

Complex calcium oscillations and the role of mitochondria and cytosolic proteins

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Abstract

Intracellular calcium oscillations, which are oscillatory changes of cytosolic calcium concentration in response to agonist stimulation, are experimentally well observed in various living cells. Simple calcium oscillations represent the most common pattern and many mathematical models have been published to describe this type of oscillation. On the other hand, relatively few theoretical studies have been proposed to give an explanation of complex intracellular calcium oscillations, such as bursting and chaos. In this paper, we develop a new possible mechanism for complex calcium oscillations based on the interplay between three calcium stores in the cell: the endoplasmic reticulum (ER), mitochondria and cytosolic proteins. The majority ($\approx 80\%$) of calcium released from the ER is first very quickly sequestered by mitochondria. Afterwards, a much slower release of calcium from the mitochondria serves as the calcium supply for the intermediate calcium exchanges between the ER and the cytosolic proteins causing bursting calcium oscillations. Depending on the permeability of the ER channels and on the kinetic properties of calcium binding to the cytosolic proteins, different patterns of complex calcium oscillations appear. With our model, we are able to explain simple calcium oscillations, bursting and chaos. Chaos is also observed for calcium oscillations in the bursting mode. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Complex calcium oscillations; Bursting; Chaos; Mitochondria

1. Introduction

Oscillations of cytosolic calcium concentration, known as calcium oscillations, play a vital role in

providing the intracellular signalling. Many cellular processes, like cell secretion or egg fertilisation for instance, are controlled by the oscillatory regime of the cytosolic calcium concentration. Since the 1980s, when self-sustained calcium oscillations were found experimentally (Cuthbertson and Cobbold, 1985; Woods et al., 1986) many further experimental works have been published

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(for review, see Goldbeter (1996) and Berridge et al. (1999)). Additionally, many theoretical studies have been carried out to explain the mechanism of calcium oscillations as well as the phenomenon of calcium waves (Meyer and Stryer, 1988; Goldbeter et al., 1990; Somogyi and Stucki, 1991; Dupont and Goldbeter, 1993; Li and Rinzel, 1994; Jafri and Gillo, 1994; Jafri and Keizer, 1997; Marhl et al., 1997, 1998a,b; Höfer 1999; Falcke et al., 1999; for review, see Goldbeter (1996)). Concerning the mechanism of calcium oscillations, it is widely agreed that the endoplasmic reticulum (ER) represents the main calcium store in the cell. Calcium release from the ER plays the predominant role in generating sustained calcium oscillations. In some mathematical models, other intracellular calcium stores, such as cytosolic proteins, are included (Jafri et al., 1992; Jafri and Gillo, 1994; Marhl et al., 1997, 1998a,b). Recently, it has become even clearer that mitochondria also play an important role in intracellular calcium signalling and do not serve only as the metabolic powerhouses of the cell (Jouaville et al., 1995; Hehl et al., 1996; Babcock et al., 1997; Golovina and Blaustein, 1997; Babcock and Hille, 1998; Jouaville et al., 1998; Ricken et al., 1998; Simpson and Russell, 1998a,b; Simpson et al., 1998; Rizzuto et al., 1998; Verkhratcky and Petersen, 1998; Drummond and Tuft, 1999). However, except in previous theoretical work by the authors Marhl et al. (1998a) and in some other studies (Meyer and Stryer, 1988; Magnus and Keizer, 1997; Selivanov et al., 1998), mitochondria are usually not included into the modelling of calcium oscillations.

In the last decade, during the period of intensive mathematical modelling of calcium oscillations, it is actually only simple calcium oscillations which have been studied, i.e. self-sustained calcium oscillations with repetitive spikes of equal amplitude. However, experimental results very often show more complex forms of self-sustained calcium oscillations as well (for review, see Borghans et al. (1997)). The most common pattern of such complex oscillations represents a periodic succession of silent and active phases. The silent phase is relatively quiescent, while during the active phase the system exhibits rapid

oscillations. This form of complex oscillations is known as bursting (cf. Vries, 1998) and the case of electrical bursting, i.e. bursting of the electric potential oscillations in excitable cells, is studied much more (e.g. Chay, 1996, 1997; for review, see Vries (1998)). The model presented by Chay (1996) is of special importance because it represents a link between the electrical bursting and calcium bursting in excitable cells. This model was the first to be radically different from previous models of bursting excitable cells, whose mechanism is based on the ion channels in the plasma membrane and on the dynamics of intracellular Ca^{2+} concentration. This model presented a possible explanation for calcium bursting oscillations in excitable cells. For non-excitable cells, Shen and Larter (1995) gave an early theoretical study of complex calcium oscillations. However, this field is relatively new and the mechanism of complex calcium oscillations, as well as its biological importance for intracellular calcium signalling, remains unexplained in many details.

