Relationship between Intracellular Calcium Store Depletion and Calcium Release-activated Calcium Current in a Mast Cell Line (RBL-1)*

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The kinetic relationship between depletion of endoplasmic reticulum calcium stores and the activation of a calcium release-activated calcium current (I_{crac}) was investigated in the RBL-1 mast cell line. The inositol trisphosphate receptor activator, inositol 2,4,5-trisphosphate ((2,4,5)IP_3), the sarcoplasmic-endoplasmic reticulum calcium ATPase inhibitor, thapsigargin, and the calcium ionophore, ionomycin, were used to deplete stored calcium. For (2,4,5)IP_3 and thapsigargin, a significant delay was observed between the initiation of calcium store depletion and the activation of I_{crac}. However, for ionomycin, little or no delay was observed. This may indicate that a specialized subcompartment of the endoplasmic reticulum functions as a regulator of calcium entry and that this compartment is relatively resistant to depletion by (2,4,5)IP_3 and thapsigargin but not to depletion by ionomycin. For all three calcium-depleting agents, the rate of development of I_{crac} once initiated, was relatively constant, suggesting an all-or-none mechanism. However, there were also clear experimental situations in which submaximal, graded depletion of stored calcium resulted in submaximal activation of I_{crac}. This complex behavior could also result from the existence of a specific subcompartment of endoplasmic reticulum regulating I_{crac}. The kinetic behavior of this compartment may not be accurately reflected by the kinetics of calcium changes in the bulk of endoplasmic reticulum. These findings add to the growing body of evidence suggesting specialization of the endoplasmic reticulum calcium stores with regard to the control of capacitative calcium entry.

Depletion of endoplasmic reticulum Ca^{2+} stores by (1,4,5)IP_3 is generally accompanied by an increase in Ca^{2+} entry across the plasma membrane. In the majority of cases, this entry seems to be signaled by depletion of the intracellular stores, a process termed capacitative calcium entry (1) or store-operated calcium entry (2). Hoth and Penner (3) first described an inward Ca^{2+} current in RBL cells that seemed to underlie, or at least contribute to, this entry. This current they designated I_{crac} for Calcium Release-Activated Calcium current. Although other distinguishable currents have been described that may represent capacitative calcium entry currents in other cell types (4), to date, I_{crac} is the best characterized electrophysiological manifestation of capacitative calcium entry. Thus, its properties and modes of regulation have received considerable scrutiny by a number of laboratories. For example, Hoth and Penner (5) observed a variable latency for the activation of I_{crac} (4–14 s) when activated by external application of ionomycin or by break-in with IP_3 in the patch pipette. These investigators assumed that release of intracellular Ca^{2+} by these two modes was essentially instantaneous and thus concluded that the latency observed reflected the time required for steps linking intracellular Ca^{2+} store depletion to plasma membrane channel activation. In a more recent report, Parekh et al. (6) described an all-or-none activation of I_{crac} by (1,4,5)IP_3, as well as a dissociation of activation of I_{crac} by IP_3 from the activation of Ca^{2+} release.

In the current studies, we have further investigated the latency for activation of I_{crac}, utilizing IP_3, the Ca^{2+}-ATPase inhibitor, thapsigargin, and ionomycin to deplete intracellular stores and have attempted to relate these latencies to observed kinetics of intracellular Ca^{2+} store depletion by these same reagents. Surprisingly, our findings suggest that the latency between depletion of calcium stores and I_{crac} activation depends on the nature of the agent used to deplete the stores. We also find that the kinetics of activation are complex, with all-or-none behavior in some but not in all instances. Our results may suggest the existence of a specialized, kinetically distinct subcompartment of the endoplasmic reticulum that functions as a regulator of capacitative calcium entry.

