Mechanism of Intracellular Calcium Transients

J. POLEDNA

Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Vlárska 5, 833 34 Bratislava, Czechoslovakia

Abstract. Calcium ions mediate extracellular signals on intracellular processes. The signalling system based on transient rises or oscillations of the cytoplasmic calcium concentration has potential advantages. The relevant mechanisms of intracellular concentration changes include calcium-induced calcium release and calcium dependent inactivation of calcium release. A model has been devised based on these processes to generate repetitive transients of the cytoplasmic calcium concentration.

Key words: Calcium oscillations — Calcium-induced calcium release — Mathematical model

Introduction

Calcium ions play an important role in numerous cellular functions, ranging from exocrine and hormone secretion through muscle and nonmuscle motility, to the activity and regulation of several important metabolic pathways. The key element in the signalling function of calcium is its reversible complexation by specific proteins (Rink and Jacob 1989).

Calcium enters the cytoplasm either from the extracellular space or from intracellular compartments which can alternately take up calcium ions from the cytoplasm by active transport and release it into the cytoplasm by passive diffusion.

Specific regulatory proteins which bind calcium must discriminate between the resting and active levels of calcium concentration. The concentration increase should be at least about one order. The discrimination can be improved by cooperation of several binding sites of a receptor. A cell keeps very low cytoplasmic concentrations of calcium ions. The lower the basal concentration, the smaller the amount of calcium ions necessary to increase the calcium concentration sufficient to fulfill the respective functions, and the less energy consumed to restore the resting conditions. Hence, there must exist a feedback mechanism, which would confine the calcium release not to exceed free calcium concentration beyond the regulation range. To restrict the calcium release, the simplest possibility is a cal-

cium dependent inactivation of calcium channels (Brehm and Eckert 1978; Poledna 1987; Schneider and Simon 1988; Poledna 1989; Valko and Zachar 1989; Parker and Ivorra 1990; Sherman et al. 1990; Nelson and Nelson 1990).

Detailed data concerning transport processes of calcium ions have been obtained from skeletal muscle preparations. The sarcoplasmic reticulum (SR) is a specialized intracellular membrane system that controls muscle contraction and relaxation by rapidly releasing and sequestering calcium. Rapid calcium fluxes are possible due to the presence of high-capacity permeation system for calcium, a ligand gated calcium release channel which plays a central role in the process of excitation-contraction coupling in skeletal muscle (Lai et al. 1988; Hymel et al. 1988). These data can be used to explain basic mechanisms of calcium signalling. On the other hand, special modes of operation, like calcium oscillations, can elucidate further calcium control in muscle cells. For instance, a repetitive calcium discharge and re-uptake, based on calcium-induced calcium release from the sarcoplasmic reticulum, can be seen in calcium overloaded myocytes (Fabiato and Fabiato 1975) or skeletal muscle fibers (Zachar et al. 1972; 1973).

The calcium release channel in the sarcoplasmic reticulum is regulated by the endogenous ligands Ca^{2+}, Mg^{2+}, and ATP. Micromolar calcium and millimolar ATP concentrations activate the channel, although the presence of both ligands is required to fully open it (Xu et al. 1989). This calcium-induced calcium release (CICR) is activated within a low calcium concentration range, and further increase of calcium concentration inhibits the release (Ikemoto et al. 1989). The basic molecular mechanism of CICR operating *in situ* is maintained in isolated SR vesicles where this process is well described.

In spite of a considerable amount of experimental data, the mechanism of generation of repetitive transients has not been explained satisfactorily. These phenomena are based on a complex system and nonlinear processes, therefore, a mathematical model would be productive. Some models describing intracellular calcium transients have been formulated (Meyer and Stryer 1988; Dupont and Goldbeter 1989; Swillens and Mercan 1990). The proposed model suggests a different mechanism which generates calcium transients. Relevant processes, which can contribute to intracellular calcium oscillations, are calcium-induced calcium release and calcium dependent calcium release inactivation.

