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Mitochondria as an important factor in the maintenance of constant amplitudes of cytosolic calcium oscillations

Marko Marhl [a,*], Stefan Schuster [a,b], Milan Brumen [a,c]

[a] *University of Maribor, Faculty of Education, Koroška cesta 160, SI-2000 Maribor, Slovenia*
[b] *Max Delbrück Center, Robert-Rössle-Str. 10, D-13125 Berlin-Buch, Germany*
[c] *Josef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia*

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Abstract

Theoretical models of intracellular calcium oscillations have hitherto focused on the endoplasmic reticulum (ER) as an internal calcium store. These models reproduced the large variability in oscillation frequency observed experimentally. In the present contribution, we extend our earlier model [Marhl et al., Biophys. Chem., 63 (1997) 221] by including, in addition to the ER, mitochondria as calcium stores. Simple plausible rate laws are used for the calcium uptake into, and release from, the mitochondria. It is demonstrated with the help of this extended model that mitochondria are likely to act in favour of frequency encoding by enabling the maintenance of fairly constant amplitudes over wide ranges of frequency.

Keywords: Calcium oscillations; Calcium signalling; ER membrane potential; Calcium-binding proteins; Mitochondria; Channels

1. Introduction

Mitochondria do not only act as important sites of free-energy transduction in living cells, but also as intracellular calcium stores [1,2]. This property of mitochondria has recently attracted renewed interest in the light of calcium-mediated intracellular signalling [3–5]. It has been shown that a change in the energy state of mitochondria can lead to modulation of the shape of Ca^{2+} oscillations and waves, which are generated by autocatalytic release of Ca^{2+} from the endoplasmic reticulum (ER) [3,6,7]. Furthermore, there is experimental evidence showing that mitochondria are often located near the mouths of channels across the ER membrane, where the local concentration of Ca^{2+} is considerably higher than the average cellular concentration [8–10].

The investigation of the time behaviour of intracellular Ca^{2+} is a prominent example of a close interplay between experimental work and modelling studies [11,12]. Numerous models of Ca^{2+} oscillations have been developed (e.g., [13–18]). In all of these models, intracellular stores such as the ER were shown to be a necessary prerequisite for the generation of self-sustained Ca^{2+} oscillations. By

* Corresponding author. University of Maribor, Faculty of Education, Department of Physics, Koroška cesta 160, SI-2000 Maribor, Slovenia. Fax: +386-62-28180; e-mail: marko.marhl@uni-mb.si

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contrast, mitochondria have not explicitly been considered in modelling studies on Ca^{2+} mediated signalling so far. The present paper is aimed at contributing to fill this gap. To this end, we elaborate on a mathematical model presented earlier [19], in which the ER as well as Ca^{2+} binding proteins in the cytosol were taken into account as the basic Ca^{2+} buffering systems. Here we include, in addition, an exchange of Ca^{2+} between the cytosol and the mitochondria. As in our earlier model, the potential difference across the ER membrane is included as a model variable.

A number of different Ca^{2+} transport systems in the mitochondrial inner membrane such as the Ca^{2+} uniporter [4,20], Na^+/Ca^{2+} exchanger [4,5], H^+/Ca^{2+} exchanger [4,20], mitochondrial transition pores (MTP) [20] and megachannels [21,22] have been described in recent years. The latter two may reflect activity of the same molecular entity [20,22]. During calcium oscillations or transients evoked by hormones inducing calcium-induced calcium release (CICR) across the ER membrane, Ca^{2+} is taken up by mitochondria via specific electrogenic uniporters very rapidly at certain cytosolic concentrations, followed by slow release back into the cytosol through Na^+/Ca^{2+} and H^+/Ca^{2+} exchangers [4,5,20,23]. In extreme situations where mitochondrial calcium load is very high, the MTPs may be opened so that a fast calcium release can occur [20]. As this release appears to be autocatalytic, the term 'mCICR' (with m standing for mitochondrial) was coined [6]. This mechanism can even be responsible for inducing calcium oscillations [7]. In the present paper, however, we will only consider calcium oscillations based on CICR out of the ER and shaped by mitochondria, rather than oscillations based on mCICR. So we will include only the calcium uniporter and exchangers (rather than the MTP). Knowledge of the kinetic properties of these transport systems is still very limited. In the present model, we use simple rate laws describing them.