EXPERIMENTAL PROCEDURES

Cell Culture and Materials—Rat basophilic leukemia cells (RBL-1, ATCC 1378-CRL, batch F-13352) were cultured as recommended by ATCC. Briefly, cells were cultured in Earle’s minimal essential medium with Earle’s salt, 10% fetal bovine serum (heat-inactivated) and 50 μg/ml streptomycin. Ionomycin and (2,4,5)IP_3 were obtained from Calbiochem. Thapsigargin was purchased from LC Laboratories.

Fura-2 Loading—The cells were allowed to attach to cover slips, were mounted in a Teflon chamber, and were incubated with 3 μM fura-2/AM (Molecular Probes) for 25 min at room temperature. The cells were then washed and bathed in normal external saline solution (see below) at room temperature for at least 10 min before [Ca^{2+}], measurements were made.

Fluorescence Measurements—The fluorescence of the fura-2 loaded cells was monitored with a photomultiplier-based system, mounted on a Nikon Diaphot microscope equipped with a Nikon 40× (1.3 N.A.) Neofluor objective. The fluorescence light source was provided by a PTI dual excitation light source equipped with a light path. The light path chopper enabled rapid interchange between two excitation wavelengths (340 and 380 nm), and a photomultiplier tube monitored the emission fluorescence at 510 nm, selected by a barrier filter (Omega). All experiments were carried out at 24 °C. Calibration and calculation of [Ca^{2+}], were carried out as described previously (7).

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1 The abbreviations used are: (1,4,5)IP_3, inositol 1,4,5-triphosphate; I_{crac}, calcium release-activated calcium current; BAPTA, 1,2-bis(2-aminoxy)ethane-N,N',N"-tetraacetic acid.

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Electrophysiology—The normal extracellular medium contained (in mM): 150 NaCl, 4.7 KCl, 1.8 CaCl2, 1.13 MgCl2, 10 glucose, and 10 HEPES (pH 7.2). Nominally Ca2+-free saline had the same composition, except no CaCl2 was added. The bath volume (0.4 ml) was rapidly exchanged with a gravity perfusion system. In the figures, exact times are indicated when new bath solution was introduced, without any correction for the dead time required for a new solution to reach the cell. When (2,4,5)IP3 was introduced into the cell for measurement of Ca2+ release, the patch pipette (2–4 MΩ, Corning glass, 7052) contained (in mM): 150 KCl, 10 NaCl, 2.10 CaCl2, 10 HEPES, 0.1 EGTA (or 0.1 BAPTA), 50 μM fur-2 free acid, and 1 MgATP (pH 7.2). For measurement of ICrac, the pipette solution was (in mM): 140 Cs-Asp, 2 MgCl2, 10 HEPES, 10 BAPTA-Cs4, and 1 MgATP (free Ca2+ ~100 nM, pH 7.2). The bath solution contained (in mM): 140 NaCl, 4.7 KCl, 10 CaCl2 (or 10 MgCl2 for Ca2+-free solution), 1.13 MgCl2, 10 glucose, and 10 HEPES (pH 7.2).

Ruptured-patch whole-cell voltage clamp was carried out as described previously (8, 9). The holding potential was 30 mV where little or no driving force for calcium entry existed. ICrac was measured from the current resulting from voltage ramps between −100 to +60 mV over a period of 160 ms executed every 5 s. The nonspecific current (the current before the induction of ICrac or the current remaining when external Ca2+ has been removed) was subtracted. All voltages were corrected for a 10-mV liquid-junction potential. Data acquisition and analysis were performed with Axopatch-1C amplifier and PCLAMP 6.1 software (Axon Instruments, Burlingame, CA). Currents were filtered at 1 kHz and digitized at 200-μs intervals. Intracellular application of (2,4,5)IP3 or external application of thapsigargin or ionomycin induced the appearance of an inward current presumed to represent ICrac, because (i) the current was strongly inwardly rectifying with a magnitude and current-voltage relationship similar to that previously described for ICrac by Hoth and Penner (3) (not shown) and (ii) the current was seen with strong intracellular calcium buffering (10 mM BAPTA) but was lost when external calcium was removed (not shown).