Some possible mechanisms explaining complex calcium oscillations in non-excitable cells have been proposed by Borghans et al. (1997) and further mathematically analysed in Houart et al. (1999). The first main characteristic observed in these models is the idea that bursting could be a consequence of changes in IP_3 production. Because IP_3 has a direct influence on the opening probability of the IP_3 -sensitive Ca^{2+} channels in the ER membrane, this approach is, of course, one of the most straightforward ways to explain the phenomenon of bursting in the case of intracellular calcium oscillations. Shen and Larter (1995) used a similar approach which also characterises the work of Olsen et al. (1999) and, recently, more detailed studies by Kummer et al. (paper in preparation, personal communication). The other mechanism explaining the complex calcium oscillations in Borghans et al. (1997) is also related to the activity of the Ca^{2+} -release channels in the ER. Here, the cytosolic calcium concentration is proposed to be the main regulator of both activation and inhibition of the Ca^{2+} -release channels in the ER. In all cases, the ER is the unique intracellular calcium store included in the model. In the same paper (Borghans et al., 1997),

a further mechanism with two intracellular calcium stores, one sensitive and one insensitive to IP_3 , is considered. As there is experimental evidence that the ER acts partly as IP_3 -sensitive and partly as IP_3 -insensitive store (Golovina and Blaustein, 1997; Simpson et al., 1998), it should be noted that the two pools could be seen as two different parts of the ER and because of the specific Ca^{2+} kinetics, cannot be easily identified with other intracellular calcium stores.

In the present paper, we give a new possible explanation of complex intracellular calcium oscillations based on calcium exchange between different calcium stores in the cell. In addition to the ER, representing the main Ca^{2+} store in the cell, we also take into account the Ca^{2+} sequestration in mitochondria and the Ca^{2+} binding to cytosolic proteins. The importance of mitochondria for the modulation of simple calcium oscillations has been analysed in our previous paper (Marhl et al., 1998a) and the role of cytosolic Ca^{2+} binding proteins for the frequency and amplitude of simple calcium oscillations has been discussed in (Marhl et al., 1997, 1998b; Schuster et al., 1998). Here, the importance of mitochondria and the cytosolic Ca^{2+} binding proteins for the complex calcium oscillations is presented. We show that the complex intracellular calcium oscillations can be explained by the specific Ca^{2+} kinetics of all three intracellular Ca^{2+} stores included in the model: the ER, mitochondria and the cytosolic Ca^{2+} binding proteins. We get simple calcium

oscillations, bursting and chaos. Chaos is also observed for calcium oscillations of the bursting type.

2. Mathematical model

The model system is schematically presented in Fig. 1. The main characteristics of the system are three different calcium stores: the ER, mitochondria and calcium binding proteins in the cytosol. We focus on calcium exchange between the cytosol and the three calcium stores, neglecting any exchange of calcium between the cytosol and the extracellular space. Considering the ER, three different calcium fluxes are included in the model: the ATP-dependent calcium uptake from the cytosol into the ER (J_{pump}), the Ca^{2+} efflux from the ER through channels following the calcium-induced calcium release (CICR) mechanism (J_{ch}) and an additional Ca^{2+} leak flux from the ER into the cytosol (J_{leak}). For the exchange of Ca^{2+} between the mitochondria and the cytosol we take into account the following Ca^{2+} fluxes: active Ca^{2+} uptake by mitochondrial uniporters (J_{in}), calcium release through Na^+/Ca^{2+} exchangers combined with a flux through the mitochondrial permeability transition pores (PTPs) in a very low-conductance state, and a very small non-specific leak flux (J_{out}).

In comparison to our previous model (Marhl et al., 1998a), some simplifications have been made,

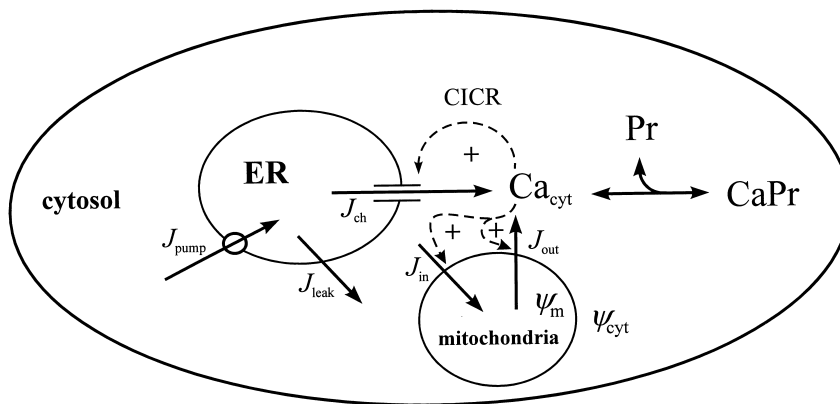


Fig. 1. Schematic presentation of the model system.

