Kinetics of Empty-Store-activated Ca\textsuperscript{2+} Influx in HeLa Cells*

(Received for publication, July 21, 1993, and in revised form, September 27, 1993)

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The intracellular Ca\textsuperscript{2+} indicator Indo-1 was used to monitor changes in cytosolic [Ca\textsuperscript{2+}] in single HeLa cells upon readmission of external Ca\textsuperscript{2+} after a short withdrawal of extracellular Ca\textsuperscript{2+} solution. HeLa cells were responsive to histamine but not to caffeine, and their thapsigargin-treated cells was higher than in control cells. Readmission of external Ca\textsuperscript{2+} after a brief withdrawal of extracellular Ca\textsuperscript{2+} resulted in a transient [Ca\textsuperscript{2+}]\textsubscript{i} rise, which then decayed to the same elevated [Ca\textsuperscript{2+}]\textsubscript{i} measured before the Ca\textsuperscript{2+} withdrawal period. The [Ca\textsuperscript{2+}]\textsubscript{i} rise was associated with an increased rate of Mn\textsuperscript{2+} entry, measured as the rate of quenching of intracellular Fura-2. The same procedure did not affect the [Ca\textsuperscript{2+}]\textsubscript{i} in control cells not pretreated with thapsigargin. The amplitude of this [Ca\textsuperscript{2+}]\textsubscript{i} transient in thapsigargin-treated cells depended on the duration of prior incubation in Ca\textsuperscript{2+}-free medium. The [Ca\textsuperscript{2+}]\textsubscript{i} rise induced by elevating the extracellular [Ca\textsuperscript{2+}] from 1.5 to 10 ms was more pronounced if the [Ca\textsuperscript{2+}]\textsubscript{i} during the initial incubation in 1.5 ms Ca\textsuperscript{2+} was first lowered by depolarizing the cells. We conclude that an empty store stimulates a Ca\textsuperscript{2+} entry pathway consisting of two components: a continuously elevated basal leak and a second component that is transient due to the high [Ca\textsuperscript{2+}]\textsubscript{i} induced inhibition of the Ca\textsuperscript{2+} entry pathway. This inhibition and the subsequent recovery from it as the [Ca\textsuperscript{2+}]\textsubscript{i} is brought to resting levels could cause the oscillatory Ca\textsuperscript{2+} entry that we recorded in a fraction of the thapsigargin-treated cells.

Ca\textsuperscript{2+}-mobilizing agonists use InsP\textsubscript{4} as second messenger to release Ca\textsuperscript{2+} from internal stores (Berridge and Irvine, 1989; Berridge, 1993). The decreased state of filling of the internal Ca\textsuperscript{2+} stores is then signaled to the plasma membrane to increase its permeability for Ca\textsuperscript{2+} (Putney, 1986, 1990). Interestingly, a similar phenomenon occurs after treatment of cells with thapsigargin, a specific inhibitor of intracellular Ca\textsuperscript{2+} pumps (Thastrup et al., 1989). The nature of the signal transfer from store to plasma membrane, as well as the exact pathway for influx, is still unknown.

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The abbreviations used are: InsP\textsubscript{4}, inositol 1,4,5-trisphosphate; InsP\textsubscript{3}, inositol 1,3,4,5-tetrakisphosphate; [Ca\textsuperscript{2+}]\textsubscript{i}, intracellular Ca\textsuperscript{2+} concentration; [Ca\textsuperscript{2+}]\textsubscript{o}, extracellular Ca\textsuperscript{2+} concentration; [Ca\textsuperscript{2+}]\textsubscript{r}, intracellular Ca\textsuperscript{2+}.

In this work, we have further characterized the time course of the [Ca\textsuperscript{2+}]\textsubscript{i} rise induced by reexposing HeLa cells that were
shortly incubated in Ca²⁺-free solution, to extracellular Ca²⁺. Takemura and Putney (1989) have observed a transient elevation of the [Ca²⁺] during the refilling process of empty stores in parotid acinar cells. Their protocol consisted of stimulating intact cells with a saturating dose of agonist in the absence of extracellular Ca²⁺, then preventing further stimulation by adding a receptor antagonist, and finally readmitting extracellular Ca²⁺ to stimulate Ca²⁺ entry. We have used a different protocol, in which store depletion was induced by a 60-min incubation in 2 μM thapsigargin. This protocol prevented Ca²⁺ sequestration into internal stores during the phase of Ca²⁺ readdition. Our results indicate that readdition of external Ca²⁺ induced a transient [Ca²⁺] rise. The transient nature of this [Ca²⁺] rise in HeLa cells was due to a time- and Ca²⁺-dependent decrease of the rate of Ca²⁺ entry. This Ca²⁺-dependent inhibition of the Ca²⁺ entry and the subsequent recovery from this inhibition could cause an oscillatory Ca²⁺ entry.