Because the intracellular solutions used for Ca2+ release and ICrac generally differed in the major cationic species (Ca2+ versus K+), some Ca2+ release experiments were carried out utilizing the Ca2+-containing solutions from the ICrac protocol (but with BAPTA reduced to 0.1 mM). No statistically significant differences in the [Ca2+]i responses were noted. For 50 μM (2,4,5)IP3, with Ca2+-containing solution, peak [Ca2+]i was 229.5 ± 15.3 nM and latency was 10.6 ± 1.1 s (n = 12); with K+-containing solution peak [Ca2+]i was 272.1 ± 19.3 and latency was 15.3 ± 1.9 s (n = 12). For 100 μM (2,4,5)IP3, with Ca2+-containing solution, peak [Ca2+]i was 385.2 ± 36.8 nM and latency was 10.6 ± 1.1 s (n = 12).

RESULTS

ICrac Is Activated after an Apparent Delay When Calcium Stores Are Emptied with (2,4,5)IP3.—The time course of activation of calcium release and inward calcium current (ICrac) was determined in single RBL-1 cells following the introduction of (2,4,5)IP3 into the cytoplasm via patch pipettes in the whole cell configuration. (2,4,5)IP3 was used to minimize effects of inositol phosphate metabolism. The cells were held at 30 mV to minimize Ca2+ entry (an approach similar to that employed by Parekh et al. (6)). After establishment of the whole cell configuration, cytosolic Ca2+ rose after a short latency as a consequence of Ca2+-being released from the intracellular stores by (2,4,5)IP3 (Fig. 1A). This initial short latency is apparently the result of the time required for IP3 to diffuse into the cell and reach a critical concentration in the vicinity of IP3 receptors. We measured ICrac activation in parallel experiments because the high levels of Ca2+ buffers required in the pipette in ICrac determinations (3) prevent observation of release of stored Ca2+. ICrac was also activated following introduction of IP3 after a significant latency following the establishment of whole cell configuration (Fig. 1B). However, at each of four different concentrations of (2,4,5)IP3 the latency for ICrac activation was greater than the latency for initiation of Ca2+ release (Fig. 1C). We assume that the filling state of the intracellular stores is initially the same in both the Ca2+ release and ICrac protocols because (2,4,5)IP3 was present in the pipette at the time of break-in, and in experiments without (2,4,5)IP3, ICrac was not activated after prolonged dialysis, and basal [Ca2+]i did not change appreciably. In the ICrac protocol, [Ca2+]i does not change because the intracellular solution contained ATP as well as sufficient added CaCl2 to keep the free [Ca2+]i in the physiological range (~100 nM). At (2,4,5)IP3 concentrations of 100 and 50 μM, the latency for ICrac activation exceeded the latency for initiation as well as for the peak of Ca2+ release (Fig. 1C), suggesting a significant delay between Ca2+ release and activation of ICrac. Both the latency for Ca2+ release and the latency for ICrac activation decreased with increasing concentration of (2,4,5)IP3, consistent with a causal relationship between store depletion and activation of ICrac (Fig. 1C). Intriguingly, the development time for ICrac (from the point where ICrac starts to be activated to the point where it is fully activated) remained relatively constant regardless of the concentration of
Means B Baseline [Ca\textsuperscript{2+}] average data from six to nine separate experiments (means ± S.E. from 4 to 16 experiments) ranges. A variable delay with similar development time for I\textsubscript{crac} activation by the four (2,4,5)IP\textsubscript{3} concentrations was determined (as in Fig. 2). These differences in Ca\textsuperscript{2+} buffering may have significant effects on the binding of (2,4,5)IP\textsubscript{3} to IP\textsubscript{3} receptors and on the amplification of Ca\textsuperscript{2+} release owing to the (2,4,5)IP\textsubscript{3} data (6)). This indicates that at least over a narrow range of IP\textsubscript{3} concentrations, activation of I\textsubscript{crac} is likely a graded function of the extent of intracellular Ca\textsuperscript{2+} store depletion (see also data below with ionomycin, Fig. 4).