whereas the calcium transport across the mitochondrial membrane is described in some more details. The purpose here is to model a basic possible mechanism for complex calcium oscillations. Therefore, in the present model the electric potential difference across the ER membrane is not taken into account and, consequently, the exchange of monovalent cations and monovalent anions between the ER and the cytosol is of no interest here. Furthermore, in the cytosol only one class of calcium binding proteins is included, the so-called buffering proteins. However, in addition to the mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchangers studied previously (Marhl et al., 1998a) a new aspect of the Ca^{2+} release through the PTPs following the mitochondrial calcium-induced calcium release (mCICR) mechanism (Ichas et al., 1994a,b; Ichas et al., 1997; Jouaville et al., 1998) is implicitly considered in J_{out} .

In the present model, there are five variables: free cytosolic calcium concentration (Ca_{cyt}), free calcium concentration in the ER (Ca_{ER}), free calcium concentration in mitochondria (Ca_{m}), the concentration of free Ca^{2+} binding sites on the cytosolic proteins (Pr) and the concentration of bounded Ca^{2+} binding sites on the cytosolic proteins ($CaPr$). The number of model variables reduces to three independent variables applying the conservation relations for the total cellular calcium Ca_{tot} ,

$$Ca_{\text{tot}} = Ca_{\text{cyt}} + \frac{\rho_{\text{ER}}}{\beta_{\text{ER}}} Ca_{\text{ER}} + \frac{\rho_{\text{m}}}{\beta_{\text{m}}} Ca_{\text{m}} + CaPr, \quad (1)$$

and for the total concentration of bound and unbound proteins Pr_{tot} ,

$$Pr_{\text{tot}} = Pr + CaPr. \quad (2)$$

Here ρ_{ER} and ρ_{m} represent the volume ratio between the ER and the cytosol and between the mitochondria and the cytosol, respectively. Assuming very fast unsaturated buffering of Ca^{2+} in the ER and mitochondrial compartments, we use constant factors β_{ER} and β_{m} for relating the concentrations of free calcium in the ER and the mitochondria to the respective total concentrations (Li et al., 1995; Smith et al., 1996).

The time dependence of the free cytosolic calcium concentration, Ca_{cyt} , is determined by Ca^{2+}

fluxes across the ER membrane, by Ca^{2+} exchange with mitochondria and by the Ca^{2+} binding to cytosolic proteins. Thus, it is described by the following equation:

$$\frac{dCa_{\text{cyt}}}{dt} = J_{\text{ch}} + J_{\text{leak}} - J_{\text{pump}} + J_{\text{out}} - J_{\text{in}} + k_- CaPr - k_+ Ca_{\text{cyt}} Pr, \quad (3)$$

where k_- and k_+ denote the off and on rate constants, respectively, of the Ca^{2+} binding.

The equation for the free calcium concentration in the ER, Ca_{ER} , is linked with the fluxes across the ER membrane as follows:

$$\frac{dCa_{\text{ER}}}{dt} = \frac{\beta_{\text{ER}}}{\rho_{\text{ER}}} (J_{\text{pump}} - J_{\text{ch}} - J_{\text{leak}}). \quad (4)$$

The equation for the free calcium concentration in mitochondria, Ca_{m} , reads:

$$\frac{dCa_{\text{m}}}{dt} = \frac{\beta_{\text{m}}}{\rho_{\text{m}}} (J_{\text{in}} - J_{\text{out}}). \quad (5)$$

As in our previous publications (Marhl et al., 1997, 1998a,b), the ATPase-mediated Ca^{2+} flux, J_{pump} , into the ER lumen is taken as a linear function:

$$J_{\text{pump}} = k_{\text{pump}} Ca_{\text{cyt}}, \quad (6)$$

where k_{pump} is the rate constant of the ATPases.

The other two Ca^{2+} fluxes across the ER membrane, the channel flux J_{ch} and the leak flux J_{leak} , are also described as in our previous publications (Marhl et al., 1997, 1998a,b), but simplified by neglecting their dependency on the ER transmembrane potential. Only the concentration gradient across the ER membrane is taken into account. The equations for both fluxes are given by:

$$J_{\text{ch}} = k_{\text{ch}} \frac{Ca_{\text{cyt}}^2}{K_1^2 + Ca_{\text{cyt}}^2} (Ca_{\text{ER}} - Ca_{\text{cyt}}), \quad (7)$$

$$J_{\text{leak}} = k_{\text{leak}} (Ca_{\text{ER}} - Ca_{\text{cyt}}), \quad (8)$$

where k_{ch} represents the maximal permeability of the Ca^{2+} channels in the ER membrane, K_1 represents the half-saturation for calcium and k_{leak} is the rate constant for Ca^{2+} leak flux through the ER membrane.