It is now commonly accepted that the information transmitted by Ca^{2+} oscillations is encoded in their frequency rather than their amplitude [11,24]. One of the aims of the present model is to examine which role is played by mitochondria in the phenomenon of frequency encoding. In particular, it will be investigated which effect mitochondria have on maintaining relatively constant amplitudes upon changes in oscillation frequency. The model is envisaged to be applicable to non-excitable cells, for example, to hepatocytes and oocytes.

2. Mathematical model

The model system is presented in Fig. 1. The main characteristics of this system are two types of internal store, notably the ER and mitochondria. As far as the ER is concerned, we consider the same processes as in our earlier model [19]. The inclusion of two different classes of calcium binding proteins studied later [25,26] is preserved in the present model as well. These classes are made up of proteins which bind calcium very fast (signalling proteins, such as the N-domains of calmodulin and troponin C) and proteins with relatively low on and off rate constants (buffering proteins, such as parvalbumin and calbindin).

Inclusion of mitochondria in the model requires one additional differential equation for the time course of mitochondrial calcium. Consequently, appropriate changes have to be made in the conservation equations for calcium, monovalent anions and monovalent cations in the cell. The following equations form the frame of the model:

$$\frac{dCa_{cyt}}{dt} = \frac{1}{1 + K_d^{II} Pr_{tot}^{II} \Big/ \left(K_d^{II} + Ca_{cyt}\right)^2} \times \left(J_{ch} - J_{pump} + J_{leak} + \frac{dPr^I}{dt} - \frac{\rho_m}{\beta_m} \frac{dCa_m}{dt} \right) \quad (1)$$

$$\frac{dPr^I}{dt} = k_-^I \left(Pr_{tot}^I - Pr^I\right) - k_+^I Ca_{cyt} Pr^I \quad (2)$$

$$\frac{dCa_m}{dt} = \frac{\beta_m}{\rho_m} \left(J_{in} - J_{out}\right) \quad (3)$$

Ca_{cyt}, Pr^I, and Ca_m represent the concentrations of free cytosolic calcium, free binding sites of the buffering proteins (see Ref. [26]), and free mitochondrial calcium, respectively. They are the main model

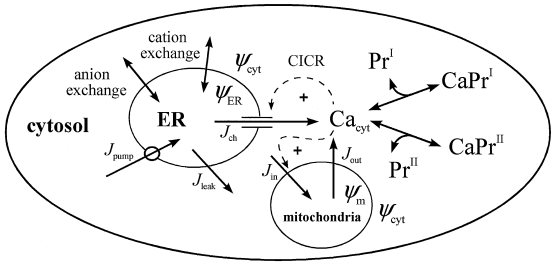

Fig. 1. Schematic presentation of the model system.

variables. Pr^{I}_{tot} and Pr^{II}_{tot} are the total concentrations of the slowly binding proteins and fast binding proteins, respectively. k^{I}_{+} and k^{I}_{-} stand for the on and off rate constants, respectively, of calcium binding to Pr^{I}. K^{II}_{d} is the dissociation constant of calcium with respect to Pr^{II}, for which the rapid-equilibrium approximation is used (cf. [27,28]). As it is known that buffering of calcium in the ER and in mitochondria is very fast and not saturated [29–31], we use constant factors β_{ER} and β_{m} for relating the free calcium concentrations in the ER and mitochondria to the respective total concentrations [27,32]. Note that all concentrations are defined with respect to the aqueous cytosolic volume. Therefore, we introduced, in Eqs. (1) and (3), a factor ρ_{m}, representing the volume ratio between the mitochondria and cytosol.