**A Delay for I\textsubscript{crac} Activity Is Also Seen when the Intracellular Ca\textsuperscript{2+} Stores Are Depleted with Thapsigargin and Ionomycin**—In the above studies, fura-2 measurement of Ca\textsuperscript{2+} release is carried out under conditions of physiological low Ca\textsuperscript{2+} buffering (0.1 mM BAPTA or 0.1 mM EGTA, see Experimental Procedures). However, I\textsubscript{crac} is necessarily measured under conditions of high Ca\textsuperscript{2+} buffering (10 mM BAPTA) to eliminate Ca\textsuperscript{2+}-activated currents and Ca\textsuperscript{2+}-dependent inactivation of I\textsubscript{crac} (3). These differences in Ca\textsuperscript{2+} buffering may have significant effects on the binding of (2,4,5)IP\textsubscript{3} to IP\textsubscript{3} receptors and on the amplification of Ca\textsuperscript{2+} release owing to the (2,4,5)IP\textsubscript{3} induced Ca\textsuperscript{2+} release (CICR) behavior of the IP\textsubscript{3} receptor (10). These factors could lead to a slower Ca\textsuperscript{2+} release by IP\textsubscript{3} under the conditions for I\textsubscript{crac} measurement, thus accounting for the longer latencies we observed. To minimize these potential problems, we investigated the time course for I\textsubscript{crac} activation following depletion of stores with thapsigargin or ionomycin. Thapsigargin is a potent inhibitor of the endoplasmic reticulum Ca\textsuperscript{2+} pump (11) and depletes Ca\textsuperscript{2+} stores by blocking Ca\textsuperscript{2+} uptake and allowing Ca\textsuperscript{2+} to passively leak out. Ionomycin is a Ca\textsuperscript{2+} ionophore that depletes the stores by either directly transporting ions or functioning as an ion channel. In neither case is it expected that Ca\textsuperscript{2+} buffers would impede the rate of Ca\textsuperscript{2+} store depletion; if anything, intracellular depletion of Ca\textsuperscript{2+} would likely be augmented. We found that a delay between Ca\textsuperscript{2+} release and I\textsubscript{crac} activation still seemed to be present when the store was depleted with thapsigargin (Fig. 3) or ionomycin (Fig. 4). As was seen for (2,4,5)IP\textsubscript{3}, the delay for activation of I\textsubscript{crac} decreased with increasing concentration of these agents. In the case of ionomycin, the development time for I\textsubscript{crac} was relatively constant for the three highest concentrations of ionomycin (58.5 ± 0.9, 62.6 ± 7.7, and 78.3 ± 2.4 s for 500, 50, and 5 nM ionomycin, respectively), and these times are comparable with the development time when I\textsubscript{crac} was activated with (2,4,5)IP\textsubscript{3}. For thapsigargin, the development times for I\textsubscript{crac} were slightly longer (75.9 ± 3.1, 105.2 ± 12.0, 115.0 ± 15.3 s for 1 μM, 100 nM and 10 nM thapsigargin, respectively). Furthermore, consistent with the (2,4,5)IP\textsubscript{3} data, the extent of I\textsubscript{crac} activation with different concentrations of thapsigargin (Fig. 3) or ionomycin (Fig. 4) was about the same as IP\textsubscript{3}. In other words, the rate of store depletion only affects the initial latency for I\textsubscript{crac} activation, not the time course of development of I\textsubscript{crac} at at least over this (2,4,5)IP\textsubscript{3} concentration range. A variable delay with similar development time for I\textsubscript{crac} at different concentrations of (1,4,5)IP\textsubscript{3} was also reported by Parekh et al. (6).

