Stimulus-Dependent Control of Inositol 1,4,5-Trisphosphate-Induced Ca^{2+} Oscillation Frequency by the Endoplasmic Reticulum Ca^{2+}-ATPase

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ABSTRACT In many cell types, receptor stimulation evokes cytosolic calcium oscillations with a frequency that depends on agonist dose. Previous studies demonstrated controversial effects of changing the activity of the endoplasmic reticulum Ca^{2+}-ATPase upon the frequency of oscillations. By numerical simulations, we found that the model of De Young and Keizer (J. Keizer and G. W. De Young, 1994, *J. Theor. Biol.* 166:431–442), unlike other models, can explain the observed discrepancies, assuming that the different experiments were performed at different stimulus levels. According to model predictions, partial inhibition of internal calcium pumps is expected to increase frequency at low stimulus strength and should have an opposite effect at strong stimuli. Similar results were obtained using an analytical estimation of oscillation period, based on calcium-dependent channel activation and inactivation. In experiments on HeLa cells, 4 nM thapsigargin increased the frequency of calcium oscillations induced by 1 and 2.5 μM histamine but had no effect on supramaximally stimulated cells. In HEp-2 cells, 2 nM thapsigargin slowed down the rapid, ATP-induced oscillations. Our results suggest that in the investigated cell types, the De Young-Keizer model based on inositol 1,4,5-trisphosphate-dependent calcium-induced calcium release can properly describe intracellular calcium oscillations.

INTRODUCTION

Oscillations of cytosolic Ca^{2+} concentration upon chemical stimulation are observed in a wide variety of electrically non-excitable cells (Berridge, 1993; Thomas et al., 1996). As a consequence of receptor activation, the information encoded in the agonist concentration is transformed into periodically changing cytosolic Ca^{2+} level. Although the way the cells decode these signals is far from known, it is suggested that information may be encoded in their frequency (Hajnóczky et al., 1995; De Koninck and Schulman, 1998). It is therefore of special importance to identify and characterize the processes that determine the period of the oscillations.

In most cell types, calcium oscillations are induced by the second messenger inositol 1,4,5-trisphosphate (IP_3) produced by the receptor-coupled phospholipase C (Clapham, 1995; Berridge, 1993), but the subsequent biochemical events that lead to periodic changes in Ca^{2+} concentration are still unclear. A possible explanation consistent with experimental data is the IP_3-dependent calcium-induced calcium release model (CICR) (Finch et al., 1991; De Young and Keizer, 1992). This model is based on periodic calcium release from and uptake into the endoplasmic reticulum. Calcium is released through the IP_3 receptor, a ligand-gated ion channel. This channel is activated if it binds IP_3 and calcium on an activating site (Marchant and Taylor, 1997), a phenomenon recently confirmed by Iino and co-workers (Miyakawa et al., 2001). Channel inactivation follows if calcium is bound to another site. The role of the other molecule involved in the model, the sarcoplasmic (endoplasmic) reticulum Ca^{2+}-ATPase is more passive. This carrier transports calcium back to the endoplasmic reticulum with a rate solely depending on the cytosolic calcium concentration (Lytton et al., 1992).

Besides this model, several other putative mechanisms have been also proposed to explain the existence of IP_3-induced calcium oscillations. These include Ca^{2+}-dependent activation of phospholipase C (Meyer and Stryer, 1991), Ca^{2+}-independent inactivation of the IP_3 receptor (Ilyin and Parker, 1994; Hajnóczky and Thomas, 1997), production of inositol 1,3,4,5-tetrakisphosphate (Zhu et al., 2000), or accelerated calcium transport through the plasma membrane (Uneyama et al., 1998; Martin and Shuttleworth, 1994).

It is likely that in a given cell several of these processes may take place simultaneously following receptor stimulation; however, distinguishing between the mechanisms may prove difficult. Mathematical modeling provides a tool to identify the molecular processes dominating other, biochemically also detectable events, by comparing the predictions of the models regarding experimental interventions made on living cells.

There are several ways to perturb cytosolic calcium homeostasis. One possibility is to alter the activity of the endoplasmic reticulum Ca^{2+}-ATPase, the molecule that controls cytosolic calcium level and therefore the calcium-controlled processes (Morgan and Jacob, 1998). Previous studies done in this field, however, apparently contradict each other. Transient overexpression of the endoplasmic reticulum Ca^{2+}-ATPase (SERCA) increased the frequency of calcium oscillations in *Xenopus laevis* oocytes (Camacho

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and Lechleiter, 1993; Lechleiter et al., 1998), whereas the same effect was observed following partial inhibition of the pumps in pancreas acinar (Petersen et al., 1993) and endothelial cells (Morgan and Jacob, 1998). In fibroblasts, inhibition of the pumps slightly increased the inter-spike period (Rossi and Kao, 1997).