MATERIALS AND METHODS

HeLa (S3) cells, which are epithelial cells derived from an epidermoid carcinoma of the cervix, were obtained from the American Type Culture Collection (Rockville, MD) and grown in Ham's F-12 medium supplemented with 10% fetal calf serum. A7r5 cells were cultured as described by Missiaen et al. (1990). The culture medium was replaced every 2–3 days. The cells were plated at a density of 2500 cells/cm² in coverglass chambers (Nunc Inc., Naperville, IL) and routinely investigated 6 days after plating. The technique for culturing BCBH1 cells and for manipulating their state of differentiation was as described by De Smedt et al. (1991a, 1991b).

Single-cell [Ca²⁺], measurements using a laser-scanning confocal fluorescence microscope (MRC-600, Bio-Rad) coupled to an inverted epifluorescence microscope (Nikon Diaphot-TMD-EF), were performed as described (Missiaen et al., 1993). Briefly, after removing the culture medium and washing the cells, they were incubated for 30 min with 5 μM Indo-1-AM dissolved in a modified Krebs solution of the following composition (mM): 135 NaCl, 5.9 KCl, 1.5 CaCl₂, 1.2 MgCl₂, 1.6 Hepes, and 11.5 glucose (pH 7.5). The cells were then incubated for another 1–1.5 h in the presence of Indo-1. During the experiment, the cells were continuously superfused with either the modified Krebs solution or a Ca²⁺-free Krebs solution containing 2 mM EGTA. The distance from the point where the fluid entered the coverglass chamber and the location of the cells which were imaged, was about 5 mm, causing some latency (5–10 s) of the various responses. K⁺-rich solutions were prepared by replacing all NaCl by KCl. All experiments were performed at room temperature. We always monitored the Ca²⁺ in 15 cells in one coverglass chamber. The [Ca²⁺], tracings shown in the various figures were representative for experiments performed on four coverglass chambers, i.e. on 60 cells. Mn²⁺ can be used as a probe for Ca²⁺ influx in a variety of cells (Halliwell and Rink, 1985). The Mn²⁺-quench experiment was performed using a photomultiplier-based system consisting of an inverted microscope (IM 10, Zeiss, Oberkochen, Germany), filter wheel, amplifier and controller (Luigs and Neumann, Germany), and photomultiplier unit (Hamamatsu, Japan). Single cells were loaded with 2 μM Fura-2 AM for 20 min at room temperature. Mn²⁺ uptake was monitored as the rate of quenching of Fura-2 fluorescence measured at the isosbestic point for the Ca²⁺-Fura-2 complex (excitation 360 nm). Measurements were corrected for autofluorescence.

RESULTS

HeLa cells contain histamine receptors, which are linked to Ca²⁺ mobilization through InsP₃ production (Tilly et al., 1990). Fig. 1A illustrates the response of a single HeLa cell to a supramaximal dose of 20 μM histamine during incubation in a 1.5 mM Ca²⁺-containing solution. Application of 20 μM histamine induced an initial rapid [Ca²⁺], rise, which then slowly declined to a plateau of elevated [Ca²⁺]. A small drop in [Ca²⁺], between the transient phase and the plateau was often observed, confirming earlier reports in HeLa cell populations (Bootman et al., 1992a) and single HeLa cells (Diarra and Sauvé, 1992). The plateau phase, which slowly declined, was curtailed by removal of extracellular Ca²⁺. When plasma-membrane Ca²⁺ influx was interrupted by omission of external Ca²⁺ and adding 2 mM EGTA prior to the histamine stimulation, only the transient [Ca²⁺] rise occurred in response to 20 μM histamine (Fig. 1B). These data confirm previous findings in HeLa cells as well as in many other cell types, that the changes in [Ca²⁺], elicited by agonists, are due partly to release of internal Ca²⁺ and partly to influx of extracellular Ca²⁺.