J_{pump} and J_{leak} represent the ATPase and leak fluxes, respectively, through the ER membrane. Simple linear rate laws are used for these fluxes [19]. J_{ch} denotes the channel flux through the ER membrane, which is characterized by CICR [19]:

$$J_{ch} = \frac{g_{Ca}}{4F^2 V_{cyt}} \Delta\tilde{\mu}^{(ER)}_{Ca} \tag{4}$$

where

$$g_{Ca} = \tilde{g}_{Ca} S Ca^2_{cyt} \Big/ \left(K^2_{Ca} + Ca^2_{cyt} \right) \tag{5}$$

Here, $\Delta\tilde{\mu}^{(ER)}_{Ca}$ denotes the electrochemical potential difference of calcium across the ER membrane, $\tilde{g}_{Ca}$ is the maximal ER membrane conductance per unit area, and S is the ER surface area. Here and below, all differences (indicated by the symbol Δ) are defined as the value in the cytosol minus the value in the organelle (ER or mitochondrion). The half-saturation constant for calcium is denoted by K_{Ca}.

There is experimental evidence about a very fast and effective calcium sequestration by mitochondria through a specific uniporter (cf. [5,10,23]) at free cytosolic calcium levels of more than about 0.5–1.0 μM (cf. [3,20,31,33]). At low cytosolic calcium, this uniporter is inhibited [20]. This has inspired us to use a step-like saturation kinetics for the Ca^{2+} influx, J_{in}, into the mitochondria. We describe it by a Hill kinetics with a large Hill coefficient, n, up to 8, taking $K_{m} = 0.7$ mM for fixing the threshold and considering that the uniporter is electrogenic:

$$J_{in} = k_{in} \frac{Ca^n_{cyt}}{K^n_{m} + Ca^n_{cyt}} \Delta\tilde{\mu}^{(m)}_{Ca} \tag{6}$$

where $\Delta\tilde{\mu}^{(m)}_{Ca}$ is the electrochemical potential difference of Ca^{2+} across the mitochondrial inner membrane,

$$\Delta\tilde{\mu}^{(m)}_{Ca} = RT \ln \frac{Ca_{cyt}}{Ca_{m}} + 2F\left(\Psi_{cyt} - \Psi_{m}\right) \tag{7}$$

Note the analogy between Eqs. (4) and (6). The free mitochondrial calcium concentration is in the range of 80–200 nM in the resting state and increases to 250–1000 nM when cytoplasmic Ca^{2+} is raised to 1–2 μM [5]. Thus, there is no considerable gradient of free Ca^{2+} across the mitochondrial membrane. Therefore, the chemical potential difference in Eq. (7) (the logarithmic term) can be neglected in comparison to the mitochondrial transmembrane potential, which amounts to about 150 mV (cf. [7]). At variance with the ER membrane, where $\Delta\Psi^{(ER)}$ is due to Ca^{2+} pumps, the potential difference across the mitochondrial inner membrane, $\Delta\Psi^{(m)}$, is established by proton pumps. Therefore, its dependence on the Ca^{2+} concentrations on either side of the membrane can approximately be neglected. An exception is when the MTPs or megachannels are opened, since these allow other ions to permeate as well. But in our study, we do not consider any opening of these pores, which seems to occur only under extreme conditions [20], and concentrate on the Ca^{2+} uniporters and exchangers. So we can assume $\Delta\Psi^{(m)}$ to be fairly constant upon the calcium oscillations evoked by CICR across the ER

