MINI-REVIEW

Regulation of calcium entry in exocrine gland cells and other epithelial cells

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Keywords **:** *calcium channels, exocrine gland cells, epithelial cells, calcium signalling*

J. Med. Invest. 56 Suppl. : 362-367, December, 2009

STORE-OPERATED CALCIUM ENTRY : THE BEGINNINGS IN EXOCRINE GLANDS

Store-operated or capacitative calcium entry is a phenomenon whereby the depletion of intracellular Ca^{2+} stores, generally from the endoplasmic reticulum, leads to the activation of plasma membrane Ca^{2+} channels (1-3). The idea developed over a decade or so from studies of the relative roles and interactions of Ca^{2+} release and Ca^{2+} entry mechanisms in salivary and lacrimal gland cells. That many cell types utilize both intracellular release of Ca^{2+} together with influx of Ca^{2+} across the plasma membrane for the generation of cytoplasmic Ca^{2+} signals have been appreciated for some time (4, 5). In salivary gland cells, it was shown that the Ca^{2+} stores released by activation of autonomic receptors (muscarinic, α-adrenergic, substance P) required Ca^{2+} influx through activated Ca^{2+} channels for their replenishment (6). In the late 1970's and early 1980's, two key findings influenced thinking on how these two modes of signaling might interact. First, it became clear that the source of intracellular Ca^{2+} for the release phase of responses was the endoplasmic reticulum (7, 8). Second, the signal for the release of Ca^{2+} from the endoplasmic reticulum was shown to be the soluble product of phospholipase C activation, inositol 1,4,5-trisphosphate (IP_3) (9, 10).

Received for publication October 17, 2009 ; accepted October 24, 2009.

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The first hint that intracellular stores might direct the activity of plasma membrane Ca^{2+} channels came from the observation in lacrimal gland cells that stores refilled rapidly following their emptying, and this rapid refilling did not require receptor activation (11). Casteels and Droogmans (12) speculated that in smooth muscle, this rapid refilling might occur through a direct route, not traversing the cytoplasm. Subsequent studies, however, showed that this could not be the case (13, 14). The general concept of store-operated entry was articulated in an hypothesis paper in Cell Calcium in 1986 (1). Subsequently, two key observations, both made using salivary gland cells, provided strong evidence for the concept.

The first was a publication essentially confirming the 1978 report showing by use of Ca^{2+} indicators that Ca^{2+} influx occurred in the absence of receptor activation, when Ca^{2+} stores were depleted (15). The second was the demonstration that depletion of Ca^{2+} stores by a mechanism independent of phospholipase C signaling quantitatively and qualitatively recapitulated the Ca^{2+} entry activated through phospholipase-linked receptors. This latter publication demonstrated for the first time the activation of Ca^{2+} entry by the SERCA pump inhibitor, thapsigargin (16). Since then, thapsigargin has come to represent the clearest pharmacological indicator for store-operated Ca^{2+} entry.

In 1992, the first demonstration of a store-operated Ca^{2+} current was published by Hoth and Penner (17). This current was measured by use of the whole-cell patch clamp mode in mast cells, and was subsequently shown to be similar in T-cells (18).

Hoth and Penner called the current I_{crac}, for *c*alcium *r*elease-*a*ctivated *c*alcium current. I_{crac} was shown to develop rather slowly (10s of seconds) following Ca^{2+} store depletion, and to be highly Ca^{2+} selective and strongly inwardly rectifying. The single channel conductance is thought to be extremely small, estimated by noise analysis to be in the fS range (18, 19). As is the case for other Ca^{2+}-selective channels, the selectivity for Ca^{2+} is lost in low divalent cation solutions, permitting measurements of larger whole-cell Na^{+} currents (20).

THE MOLECULAR COMPONENTS OF SOCE

For a full twenty years following the first formulation of the concept of store-operated Ca^{2+} entry, investigations moved in fits and starts attempting to resolve two fundamental questions : what is the nature of the signal from the endoplasmic reticulum, and what is the identity of the Ca^{2+} channel? Numerous candidates for the signaling mechanism came and went, including cyclic GMP, arachidonic acid metabolites, inositol 1,3,4,5-tetrakisphosphate, and the IP_3 receptor to name a few (3). One idea, that a diffusible substance termed "calcium influx factor" or CIF has received continuing support from a limited number of laboratories (21-24). While the role of such a factor is possible within the context of the Ca^{2+} sensor STIM1 (discussed below), the major impediment to understanding the function of CIF is the lack of knowledge of its structure. This prevents the majority of laboratories from following up on the published findings of a few, since its formation and action can only be investigated through use of tedious methods of partial purification and reconstitution.

Remarkably, in 2005 the powerful use of RNAi-based genetic screens revealed the endoplasmic reticulum Ca^{2+} sensor, and one year later, the SOC channel. The Ca^{2+} sensor, STIM1, was reported by two laboratories within a few weeks of one another (25, 26). STIM1, and in vertebrates it's close relative STIM2, are single pass membrane proteins. STIM1 is found in the endoplasmic reticulum and plasma membrane, while STIM2 appears to be exclusively in the endoplasmic reticulum (27). The function of STIM1 in the plasma membrane, at least in the context of SOCE, is unknown since constructs incapable of reaching the plasma membrane are fully capable of supporting SOCE (28). There is evidence that STIM1 in the plasma membrane plays a role in the function of non-store-operated arachidonic acid gated channels (29). Much of the key domain structure of STIM1 is known (Fig. 1). The N-terminus is

