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Mitochondria regulate the amplitude of simple and complex calcium oscillations

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Abstract

In a mathematical model for simple calcium oscillations [Biophys. Chem. 71 (1998) 125], it has been shown that mitochondria play an important role in the maintenance of constant amplitudes of cytosolic Ca^{2+} oscillations. Simple plausible rate laws for Ca^{2+} fluxes across the inner mitochondrial membrane have been used in this model. Here we show that it is possible to use the same rate laws as a plug-in element in other existing mathematical models and obtain the same effect on amplitude regulation. This result appears to be universal, independent of the type of model and the type of Ca^{2+} oscillations. We demonstrate this on two models for spiking Ca^{2+} oscillations [J. Biol. Chem. 266 (1991) 11068; Cell Calcium 14 (1993) 311] and on two recent models for bursting Ca^{2+} oscillations; one of them being a receptor-operated model [Biophys. J. 79 (2000) 1188] and the other one being a store-operated model [BioSystems 57 (2000) 75].

Keywords: Calcium oscillations; Mitochondria; Frequency encoding

1. Introduction

Oscillatory changes in free cytosolic calcium concentration are known as calcium oscillations. They play an important role in cell signalling. Many cellular processes, e.g. egg fertilization or cell secretion, are regulated by agonist evoked Ca^{2+} oscillations. In dependence on the type of agonist as well as on its concentration, different patterns and frequencies of Ca^{2+} oscillations appear. In some cells, time intervals between two Ca^{2+} spikes change from a few seconds to several minutes or even tens of minutes (for a review see [1]). However, in contrast to the large changes in frequency, the amplitude of Ca^{2+} oscillations remains nearly constant in many cell types. This likely allows the cell to encode information in the frequency of Ca^{2+} oscillations. This was already

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suggested in some early papers [2]. However, experimental evidence was lacking until recently. Now, it has been shown that altering the frequency of Ca^{2+} oscillations affects several cellular processes like gene expression [3,4] and enzymatic activities ([5,6], cf. [7–9]).

Because of these observations, almost all theoretical models for intracellular Ca^{2+} oscillations show frequency encoding explicitly or at least implicitly. However, in most cases the changes of frequency are also accompanied by large unphysiological changes in the maximum amplitude of free cytosolic Ca^{2+} concentration. This indicates that knowledge about the mechanism for amplitude regulation of Ca^{2+} oscillation is lacking and this has the consequence that the mechanism for frequency encoding remains poorly understood.

Meyer and Stryer [10] were the first to discuss the amplitude regulation of Ca^{2+} oscillations by inclusion of mitochondria as intracellular Ca^{2+} stores. Mitochondria are also considered in a model of Chay [11]; however, here, they are not considered to play a role in the amplitude regulation of Ca^{2+} oscillations. Both models assume the mitochondrial Ca^{2+} concentration to be constant. Recently, Marhl et al. [12] showed for the first time a role of mitochondria in the regulation of the amplitude during Ca^{2+} oscillations. In this study, mitochondria act as buffers effectively limiting the amplitude of Ca^{2+} oscillations and keeping the amplitude constant. This was shown with one specific model and the effect was not verified for any other model so far.

The lack of consideration of mitochondria in most models is astonishing, since experiments have shown that mitochondria play an important role in shaping Ca^{2+} oscillations [13–20]. Mitochondria are located in close proximity to the ER release sites [16,17,19–26] which makes them a good candidate for sequestration of the released Ca^{2+} from the ER. Indeed, in chromaffin cells it has been observed that mitochondria can absorb up to 80% of the entire Ca^{2+} released from the ER [27]. Mitochondrial uptake is very fast and becomes activated, if the average concentration of the free cytosolic calcium rises over a level of approximately 0.5 μM (cf. [28–32]). This kinetics is known as the rapid-mode of mitochondrial calcium uptake (RAM, for review see [33]) and its calcium concentration dependence appears to be step-wise. Ca^{2+} release from mitochondria is much slower and prolonged and taken together, the special kinetics of the uptake and release could play a crucial role in amplitude regulation of Ca^{2+} oscillations.

Based on experimental evidence, Marhl et al. [12] have shown an example of a mathematical model, in which mitochondria indeed support the maintenance of a nearly constant amplitude over the whole oscillatory range with a broad frequency spectrum. In the model, simple plausible rate laws for mitochondrial Ca^{2+} influx and efflux have been used with a surprisingly positive effect on the constancy of the amplitude. However, since the effect was only observed in one specific model and there is a need for finding universal effects in order to make theoretical results far-reaching, it is important to verify the observations with other models.

Using the same kinetic equations for Ca^{2+} exchange across the inner mitochondrial membrane as in Marhl et al. [12] we show that they can be inserted in a modular way in other mathematical models for spiking and bursting Ca^{2+} oscillations. Here they generate similar effects on oscillation amplitude as in the original paper. We consider very different types of mathematical models (receptor- and store-operated, one- and two-pool models) as well as different types of Ca^{2+} oscillations (spiking and bursting). Two early one-pool models [34,35] are chosen to demonstrate the effect of mitochondria on amplitude regulation of spiking Ca^{2+} oscillations. The two-pool model of Goldbeter et al. (cf. [35,36]) is also discussed. Furthermore, the amplitude regulation of bursting Ca^{2+} oscillations is demonstrated for a receptor-operated model [37] and for a store-operated model [38]. For each model two bifurcation diagrams are presented: the first one being calculated for the model without consideration of mitochondria and the second one corresponding to the model with the calcium concentration in the mitochondria as an additional variable.