Assuming that in at least part of these cells identical mechanisms are responsible for calcium oscillations, these controversial results have not been explained yet. Mathematical modeling suggested the cause be the different type of pumps overexpressed in oocytes (Jafri and Keizer, 1995), but more recent experiments confirmed the effect with SERCA 2b, the isoform originally present in those cells (Camacho and Lechleiter, 1995; Lechleiter et al., 1998). Petersen et al. (1993) suggested that the difference results from cells oscillating at different frequencies in the individual experiments. In our study we further explored this possible explanation by using numerical simulations, analytical calculations, and $[Ca^{2+}]_i$ measurements in human cell lines.

MATERIALS AND METHODS

Numerical simulations

Numerical solutions of the differential equations were calculated with MATLAB (MathWorks, Natick, MA), using a built-in algorithm based on a modified Rosenbrock formula. Relative error tolerance was set to 10^{-6}.

Simulations were carried out by using both the original parameters of the models and parameters arbitrarily altered by 15–20%. Calculations in both cases gave qualitatively similar results. The three-variable model of De Young and Keizer was used, with the parameter c_2 set to 0.25 μM^{-1} (Keizer and De Young, 1994).

Materials

Fluo-3/AM, thapsigargin, and Pluronic F-127 were purchased from Molecular Probes (Eugene, OR) Histamine, ATP, HEPES, dimethylsulfoxide, RPMI 1640, and fetal calf serum (FCS) were purchased from Sigma (St. Louis, MO). Other reagents were of analytical grade. Fluo-3/AM was dissolved in dimethylsulfoxide containing 0.1% (w/v) Pluronic F-127. Thapsigargin stock solution was made in 95% ethanol, stored at −20°C, and used for about a week. Dilutions in distilled water were made on the day of the experiments.

Cell culture

HeLa and HEp-2 cells were seeded and cultured in 35-mm-diameter plastic petri dishes (10^5 cells/petri dish) for 20–28 h before experiments, in RPMI 1640 culture medium supplemented with 10% FCS, 50 U/ml penicillin, and 50 μg/ml streptomycin. Dye loading and subsequent procedures were carried out in the following solution (in mM): 140 NaCl, 5 KCl, 1 Na_2HPO_4, 0.5 $MgSO_4$, 5 glucose, 10 HEPES (pH 7.2). The solution also contained 5 mM $CaCl_2$ in the case of HeLa cells (see Results) and 1 mM $CaCl_2$ in experiments with HEp-2 cells. In experiments without extracellular calcium, $CaCl_2$ was omitted from the medium. Thapsigargin treatment did not affect cell viability as tested using Trypan Blue exclusion 6 h after treatment.

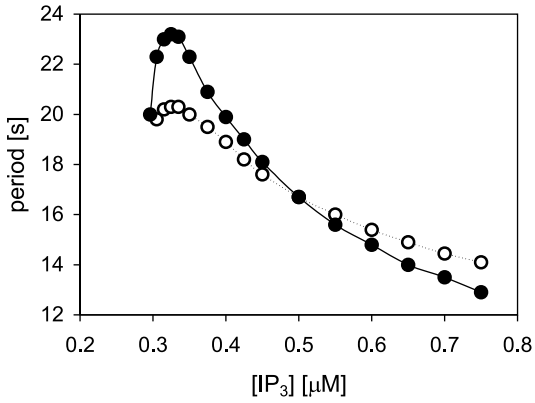

FIGURE 1 The period of oscillations calculated with the three-variable model of De Young and Keizer as a function of IP_3 concentration. SERCA maximal rates are 0.85 $\mu M\ s^{-1}$ (●) and 0.55 μMs^{-1} (○).

Dye loading and fluorescence measurements

Before dye loading, unattached cells were removed by washing the petri dishes twice with the above solution. Cells were then incubated in 3 μM fluo-3/AM at room temperature (19–21°C) for 30 min and washed again twice. The final volume was 3 ml. The petri dishes were placed onto the stage of a Nikon TE 300 inverted microscope attached to an MRC 1024 laser scanning confocal system (Bio-Rad, Hertfordshire, UK). The sample was illuminated with an argon ion laser at 488 nm through a 20× Plan Fluor objective, and the fluorescence above 515 nm was detected. Images were taken at 0.5-s intervals. Average fluorescence intensity from selected regions, each containing one cell, was calculated with Time Course software (Bio-Rad, Hertfordshire, UK). Because of the significant uncertainty of the K_D and F_{max}/F_{min} values of fluo-3 in the cytosol (Harkins et al., 1993), no calibration to absolute calcium concentration was carried out, and data were expressed as the average fluorescence intensity (F) normalized with the basal fluorescence (F_0). During measurements, reagents were added to the extracellular medium, and the content of the petri dish was mixed with another pipette to achieve homogeneous distribution. All measurements were made at room temperature.