Thapsigargin releases Ca²⁺ from internal stores by specifically inhibiting the intracellular Ca²⁺ pumps (Thastrup et al., 1989). Addition of 2 mM thapsigargin to HeLa cells incubated in a 1.5 mM Ca²⁺-containing solution, also increased the [Ca²⁺], (Fig. 1C). A sudden removal of extracellular Ca²⁺ produced an immediate drop in [Ca²⁺], in the thapsigargin-treated cells. When applied to cells exposed to a Ca²⁺-free solution, thapsigargin only produced a transient [Ca²⁺], rise (Fig. 1D). These findings indicate that part of the thapsigargin response in Ca²⁺-containing solution must represent Ca²⁺ entry, confirming the original observations of Takemura et al. (1989).

All the known types of internal Ca²⁺ pumps, expressed in COS cells, are thapsigargin-sensitive (Lyttton et al., 1991), but studies in intact cells have suggested that, under some conditions, not all stores are affected by thapsigargin, e.g. the caffeine-sensitive store of some cell types does not seem to react to thapsigargin (Law et al., 1990; Robinson and Burgoyne, 1991; Foskett and Wong, 1991). It was therefore necessary to check whether thapsigargin indeed depleted the stores under our
Following the top of the \([Ca^{2+}]_i\) transient and once the \([Ca^{2+}]_i\) effect on the \([Ca^{2+}]_j\) of withdrawing external \(Ca^{2+}\) immediately decrease of the \([Ca^{2+}]_j\) following the initial \([Ca^{2+}]_i\) peak would affect the plasma membrane. We therefore investigated whether the presence of thapsigargin represented the balance between the rate of \(Ca^{2+}\) entry into the cell and the rate of \(Ca^{2+}\) extrusion across the plasma membrane. We can assume that the measured free \([Ca^{2+}]_i\) in the presence of thapsigargin represented the balance between the rate of \(Ca^{2+}\) entry into the cell and the rate of \(Ca^{2+}\) extrusion across the plasma membrane. We therefore investigated whether the decrease of the \([Ca^{2+}]_i\), following the initial \([Ca^{2+}]_i\) peak would be due to a delayed activation of the \(Ca^{2+}\) extrusion mechanism at the level of the plasma membrane, or to an inactivation of a component of the \(Ca^{2+}\) entry mechanism. Fig. 4 represents the effect on the \([Ca^{2+}]_i\) of withdrawing external \(Ca^{2+}\) immediately following the top of the \([Ca^{2+}]_i\) transient and once the \([Ca^{2+}]_i\) transient was over. The rate of the \([Ca^{2+}]_i\) decrease was used to estimate the rate of \(Ca^{2+}\) extrusion across the plasma membr.
Fig. 5. Effect of a short lasting withdrawal of extracellular Ca\(^{2+}\) on the subsequent rate of Mn\(^{2+}\) uptake in a thapsigargin-pretreated cell. In a, a Fura-2 loaded cell was preincubated with 2 μM thapsigargin for 1 h in a 1.5 mM Ca\(^{2+}\)-containing modified Krebs solution. 1.5 mM Mn\(^{2+}\) was added as indicated by the arrow in the continuous presence of 1.5 mM Ca\(^{2+}\). The cell in b was similarly preincubated in 2 μM thapsigargin and 1.5 mM Ca\(^{2+}\). External Ca\(^{2+}\) was then removed and 2 mM EGTA added for a 10-min period (the first 500 s are not shown). The [Ca\(^{2+}\)], was then restored to 1.5 and 1.5 mM Mn\(^{2+}\) was added at the same time (arrow). The traces are representative of three experiments.

Mn\(^{2+}\) can be used as a probe for Ca\(^{2+}\) influx in a variety of cells (Hallam and Rink, 1985). We have measured the unidirectional uptake of Mn\(^{2+}\) into single Fura-2-loaded HeLa cells to investigate whether the decrease of the [Ca\(^{2+}\)]\(_i\), following the initial [Ca\(^{2+}\)]\(_i\) peak represented the inactivation of a component of the Ca\(^{2+}\) entry pathway. We have studied the effect of a short lasting withdrawal of extracellular Ca\(^{2+}\) on the subsequent rate of Mn\(^{2+}\) uptake in the presence of 1.5 mM Ca\(^{2+}\) in a thapsigargin-pretreated cell (Fig. 5). The rate of Fura-2 quenching induced by 1.5 mM Mn\(^{2+}\) added after a 1-h exposure to a 1.5 mM Ca\(^{2+}\) containing modified Krebs solution, is shown in Fig. 5a. Fig. 5b shows that the same procedure induced a much more accelerated rate of Mn\(^{2+}\) quenching if the Mn\(^{2+}\) and Ca\(^{2+}\) addition was preceded by a 10-min incubation in a Ca\(^{2+}\)-free medium. The very pronounced [Ca\(^{2+}\)]\(_i\) rise induced by a short preincubation in Ca\(^{2+}\)-free medium (Fig. 3A) was therefore associated with an increased rate of Mn\(^{2+}\) entry. This finding indicates that the rate of Ca\(^{2+}\) entry is highest immediately following the incubation in Ca\(^{2+}\)-free medium and that this rate progressively decreases leading to the declining phase of the transient.

