Calcium influx and its control by calcium release

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Changes in the concentration of intracellular Ca^{2+} are crucial for signal transduction in virtually every cell. In the past year, more of the diversity of receptor-mediated Ca^{2+} influx mechanisms has been shown, and it has been disclosed that one of the most effective Ca^{2+} influx pathways, known as 'capacitative Ca^{2+} entry', occurs via Ca^{2+}-selective ion channels in the plasma membrane that are activated following depletion of intracellular Ca^{2+} stores. Although the exact activation mechanism of capacitative Ca^{2+} entry still remains a mystery, the identification of plasma membrane currents following store depletion and the characterization of their biophysical properties opens the possibility of unraveling the features and molecular components of the phenomenon of capacitative Ca^{2+} entry.

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Introduction

Changes in the concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) are involved in countless cellular responses of both electrically excitable cells, which contain voltage-gated ion channels, and non-excitable cells, which lack these and typically respond to hormonal stimulation. As diverse are these cells' functions, are the time scales over which changes in $[Ca^{2+}]_i$ take place. Different mechanisms are employed to achieve rises in $[Ca^{2+}]_i$ that can last a few milliseconds for triggering transmitter release, several seconds for activating Ca^{2+}-dependent enzymes, or minutes and hours for initiating proliferation and gene expression. The source of Ca^{2+} can be either, intracellular—stored in organelles and usually mobilized by the second-messenger inositol 1,4,5-trisphosphate [1••], or extracellular—entering across the plasma membrane through Ca^{2+} conducting channels [2]. Ca^{2+}-permeable ion channels may be classified according to their gating mechanism as voltage-, receptor- or second-messenger-activated [2]. It has been hypothesized for some time that, in addition to these ion channels, Ca^{2+} influx may also be controlled by the filling state of intracellular Ca^{2+} stores—the phenomenon termed capacitative Ca^{2+} entry [3–5]. The ion channels involved in capacitance Ca^{2+} entry are activated by an unknown mechanism following depletion of intercellular Ca^{2+} stores. As voltage-activated Ca^{+} channels and receptor-mediated Ca^{2+} influx have been extensively reviewed (see [2,6]), this discussion will focus mainly on the implications of Ca^{2+} influx activated by store depletion.

Ca^{2+}-permeable ion channels

The best characterized mechanism of Ca^{2+} influx is brought about by voltage-operated Ca^{2+} channels (VOCs). These are Ca^{2+}-selective pores that normally activate for milliseconds during action potentials, and can be found in neurons, muscle cells, and endocrine cells. Their peak amplitudes are typically around 5–50 pA/pF^{-1}, which is large enough to be easily recorded in patch-clamp experiments. Based on their voltage-dependence and pharmacological profile at least four types of voltage-gated Ca^{2+} channels appear to exist, the L-, N-, T-, and P-type.

Receptor-operated Ca^{2+}-permeable ion channels (ROCs) are directly gated by a ligand and are found in excitable cells (which respond to excitatory neurotransmitters e.g. acetylcholine, glutamate, serotonin) as well as in non-excitable cells (responding to agonists such as ATP, histamine). These channels are usually referred to as non-selective cation channels: they are not nearly as selective for Ca^{2+} as VOCs. Current amplitudes are usually in the range of 2–20 pA/pF^{-1}. As most of the current is carried by monovalent ions, the contribution of these cation channels to Ca^{2+} influx has not been well defined.

Second-messenger-operated Ca^{2+}-permeable ion channels (SMOCs) share many properties with ROCs. The main difference is that SMOCs are gated from the intracellular cytosolic side, by G-proteins or second messengers, secondary to receptor stimulation. SMOCs are found in olfactory neurons and photoreceptor cells where cyclic nucleotides (cAMP and cGMP) mediate the activation

Abbreviations

I_{CRAC}—Ca^{2+} release-activated Ca^{2+} current; **IP_3**—inositol 1,4,5-trisphosphate; **IP_4**—inositol 1,3,4,5-tetrakisphosphate; **ROC**—receptor-operated Ca^{2+}-permeable ion channel; **SMOC**—second-messenger-operated Ca^{2+}-permeable ion channel; **VOC**—voltage-operated Ca^{2+} channel.