The extent of intracellular release of Ca\textsuperscript{2+} was determined in parallel experiments in which the status of intracellular stores was assessed by application of the calcium ionophore, ionomycin, 5 min after breaking into the cells (by which time I\textsubscript{crac} had reached its plateau level, Fig. 2A). Despite differing latencies for initiation of Ca\textsuperscript{2+} release and I\textsubscript{crac}, at all but the lowest concentration of (2,4,5)IP\textsubscript{3} (10 μM), the magnitude, both of intracellular release of Ca\textsuperscript{2+} and of I\textsubscript{crac}, was relatively constant (Fig. 2, B and C). At 10 μM (2,4,5)IP\textsubscript{3}, however, both intracellular release and steady-state I\textsubscript{crac} were clearly less than maximal (Fig. 2, B and C). In experiments with still lower concentrations of (2,4,5)IP\textsubscript{3} (5 μM for example), a proportion of cells did not respond with release or with I\textsubscript{crac} activity. Thus, over this range of concentrations of (2,4,5)IP\textsubscript{3}, I\textsubscript{crac} activation seems all-or-none as described previously for (1,4,5)IP\textsubscript{3} (6). However, this seems to result from the fact that most concentrations of IP\textsubscript{3}, which are sufficient to induce release of Ca\textsuperscript{2+}, induce an all-or-none release of Ca\textsuperscript{2+}. At 10 μM (2,4,5)IP\textsubscript{3}, where release is submaximal, steady-state I\textsubscript{crac} activation is also submaximal (similar finding was reported for I\textsubscript{crac} by Parekh et al. (6)). This indicates that at least over a narrow range of IP\textsubscript{3} concentrations, activation of I\textsubscript{crac} is likely a graded function of the extent of intracellular Ca\textsuperscript{2+} store depletion (see also data below with ionomycin, Fig. 4).

**FIG. 2.** Relationship between the extent of Ca\textsuperscript{2+} store depletion and activation of I\textsubscript{crac}. A. Protocol used. 300 s after breaking into fura-2-loaded cells with pipettes containing one of the four concentrations of (2,4,5)IP\textsubscript{3}, a high (5 μM) concentration of ionomycin was added to assess the content of intracellular stores. In this example, the dashed line indicates a control experiment (no (2,4,5)IP\textsubscript{3} in the pipette), and the solid line indicates the result with 25 μM (2,4,5)IP\textsubscript{3} in the pipette. B, average data from six to nine separate experiments (means ± S.E.). Baseline [Ca\textsuperscript{2+}], was not subtracted and is indicated by the arrow. C, the result from parallel experiments in which the extent of I\textsubscript{crac} activation by the four (2,4,5)IP\textsubscript{3} concentrations was determined (as in Fig. 1B). Means ± S.E. from 4 to 16 experiments.

For experiments measuring Ca\textsuperscript{2+} release owing to ionomycin or thapsigargin, we used intact rather than dialyzed cells. This is because at low concentrations of ionomycin, when release is gradual, the rise in [Ca\textsuperscript{2+}], associated with release is difficult to detect presumably owing to constant exchange of the Ca\textsuperscript{2+}-associated dye through the pipette. With maximal concentrations of ionomycin where this is less of a problem, the magnitude and extent of the response to ionomycin are comparable (compare Fig. 5 with Fig. 2). Because ionomycin and thapsigargin deplete Ca\textsuperscript{2+} stores by passive means, we presume that the rates of depletion will be similar under the different conditions, but the data in intact cells give a more accurate reflection of the extent of depletion.
except at the lowest concentration of ionomycin (0.5 nM, Fig. 4).