In order to find out whether the trigger for activation of the entry mechanism following the incubation in Ca\(^{2+}\)-free medium was the decreased [Ca\(^{2+}\)]\(_i\), or the decreased [Ca\(^{2+}\)], we have tested the effect of different [Ca\(^{2+}\)]\(_i\), (Fig. 6). In Fig. 6A, plasma-membrane Ca\(^{2+}\) entry in a thapsigargin-pretreated HeLa cell was increased by raising [Ca\(^{2+}\)], from 1.5 to 10 mM. Thereupon, the cell was incubated in a Ca\(^{2+}\)-free medium for 150 s, and then challenged again with the same 10 mM Ca\(^{2+}\)-containing solution. This second application of 10 mM Ca\(^{2+}\) produced a more pronounced [Ca\(^{2+}\)]\(_i\) rise than the first application, indicating that the entry mechanism must have been partially inactivated during the first 10 mM Ca\(^{2+}\) application. In Fig. 6B, a rather similar protocol has been followed, except that the first 10 mM Ca\(^{2+}\) challenge was preceded by a 5-min incubation in a medium containing 1.5 mM Ca\(^{2+}\) and 140 mM K\(^+\) to depolarize the cell and hence to decrease the driving force for Ca\(^{2+}\) ions across the plasma membrane. The free [Ca\(^{2+}\)], during this incubation in depolarizing medium (Fig. 6B) was therefore lower than during incubation in normal medium (Fig. 6A). Subsequently raising the [Ca\(^{2+}\)], to 10 mM in the 5.9 mM K\(^+\)-containing solution after incubation in this depolarizing solution, produced a [Ca\(^{2+}\)]\(_i\) rise of similar magnitude to that following an incubation in the EGTA-containing Ca\(^{2+}\)-free medium (Fig. 6B). The [Ca\(^{2+}\)]\(_i\) rise upon raising the [Ca\(^{2+}\)], from 1.5 to 10 mM was therefore higher if the [Ca\(^{2+}\)], was initially lowered by raising the external K\(^+\) concentration. These data suggest that it was cytosolic Ca\(^{2+}\), and not external Ca\(^{2+}\), which inactivated the entry mechanism.

The amplitude of the [Ca\(^{2+}\)]\(_i\) rise upon readdition of external Ca\(^{2+}\) to thapsigargin-pretreated HeLa cells bathed in Ca\(^{2+}\)-free solution depended on the duration of the prior incubation in Ca\(^{2+}\)-free solution (Fig. 7). A brief withdrawal of external Ca\(^{2+}\) was followed by a much less pronounced [Ca\(^{2+}\)]\(_i\) rise than a long-lasting omission of extracellular Ca\(^{2+}\). Note that under both conditions, the [Ca\(^{2+}\)], dropped to the same level. These results could point to a time-dependent activation of the entry mechanism upon lowering [Ca\(^{2+}\)].

Our finding of a time-dependent inactivation of the entry mechanism at high free [Ca\(^{2+}\)], and a time-dependent reactivation at low [Ca\(^{2+}\)], implies that thapsigargin-treated HeLa cells should have the capability to oscillate. Fig. 6A illustrates that such oscillatory behavior was occasionally observed. About 20% of the cells oscillated in the presence of thapsigargin. We always monitored 15 cells in a field. We never observed two cells, including neighboring cells, oscillating in synchrony. For comparison, a typical Ca\(^{2+}\) oscillation induced by 1 μM histamine in a HeLa cell not pretreated with thapsigargin (Tilly et al., 1990; Bootman et al., 1992b; Missiaen et al., 1993) is shown in Fig. 8A. The thapsigargin-induced Ca\(^{2+}\) oscillation had a lower amplitude, exhibited a slower rising phase and individual spikes in the oscillation lasted longer than the histamine-induced ones.