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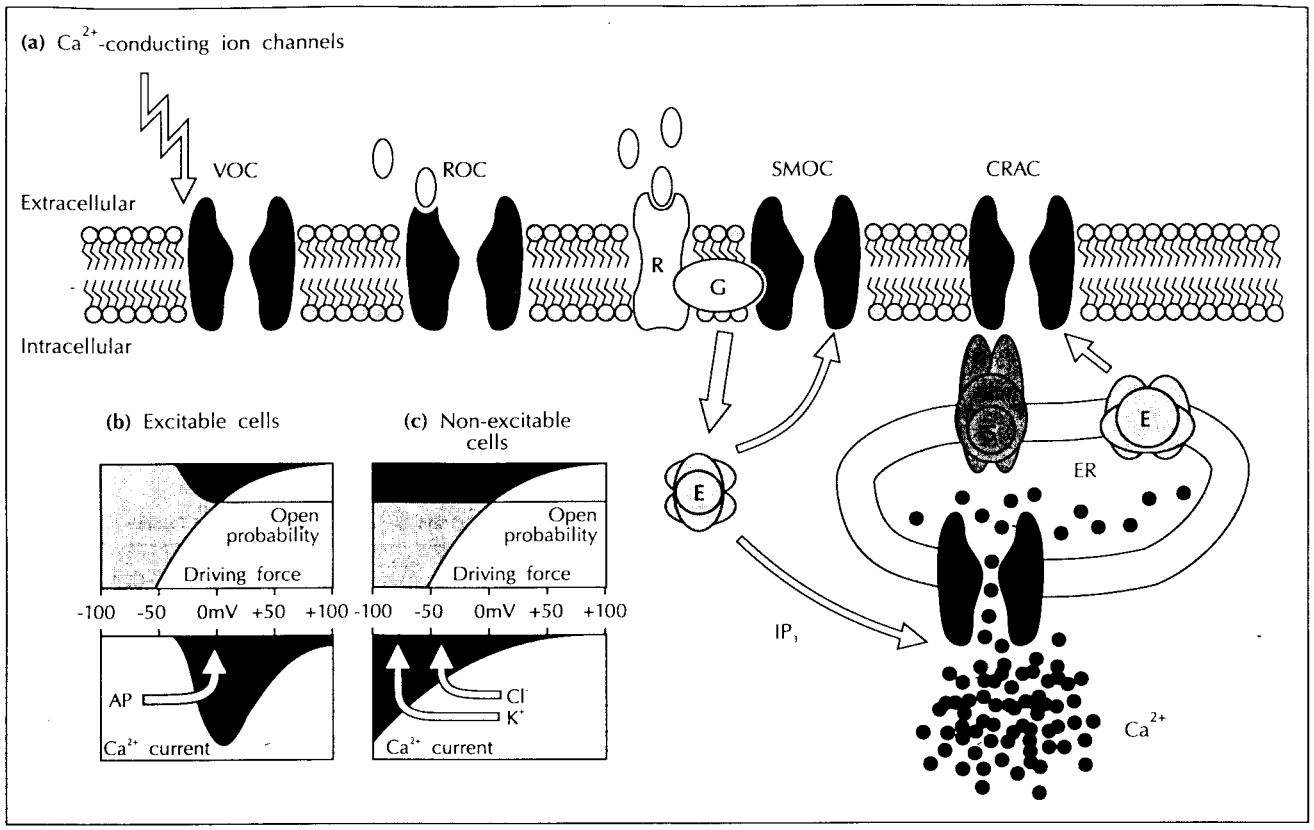