Model and Results

Let calcium concentrations in the reticulum be c_r and that in the cytoplasm c. When the ratio of effective volumes (taking into account low affinity binding sites) of the cytoplasm and the reticulum is δ, then

$$c_r = C + (C - c)\delta \quad \text{and} \quad c_r - c = (1 + \delta)(C - c) \tag{1}$$

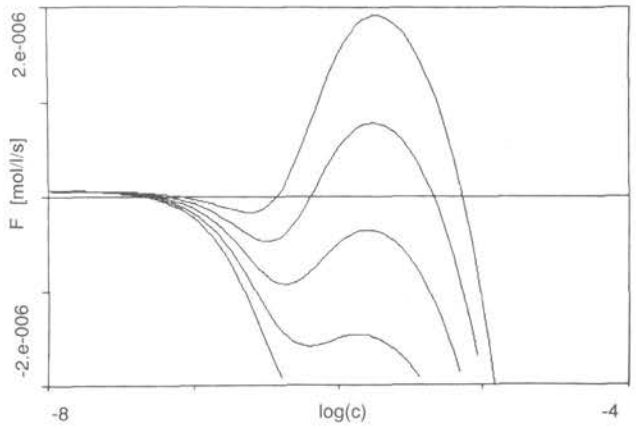

Figure 1. Changes in the shape of calcium flux. The inactivation parameter, w, is varied between 0.6 and 1. For the other parameters see Table 1, column a. Abscissa: logarithm of calcium concentration in mol/l.

where C is the average free calcium concentration in a cell as a whole.

The calcium releasing channel is controlled by a receptor which binds h calcium ions. Calcium binding is described by

$$\frac{\mathrm{d}r}{\mathrm{d}t} = k_+ c^h (R - r) - k_- r \tag{2}$$

where R is the total number of receptors. For a steady state,

$$r_s = \frac{Rc^h}{c^h + A^h}, \quad \text{where} \quad A^h = \frac{k_-}{k_+} \tag{3}$$

Then, the channel permeability is

$$p_c = \frac{Qc^h}{c^h + A^h} w \tag{4}$$

where w corresponds to channel inactivation, which may be calcium dependent, and Q is the maximal permeability.

The calcium flow between the intravesicular and the cytoplasmic compartments is

$$F = -P\frac{c^f}{c^f + B^f} + \left(L + Q\frac{c^h}{c^h + A^h} w\right)(C - c)(1 + \delta) \tag{5}$$

where L denotes leak permeability, and the first term represents the calcium pump with a maximal flow P. According to experimental data, cooperativity expressed

by f and h should be $f = 2$ and $h = 2$. Since w varies slowly, it is helpful to examine how the calcium flow depends on c when w is treated as a parameter (Fig. 1). The concentrations corresponding to zero crossings of this flow represent steady states of the system. For $B < A$, there is an intermediate range of w values for which F generates three steady states, with the middle point being unstable and the others being stable. The left interval between zero crossings corresponds to the net flux into the vesicles, and the displaced calcium concentration returns to the left resting steady state. The right interval represents the net flux from the vesicles, and the displacement to this values is self-regenerative, moving calcium concentration to the right steady state point. This process describes the calcium-induced calcium release.

The channel with the binding sites occupied slowly inactivates. This is described by decreasing w and by fusion of two intersections of F with the zero line and their disappearing. Concentration c returns to the resting state and calcium dissociates from binding sites.

The equation which describes the calcium concentration dynamics of the proposed model is given by

$$\frac{\mathrm{dc}}{\mathrm{dt}} = -P\frac{c^2}{c^2 + B^2} + \left(L + Q\frac{c^2}{c^2 + A^2}w\right)(C - c)(1 + \delta) \tag{6}$$

It states that the only source or sink of calcium ions are intracellular vesicles. The simplest description of w behavior, which depends on calcium concentration, is

$$\frac{\mathrm{dw}}{\mathrm{dt}} = \frac{w_0 - w}{\tau} \tag{7}$$

$$w_0 = 1 - \frac{c}{c + K} = \frac{K}{c + K}$$

$$\tau = \frac{1}{k_{i+}(c + K)}$$

where w_0 represents calcium dependent steady state, τ is the time constant, and K is the equilibrium constant of calcium binding.

