} by thapsigargin, suggesting that Ca^{2+} store depletion by the IP_3R might directly regulate SOCE through a mechanism independent of STIM-activation upon IP_3-mediated ER-store Ca^{2+} release (Venkiteswaran and Hasan, 2009; Chakraborty et al., 2016).

An initial understanding of the cellular mechanism for attenuated SOCE in ER-Ca^{2+} store-depleted neurons from

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IP_3R mutants was obtained in a study from *Drosophila* neurons where STIM–Orai interactions (measured by co-immunoprecipitation) appeared weaker (Chakraborty et al., 2016). However, in the same study, normal SOCE was observed in DT-40 cells (an avian immune cell line) with a simultaneous knockout of the three vertebrate IP_3R isoforms, indicating that regulation of SOCE by the IP_3R independent of ER-Ca^{2+} release may be specific to invertebrates. The co-regulation of ER-Ca^{2+} release and store-operated Ca^{2+} entry in non-excitable immune cells was proposed earlier through conformational coupling between the IP_3R regulation and SOCE components (Ma et al., 2000), but subsequent data from studies using pharmacological reagents did not support direct regulation of store-operated Ca^{2+} entry by the IP_3R in vertebrate cells (Prakriya and Lewis, 2001). Several other studies also demonstrated normal or near-normal SOCE in a human epithelial cell line (HEK) with complete deletion of all three IP_3R subtypes (Bartok et al., 2019; Yue et al., 2020).

A better understanding of these contradictory results has come from the results of a recently published paper (Chakraborty et al., 2023), where the authors created an inducible knock-down for the IP_3R1 in a human embryonic stem cell line that enabled a generation of differentiated neural precursor cells and neurons. Upon knockdown of IP_3R1 (the predominant isoform in mammalian neurons) for periods of a month or less, SOCE was attenuated to approximately 50% of control levels in both neurol progenitor cells and differentiated neurons. Either genetic knock-outs of all three IP_3R isoforms over prolonged periods (as in the earlier studies) lead to upregulation of compensatory mechanism(s) for SOCE or regulation of SOCE by IP_3Rs differs in neurons as compared to other cell types or a combination of both of the above possibilities. For a mechanistic understanding of this phenomenon, further experiments were performed in the human neuroblastoma cell line SH-SY5Y. Here too knockdown of IP_3R1, performed over 2–3 weeks, led to strongly attenuated SOCE accompanied by reduced STIM–Orai contacts that could be rescued by overexpression of either the rat IP_3R1 or IP_3R3. Interestingly, SOCE and STIM–Orai contacts were also rescued by a pore-dead version of the rat IP_3R1, but not by a ligand-binding deficient mutant form of IP_3R1. Importantly, SOCE in IP_3R1 knockdown SHSY5Y cells could be rescued by overexpression of either STIM1 or the ER–PM contact-enhancing protein ESyt1. Based on these observations, the authors suggest that IP_3 binding to the IP_3R1 enables better contact formation between the ER-localized STIM1 and PM-localised Orai1, independent of ER-Ca^{2+} release. IP_3 binding to the IP_3R thus appears to be a first step in inducing STIM–Orai coupling prior to depletion of ER-Ca^{2+}.

What is the source of IP_3 when ER-store depletion is by thapsigargin, a pharmacological agent that does not induce IP_3 formation, but which was used for measuring SOCE in the experiments described above. An inhibitor for the G-protein that activates PLCβ for PIP2 hydrolysis and formation of IP_3 mirrored the effect of reduced SOCE in SHSY5Y cells indicating that ambient Gαq signaling generates IP_3 in resting neuronal cells. In support of this idea generation of agonist-induced IP_3 restored SOCE in IP_3R1 knockdown SHSY5Y cells with partially depleted ER-Ca^{2+} stores. SOCE in neuronal cells thus has a dual dependence on IP_3R; that is, IP_3 binding and ER-Ca^{2+} release. What about non-excitable cells? SOCE in HEK cells remains similar to control either by IP_3R1 knockdown or by inhibition of Gαq but when the two are presented together HEK cells also show reduced SOCE. These observations suggest that the extent to which IP_3Rs tune STIM–Orai interactions and SOCE may be determined by the differential strength of cell type-specific IP_3 signaling. In neuronal cells, reduction of either IP_3R1 or basal IP_3 is sufficient to unveil the dual contribution of IP_3R for regulation of SOCE, whereas non-excitable cells (such as HEK and DT-40) require reduced levels of both IP_3 and IP_3R to unveil this contribution. Normal SOCE in response to pharmacological depletion of ER-Ca^{2+} stores observed in HEK cells with a complete knockout of all three IP_3R subtypes is possibly due to compensatory mechanisms that create more ER–PM junctions.

The mechanism by which IP_3-binding to the IP_3R enhances STIM–Orai interactions and SOCE in the absence of ER-Ca^{2+} release remains to be identified. Rescue of SOCE in the IP_3R1^{KD} by the ER–PM contact protein Esyt1 supports the idea that IP_3-binding enhances ER–PM contacts. Recent cryo-electron microscopy studies have demonstrated conformational changes in the N-terminus domains of the IP_3R upon ligand binding (Fan et al., 2022; Schmitz et al., 2022). Such conformational changes could enhance ER–PM contacts and poise STIM1 and Orai1 for channel opening upon ER-Ca^{2+} depletion. In the absence of ligand binding to the IP_3R1, STIM1 movement to the ER–PM junction is attenuated upon stimulation of agonist induced SOCE (Chakraborty et al., 2023). There is, however, no convincing evidence to support direct IP_3R–STIM1 interactions during SOCE. Indeed, wild-type and ligand-binding mutant versions of the IP_3R1 show similar localization at the ER–PM junction before, during, and after agonist-induced SOCE. Rescue of SOCE in IP_3R1^{KD} cells by STIM1 suggests that SOCE in neuronal cell soma may be limited by STIM1 expression and IP_3-bound IP_3Rs might enhance STIM1 presence at the ER–PM junction and thus help raise SOCE. Alternately, or in addition, IP_3-bound IP_3Rs could support faster formation of ER–PM junctions. While STIM and Orai are undoubtedly the core components of SOCE, many additional proteins modulate their interactions, and yet other proteins/lipids contribute by regulating the assembly of ER–PM contacts. In summary, though a complete understanding of how IP_3-bound IP_3Rs enhance SOCE is lacking, it is likely through an intermediate that enhances STIM1 presence at ER–PM contact sites upon IP_3-binding, possibly by altering membrane conformation. The nature of this intermediate is

yet to be identified. Candidates worth investigating are the Septin family of cytoskeletal proteins, amongst which Septin 7 was identified earlier as a negative regulator of SOCE in *Drosophila* (Deb et al., 2016) and mouse (Dhanya and Hasan, 2021) neurons, whereas Septin 2/4 are positive regulators of SOCE (Deb and Hasan, 2016).

At a systemic level, it is of interest that *Drosophila* mutants for the IP_3R, STIM, and Orai exhibit similar phenotypes frequently deriving from the same neurons suggesting that neuronal SOCE is coupled closely to IP_3R function. Neuromodulators that stimulate IP_3-Ca^{2+} probably also trigger neuronal SOCE in specific neuronal classes where together the two modes of intracellular Ca^{2+} signaling contribute to neuronal function over and above the maintenance of neuronal Ca^{2+} homeostasis. The contribution of IP_3/Ca^{2+}/SOCE for modulating neuronal functions over a range of time periods from seconds to hours needs further investigation.

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