Fig.1. Ca^{2+} influx through Ca^{2+} ion channels. (**a**) Schematic representation of the various types of Ca^{2+}-conducting ion channels and their gating mode. VOCs are activated by depolarization, ROCs are activated by extracellular ligands, and SMOCs are activated either by G-proteins (G) coupled to receptors (R), or by second messengers generated by effector enzymes (E). Ca^{2+} release-activated Ca^{2+} channels (CRACs) are gated by an unknown mechanism following depletion of intracellular Ca^{2+} stores—presumably the endoplasmic reticulum (ER)—by IP_3. The luminal Ca^{2+} content is registered by a putative Ca^{2+} sensor (S) which transmits a signal to the plasma membrane channels. This could be a direct coupling or via a generation of messengers (possibly through an intermediate effector protein, (E). The inset shows the relationship between membrane voltage, electrochemical driving force, and Ca^{2+} influx for electrically (**b**) excitable cells (containing VOCs) and (**c**) non-excitable cells (containing CRACs). In both cell types, the driving force is determined by the concentration gradient of Ca^{2+} and the voltage across the plasma membrane, being strongest at hyperpolarized potentials. In excitable cells, VOCs are closed at resting membrane potentials (~ -70 mV) and open during action potentials (AP). Their open probability is seen to increase with depolarizing voltages. The resulting Ca^{2+} current is the product of driving force and open probability; it shows a bell-shaped current-voltage relationship. In non-excitable cells, the open probability of CRACs is dependent on the filling state of Ca^{2+} stores and independent of voltage. Ca^{2+} influx is only determined by the driving force and the resulting Ca^{2+} current has an inwardly rectifying shape. Additional activation of Ca^{2+}- and/or voltage-dependent K^+ and Cl^- channels hyperpolarizes the plasma membrane to provide the necessary driving force for Ca^{2+} influx.

of cation channels. There is also evidence that second messengers such as $[Ca^{2+}]_i$, protein kinases and inositol phosphates may be involved in the activation or modulation of plasma membrane ion channels.

The gating mode of these three types of Ca^{2+}-permeable ion channels, as well as that of the Ca^{2+} release-activated Ca^{2+} channels described below is illustrated in Fig. 1., which also shows how membrane voltage, electrochemical driving force and Ca^{2+} influx are related in both excitable and non-exictable cells.

Capacitative calcium influx — models and mechanisms

The notion that Ca^{2+} influx might be controlled by the filling state of intracellular Ca^{2+} stores comes from a number of observations (reviewed in [5]): first, Ca^{2+} release and Ca^{2+} influx are sequentially activated by inositol 1,4,5-trisphosphate (IP_3)-generating agonists to produce biphasic changes in $[Ca^{2+}]_i$; second, IP_3 can mimic receptor stimulation in both Ca^{2+} release and influx; third, refilling of Ca^{2+} stores depends on the concentration of extracellular Ca^{2+}; fourth, Ca^{2+} oscillations do not persist during prolonged removal of extracellular Ca^{2+}; fifth, microsomal Ca^{2+} pump inhibitors (e.g. thapsigargin and 2,5-di-*tert*-butylhydroquinone) that prevent re-uptake of Ca^{2+} into stores induce Ca^{2+} influx; sixth, removal and subsequent re-admission of extracellular Ca^{2+}, or excessive buffering of $[Ca^{2+}]_i$, causes Ca^{2+} influx; and seventh, ionomycin, a Ca^{2+} ionophore, also releases Ca^{2+} from internal stores and induces Ca^{2+} influx in addition to its ionophore-related effects.

Based on these findings, several models have been proposed to account for the observed phenomena. Common to all of these models is the notion that Ca^{2+} influx is triggered by emptying of intracellular Ca^{2+} stores,

termed capacitative Ca^{2+} entry. Initially it was thought that the intracellular stores were refilled directly by extracellular Ca^{2+}, without passing through the cytosolic compartment [4]. This model was modified as better methods of assessing $[Ca^{2+}]_i$ became available [5] and showed that an increase in cytosolic $[Ca^{2+}]_i$ is associated with capacitative Ca^{2+} entry. This suggested that the intracellular Ca^{2+} stores somehow activate Ca^{2+} channels in the plasma membrane, and that Ca^{2+} first enters the cytosol and is then taken up into the storage organelles.

This model was further refined by a hypothesis that drew an analogy between capacitative Ca^{2+} influx and skeletal muscle excitation–contraction coupling [7]. This coupling is thought to involve a direct protein–protein interaction of plasma membrane Ca^{2+} channels (dihydropyridine receptors) that serve as voltage sensors to communicate with intracellular Ca^{2+} release channels (ryanodine receptors) (reviewed in [8]). By analogy, capacitative Ca^{2+} entry might encompass the reciprocal process in which Ca^{2+} release channels (IP_3 receptors), on storage organelles, signal to plasma membrane Ca^{2+} channels. Like the muscle cell ryanodine receptor [9], the IP_3 receptor is sensitive to luminal Ca^{2+} concentration [10,11] and could, therefore, act as a Ca^{2+} sensor. The identity of the plasma membrane Ca^{2+} channel it would be associated with is not known, but it has been postulated that inositol 1,3,4,5-tetrakisphosphate (IP_4) receptors may be possible candidates, based on the finding that in some cells IP_4 enhances Ca^{2+} influx [12•,13,14]. Currently, the role of IP_4 in capacitative Ca^{2+} entry is a matter of controversial discussion [7,15–17].