Since the system is two-dimensional and autonomous, its solutions can be studied geometrically in a phase space which spans both dynamics-relevant variables, c and w. A point in phase space corresponds to the state of the system. The time evolution of the system is represented by a curve in phase space, the trajectory. The right sides of equations (6) and (7) supply, in each point of the plane (c, w), a tangent to the trajectory. I.e., starting from a point defined as initial condition, the course of the trajectory is determined by a tangent in each successive point of the phase space reached. Usually, it is sufficient to draw curves, nullclines, for which dc/dt and/or dw/dt are zero, and directions of tangents in their neighborhood. By studying the graphs of these curves, we obtain a clear picture of how the

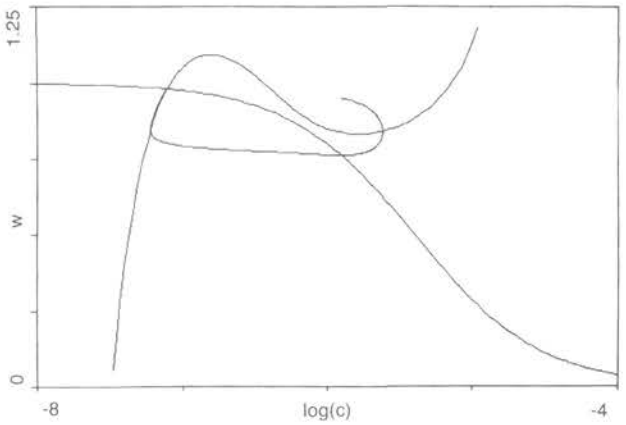

Figure 2. The phase portrait for the system of equations (6) and (7). Both nullclines have been drawn, the nonmotonic (8) and the monotonically decreasing (9), which intersect in the steady state point. The trajectory is the response to a sudden increase of the cytoplasmic calcium concentration from the resting state. It returns back to the steady state point and represents the calcium-induced calcium release. For the parameters see Table 1, column a. Abscissa: logarithm of calcium concentration in mol/l.

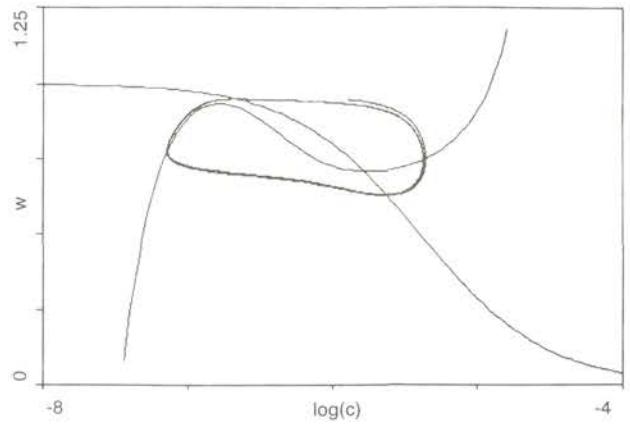

Figure 3. The phase portrait for the system of equations (6) and (7), where the parameter C is raised. The trajectory shows oscillations of the cytoplasmic calcium. For the parameters see Table 1, column b. Abscissa: logarithm of calcium concentration in mol/l.

solutions behave generally and how this behavior corresponds quite closely to the experimentally observed physiological phenomena.