There is experimental evidence of a very fast and effective calcium sequestration by mitochon-

dria through a specific uniporter (cf. Hehl et al., 1996; Babcock et al., 1997; Applegate et al., 1997) at free cytosolic calcium levels of more than $\approx 0.5\text{--}1.0\ \mu\text{M}$ (cf. Jouaville et al., 1995; Bernardi and Petronilli, 1996; Hehl et al., 1996; Herrington et al., 1996; Hoth et al., 1997; Ricken et al., 1998). Therefore, for the mitochondrial Ca^{2+} uptake by uniporters, J_{in} , a step-like kinetics is considered (Marhl et al., 1998a).

$$J_{\text{in}} = k_{\text{in}} \frac{Ca_{\text{cyt}}^8}{K_2^8 + Ca_{\text{cyt}}^8} \quad (9)$$

where k_{in} represents the maximal permeability of the uniporters in the mitochondrial membrane and K_2 represents the half-saturation for calcium. As in our previous publication (Marhl et al., 1998a), in factor k_{in} the constant value of the mitochondrial transmembrane potential, $\Delta\psi^{(\text{m})}$, is implicitly included. The potential difference $\Delta\psi^{(\text{m})}$ is usually strongly changed only by fast release of calcium through the PTP. Note that under normal physiological conditions just a slow release of calcium from the mitochondria takes place (Bernardi and Petronilli, 1996; Eriksson et al., 1999; Svichar et al., 1999). In the publication (Marhl et al., 1998a) we explicitly speak about the $\text{Na}^+/\text{Ca}^{2+}$ and $\text{H}^+/\text{Ca}^{2+}$ exchangers as providers of this slow calcium release.

For the sake of generality, in addition to the $\text{Na}^+/\text{Ca}^{2+}$ and $\text{H}^+/\text{Ca}^{2+}$ exchangers also PTPs are considered here. Their role is often correlated with the mitochondrial CICR (mCICR) as a possible candidate for the calcium spike modulation (Ichas et al., 1994a,b, 1997; Jouaville et al. 1998). It seems to be of special importance in the study of complex calcium oscillations. Therefore, we take into account a mCICR-like kinetic of the PTPs, whereby we propose that the PTPs can only be transiently opened in a very low-conductance state and so have minimal consequence for changing the transmembrane potential $\Delta\psi^{(\text{m})}$ (Babcock and Hille, 1998; Simpson and Russell, 1998a). Such modelling seems to be reasonable for biological systems with mitochondrial populations, as in our case. There is much experimental evidence that mitochondria

are clustered in the neighbourhood of the ER Ca -channels sites (Drummond and Fay, 1996; Golovina and Blaustein, 1997; Spacek and Harris, 1997; Applegate et al., 1997; Rizzuto et al., 1998). In contrast, it is experimentally shown that large openings of the PTP with consequent fast electric potential depolarisations are rather specific only for individual mitochondria (Hüser et al., 1998) or generally in some extreme physiological conditions, as in the case of apoptosis as a type of cell death (Simpson and Russell, 1998a).

Combining the calcium release through $\text{Na}^+/\text{Ca}^{2+}$ exchangers, the flux through PTPs in a very low-conductance state comparable to that of the $\text{Na}^+/\text{Ca}^{2+}$ exchangers, and a very small non-specific leak flux, the mitochondrial Ca^{2+} efflux, J_{out} , can be expressed as:

$$J_{\text{out}} = \left(k_{\text{out}} \frac{Ca_{\text{cyt}}^2}{K_3^2 + Ca_{\text{cyt}}^2} + k_{\text{m}} \right) Ca_{\text{m}}. \quad (10)$$

The constant k_{out} is the maximal rate for calcium flux through $\text{Na}^+/\text{Ca}^{2+}$ exchangers and PTPs in a very low-conductance state. The rate constant k_{m} stands for the non-specific leak flux. Note that the mitochondrial Ca^{2+} release is experimentally observed to be of ten to 100 times slower than the uniporter uptake (Bassani et al., 1998; Falcke et al., 1999) and that under normal physiological conditions, no explicit evidence for the PTPs opening exists at all (Eriksson et al., 1999; Svichar et al. 1999). Therefore, the expression for J_{out} implicitly includes both the kinetics of the $\text{Na}^+/\text{Ca}^{2+}$ exchangers and the kinetics of the PTPs in low-conductance state with only one rate constant determining the maximal calcium efflux from the mitochondria. The constant K_3 represents the half-saturation for calcium.

Model parameters used in our calculations are listed in Table 1. Most of the parameter values are taken from measurements reported in the literature and were already discussed in detail in our previous papers (Marhl et al., 1997, 1998a,b). The values used here are in agreement with those used in our previous publications. However, the modifications of model equations have to be taken into account to see the analogy of the values.