A less direct mechanism of activation of capacitative Ca^{2+} entry could involve intermediate signalling messengers released from the storage organelles. At present, there is only one candidate, cGMP, which has been reported to induce Ca^{2+} entry in pancreatic acinar cells [18]. Intracellular perfusion of these cells with cGMP activates a Ca^{2+} current with similar characteristics to Ca^{2+} release-activated Ca^{2+} calcium currents [19], although this appears difficult to reconcile with data from the same laboratory showing the nucleotide's inability to raise $[Ca^{2+}]_i$ in these cells [20]. At present, there seems to be no evidence that cGMP serves as a messenger for capacitative Ca^{2+} entry in other cellular systems. In our own studies on mast cells, we could not find any effect of either cGMP or cAMP on Ca^{2+} release-activated Ca^{2+} currents (unpublished data). Cytochrome P450 has also been proposed to mediate capacitative Ca^{2+} entry, based on the similar sensitivity of Ca^{2+} influx and cytochrome P450 to the inhibition by imidazole antimycotics [21]. This has been questioned, however, as there is also a similar inhibition of VOCs by these compounds [22]. Another model invokes a kinase–phosphatase cycle [23] whereby elevated cytosolic Ca^{2+}, occuring during store depletion, increases Ca^{2+} permeability via stimulation of a tyrosine kinase, while a high phosphatase activity is maintained by replenished Ca^{2+} stores.

Thus, although it is now generally accepted that capacitative Ca^{2+} influx is a wide-spread phenomenon, the various components of this signalling system are still a matter for speculation. Recent work on *Drosophila* photoreceptors might provide a clue to the identification of an ion channel linked to the inositide signalling pathway [24,25•,26]. The *trp* gene codes for a Ca^{2+}-permeable channel which is activated after light-induced IP_3 production and causes a sustained inward current [25•]; in *trp* mutants the response is transient. The light response is reminiscent of biphasic $[Ca^{2+}]_i$ changes in mammalian cells following receptor stimulation, and the sustained current in photoreceptors could involve capacitative Ca^{2+} influx. Activation of these Ca^{2+} channels by store depletion awaits confirmation, however, as a direct gating by IP_3, as seen in olfactory neurons [27•], cannot be dismissed.

Ca^{2+} release-activated Ca^{2+} currents

For a long time, the data in support of the capacitative Ca^{2+} entry hypothesis were rather indirect, having been mainly generated by complex protocols designed to empty and replenish Ca^{2+} stores while monitoring $[Ca^{2+}]_i$ with the Ca^{2+} indicator dye fura-2, and using Mn^{2+} to assess Ca^{2+} influx (for examples, see [28–30,31•]; Mn^{2+} appears to use the same influx pathways as Ca^{2+} but produces a quench of fura-2 fluorescence. Unambiguous interpretation of Mn^{2+} quenching experiments is difficult, both for technical reasons [31•] and because Mn^{2+} is capable of leaking through VOCs [32] as well as SMOCs and Ca^{2+} release-activated channels [12•,33•,34•]. Electrophysiological data on capacitative Ca^{2+} entry mainly screened indirect markers of cytosolic $[Ca^{2+}]_i$, such as Ca^{2+}-activated K^+ or Cl^- channels [14, 16, 35]. Few studies showed more direct evidence for a highly selective Ca^{2+} current activated by agonists or IP_3 [36,37].

Because of its small amplitude of 0.1–2.0 pA/pF^{-1}, it was not until last year that direct electrophysiological identification of a Ca^{2+} current activated by store depletion was achieved in mast cells [38••]. This influx pathway, designated as Ca^{2+} release-activated Ca^{2+} current (I_{CRAC}), appears to be as Ca^{2+}-selective as VOCs and, although the gating of this influx pathway is independent of membrane voltage, there is, nevertheless, a strong dependence of Ca^{2+} influx on the driving force exerted by the membrane potential, i.e. the influx rate increases with hyperpolarization and decreases with depolarization (see Fig.1c).