RESULTS

The effect of altering SERCA activity on the behavior of four mathematical models describing intracellular calcium oscillations has been examined performing numerical simulations. Three of these models, the model of Goldbeter et al. (1990), that of Meyer and Stryer (1991), and that of Atri et al. (1993) predicted that partial inhibition of the endoplasmic reticulum Ca^{2+}-ATPase increases the frequency of the oscillations regardless of stimulus level. In the model of De Young and Keizer (De Young and Keizer, 1992; Keizer and De Young, 1994), however, changing the Ca^{2+}-ATPase activity changed the frequency of the oscillations in a stimulus-dependent way. Fig. 1 shows the dependence of oscillation period on stimulus level at two different maximal rates of the Ca^{2+}-ATPase calculated with the model. Except for very low IP_3 levels, increasing IP_3 concentration increased the frequency at a given SERCA rate. Surprisingly, however, the curves at different SERCA rates crossed each

other, and the sign and magnitude of their difference depended on IP_3 level. Whereas a decrease in Ca^{2+}-ATPase activity increased the frequency at low stimulus level, an opposite effect was found at high IP_3 concentration.

We also tried to explain literature data by assuming that at constant stimulus level another parameter changed from experiment to experiment, and we performed calculations after altering other parameters. These calculations, however, resulted in an increase in frequency upon SERCA inhibition in all models irrespective of parameter values (data not shown).

Now we searched for an explanation of the striking stimulus-dependent behavior of the De Young-Keizer model. Oscillations in this model are caused by repetitive activation and inactivation of the IP_3 receptor, with cytosolic calcium concentration, fraction of open receptors, and the fraction of only IP_3-bound receptors as variables. Two phases of the calculated oscillation cycle were arbitrarily distinguished, as illustrated in Fig. 2 *A*. The time period during which the fraction of open receptors increased was called activation phase, whereas the phase where this fraction decreased was termed inactivation phase. As both processes involve calcium-dependent reactions, changing Ca^{2+}-ATPase activity should indirectly affect the duration of both of them. As it can be seen in Fig. 2, *B* and *C*, lowering Ca^{2+}-ATPase activity shortens the duration of activation and lengthens the inactivated phase independent of the IP_3 level. The different relative weights of these phases at different IP_3 concentrations result in an opposite dependence of their sums at low and high IP_3 levels.

To examine which features of the complex model result in this observation, we approximated the period of calcium oscillations in an analytically tractable way. Although not all assumptions of this simplified model are consistent with experimental results, they are required to analytically solve the equations and crudely estimate the dependence of oscillation period on different parameters. We estimated the period as the sum of the duration of three processes: a lag phase involving the initiation of calcium-induced calcium release, the fast upstroke of this autocatalytic process, and finally calcium resequestration in a refractory phase. We assumed that calcium release is terminated by an undefined, calcium-dependent process that is close to equilibrium during the refractory phase. The detailed description of this model is found in the Appendix.

Fig. 3 shows the results of this analytical calculation using the parameters given in Table 1. Here SERCA activity is represented as parameter k_p, whereas stimulus strength is given by k_a. Increasing k_a decreases the length of the oscillation period, and lowering k_p increases or decreases frequency at low or high k_a values, respectively.

To test theoretical predictions, we investigated the effect of submaximal doses of thapsigargin on agonist-induced calcium oscillations in human epithelial cell lines.