Activation of I_{crac} Only Requires a Minimal Depletion of the Ca^{2+} Stores, and Full Activation of I_{crac} Does Not Require Full Depletion of the Store—For IP_{3}, thapsigargin, and ionomycin, there seems to be a significant delay between the release of intracellular Ca^{2+} and activation of I_{crac}. This could result either from an interval of time required to release and/or synthesize some signaling messenger or from the need to deplete intracellular stores below some critical level before the activation process begins. From inspection of the data in Figs. 3 and 4, it seems that for both agents release of Ca^{2+} is well under way prior to the activation of I_{crac}. However, the amount of Ca^{2+} needed to increase [Ca^{2+}]_{i} into the 100–300 nM range is potentially very small in comparison with the total Ca^{2+} content of intracellular stores. Thus, we attempted to determine more quantitatively the extent of depletion required to activate I_{crac} by estimating the Ca^{2+} store content at the time when I_{crac} is initially activated. The latency and time course of activation of I_{crac} for 1 μM thapsigargin and 5 nM ionomycin are very similar (Fig. 4). In parallel experiments with intact RBL-1 cells, we added a high dose of ionomycin (5 μM) 50 s after treatment with either 1 μM thapsigargin or 5 nM ionomycin, corresponding to the time of initiation of I_{crac} (see Fig. 3), to assess the Ca^{2+} content of the stores and compared it with that of control cells that had not been exposed to either agent. As shown in Fig. 5, at the time when I_{crac} was initiated by 1 μM thapsigargin, significant release of Ca^{2+} had already occurred. 100 s later, when I_{crac} activation was maximal, a small but significant amount of stored Ca^{2+} remained in the cells. Thus, for thapsigargin, it seems that either a significant amount of Ca^{2+} must be released before the initiation of I_{crac} or a significant amount of time is required for steps linking Ca^{2+} store depletion to I_{crac} activation.

Surprisingly, when ionomycin was used to deplete intracellular stores, a different result was obtained. 50 s after application of 5 nM ionomycin, despite the fact that a discernible
elevation in \([Ca^{2+}]_i\) had occurred, the \(Ca^{2+}\) content of the stores seemed to be about the same in ionomycin-treated and control cells (Fig. 6). Thus, the stores are apparently only slightly depleted at 50 s with 5 nM ionomycin. This result suggests that when ionomycin is used to deplete stored \(Ca^{2+}\) only a very small reduction is required to initiate activation of \(I_{\text{crac}}\). It also indicates that for ionomycin, the delay in activating \(I_{\text{crac}}\) may not reflect a longer delay than that required to significantly reduce the \(Ca^{2+}\) content of intracellular \(Ca^{2+}\) stores.

At 150 s following addition of 5 nM ionomycin, \(I_{\text{crac}}\) was fully activated (Fig. 4). When we used the same strategy to assess the store content at this time, we found that the \(Ca^{2+}\) stores were depleted by about 50% (Fig. 6). This result suggests that \(I_{\text{crac}}\) can be fully activated with only partial depletion of intracellular \(Ca^{2+}\) stores.

**DISCUSSION**

The temporal relationship between the discharge of intracellular stores of \(Ca^{2+}\) and the activation of capacitative calcium entry is key to understanding the nature of the signaling process. However, it is difficult to determine these two parameters under similar conditions because the very high \([Ca^{2+}]_i\), buffering required to detect \(I_{\text{crac}}\) prevents detection of \(Ca^{2+}\) release to the cytoplasm. To minimize this problem, we have relied on comparisons among three agents that cause depletion of endoplasmic reticulum \(Ca^{2+}\) stores by clearly distinct mechanisms: IP3 by activating a membrane receptor/ion channel; thapsigargin, which blocks the SERCA pumps that accumulate \(Ca^{2+}\) in the endoplasmic reticulum; and ionomycin, which passively transports \(Ca^{2+}\) down its concentration gradient. Although we expect experimental conditions such as \(Ca^{2+}\) buffering to affect release of \(Ca^{2+}\) by IP3, we expect this to be much less of a factor than thapsigargin and ionomycin. Nonetheless, we cannot know with absolute certainty that this is so. Ideally, one would like to be able to assess the \(Ca^{2+}\) content of intracellular stores in the experiments in which \(I_{\text{crac}}\) is measured. Recently described technologies may permit such a determination in the near future (12).