Some of the salient features of this I_{CRAC} Ca^{2+} current as measured in mast cells [34•,38••,39•] are: first, activation only seems to require depletion of intracellular stores (e.g. using IP_3, ionomycin, or Ca^{2+} chelators) but there seems to be no direct requirement for inositol phosphates (IP_3 or IP_4), increases in $[Ca^{2+}]_i$, cGMP or cAMP; second, the current shows inward rectification with reversal potentials > +50 mV; third,current amplitudes depend on $[Ca^{2+}]_o$ (apparent k_d is 3.1 mM); fourth, selectivity is similar to VOCs. The selectivity is lost upon removal of all divalent ions from the bath solution, allowing Na^+ ions to permeate; fifth, Ba^{2+} and Sr^{2+} are

less permeable than Ca^{2+}; sixth, there is a small Mn^{2+} permeability less than 10% of that with Ca^{2+}; seventh, the blocking efficacy of divalent and trivalent ions is $La^{3+} > Zn^{2+} > Cd^{2+} > Be^{2+}$, Co^{2+}, $Mn^{2+} > Ni^{2+} > Sr^{2+}$, Ba^{2+} (Ba^{2+}, Sr^{2+}, and Mn^{2+} cause a permeation block); and eighth, single-channel conductance is estimated to be below 1 pS; in Jurkat T cells, a conductance of 10 fS was estimated from noise analysis [40•].

Ca^{2+} release-activated Ca^{2+} currents appear to be widely distributed, being found in almost all non-excitable cells that we have tested so far, including rat peritoneal mast cells, rat basophilic leukemia cells (RBL-2H3), hepatocytes, dissociated thyrocytes, Swiss 3T3 fibroblasts, and L-60 cells (a human leukemia cell line) [41]. Recent evidence suggests that a Ca^{2+} current with almost identical properties to I_{CRAC} is also present in MDCK cells (an epithelial cell line from kidney) (P Dietl, abstract M, 37th Annual Meeting of the Biophysical Society, Washington DC, February 1993), *Xenopus* oocytes [43], pancreatic acinar cells [19], hepatocytes (J Duszynsky, M Elensky, JY Cheung, *et al.*, abstract W, Annual Meeting of the Biophysical Society, Washington DC, February 1993), macrophages (A Malayev, DJ Nelson, abstract M, Annual Meeting of the Biophysical Society, Washington DC, February 1993), Jurkat T cells [40•,43], and in A431 human epidermoid carcinoma cells (A Lückhoff and D Clapham, personal communication). Interestingly, the last two happen to be cells in which a direct gating of cation channels by IP_3 has also been reported [44,45].

Other mechanisms of Ca^{2+} influx

While capacitative Ca^{2+} entry is recognized as being a feature of most non-excitable cells, there may be alternative Ca^{2+} influx pathways. In most cells, there are also ROCs and/or SMOCs. The relative roles of these mechanisms and their expression in various cell types need further characterization.

As IP_3 receptors are found in the plasma membrane of some cells [46,47], inositol phosphates may gate or modulate plasma membrane channels directly and independently of store depletion. Ion channels activated by IP_3 have been identified in Jurkat T cells [44], A431 cells [45], and olfactory neurons [27•]. These channels have considerably larger single-channel amplitudes, with conductance in the pS range, and are less selective for Ca^{2+} than are Ca^{2+} release-activated channels. All of these studies monitored IP_3-activated plasma membrane channels in excised membrane patches. As mentioned above, because lymphocytes and A431 cells also possess Ca^{2+} release-activated Ca^{2+} currents, it needs to be determined whether these observation have a physiological function or whether patch-excision disrupts the capacitative influx mechanism and alters the ion channels involved such that they become IP_3-sensitive. This latter possibility would reconcile the presence of IP_3-gated channels in excised patches with the apparent absence of these channels in whole-cell experiments [40•,43]. Further complexity is added by studies in A431 cells, where there also seem to be Ca^{2+}-selective channels that are activated by growth factors acting through phospholipase A_2 activation [48].