We finally investigated whether the transient [Ca\(^{2+}\)]\(_i\) rise that we observed upon readdition of external Ca\(^{2+}\) to a thapsigargin-pretreated HeLa cell (Fig. 9A) also occurred in other cell
Thapsigargin-stimulated Ca²⁺ Entry in HeLa Cells

The main finding in this work is that reexposure of HeLa cells with functionally depleted Ca²⁺ stores to extracellular Ca²⁺ induced a large transient increase in [Ca²⁺]. This transient occurred in the presence of thapsigargin, i.e., under conditions where the histamine-sensitive store was proven to be empty, and could therefore not represent the delayed sequestration of cytoplasmic Ca²⁺ into the internal stores. There are two possible explanations for this transient peak. A first possibility is that the rapid decline of the [Ca²⁺] transient is due to a delayed activation of the Ca²⁺ extrusion at the level of the plasma membrane. An enhanced extrusion following an initial [Ca²⁺] transient has been reported for smooth-muscle cells, but this activation diminished over a period of 30 s (Becker et al., 1989). The experiment represented in Fig. 4, showing that the rate of Ca²⁺ extrusion following the [Ca²⁺] transient was not higher than on top of the [Ca²⁺] transient, does not favor this possibility. A second possibility is that the Ca²⁺ entry mechanism became inactivated. The Mn²⁺ uptake experiment (Fig. 5) favors the concept of a slow Ca²⁺-dependent inactivation of the entry of divalent cations.

The way in which the state of filling of the store, or of a part thereof (Shuttleworth and Thompson, 1992), is signaled to the plasma membrane is not resolved yet, and it is therefore not clear how Ca²⁺ might act. The transient nature of the [Ca²⁺] transient, upon readdition of extracellular Ca²⁺ was not primarily caused by time-dependent changes in membrane potential, since the [Ca²⁺] transient also occurred in a voltage-clamped cell at −40 mV. However, secondary changes in membrane potential as a result of the [Ca²⁺] rise may modulate the amplitude of the [Ca²⁺] transient. The inactivation of the entry mechanism by high Ca²⁺, and its reactivation by low Ca²⁺, could represent a direct effect of Ca²⁺ on the Ca²⁺ entry channel. It should be pointed out that the [Ca²⁺] in the vicinity of the plasma membrane, and especially in the mouth of the channel, is much higher than the [Ca²⁺] measured in the bulk of the cytoplasm (Stern, 1992). The inactivation of a Ca²⁺-permeable channel by cytosolic Ca²⁺ would be in line with observations in mast cells by Hoth and Pemper (1992), who observed that the Ca²⁺-release activated current could only be recorded in the presence of a very low [Ca²⁺]. The InsP₃-activated plasma-membrane Ca²⁺ current in Jurkat T cells also inactivated in the presence of high [Ca²⁺] (McDonald et al., 1993). The time course of the inactivation in Jurkat T cells (30–40 s) is in the

![Figure 7](image)

**Fig. 7.** Effect of varying the time of incubation in Ca²⁺-free medium on the amplitude of the subsequent [Ca²⁺] spike in a thapsigargin-pretreated HeLa cell. [Ca²⁺], (expressed in mM) was varied as indicated below the tracing. Note that the [Ca²⁺] decreased to the same level during the short and long incubation in Ca²⁺-free medium.

![Figure 8](image)

**Fig. 8.** Ca²⁺ oscillations in HeLa cells. The cell in A was pretreated with 2 μM thapsigargin in a 1.5 mM Ca²⁺-containing solution for 1 h. The oscillatory response was observed in about 20% of the thapsigargin-pretreated cells. B illustrates, for comparison, an oscillation induced by 1 μM histamine in a control cell not pretreated with thapsigargin.