In experiments utilizing (2,4,5)IP3 as an activator of intracellular IP3 receptors, we observed a clear distinction between
the time required for detectable release of stored Ca\(^{2+}\) and that required for activation of \(I_{\text{crac}}\). That is, significantly shorter intervals were required for mobilization of intracellular Ca\(^{2+}\) stores than for activation of \(I_{\text{crac}}\). As discussed above, because different Ca\(^{2+}\) buffering conditions were necessarily used for these two determinations, it is not clear whether the latency for release of Ca\(^{2+}\) was identical in the two experimental conditions. In fact, one might expect augmentation of the Ca\(^{2+}\) release process with minimal Ca\(^{2+}\) buffering through calcium-induced calcium release. However, if that were the case, one might also expect that concentrations of (2,4,5)IP\(_3\) inducing only partial depletion of stores with high concentrations of intracellular buffers might cause complete depletion with lower buffer concentrations. From the data in Fig. 2, this does not seem to be the case; 25–100 \(\mu\)M (2,4,5)IP\(_3\) induced maximal responses in the high and low buffer conditions (\(I_{\text{crac}}\) in the former, depletion in the latter), whereas 10 \(\mu\)M (2,4,5)IP\(_3\) induced a partial activation with both buffer conditions.

Differences in Ca\(^{2+}\) buffering should be less of an issue for experiments utilizing thapsigargin or ionomycin. These agents deplete Ca\(^{2+}\) stores by distinct and passive mechanisms, such that cytoplasmic Ca\(^{2+}\) buffering should affect the kinetics of depletion minimally if at all. From inspection of the time courses of \(I_{\text{crac}}\) activation and of [Ca\(^{2+}\)]\(_i\), increase in Figs. 3 and 4, it seems that Ca\(^{2+}\) release from the endoplasmic reticulum does indeed precede \(I_{\text{crac}}\) by some tens of seconds. However, the data in Fig. 6 show that for low concentrations of ionomycin at least, the level of cytoplasmic Ca\(^{2+}\) can be a poor indicator of the extent of depletion of Ca\(^{2+}\) stores. Despite a significant rise in cytoplasmic Ca\(^{2+}\) 50 s after addition of 5 nM ionomycin, the total stored Ca\(^{2+}\) content of the endoplasmic reticulum was changed minimally. These results indicate that especially with low concentrations of Ca\(^{2+}\)-depleting agents, changes in [Ca\(^{2+}\)]\(_i\) can misrepresent the extent of changes in intracellular Ca\(^{2+}\) stores. Thus, for this concentration of ionomycin, and in contrast to the findings with (2,4,5)IP\(_3\) and thapsigargin, there may be very little delay between the fall in Ca\(^{2+}\) content of the endoplasmic reticulum and the initiation of \(I_{\text{crac}}\). In an earlier report, McDonald et al. (13) reported minimal latency for \(I_{\text{crac}}\) activation when (1,4,5)IP\(_3\) was rapidly released within Jurkat T cells by flash photolysis.