During the past year, further evidence has accumulated that IP_4 may also be involved in Ca^{2+} influx. Although IP_4 is unable to activate Ca^{2+} influx by itself, it may enhance Ca^{2+} entry in conjunction with other factors. In endothelial cells, IP_4 increases the open probability of Ca^{2+}-activated cation channels [12•]. In *Xenopus* oocytes, pancreatic acinar cells, and lacrimal gland cells, however, the additional presence of IP_3 is required to produce the synergistic enhancement of Ca^{2+} influx by IP_4 [13,14,35]. The findings in endothelial cells point towards a role for IP_4 in fine-tuning the activity of Ca^{2+}-activated cation channels. The results in pancreatic acinar cells, lacrimal gland cells, and oocytes were obtained by monitoring Ca^{2+}-activated K^+ and Cl^- currents as an indirect measure of Ca^{2+} entry, and further experiments may shed light on the mechanism by which IP_4 acts in these cells. IP_4-modulated Ca^{2+} influx may not be a general phenomenon, because in a variety of other cell types no Ca^{2+}-influx-related actions of IP_4 could be detected: in rat peritoneal mast cells and RBL-2H3 cells, IP_4 had no effect on I_{CRAC} nor did it interfere with non-selective cation channels [36].

Evidence for activation of a Ca^{2+} influx pathway independent from store depletion comes from smooth muscle cells [49]. In these cells, Ca^{2+} entry induced by platelet-derived growth factor does not appear to involve heparin-sensitive IP_3 receptors, but rather ongoing receptor occupancy and phosphatidylinositol 4,5-bisphosphate seem to be required. A Ca^{2+} influx pathway independent of inositol phosphates and release of intracellular Ca^{2+} has been demonstrated in fibroblasts transfected with chimeric muscarinic receptors [50•]. The capacitative Ca^{2+} influx pathway may also be modulated independently from phosphoinositide hydrolysis as suggested in Jurkat T cells, where the thapsigargin-induced entry of Ca^{2+} is antagonized by cholera toxin [51]. In other cells, however, cholera toxin interferes with agonist-mediated inositol phosphate production and Ca^{2+} release [52].

Perspectives

All Ca^{2+}-permeable ion channels contribute to changes in $[Ca^{2+}]_i$ when activated. In excitable cells, VOCs trigger vesicular release and muscle contraction, and ROCs may serve to depolarize the cell to the threshold of action potential generation. In glutamate receptor channels of the *N*-methyl-D-aspartate (NMDA) type, Ca^{2+} influx may be involved in the process of long-term potentiation. In non-excitable cells, ROCs may allow for a limited Ca^{2+} influx during the presence of an agonist. SMOCs may produce sustained changes in $[Ca^{2+}]_i$ and prolong the effects of short agonist exposure. It is likely that all of these ion channels are under the positive and negative feedback control of a variety of messenger systems, allowing the cell to respond in an appropriate fashion.

The major function of capacitative Ca^{2+} influx appears to consist of replenishing empty intracellular Ca^{2+} stores. But some additional features may be worth considering. Because this influx pathway is so selective, it provides the major contribution to the plateau phase of elevated $[Ca^{2+}]_i$ following receptor stimulation. At least for mast cells, this plateau phase may serve to increase the rate of G protein-dependent exocytosis. A concomitant activation of cation channels via G proteins adds to Ca^{2+} influx but generally contributes less than 30 % of the total Ca^{2+} influx [33•].

A second interesting feature of capacitance Ca^{2+} entry is its possible involvement in the encoding of cellular responses to varying agonist concentrations. In many cells, exposure to low concentrations of agonists leads to Ca^{2+} oscillations, whereas high concentrations trigger additional Ca^{2+} influx. This might dramatically alter the response of a cell to an agonist in a concentration-dependent manner. There may also be a temporal integration of receptor occupancy. Cellular responses following receptor stimulation by high-affinity ligands might be encoded by the exposure time to the agonist rather than by its concentration. This would also result in oscillatory Ca^{2+} release, and prolonged receptor stimulation could eventually lead to a switch from Ca^{2+} oscillations to capacitative Ca^{2+} entry due to the continued drain of small amounts of Ca^{2+} from the stores with each Ca^{2+} transient.

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