Table 1
Model parameters for which all results are calculated unless otherwise stated

Parameter	Value
<i>Total concentration</i>	
Ca_{tot}	90 μM
Pr_{tot}	120 μM
<i>Geometric parameters</i>	
ρ_{ER}	0.01
ρ_{m}	0.01
β_{ER}	0.0025
β_{m}	0.0025
<i>Kinetics parameters</i>	
k_{ch}	4100 s^{-1}
k_{pump}	20 s^{-1}
k_{leak}	0.05 s^{-1}
k_{in}	300 $\mu\text{M s}^{-1}$
k_{out}	125 s^{-1}
k_{m}	0.00625 s^{-1}
k_{+}	0.1 $\mu\text{M}^{-1} \text{s}^{-1}$
k_{-}	0.01 s^{-1}
K_1	5 μM
K_2	0.8 μM
K_3	5 μM

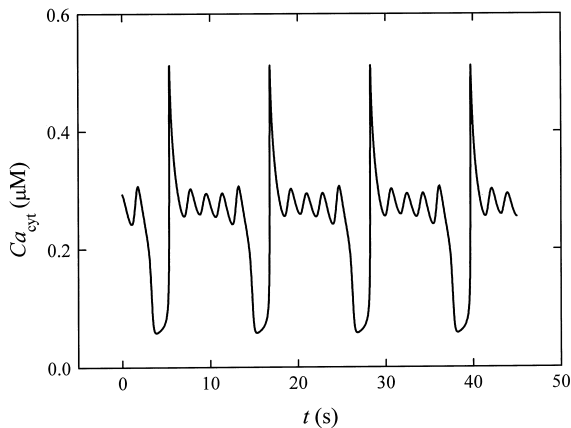


Fig. 2. Calcium bursting oscillations for the reference case (see Table 1 for parameter values).

3. Results

To simulate the intracellular calcium oscillations, the model equations are integrated numerically. For different sets of model parameters, we get various types of calcium oscillations, from

simple calcium oscillations to bursting and chaos. Here, we focus on complex calcium oscillations and therefore simple calcium oscillations are not shown. Parameter values, giving the simplest case of bursting are referred to as reference case and are listed in Table 1. It should be noted that all results are calculated for the parameter values given in Table 1 unless otherwise stated.

The results of numerical integration for the reference case are shown in Fig. 2. We plot the cytosolic calcium concentration (Ca_{cyt}) versus time (t) as a characteristic result, which enables a direct comparison with usual experimental measurements. Fig. 2 shows a typical example of bursting of intracellular calcium oscillations. The superposition of high frequency oscillations to the basic calcium spikes is well expressed.

A closer look on this reference case allows analysis of the mechanism providing the calcium bursting oscillations. Fig. 3a shows time courses of model variables for one cycle, i.e. between two basic spikes. For a better explanation of the mechanism of bursting we divide the whole cycle into three different phases. In Phase I, the Ca^{2+} release from the ER is the dominating process. This leads to the rapid increase of cytosolic and mitochondrial calcium. Taking into account the Ca^{2+} buffering in the mitochondrial matrix (see factor β_{m} in Eqs. (1) and (5)), we can realise that the largest fraction of the released calcium ($\approx 80\%$) is sequestered in the mitochondria, which is in good agreement with recent experimental observations (Babcock and Hille, 1998). At the end of calcium sequestration in mitochondria, Phase I is finished and a slow release of calcium from the mitochondria begins (Phase II). In agreement with experimental observations, calcium release from mitochondria is much slower than its uptake (Bernardi and Petronilli, 1996; Hehl et al., 1996; Bassani et al., 1998; Babcock and Hille, 1998; Falcke et al., 1999). The importance of this slow Ca^{2+} release is in Ca^{2+} transferring from mitochondria to the cytosolic proteins. This is a long-term process accompanied by much faster Ca^{2+} exchange between the ER and the cytosolic proteins causing small calcium spikes of bursting oscillations. Therefore, the kinetic properties of the ER channel as well as Ca^{2+} binding dynamics

of proteins are of crucial importance for the generation of these high-frequency calcium oscillations between the main spikes characterising the bursting oscillations. So, for example, slight changes of binding parameter k_{on} significantly influence the number of bursts, whereas a more drastic parameter changing results as simple calcium oscillations. In this case, the Ca^{2+} is transferred rather directly from mitochondria to the ER (not shown here). At the end of the bursting phase (Phase II), the silent phase (Phase III) begins, during which the dissociation of the CaPr complex is the major process. The dissociation rate determines the duration of the silent phase.