What then is the meaning of the delay between Ca\(^{2+}\) store depletion and \(I_{\text{crac}}\) activation seen with (2,4,5)IP\(_3\) and thapsigargin? As discussed above, for (2,4,5)IP\(_3\), the difference may reflect differences in rates of Ca\(^{2+}\) discharge with the two different intracellular Ca\(^{2+}\) buffering systems used. But perhaps a more fundamental difference among these three modes of Ca\(^{2+}\) release is that only ionomycin can be assumed to release Ca\(^{2+}\) in a spatially nonspecific manner throughout the cell. In other words, because of its presumed mechanism of action, we expect ionomycin to release Ca\(^{2+}\) from all components or regions of the endoplasmic reticulum with similar facility. However, for (2,4,5)IP\(_3\) and thapsigargin, this may not be the case. (2,4,5)IP\(_3\) will cause activation of IP\(_3\) receptor channels and cause discharge of Ca\(^{2+}\) only at the specific sites where these receptors are located. Thapsigargin will lead to depletion of stores by passive leak of Ca\(^{2+}\) following inhibition of SERCA pumps. Virtually nothing is known about the channels mediating this presumably IP\(_3\)-insensitive or basal movement of Ca\(^{2+}\), but such sites could also be localized in a nonhomogeneous manner in specific regions of the endoplasmic reticulum. Thus, the current findings indicate that a specific subfraction of the endoplasmic reticulum regulates the Ca\(^{2+}\) channels underlying \(I_{\text{crac}}\) and that as Ca\(^{2+}\) is depleted from the endoplasmic reticulum through IP\(_3\) receptors, or through the leak pathway involved with thapsigargin action, this specific subcompartment is more slowly depleted than the majority of the endoplasmic reticulum. To our knowledge, this is the first evidence for differential effects of thapsigargin in subcompartments of the endoplasmic reticulum. The suggestion of a specific subfraction of endoplasmic reticulum involved in the regulation of \(I_{\text{crac}}\) is consistent with the finding that for both ionomycin and thapsigargin, full depletion of the endoplasmic reticulum store is not required for maximal activation of \(I_{\text{crac}}\). In at least one earlier report, specialization of the endoplasmic reticulum Ca\(^{2+}\) stores with respect to regulation of capacitative Ca\(^{2+}\) entry has been suggested (14). It was suggested that the subfraction of endoplasmic reticulum coupled to capacitative calcium entry was at most 30% of the total thapsigargin-sensitive Ca\(^{2+}\) stores.

The current findings indicate that the kinetics of activation of \(I_{\text{crac}}\) are complex. Over certain concentration ranges with all three modes of activation, the time course of \(I_{\text{crac}}\) activation is relatively constant. This may simply result from the fact that in most experimental situations, release of Ca\(^{2+}\) occurs more rapidly than the steps involved in signaling \(I_{\text{crac}}\). This may also be suggestive of an all-or-none mechanism of activation, as proposed by Parekh et al. (6). However, graded activation of \(I_{\text{crac}}\) was observed with 10 \(\mu\)M (2,4,5)IP\(_3\), as well as in an earlier utilizing cyclopiazonic acid to deplete endoplasmic reticulum calcium (14). As in the current study, Parekh et al. (6) concluded that a small component of the total intracellular Ca\(^{2+}\) stores regulates \(I_{\text{crac}}\) but this conclusion was based on findings that low concentrations of (1,4,5)IP\(_3\) seemed to activate Ca\(^{2+}\) release without activating Ca\(^{2+}\) entry. In our study, we have exclusively utilized (2,4,5)IP\(_3\), a poorly metabolizable analog of (1,4,5)IP\(_3\), to avoid possible complications of differential metabolism under different experimental conditions. With (2,4,5)IP\(_3\) as the mobilizing ligand, no dissociation between the concentrations required for release and those required for \(I_{\text{crac}}\) activation were observed (Fig. 2). However, it is the differential latencies observed for ionomycin, thapsigargin, and (2,4,5)IP\(_3\) that lead us to propose a specialized, quantitatively minor component of the endoplasmic reticulum as the site of control of capacitative calcium entry and \(I_{\text{crac}}\). If in fact a small compartment of the endoplasmic reticulum is responsible for regulation of \(I_{\text{crac}}\) it is possible that the kinetics of \(I_{\text{crac}}\) activation reflect the kinetic behavior of this small pool of Ca\(^{2+}\) and that these kinetics are not accurately reflected by the average time course of changes in cytoplasmic and stored Ca\(^{2+}\) in the cell. Future work must concentrate on experimental dissection of these functionally distinguishable subcompartments of endoplasmic reticulum Ca\(^{2+}\) stores if we are to fully understand how the plasma membrane Ca\(^{2+}\) movements underlying \(I_{\text{crac}}\) and capacitative calcium entry are regulated.

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Relationship between Intracellular Calcium Store Depletion and Calcium Release-activated Calcium Current in a Mast Cell Line (RBL-1)

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doi: 10.1074/jbc.273.31.19554

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