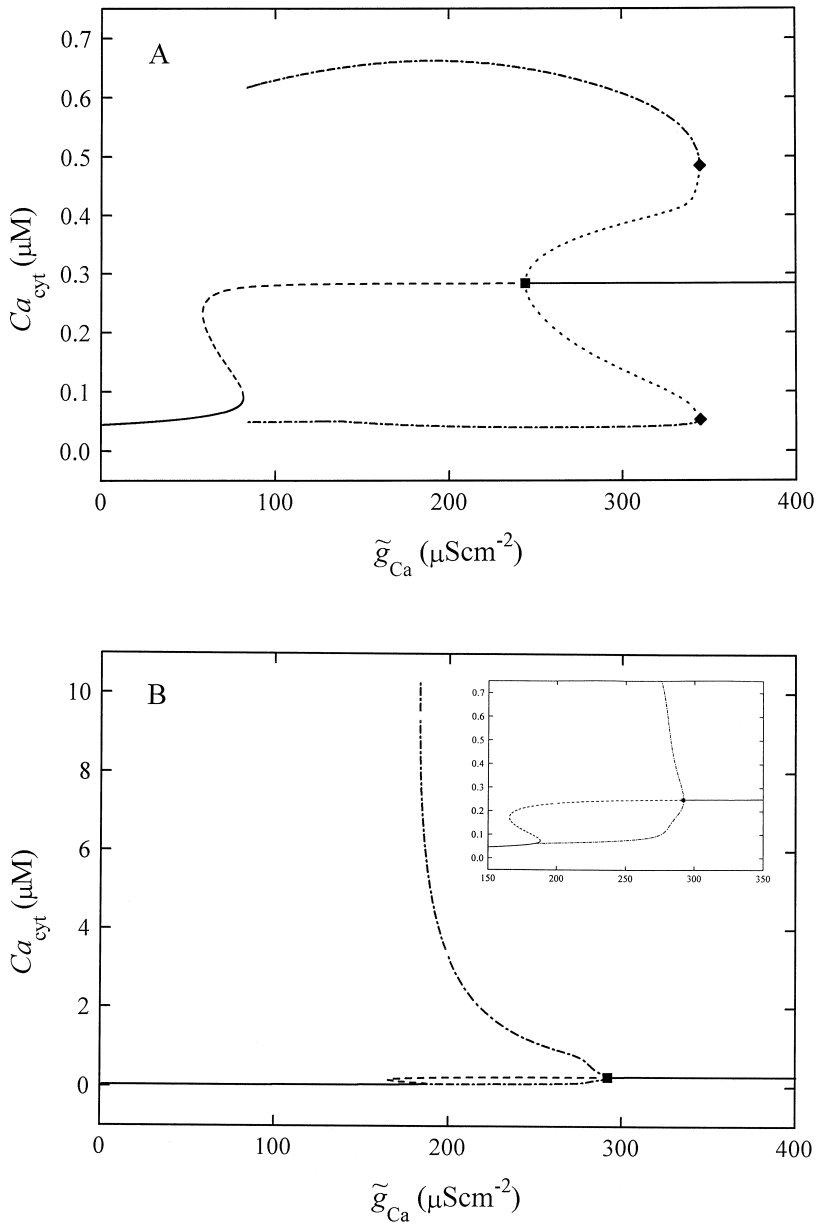

Fig. 2. Bifurcation diagram computed by the program AUTO [34] for the model under consideration with $\tilde{g}_{Ca}$ as the bifurcation parameter. (A) Model with inclusion of mitochondria, (B) model without mitochondria. Solid and dashed lines represent stable and unstable steady states, respectively. Dash-dotted and dotted lines indicate the amplitudes of stable and unstable limit cycles, respectively. Hopf bifurcations (■) and the saddle-node bifurcation of the limit cycle (♦) are indicated. The parameter values concerning mitochondria are $\beta_m = 0.0001$, $\rho_m = 0.06$, $k'_{in} = 100\ \mu M\ s^{-1}$, $k_{out} = 0.2\ s^{-1}$, and $n = 8$. The case without mitochondria (B) is modelled by putting $k_{in} = 0$, $k_{out} = 0$ and changing the total concentration Ca_{tot} to the value of 80 μM (instead of 300 μM in A). Since the present contribution concerns the role of mitochondria, and just the frame of the model equations is presented, we do not list the other parameter values here. They are available from the authors on request. Inset in B: Amplification of part of the plot.

membrane. Accordingly, we can simplify Eq. (6) to give

$$J_{in} = k'_{in}\frac{Ca_{cyt}^{n}}{K_m^{n} + Ca_{cyt}^{n}},\quad k'_{in} = 2Fk_{in}\Delta\Psi^{(m)} \qquad (8a,b)$$

The slow release of Ca^{2+} out of the mitochondria through the Na^{+}/Ca^{2+} and H^{+}/Ca^{2+} exchangers (cf. [4,5,20,23,33]) is here modelled by a simple linear dependence on the mitochondrial Ca^{2+} concentration:

$$J_{out} = k_{out}Ca_m \qquad (9)$$

As these exchangers operate in an electroneutral way, their rates do not depend on the transmembrane potential.