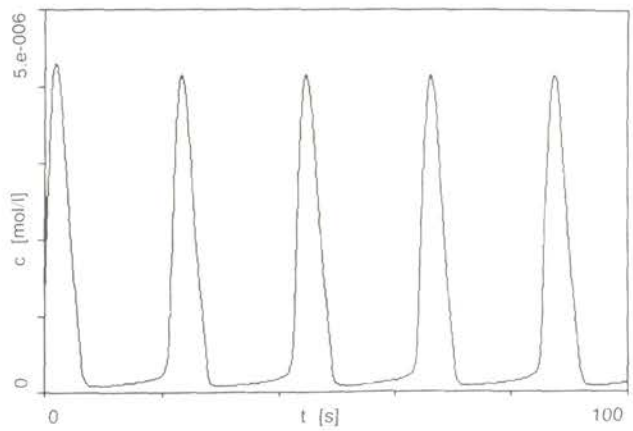

Figure 4. Illustration of the intracellular calcium oscillations corresponding to the phase portrait shown in Fig. 3. This is a numerical solution to the system (6) and (7). For the parameters see Table 1, column b.

Table 1. Parameters for simulations.

Parameter	a	b
P/Q [mol/l]	5.0×10^{-4}	5.0×10^{-4}
L/Q [1]	5.0×10^{-3}	5.0×10^{-3}
$\delta+1$ [1]	21.0	21.0
A [mol/l]	5.0×10^{-7}	5.0×10^{-7}
B [mol/l]	4.0×10^{-7}	4.0×10^{-7}
K [mol/l]	4.0×10^{-6}	4.0×10^{-6}
C [mol/l]	3.1×10^{-5}	3.6×10^{-5}
$1/k_{i+}$ [mol.l^{-1}.s]	2.0×10^{-5}	2.0×10^{-5}
Q [s^{-1}]	2.0×10^{-2}	2.0×10^{-2}

For the steady states of the Eqs. (6) and (7) there are nullclines

$$w = \left(\frac{P}{Q(1+\delta)} \frac{c^2}{(c^2+B^2)(C-c)} - \frac{L}{Q} \right) \frac{c^2+A^2}{c^2} \tag{8}$$

$$w = \frac{K}{c+K} \tag{9}$$

For $B < A$, the nullcline (8) can be nonmonotonic. There exists an interval where three different values of c correspond to each w. This reflects the process of CICR. The intersection of nullclines is a steady state point. When it lies on the ascending limb of the nullcline (8), it is stable and represents the resting state. It attracts all

trajectories appearing in its neighborhood. The phase portrait (Fig. 2) shows the case of calcium stimulated release and return to the resting state. If C increases, the nullcline (8) moves downward and the intersection shifts on the descending part of the nullcline. We have seen, by analyzing the flux, that the descending part of the nullcline (8) is unstable. For the derived system of equations, the trajectories approach the closed attractor surrounding the steady state point (Fig. 3). Such a closed attractor is called a stable limit cycle, which corresponds to unceasing oscillations (Fig. 4). The frequency can be controlled by parameters corresponding to external conditions. These examples use reasonable parameters summarized in Table 1. They are in the range of experimentally obtained data (Gilchrist et al. 1990; Kirtley et al. 1990; Nelson and Nelson 1990; Soler et al. 1990).

If C increases above a particular value, a periodic solution disappears, that is, the steady state point moved on the ascending limb of the nullcline. The steady state is stable at high calcium levels.

Discussion

There is an enormous variety of oscillatory patterns between the cells and also within an individual cell, responding to different agonists. Most frequently, transient calcium oscillations are induced by activating those receptors which act through phosphoinositide pathway (Berridge 1990). All types of calcium transients may have a common essential mechanism.

Several models have been suggested describing intracellular calcium transients phenomena. A molecular model that accounts for periodic calcium spiking induced by a constant stimulus was proposed by Meyer and Stryer (1988). In their model, the messengers $InsP_3$ and cytosolic calcium ions are crosscoupled. The stimulation of receptor-activated phospholipase C by released calcium ions leads to positive feedback. Osipchuk et al. (1990) showed that calcium infusion into single internally perfused mouse pancreatic acinar cells can generate repetitive calcium spikes near the cell membrane similar in nature to those invoked by $InsP_3$ infusion. It therefore now seems unlikely that the calcium oscillations are due to pulsatile $InsP_3$ formation.