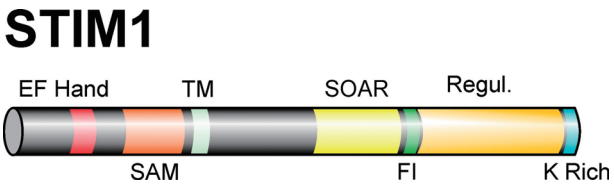

Figure 1. The domain structure of STIM1 includes a calcium sensing EF Hand, a sterile alpha motif (SAM), a single transmembrane domain (TM) and Orai-interacting SOAR domain, a regulatory (Regul.) domain in which numerous phosphorylation sites reside, and a C-terminal basic region that may be involved in interactions with plasma membrane acidic phospholipids.

directed towards the lumen of the endoplasmic reticulum. Therein lies the Ca^{2+} binding domain, an unpaired EF-hand. Immediately downstream is a sterile alpha motif (SAM) domain which is known to mediate protein-protein interactions, and interestingly also protein RNA interactions. When Ca^{2+} dissociates from the EF hand, this causes a conformational change in the EF-hand and SAM domains causing them to interact, initially dimerize and then to oligomerize (26, 30-32). STIM1 then aggregates in discrete subplasmalemmal sites where it apparently can interact directly with Orai channel molecules and activate them (33, 34). This is accomplished through a coiled-coiled domain first described by Yuan, *et al.* as a SOAR (STIM-Orai activating region) domain (35), and rapidly confirmed by three additional laboratories (34, 36, 37).

The evidence is very strong that Orai proteins constitute the pore forming subunits of the store-operated or CRAC channel. Overexpression of STIM1 and Orai1 produces huge I_{crac} (28, 38-40). Mutation of a glutamate at position 106 in human Orai1 to alanine or glutamine results in an inactive channel, while the more conservative mutation to aspartate results in a channel with altered selectivity (41-43).

ROLE OF SOCE IN CALCIUM OSCILLATIONS

In most non-excitable cells, including exocrine gland cells, activation of Ca^{2+}-mobilizing receptors

does not produce a sustained elevation in $[Ca^{2+}]_i$, but rather a series of Ca^{2+} spikes superimposed on a steady baseline. This phenomenon is generally referred to as Ca^{2+} oscillations (44, 45). The process of repetitive Ca^{2+} oscillations in epithelial cells was first inferred from fluctuations in chloride current by Berridge (46), and first directly demonstrated in hepatocytes by Cobbold (47). The major hallmark of these regenerative cytoplasmic Ca^{2+} spikes is their constant amplitude but variable frequency as a function of stimulus strength (45, 47). Such behavior is typical of an excitable process and requires some kind of positive feedback process to produce all-or-none rises in $[Ca^{2+}]_i$ together with a shut-off or depletion mechanism to limit the size of the spikes. Despite decades of research, there is as yet no general consensus as to the nature of the key elements underlying cytoplasmic Ca^{2+} oscillations. This may be because multiple mechanisms exist that play different roles depending on the cell type and nature of the activating signal. There are two general models for cytoplasmic Ca^{2+} oscillations. One involves a positive feed back by Ca^{2+} on phospholipase C, causing fluctuations in IP_3 levels (47-49). With this model, IP_3 levels oscillate and Ca^{2+} signals reflect these changes in IP_3. In the alternative view, IP_3 would remain constant, and the positive feed back would arise from Ca^{2+}-induced activation of the IP_3 receptor.

The maintenance of Ca^{2+} oscillations requires influx of extracellular Ca^{2+} (45). In addition, it has been demonstrated that influx of Ca^{2+} during oscillations is primarily responsible for the activation of downstream responses, such as gene expression (50). Thus, it is important to understand the nature of this Ca^{2+} influx mechanism. There has been some controversy regarding this issue ; although it is well accepted that maximal concentrations of agonists activate Ca^{2+} entry through the store-operated mechanism, it has been suggested that with low, more physiological concentrations of agonists, other non-store-operated entry pathways may be more significant (51).

We examined the Ca^{2+} entry supporting Ca^{2+} oscillations in a kidney cell line by using a combination of pharmacological and molecular criteria (52, 53). The data strongly indicate that it is the classical store-operated mechanism that supports these oscillations. Specifically : the oscillations were blocked by agents known to block store-operated channels, and in the same and unique concentration ranges wherein store-operated channels are affected (52) ; and oscillations were blocked by RNAi knockdown of either the Ca^{2+} sensor, STIM1, or the SOC channel subunit, Orai1 (53). Interestingly, the oscillations were blocked by knockdown of STIM1, but were unaffected by knocking down STIM2, despite the fact that STIM2 is expected to be more active with small reductions in Ca^{2+} store content (54). This suggests that Ca^{2+} oscillations are capable of transiently lowering store content in critical sites into the range sensed by STIM1, and that STIM1 may thus be specially adapted to interacting with Orai channels to produce effective activation of downstream signals (54).

CONCLUSION

In exocrine gland cells, Ca^{2+} signalling underlies the activation and control of secretory processes. A major component of these Ca^{2+} signals is the entry of Ca^{2+} across the plasma membrane through store-operated channels. In recent years, much has been learned of the molecular nature of store-operated channels, composed of Orai subunits as well as the Ca^{2+} sensors, STIM1 and 2, that initiate store-operated signaling. We look forward to continuing studies of the functions and regulation of these key Ca^{2+} signaling proteins and to a better understanding of their roles in exocrine physiology.

ACKNOWLEDGEMENTS

Work discussed in this review originating in the authors' laboratory was supported by the Intramural Program, National Institutes of Health.

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