In analysing the mechanism of calcium bursting oscillations, a picture of the limit cycle is also very

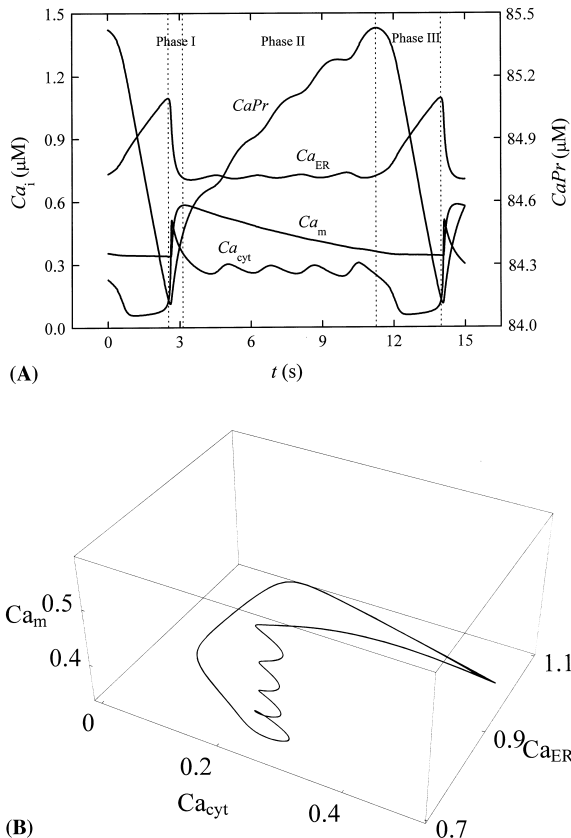


Fig. 3. Analysis of bursting oscillations for the reference case. (a) Time courses of Ca_{cyt} , Ca_{ER} , Ca_m and $CaPr$ for one cycle. The concentration of unbound Ca^{2+} binding sites (Pr) is simply related with $CaPr$ (Eq. (2)) and not shown explicitly; (b) Limit cycle in 3D-phase space of Ca_{cyt} , Ca_{ER} and Ca_m (in μM).

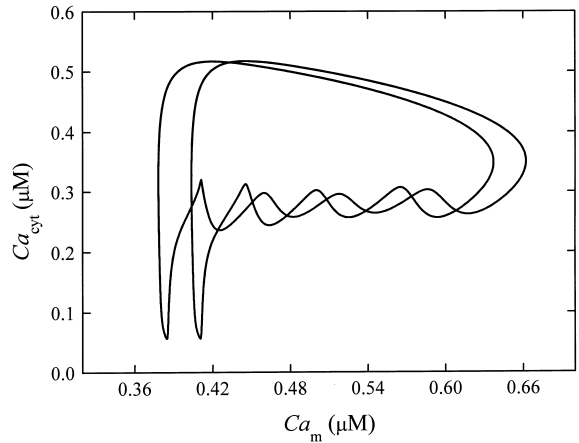


Fig. 4. Two-folded limit cycle of Ca^{2+} bursting oscillations. Parameter values differing from those listed in Table 1, $k_{ch} = 4000 \text{ s}^{-1}$.

helpful. The limit cycle for the reference case is shown in 3D-phase diagram of Fig. 3b. Note, that due to conservation relations (Eqs. (1) and (2)) our system is actually a 3D one. In particular, Fig. 3b gives good insight into the origin of superposed high frequency oscillations. These oscillations can be recognised by the vertical spiral, which is the consequence of a slow decline of mitochondrial calcium accompanied by the fast cytosolic exchange of calcium between the ER and the cytosolic proteins.

For the above discussion, we have chosen a set of model parameters that leads to simple limit cycle oscillations. In other regions of the channel permeability, k_{ch} , more complex behaviour is observed. Fig. 4 shows an example of a 2-fold limit cycle in phase plane Ca_{cyt} versus Ca_m . In this case, we have bursting oscillations with two different patterns exchanging periodically in time.

Although a more detailed analysis of the dynamic behaviour of the system in different regions of the parameter space is left for a sequel paper, it should be mentioned that period doubling, shown in Fig. 4, represents a typical transition to chaos. To demonstrate an example of chaotic behaviour of our system, we show the phase plane plot Ca_{cyt} versus Ca_m for $k_{ch} = 2950 \text{ s}^{-1}$ in Fig. 5a. The return map in Fig. 5b indicates strong evidence for deterministic chaos. In this figure, the succes-