An alternative view holds that the mechanism responsible for the oscillations appears to be the calcium-induced calcium release from intracellular stores (Dupont and Goldbeter 1989). The model considered relies on the hypothesis that an external stimulus triggers the synthesis of a certain amount of $InsP_3$ that induces the release of calcium from an $InsP_3$-sensitive pool. Cytosolic calcium is pumped into an $InsP_3$-insensitive compartment, calcium in this compartment is released into the cytosol in a process activated by cytosolic calcium. The model involves two variables, namely, the concentration of calcium in cytosol and that in the $InsP_3$-insensitive store. These two variables are closely coupled and dim the mechanism

of repetitive calcium transients. Peres (1990) showed that mouse oocytes possess intracellular stores that are able to discharge Ca ions into the cytoplasm in response to $InsP_3$. The role of the $InsP_3$-induced Ca release appears to be that of gradually raising the calcium level up to the point where Ca-induced Ca release is triggered. These observation, therefore, produce evidence supporting the oscillatory model of Dupont and Goldbeter (1989) and Berridge (1990), which is based on the interplay between $InsP_3$-sensitive and Ca-sensitive stores. However in order to produce oscillations, this model requires a continuous generation of $InsP_3$, which does not appear essential in the experiments made by Peres (1990). Moreover, the CICR-positive feedback is not terminated by depletion of available calcium and therefore it appears necessary to hypothesize the existence of a spontaneous inactivation process of the CICR mechanism.

Another model has been proposed by Swillens and Mercan (1990). Only two pools of calcium are considered, namely the cytosol and an $InsP_3$-sensitive store. Calcium flux from intracellular stores is inhibited by intra-vesicular calcium. Positively cooperative inhibition of calcium flux by intravesicular calcium leads to abrupt switch between a phase of slow calcium increase and the sharp calcium spike. Direct experimental evidence in favor of the proposed mode of calcium action is lacking.

The presented model is based on assumptions about calcium-induced calcium release and calcium dependent inactivation of calcium release, which have been well documented. Their properties have been derived from experiments on SR vesicles and skinned muscle fibers. A population of membrane vesicles obtained from cisternal-junctional SR was capable of exhibiting calcium-induced calcium release. A leakage pathway can be triggered by increasing calcium concentration in the medium from 10^{-8} to 10^{-6} mol/l. The resulting efflux proceeded with rate constants of the order of $10\ s^{-1}$ or higher (Kirtley et al. 1990)

Nelson and Nelson (1990) suggested that two calcium regulatory sites might be functional for CICR from SR. A heavy skeletal muscle SR fraction was actively loaded stepwise with calcium until calcium-induced calcium release occurred. The total calcium load at which release occurred is postulated to be regulated by an intraluminal low affinity receptor. The critical concentration of calcium required extraluminally was determined, and it averaged $2.14 \pm 0.24\ \mu$mol/l. Inactivation of the calcium channel is influenced by the extraluminal calcium concentration and holds the amount of calcium released constant, regardless of the calcium preload. The CICR cannot occur until a critical load of calcium is obtained within the SR. However, the existence of an intraluminal receptor is not necessary, as shown by the model. These experiments confirm the role of parameter C as a bifurcation parameter. When the system is perturbated by an increase of the intracellular calcium concentration, the value of C determines that the system oscillates, responses with CICR or simply returns to the resting state.

Also, the model is able to explain the effect of caffeine, which can induce oscillations in some cells (Kristian et al. 1991). The effect of caffeine lies in an enhancement of membrane permeability for calcium, which can reflect the parameter Q. This parameter shifts the nullcline (8) in appropriate direction.

Evidence for the existence of CICR in non-muscle cells is based mainly on observations on intact cells: they mobilize calcium from internal stores when calcium is introduced into the cytoplasm either by injection or by enhanced influx across the plasma membrane (Berridge 1990, Kristian et al. 1991).

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