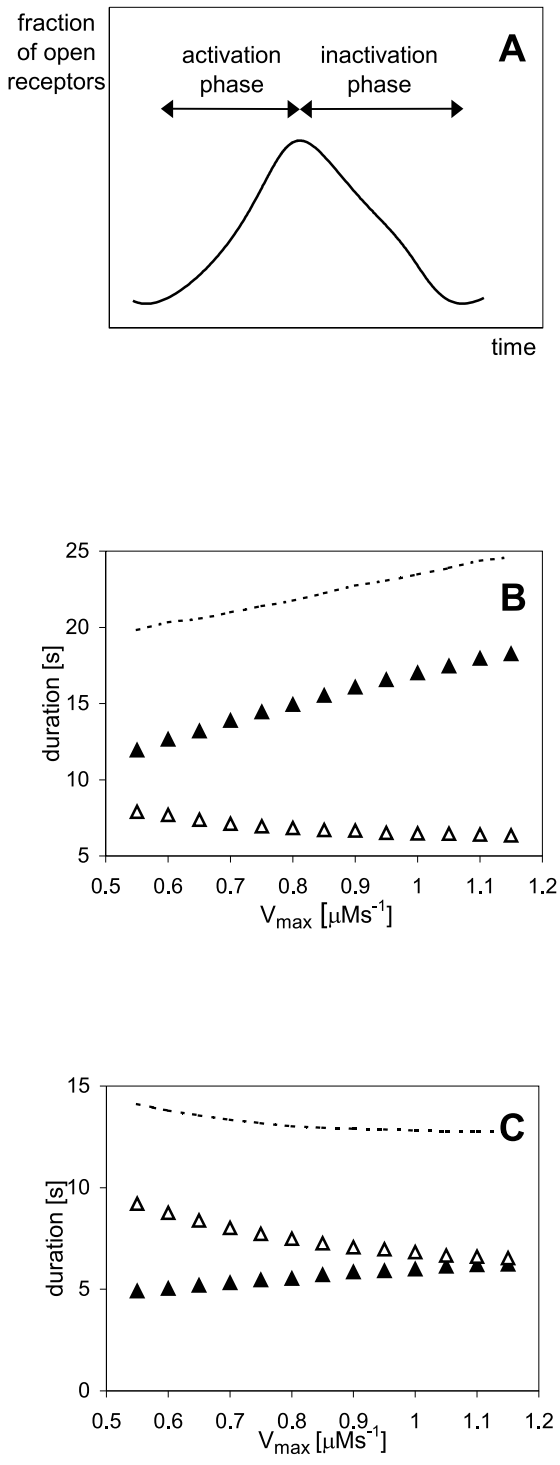

FIGURE 2 (*A*) The definitions of activation and inactivation phase of the time course of the open receptor fraction calculated with the De Young-Keizer model. (*B* and *C*) The dependence of the duration of activation phase (▲), inactivation phase (△), and their sum, the period (— — —) on maximal SERCA rate at low (0.35 μM, *B*), and high (0.75 μM, *C*) IP_3 concentration.

In HeLa cells, 5-min treatment with 4 nM thapsigargin did not increase cytosolic calcium level (similar to Missiaen et al., 1994). Transient or steady increases in calcium level were observed in a fraction of cells 5–15 min after thapsigargin addition. To test the effect of the 5-min treatment, thapsigargin was washed off and cells were incubated in nominally calcium-free medium for 30 min and subsequently stimulated with 100 μM histamine. The amplitude of the calcium peak was 7.1 $\pm$ 0.2 (expressed relative to basal fluorescence, mean $\pm$ SE) in control cells where only vehicle was added and 2.9 $\pm$ 0.3 in thapsigargin-pretreated cells, indicating partial inhibition of calcium sequestration.

In the next series of experiments, the effect of thapsigargin on the frequency of histamine-induced intracellular calcium oscillations was studied. At 70–80 s after the addition of 4 nM thapsigargin, cells were stimulated with different concentrations of histamine, an agonist known to induce calcium oscillations in HeLa cells (Sauve et al., 1991). Changes in intraluminal calcium concentration during this period and their effect on calcium release were considered negligible. To achieve greater oscillation frequencies (see later), extracellular calcium concentration was set to 5 mM (Bootman et al., 1996). Except for the highest concentration used, histamine induced oscillations in a similar fraction of thapsigargin-treated and control cells. Average peak-to-peak time was determined from the first two to six spikes, in cells where no significant irregularities in period were observed. Our results are summarized in Table 2 and Fig. 4, *A* and *B*. Thapsigargin treatment decreased the period of oscillations at low histamine concentration (1 and 2.5 μM) but did not alter oscillation frequency at maximal (100 μM) and supramaximal (500 μM) histamine dose. To rule out that oscillations at low histamine level are accelerated by any thapsigargin-induced calcium influx (Missiaen et al., 1994; Girard and Clapham, 1993), experiments were carried out also in calcium-free medium. In control cells, 1 μM histamine usually induced one peak or, rarely, very slow oscillations, whereas in 25% of thapsigargin-treated cells, oscillations were observed with a mean period of 72.5 $\pm$ 9.5 s ($n = 10$).

Although these results met our expectations, in HeLa cells no further increase in frequency could be achieved by increasing histamine concentration to test our predictions at greater stimulus levels. We therefore investigated the effect of thapsigargin on a cell type displaying more frequent oscillations. We chose HEp-2 cells, where extracellular nucleotides induce periodic calcium release from IP_3-sensitive and ryanodine-insensitive pools with greater frequencies than in HeLa cells (Visegrády et al., 2000).