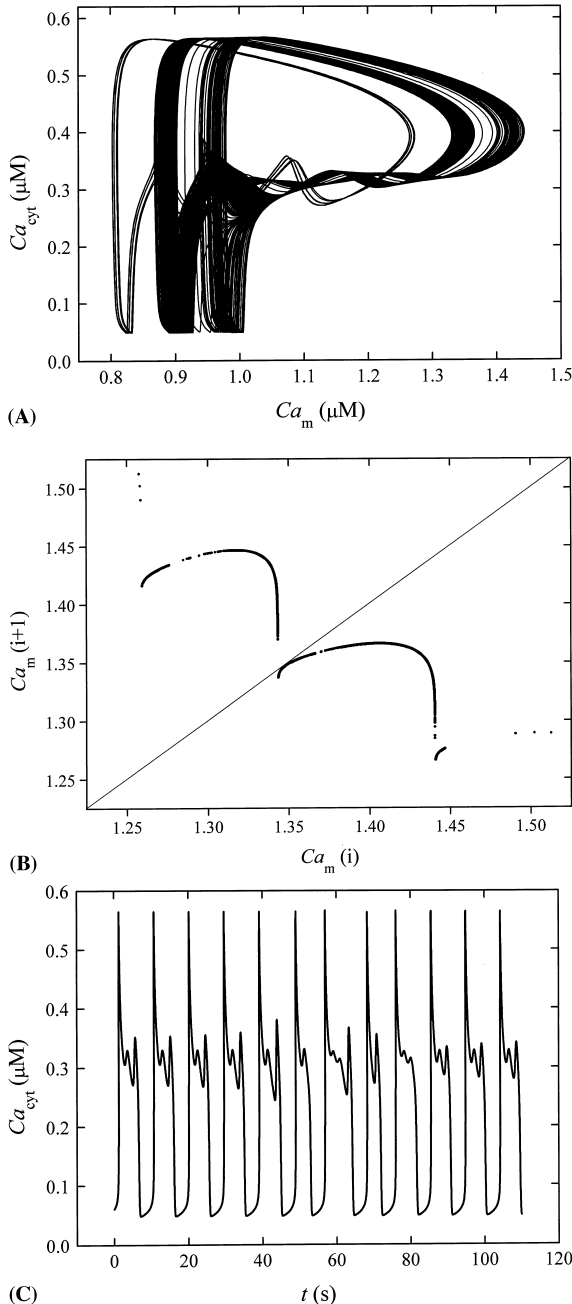


Fig. 5. Chaotic behaviour of Ca^{2+} bursting oscillations: (a) projection of trajectory in phase plane Ca_{cyt} versus Ca_m ; (b) return map for Ca_m (in μM); and (c) time course of Ca_{cyt} . Parameter values differing from those listed in Table 1, $k_{\text{ch}} = 2950 \text{ s}^{-1}$.

sive maxima of Ca_m are plotted against their predecessors. If these maxima are plotted for an infinitely long time series, a continuous curve is obtained. Two important characteristics of the chaotic behaviour in our model should be pointed out. First, note that this is an example of chaotic bursting and second, the amplitudes of main calcium spikes remain nearly constant all the time. This is convincingly demonstrated in the time course of cytosolic calcium depicted in Fig. 5c.

4. Discussion

In the paper a possible mechanism explaining complex calcium oscillations is presented, which gives a new complementary aspect to the existing theoretical studies of complex intracellular calcium oscillations (Shen and Larter, 1995; Borghans et al., 1997; Houart et al., 1999; Olsen et al., 1999). We show that complex calcium oscillations arise as a consequence of Ca^{2+} exchanging between the cytosol and three different intracellular calcium stores: the ER, mitochondria and cytosolic proteins. For Ca^{2+} exchange between the cytosol and a particular calcium store, simple plausible rates are used. We take into account the CICR from the ER, the ATP-ase active transport into the ER, the uniporter Ca^{2+} uptake by mitochondria, slow release from the mitochondria and Ca^{2+} binding to the cytosolic proteins. With the model, we get simple calcium oscillations, bursting and chaos. Moreover, the chaotic behaviour of calcium bursting oscillations is obtained. All model results are in agreement with known experimental observations. The cytosolic calcium transients have a typical spike-like form, reasonable frequencies and amplitudes. We emphasise that the amplitudes of main calcium spikes remain nearly constant in the whole range of the oscillatory regime. It is valid for all types of calcium oscillations, i.e. for simple calcium oscillations, for regular bursting, as well as for chaotic bursting calcium oscillations. As explained in our previous publication (Marhl et al., 1998a), the constancy of amplitudes of calcium oscillations is a consequence of the specific mitochondrial kinetics.

The functioning of the intracellular calcium stores considered in the model agrees with known experimental observations. The fast calcium release from the ER, characterised by the CICR kinetics, is confirmed by many experiments and used in almost all mathematical models for calcium oscillations (cf. Goldbeter, 1996; Heinrich and Schuster, 1996). Calcium binding to cytosolic proteins was also well studied experimentally. A short review of these works and their importance for calcium oscillations is given in our earlier publications (Schuster et al., 1998; Marhl et al., 1998b). The inclusion of mitochondria into the modelling of calcium oscillations is also motivated by many experimental works showing the importance of mitochondria in cell signalling (Jouaville et al., 1995; Hehl et al., 1996; Babcock et al., 1997; Golovina and Blaustein, 1997; Babcock and Hille, 1998; Jouaville et al., 1998; Ricken et al., 1998; Simpson and Russell 1998a,b; Simpson et al., 1998; Rizzuto et al., 1998; Drummond and Tuft, 1999). It becomes clear that mitochondria actively sequester the Ca^{2+} released from the ER (Hehl et al., 1996; Babcock et al., 1997; Applegate et al., 1997; Simpson and Russell, 1998a; Rizzuto et al., 1998; Drummond and Tuft, 1999). Some recent experiments also predict more accurate values, namely that $\approx 80\%$ of calcium released from the ER is cleared first into the mitochondria (Babcock and Hille, 1998). Our results confirm this value with surprisingly high accuracy.