3. Results

To study transitions between stable steady states and oscillations, we take the maximal ER channel conductivity, $\tilde{g}_{Ca}$, as a free parameter of the model. Changes in the value of this parameter correspond to changes in agonist stimulation of the cell. In Fig. 2 the bifurcation diagrams for this parameter are shown for the cases with and without mitochondria. In the latter case, the total concentrations of monovalent anions and cations are reduced according to the volume ratio ρ_m, because the concentrations are defined with respect to the aqueous cytosolic volume. In contrast, the diminution of the total calcium concentration Ca_{tot} in the absence of mitochondria indicated in the legend to Fig. 2 takes into account both the volume effect and the missing buffering capacity of mitochondrial Ca^{2+} buffers (expressed by the factor β_m). One can see in Fig. 2 that when mitochondria are included in the model, in the whole region of the free parameter $\tilde{g}_{Ca}$ corresponding to an oscillatory regime, the amplitude of spikes and their base level have almost constant values. This means that, as $\tilde{g}_{Ca}$ is gradually changed, the calcium oscillations suddenly appear, then they are maintained with a fairly constant amplitude, and then suddenly disappear. In contrast, in the case without mitochondria, the amplitude undergoes enormous changes upon variation of $\tilde{g}_{Ca}$, reaching values way above the peaks in the case with mitochondria. This is partly due to the fact that the Hopf bifurcation is subcritical in Fig. 2A (the amplitude grows beginning with finite values) and supercritical in Fig. 2B (the amplitude grows beginning with zero, see also Ref. [19,26]).

As opposed to amplitude, changes in frequency are relatively large upon variation in $\tilde{g}_{Ca}$. Fig. 3 shows calcium oscillations for two different values of $\tilde{g}_{Ca}$, again comparing the cases with and without mitochondria. One can see that when mitochondria are included, not only the amplitude but also the shape of the calcium spikes remains practically unchanged in spite of large changes in frequency. Furthermore, for a given value of $\tilde{g}_{Ca}$ a much larger period of oscillations is observed in the presence of mitochondria then in their absence. So, a further effect of mitochondria is a considerable shift of oscillation frequency to lower values. This is due to the additional time delay caused by the uptake of calcium into, and release from, the mitochondria.

Moreover, we have changed the Hill coefficient in Eq. (6), thus simulating different shapes of step-like kinetics of the Ca^{2+} uptake by mitochondria. If the coefficient is reduced from 8 to 6 or less, the variations in amplitude upon variation of $\tilde{g}_{Ca}$ become increasingly larger (not shown).

4. Discussion

Extending a model of Ca^{2+} oscillations in non-excitable cells presented earlier [19], we have outlined how the effect of mitochondria in shaping these oscillations can be elucidated by numerical simulation. We have shown that Ca^{2+} sequestration by mitochondria leads to reasonably constant amplitudes of Ca^{2+} oscillations over wide ranges of oscillation frequency, due to cutting the peaks at about the threshold level of fast Ca^{2+} uptake by mitochondria. It is worth noting that this uptake is fast enough to occur in the upstroke of each Ca^{2+} peak, which is in accordance with experimental observations [23]. The release of Ca^{2+} from the mitochondria through specific exchangers is slower, but still fast enough to proceed in each intermediate phase between two peaks in a way that allows a periodic behaviour.

While several of the cited references on mitochondria concern oocytes and hepatocytes, some oth-

ers concern excitable cells (neurons and cardiac cells). It can be supposed that the properties of mitochondria relevant for our study do not differ very much in different cell types.