Because 4 nM thapsigargin elevated cytosolic calcium level in a fraction of HEp-2 cells, 2 nM thapsigargin was used instead. A 5-min pretreatment with thapsigargin before a 30-min incubation in calcium-free medium reduced the amplitude of the calcium signal induced by 100 μM ATP from 10.5 $\pm$ 0.4 in control cells to 6.2 $\pm$ 0.2 (determined as relative change to basal fluorescence).

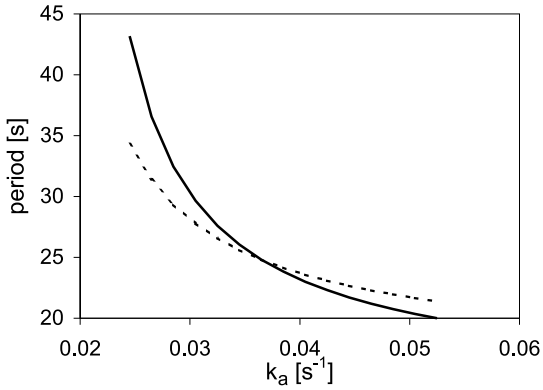

FIGURE 3 Analytically estimated duration of oscillation period as a function of k_a at $k_p = 0.225\ s^{-1}$ (——) and $0.185\ s^{-1}$ (— — —).

In HEp-2 cells, thapsigargin pretreatment slightly changed the mean period of calcium oscillations induced by 20 μM ATP from 15.4 $\pm$ 0.4 s ($n = 40$) to 17.2 $\pm$ 0.4 s ($n = 45$) (significantly different at $p < 0.05$ using Student's *t*-test). Fig. 4 *C* shows representative traces of oscillations from thapsigargin-treated and control cells.

The effect of thapsigargin on oscillation frequency was compared with the analytically estimated predictions. Fig. 5 shows our data measured in HeLa and HEp-2 cells together with the calculated values presented in Fig. 3. Values are plotted as frequency change as a function of control frequency.

DISCUSSION

Previous studies on different cells demonstrated contradictory effects of modulating endoplasmic reticulum Ca^{2+}-ATPase activity upon the frequency of intracellular calcium oscillations. According to literature data, it is probable that in some of the investigated cell types, similar mechanisms result in cytosolic calcium oscillations (Wakui et al., 1989; Lechleiter and Clapham, 1992). We investigated which of the models proposed to describe calcium oscillations can explain the experimental data.

Numerical calculations with the model of De Young and Keizer (De Young and Keizer, 1992; Keizer and De Young, 1994), based on IP_3-dependent CICR, predicted, in contrast to other models, that the effect of inhibiting SERCA depends on the stimulus level (Fig. 1). Further studies demonstrated that in this model decreasing the maximal SERCA rate accelerates the initiation of calcium spikes (similarly to Petersen and Dupont (1994)) but lengthens the period during which the fraction of open receptors decreases (Fig. 2).

These effects could be explained by the analysis of a simplified reaction scheme describing calcium release from, and uptake into a store, and calcium binding to the IP_3

TABLE 1 Parameters used in the analytical approximation of oscillation period

Parameter	Value	Meaning
k_p	Varied	Apparent first order rate constant of calcium extrusion
k_{ch}	5 s^{-1}	Apparent rate constant of calcium release
k_a	Varied	Apparent first order rate constant of calcium association to IP_3 receptor
k_d	0.4 s^{-1}	Ca^{2+} dissociation rate constant from IP_3 receptor
K_M	250 nM	SERCA Michaelis constant
$[Ca^{2+}]_i(0)$	100 nM	Resting $[Ca^{2+}]_i$
$[Ca^{2+}]_i(t^*)$	800 nM	$[Ca^{2+}]_i$ at start of resequestration
$RC(0)$	0.05 nM	Initial concentration of calcium-bound IP_3 receptors
K	2 s	Time duration of fast calcium release phase

receptor. With these assumptions, partially inhibiting the Ca^{2+}-ATPase, which provides a parallel reaction route for cytosolic calcium beside channel activation, should shorten the time required for calcium to activate sufficient IP_3 receptors. In the refractory phase SERCA inhibition slows down the removal of dissociated calcium from the cytosol and thus lengthens the duration of calcium-dependent inhibition (Fig. 3).

Based on these observations, we propose that stimulus dependence in the effect of SERCA inhibition can occur in cells where IP_3-dependent CICR induces intracellular calcium oscillations, because at low and high stimulus levels, different processes (e.g., activation and elimination of inhibition, respectively) are dominant in determining the oscillation period. At weak stimuli a slow build-up of open receptors, whereas at high stimulus levels the termination of inhibition, is the rate-limiting phase preceding a calcium spike. This explanation is consistent with recent experimental data (Marchant and Parker, 2001).