![Figure 9](image)

**Fig. 9.** Effect of a short incubation in Ca²⁺-free medium on the subsequent [Ca²⁺] rise upon Ca²⁺ readmission in thapsigargin-pretreated A7r5 and BC₃H₁ cells. [Ca²⁺], (in mM) was varied as indicated below the tracings. A illustrates, for comparison, the [Ca²⁺], in a HeLa cell. B is an A7r5 smooth-muscle cell. The lower two panels represent a BC₃H₁ muscle cell, either in its differentiated form (C) or its undifferentiated form (D).
same range as observed in our study. The inactivation of the entry mechanism by high Ca$^{2+}$, and its reactivation by low Ca$^{2+}$, could also represent a Ca$^{2+}$ effect on the production or metabolism of a putative messenger, or represent an effect of Ca$^{2+}$, on a critical phosphorylation or dephosphorylation step (Parek et al., 1983; Randriamamitsa and Tsien, 1993). The time dependence of the activation (Fig. 7) is in the expected range for such phosphorylation or dephosphorylation events. We cannot finally exclude that the inhibitory effects of Ca$^{2+}$, would be exerted from within the stores, even though they are functionally empty, since the free luminal [Ca$^{2+}$] under these conditions should be similar as the Ca$^{2+}$,. However, it is conceivable that the primary control of "capacitative" Ca$^{2+}$ entry by luminal Ca$^{2+}$ occurs in a range of much higher luminal [Ca$^{2+}$] than the range of 204–134 nm Ca$^{2+}$ (Fig. 3A).

The simplest explanation of our data is that empty stores induce two changes at the level of the plasma membrane. The first would result in an increased rate of Ca$^{2+}$ entry, contributing to the steady-state elevation of the [Ca$^{2+}$] in thapsigargin-treated cells incubated in the presence of 1.5 mm Ca$^{2+}$. The second component is a rapidly inactivating entry pathway under control of cytosolic Ca$^{2+}$. The latter pathway was transiently activated by a short incubation in Ca$^{2+}$-free medium. Interestingly, the second component was not present in some cell types and even seemed differentiation-dependent, at least in BCC H2 cells (Fig. 9).

Our occasional observation of a Ca$^{2+}$ oscillation in thapsigargin-treated cells was in a certain way unexpected, since current models to explain oscillations in non-excitable cells all rely on the presumed periodic release and reuptake of Ca$^{2+}$ from internal stores (Rooney et al., 1990). Periodically increasing its permeability for Ca$^{2+}$. It remains to be determined if empty stores, even though they were depleted and, as a consequence, that the measured Ca$^{2+}$, represented the balance between the rate of Ca$^{2+}$ entry and efflux have been observed during each [Ca$^{2+}$, spike of an oscillation in single pancreatic acinar cells) (Replink et al., 1992). It remains to be determined whether the pump activity at a fixed [Ca$^{2+}$, will also be affected by agonists. Experiments on the purified Ca$^{2+}$ pump protein have shown that the agonist-induced breakdown of phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate would tend to inhibit the pump (Choquette et al., 1984; Missiaen et al., 1989), while the activation of the protein kinase C branch of the signaling pathway would stimulate it (Smallwood et al., 1988). The rise in cyclic GMP concentration, which often accompanies agonist stimulation (Pandol and Schoeffield-Payne, 1990), would also stimulate the Ca$^{2+}$ pump (Vrolix et al., 1988). The net effect of agonists on the rate of Ca$^{2+}$ extrusion in intact cells is less clear. Experiments on intact cells have suggested that Ca$^{2+}$-mobilizing agents stimulate Ca$^{2+}$ extrusion at the level of the plasma membrane (Duddy et al., 1989; Zhang et al., 1992). However, possible compartmentalization of the dye could seriously affect the interpretation of such experiments, at least in hepatocytes (Glenon et al., 1992). In the present work, we have found that histamine failed to affect the elevated steady-state [Ca$^{2+}$,] in the presence of thapsigargin (Fig. 2B). The lack of any [Ca$^{2+}$,] rise in response to histamine indicates that the internal stores were depleted and, as a consequence, that the measured Ca$^{2+}$,, represents the balance between the rate of Ca$^{2+}$ entry and extrusion across the plasma membrane. The lack of effect of histamine on the steady-state [Ca$^{2+}$,], under these conditions seems to suggest that this agonist had only a minor effect on the Ca$^{2+}$ extrusion system in HeLa cells.

In conclusion, depleting the internal stores with thapsigargin increased the rate of Ca$^{2+}$ entry into the cell, as judged from the increased resing Ca$^{2+}$,. A short period of lowering [Ca$^{2+}$,], induced a dramatic activation of the entry mechanism. Our findings are compatible with the hypothesis that some component of the entry mechanism is inactivated by high [Ca$^{2+}$,], and, therefore, that a low [Ca$^{2+}$,], is needed to fully activate the Ca$^{2+}$ entry pathway.
Thapsigargin-stimulated Ca\(^{2+}\) Entry in HeLa Cells

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