The property of Ca^{2+} amplitudes to be nearly independent of oscillation frequency is likely to allow the cell to encode information almost exclusively in the frequency. Thus, our results appear to be of interest in the light of the intensely discussed feature of frequency encoding (cf. [11,24,28]). It is worth noting, however, that this feature should still be considered as a hypothesis. We do not wish to exclude completely that information is encoded in a complex way both in frequency and amplitude. At

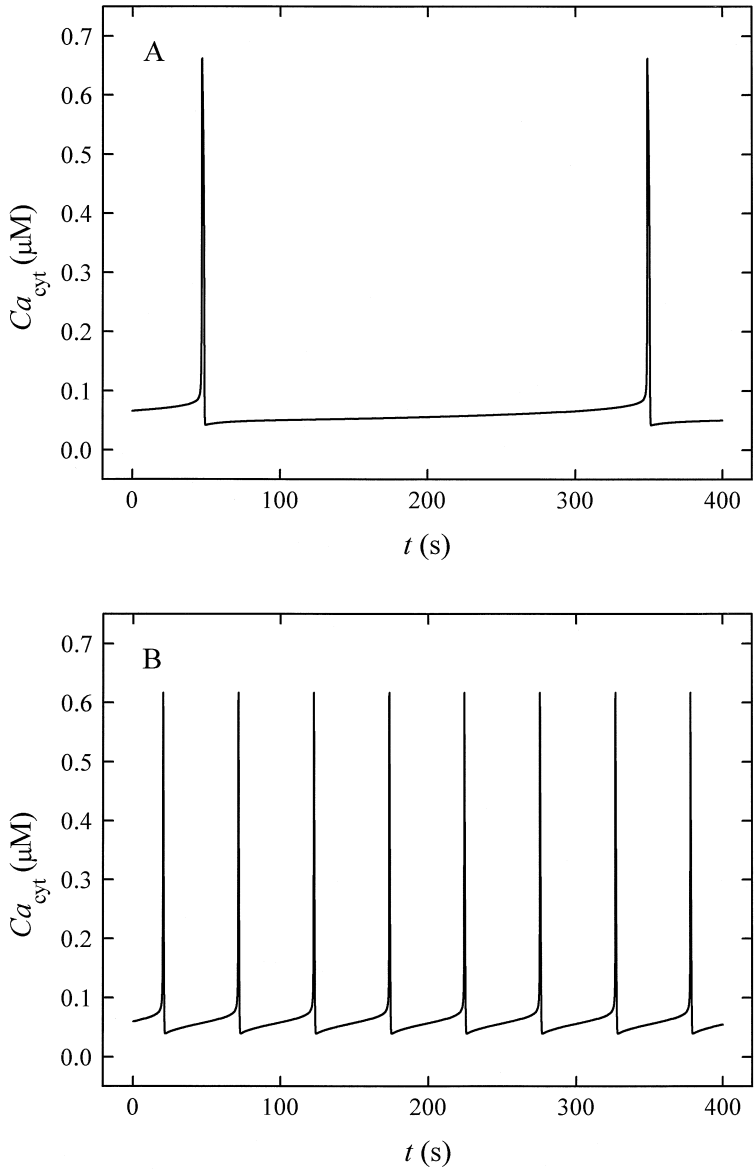

Fig. 3. Numerical simulation of calcium oscillations for the model with inclusion of mitochondria (A,B) and the model without mitochondria (C,D). Parameter values are as in Fig. 2, and $\tilde{g}_{Ca} = 190\ \mu S\ cm^{-2}$ (A,C), $290\ \mu S\ cm^{-2}$ (B,D). Inset in D: Amplification of part of the plot.