Experiments performed on agonist-stimulated HeLa and HEp-2 cells showed that the effect of partial SERCA inhibition by thapsigargin depended on agonist dose and thus on oscillation frequency as well (Fig. 4). Thapsigargin, a specific and irreversible inhibitor of SERCA pumps (Lytton et al., 1991) reduces SERCA activity in a way similar to the simulations. In HeLa cells, at low histamine dose (frequency: 0.5–1.5 spikes/min) thapsigargin shortened the interspike period independent of extracellular calcium influx, whereas at supramaximal dose (2 spikes/min) it had no effect on the frequency. In ATP-stimulated HEp-2 cells that displayed more frequent oscillations (4 spikes/min), SERCA inhibition slightly decreased the frequency.

Although observed in different cell types, these experimental findings still confirm our expectations. HeLa cells, like *Xenopus* oocytes, are well-studied prototypes for the IP_3-dependent CICR mechanism (Bootman et al., 1994; Barrero et al., 1997). In HEp-2 cells, extracellular nucleotides induce calcium oscillations from IP_3-sensitive internal pools (Visegrády et al., 2000). The weak dependence of the oscillation frequency in HEp-2 cells on ATP dose may result from the signal transduction pathway enabling calcium oscillations only at saturating agonist dose.

Based on IP_3-dependent CICR, we can now explain discrepancies in the literature. In weakly stimulated pancreatic acinar and endothelial cells, partial SERCA inhibition increased the frequency (Petersen et al., 1993; Morgan and Jacob, 1998), whereas SERCA overexpression, the opposite of inhibition, had the same effect in fast oscillating *Xenopus* oocytes (Lechleiter et al., 1998). Our suggestion is supported by the fact that in pancreas cells and oocytes, the frequency change decreased with increasing or decreasing stimulus levels, respectively (Petersen et al., 1993; Camacho and Lechleiter, 1993), as predicted by our calculations.

Our proposal does not exclude other mechanisms that may play a role during agonist stimulation in the investigated cells. In pancreas acinar cells, CICR through ryanodine receptors is supposed to take place during oscillations (Wakui et al., 1990), whereas in HeLa cells, Ca^{2+}-dependent phospholipase C activity (Bootman et al., 1996) or altered calcium flux through the plasma membrane (Uneyama et al., 1998; Missiaen et al., 1994) may also affect calcium oscillations. Nevertheless, these processes are generally not prerequisite for the existence of oscillations, and present mathematical models based on them cannot explain a decreased or unchanged frequency of calcium oscillations after partial SERCA inhibition.

Our explanation based on IP_3-dependent CICR, in contrast, is obviously not valid for fibroblasts, where SERCA inhibition further slowed down already low-frequency oscillations (Rossi and Kao, 1997). This is consistent with

TABLE 2 Mean period of calcium oscillations induced by various concentrations of histamine in HeLa cells treated with 4 nM or no thapsigargin

	Histamine			
	1 μM	2.5 μM	100 μM	500 μM
Control	106.2 ± 9.0 s (n = 29)	51.8 ± 3.9 s (n = 42)	30.4 ± 1.8 s (n = 66)	29.3 ± 2.6 s (n = 14)
Thapsigargin treated	87.0 ± 4.9 s* (n = 44)	40.9 ± 3.1 s* (n = 39)	26.7 ± 1.0 s (n = 48)	27.4 ± 2.8 s (n = 7)

Values represent mean ± SE, n denotes the number of cells data were taken from.
*Significantly differs from control at $p < 0.05$ using Student's t-test.