In the model we show that, in addition to the fast Ca^{2+} uptake by mitochondria, a very slow Ca^{2+} release back to the cytosol is of crucial importance for understanding the mechanism of complex calcium oscillations. This calcium kinetics is in agreement with known experimental evidences showing that under normal physiological conditions Ca^{2+} efflux from the mitochondria is much slower than Ca^{2+} uptake by uniporters (Hehl et al., 1996; Bernardi and Petronilli, 1996; Babcock et al., 1997; Bassani et al., 1998; Verkhratsky and Petersen, 1998; Falcke et al., 1999). In our previous study (Marhl et al., 1998a), this calcium release from mitochondria was modelled explicitly by taking into account only the $\text{Na}^+/\text{Ca}^{2+}$ exchangers and no PTPs. Recent experimental results show that at physiological con-

ditions of normal cellular functioning the Ca^{2+} release occur via the $\text{Na}^+/\text{Ca}^{2+}$ exchangers rather than via the PTPs (Simpson and Russell, 1998b; Svichar et al., 1999; Eriksson et al., 1999).

However, since Ichas and collaborators have shown that PTPs possess the mCICR kinetics (Ichas et al., 1994a,b, 1997; Jouaville et al., 1998), which could serve as a calcium spike modulator, in this study of complex calcium oscillations we include also a mCICR-like kinetic of the PTPs in the model, whereas we consider just transient openings of the PTPs in a very low-conductance state. This is in agreement with the evidence that for PTPs several subconductance states are proposed (Simpson and Russell, 1998a; Eriksson et al., 1999). Although little experimental evidence exists about their regulation (Eriksson et al., 1999), it is well believed that at normal physiological conditions the PTPs should not be opened with large conductivities (Simpson and Russell, 1998a,b). Note that huge openings of the PTPs do not cause only the release of large quantities of matrix calcium but also activate a release of cell death factors from the mitochondrial matrix. There are several examples of different mitochondrial matrix proteins or some intermembrane mitochondrial proteins such as cytochrome *c*, to be released from mitochondria via the PTPs and they appear to act as a direct activator of apoptosis (Simpson and Russell, 1998a). Larger openings of PTPs with consequent fast electrical potential depolarisations are rather specific for individual mitochondria experiments and less specific in the case of mitochondrial populations, such as our case in the neighbourhood of the ER Ca-channels sites (Hüser et al., 1998).

Model predictions show that at the very low conductivity of PTPs considered in the model, the specific mCICR-like kinetics of calcium efflux from the mitochondria seems to be of no crucial importance for complex calcium oscillations. Thus, we get complex calcium oscillations for a simple linear type of Ca^{2+} efflux from the mitochondria as well (not shown here, but the reader can check this by exchanging the expression $\text{Ca}_{\text{cyt}}^2 / (K_3^2 + \text{Ca}_{\text{cyt}}^2)$ in Eq. (10) with a constant factor of 0.004, for example). These model predictions can be seen in agreement with some most recent ex-

periments for excitable as well as for non-excitable cells, showing that PTPs do not participate in the normal physiological regulation of calcium signals (Eriksson et al., 1999; Svichar et al., 1999). Additionally, in accordance to some experimental observations showing that PTPs may not be present in all mitochondria (Simpson and Russell, 1998a), our model could even be seen as a yet more general one, giving an explanation of complex calcium oscillations in a larger variety of different cell types.

In future studies of the basic mechanism providing complex calcium oscillations, it will be important to focus on more detailed description of the intracellular Ca^{2+} stores considered, rather than on attempts to include other Ca^{2+} stores. On the basis of known experimental results, an inclusion of further Ca^{2+} stores seems to be of no special importance for explaining the basic mechanism providing complex calcium oscillations. For example, it has recently been shown that changes in nucleus Ca^{2+} concentration are very small in comparison to the other intracellular calcium stores (Xiong and Ruben, 1998). Additionally, the Ca^{2+} concentration in nucleus changes simply concomitantly with the transient elevations of the cytosolic calcium. Therefore, from the point of view of mathematical modelling, the nucleus, in responding to calcium signals, plays a role similar to that played by signalling proteins (Marhl et al., 1998a,b), which have been excluded from the present model.

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