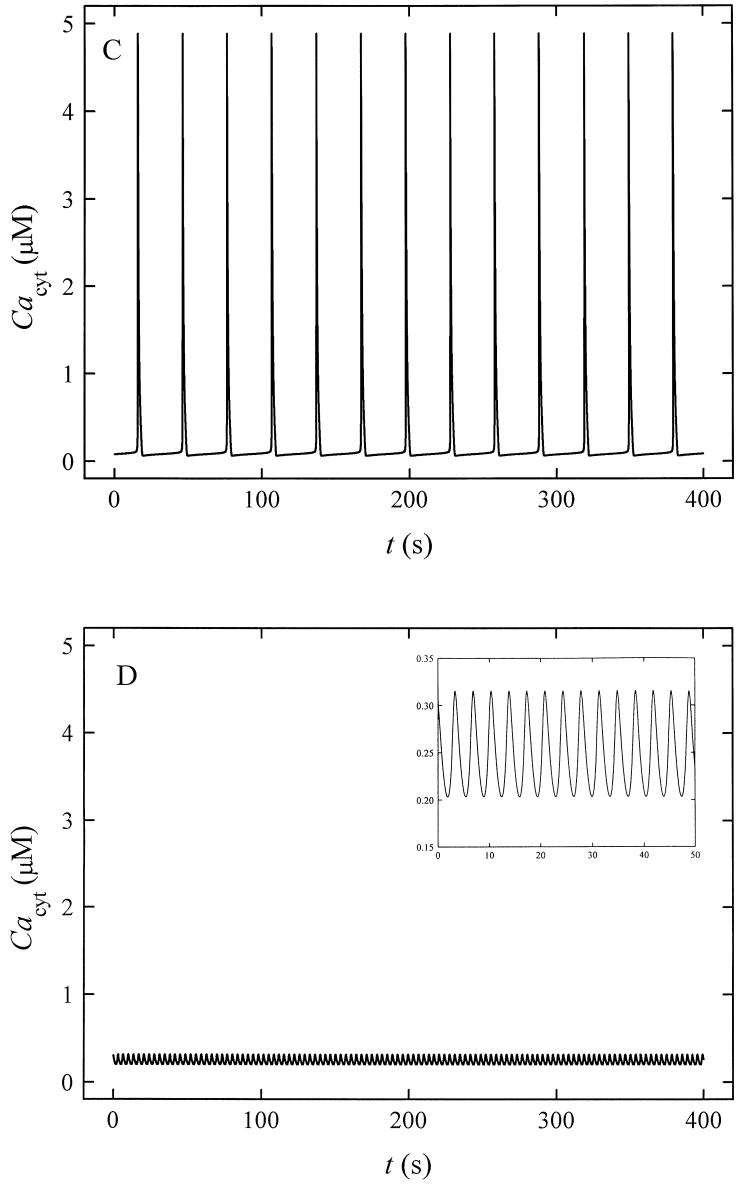

Fig. 3 (continued).

any event, it can be argued that a very efficient Ca^{2+} uptake by mitochondria at the given threshold plays an important role in regulating the amplitude of frequency encoded signals.

The effect of mitochondria on the amplitudes of Ca^{2+} oscillations consists not only in maintaining their constancy, but also in lowering the Ca^{2+} peaks. A physiological advantage of this is that the harmful effects of high Ca^{2+} levels, such as the risk of formation of insoluble Ca^{2+} phosphates (cf. [35]) is reduced.

We have devised our model to parallel, by theoretical studies, the growing body of experimental data on the role of mitochondria in the intracellular Ca^{2+} balance. It is conceived of as a basis for further theoretical work. In fact, the model is based on a

number of approximations which may appear to some extent as oversimplifications. For example, representing the uptake kinetics of mitochondria with respect to Ca^{2+} by a sigmoidal function with a sharp transition is only a rough approximation. However, it is in qualitative accordance with the experimentally established fact that Ca^{2+} is taken up by mitochondria at free cytosolic Ca^{2+} concentrations of more than about 0.5–1.0 μM (see above). On the other hand, the Hill kinetics used shows pronounced saturation, while Hoth et al. [31] reported that Ca^{2+} uptake by mitochondria did not readily saturate. However, taking into account an increased Ca^{2+} sequestration at higher Ca^{2+} levels would increase the effect of amplitude limitation even further.

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