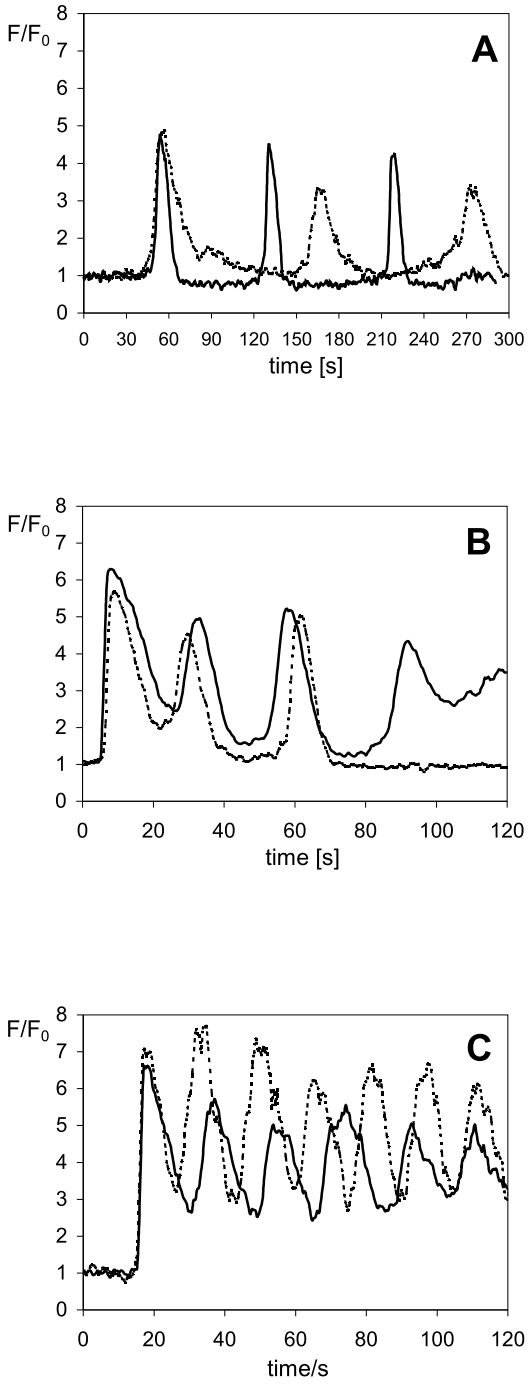

FIGURE 4 Calcium oscillations induced by 1 (*A*) and 100 (*B*) μM histamine in HeLa cells and 20 μM ATP in HEp-2 cells (*C*). Representative oscillations in cells treated with 4 nM (HeLa cells) or 2 nM (HEp-2 cells) thapsigargin (——) compared with control cells (— — —).

experiments suggesting that in these cells a different mechanism be responsible for agonist-induced calcium oscillations (Harootunian et al., 1991).

Finally, it is remarkable that the model of Atri et al. (1993), which is very similar to that of De Young and Keizer, but assumes equilibrium binding of calcium to the

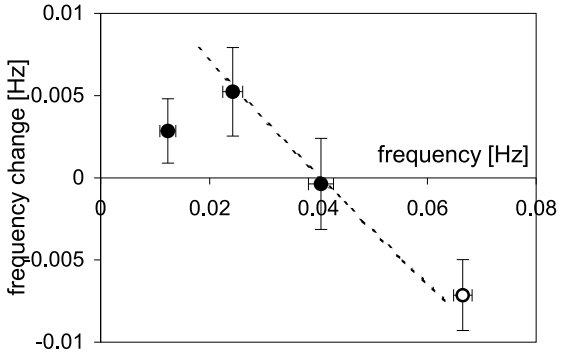

FIGURE 5 The change of frequency as a function of initial frequency, calculated from experiments summarized in Table 2 and text, and analytical calculation shown in Fig. 3. Note that (mean frequency) $>$ (mean period)$^{-1}$. Data are from HeLa cells (1, 2.5, and 100 μM histamine) (●), HEp-2 cells (○), and calculation (— — —). Bars represent SEM.

activating site on the IP_3 receptor, does not show a stimulus-dependent effect of SERCA inhibition. The time dependence of the binding of calcium to these sites seems to be important in describing calcium oscillations.

In conclusion, we found a theoretical explanation for controversial literature data regarding the effect of SERCA inhibition upon the frequency of intracellular calcium oscillations in different cell types. Based on previous observations, we assumed that in these cells similar mechanisms result in agonist-induced periodic calcium changes. By mathematical modeling and analytical calculation we have shown that the IP_3-dependent calcium-induced calcium release model can explain the stimulus-dependent effect of changing SERCA activity. Measurements on agonist-stimulated HeLa and HEp-2 cells confirmed our expectations. Our results indicate that in these cell types this process may play a predominant role in sustaining periodic changes in cytosolic calcium level after receptor stimulation.

APPENDIX

Analytical approximation of the oscillation period

We calculated the period of oscillations as the sum of the characteristic time of slow channel activation preceding the autocatalytic upstroke, the duration of rapid calcium release, and the time constant of the falling phase, where only calcium extrusion is taken into account. We assumed that the duration of the fast rising phase is relatively short, depends solely on channel conductance, and is independent from parameters such as maximal pump rate or $[IP_3]$.

Estimation of the time constant of calcium-induced calcium release

A simplified model of IP_3-dependent calcium-induced calcium release was constructed using the following assumptions.

Calcium transport takes place between the cytosol and the endoplasmic reticulum. Calcium buffers and control by luminal calcium concentration are neglected. Calcium is released from the endoplasmic reticulum by the

open IP_3 receptor with a rate J_{ch}, and it is pumped back by the Ca^{2+}-ATPase with J_p:

$$\frac{d[Ca^{2+}]_i}{dt} = J_{ch} - J_p \quad (1)$$

$$J_{ch} = P \times RC \times ([Ca^{2+}]_{ER} - [Ca^{2+}]_i),$$

where $[Ca^{2+}]_i$ is the cytoplasmic free calcium ion concentration, P is the permeability of the IP_3 receptor, RC is the number of open receptors related to a given volume of cytoplasm, and $[Ca^{2+}]_{ER}$ is the luminal calcium concentration. Assuming that $[Ca^{2+}]_{ER} \gg [Ca^{2+}]_i$ and that $[Ca^{2+}]_{ER}$ is constant in the initiation phase (Barrero et al., 1997), we get

$$J_{ch} = k_{ch} RC,$$

where $k_{ch} = P[Ca^{2+}]_{ER}$

To approximate the pump rate, we assume Michaelis-Menten kinetics with a maximal rate of V_{max} and that $[Ca^{2+}]_i \approx K_M$. Thus,

$$J_p = V_{max} \times \frac{[Ca^{2+}]_i}{[Ca^{2+}]_i + K_M} \approx \frac{V_{max}}{2K_M} \times [Ca^{2+}]_i$$

$$= k_p \times [Ca^{2+}]_i. \quad (2)$$

The number of open receptors changes as calcium reacts with receptors that already bound IP_3 (R) with a rate constant k_+ and as open receptors dissociate to R and $[Ca^{2+}]_i$.

$$\frac{dRC}{dt} = k_+ R \times [Ca^{2+}]_i - k_d RC$$

Assuming that in the lag phase R is constant, we introduce the parameter $k_a = k_+ R$. Note that through R, k_a includes stimulus level, as the fraction of IP_3-bound receptors depends on cytosolic IP_3 concentration and thus stimulus strength.

$$\frac{dRC}{dt} = k_a \times [Ca^{2+}]_i - k_d RC \quad (3)$$

Solving the differential Eqs. 1 and 3 yields

$$[Ca^{2+}]_i(t) = C_1 e^{k_1 t} + C_2 e^{k_2 t}$$

$$RC(t) = RC_1 e^{k_1 t} + RC_2 e^{k_2 t}, \quad (4)$$

where $k_2 < 0$, $|k_2| > k_1$, and $k_1 = (-k_p - k_d + \sqrt{(k_d - k_p)^2 + 4k_a k_{ch}})/2$.

If $t > |k_2^{-1}|$ and $k_1 > 0$, $[Ca^{2+}]_i(t) \approx C_1 e^{k_1 t}$. The characteristic time constant for this process, τ_1 can be defined as

$$[Ca^{2+}]_i(\tau_1) = e \times [Ca^{2+}]_i(0)$$

$$\tau_1 = \frac{1}{k_1} \ln\left(\frac{e \times [Ca^{2+}]_i(0)}{C_1}\right). \quad (5)$$

Estimation of the time constant of calcium uptake

The following assumptions were used to determine the time constant of calcium extrusion from the cytosol: 1) no calcium release takes place in this phase, and 2) the calcium pump displays Michaelis-Menten kinetics (the pump actually displays Hill kinetics (Lytton et al., 1992); however, for analytical solution of the autocatalytic phase, Michaelis-Menten kinetics has been already considered).

$$\frac{d[Ca^{2+}]_i}{dt} = -V_{max} \frac{[Ca^{2+}]_i}{K_M + [Ca^{2+}]_i}$$

The implicit solution is

$$[Ca^{2+}]_i(t) + K_M \ln([Ca^{2+}]_i(t))$$

$$= [Ca^{2+}]_i(t^*) + K_M \ln([Ca^{2+}]_i(t^*)) - V_{max}(t - t^*),$$

where t^* is the time point when the pumping is started. Because this process represents calcium falling back to initial level after the lag phase and rapid release, the characteristic time τ_2 will be defined as

$$[Ca^{2+}]_i(t^* + \tau_2) = [Ca^{2+}]_i(t^*)/e^2.$$

Recalling from Eq. 2 that $V_{max} = 2K_M k_p$,

$$\tau_2 = \frac{1}{k_p} + \frac{1}{2K_M k_p} \times \frac{e^2 - 1}{e^2} \times [Ca^{2+}]_i(t^*). \quad (6)$$

The approximated period can be calculated from Eqs. 5 and 6 as

$$T = \tau_1 + \tau_2 + K,$$

where K is the time duration of the rapid upstroke of calcium release.

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