2. General model scheme

A general scheme for signal transduction via Ca^{2+} is presented in Fig. 1. The system consists of a cell surrounded by extracellular medium with constant Ca^{2+} concentration. After the binding of an agonist to the extracellular side of a membrane-bound receptor molecule, the G_α subunit at the intracellular side of the receptor-coupled G protein is activated. The activation and inactivation dynamics of the G_α subunit are denoted as $J_{G_\alpha,act}$ and $J_{G_\alpha,inact}$, respectively. The activated G protein in turn stimulates a phospholipase C (PLC) the activation and inactivation dynamics of which are denoted as $J_{PLC,act}$ and $J_{PLC,inact}$, respectively. The PLC catalyses the hydrolysis of the membrane lipid phosphatidyl inositol-4,5-bisphosphate to form inositol-1,4,5-trisphosphate (IP_3), which binds to specific receptors at the membrane of, e.g. the endoplasmic reticulum (ER). This binding leads to the opening of Ca^{2+} channels and to a massive release of Ca^{2+} from the ER ($J_{ER,ch}$) which is much larger than the non-specific leak flux ($J_{ER,leak}$). In most models, this release is further enhanced by calcium, the so-called calcium-induced calcium release (CICR). Calcium is pumped back into the ER by ATP-ases ($J_{ER,pump}$). In addition to the ER, mitochondria are considered as important intracellular Ca^{2+} stores, characterized by a Ca^{2+} uptake ($J_{m,in}$) and a Ca^{2+} release ($J_{m,out}$). In the cytosol Ca^{2+} binding to proteins is taken into account. The kinetics for this process are specified by rate constants for binding (k_{on}) and dissociation (k_{off}). Calcium exchange across the plasma membrane is taken into account by a Ca^{2+} influx (J_{in}) and a Ca^{2+} efflux (J_{out}). The Ca^{2+} influx can be influenced by the activated G_α subunit, the IP3 concentration or by the emptying of intracellular stores, the so-called capacitive influx, whereas the Ca^{2+} efflux is catalyzed by ATP dependent calcium pumps.

We use the general model scheme, represented in Fig. 1 as a framework for studying existing models for intracellular Ca^{2+} oscillations [34,35,37,38]. All processes, which are included in a particular model, are part of this general model scheme. However, descriptions of some processes differ from one model to another and the respective mathematical terms also vary and will be specified in the later sections. For all models the same mitochondrial Ca^{2+} dynamics are used as a plug-in element. This corresponds to the one originally proposed by Marhl et al. [12]:

$$\frac{dCa_m}{dt} = J_{m,in} - J_{m,out}, \tag{1a}$$

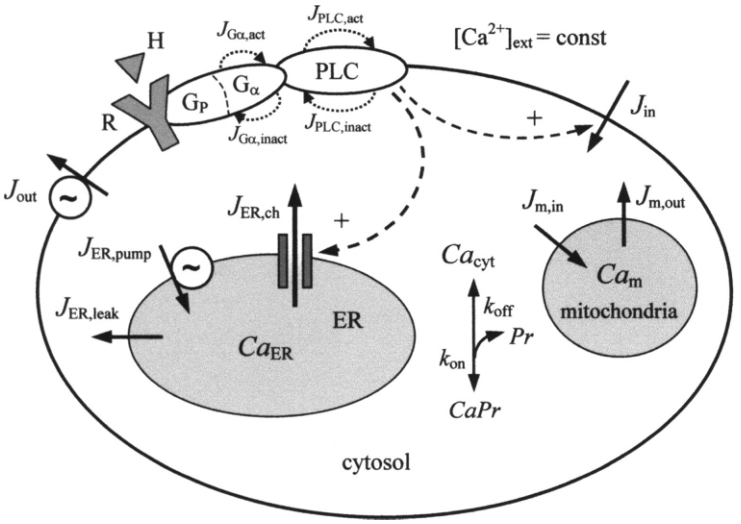

Fig. 1. Schematic presentation of the model systems.

where

$$J_{m,in} = k_{m,in} \frac{Ca_{cyt}^{n}}{K_{m}^{n} + Ca_{cyt}^{n}} \quad (1b)$$

and

$$J_{m,out} = k_{m,out} Ca_{m}. \quad (1c)$$

The Ca^{2+} influx into mitochondria ($J_{m,in}$) is modelled by step-like saturation kinetics [Eq. (1b)] with a large Hill coefficient n, of up to 8, in order to correspond to the RAM mechanism described above. In Fig. 2 the steepness of the Ca^{2+} influx kinetics is shown in dependence on the Hill coefficient n. The simple linear $J_{m,out}$ represents Ca^{2+} release through the Na^{+}/Ca^{2+} and H^{+}/Ca^{2+} exchangers.

3. Mathematical models and results

The model proposed by Somogyi and Stucki [34] is a store-operated model consisting of three main compartments: the cytosol, the ER, and the extracellular medium. The extracellular Ca^{2+} concentration is assumed to be constant, whereas changes of free Ca^{2+} concentration in the cytosol and in the ER are considered in the following way:

$$\frac{dCa_{cyt}}{dt} = J_{ER,ch} - J_{ER,pump} + J_{ER,leak} + J_{in} - J_{out}, \quad (2a)$$

$$\frac{dCa_{ER}}{dt} = J_{ER,pump} - J_{ER,leak} - J_{ER,ch}, \quad (2b)$$

where

$$J_{ER,ch} = k_{ER,ch} \frac{Ca_{cyt}^{4}}{K_{ch}^{4} + Ca_{cyt}^{4}} Ca_{ER}, \quad (2c)$$

$$J_{ER,pump} = k_{ER,pump} Ca_{cyt}, \quad (2d)$$

$$J_{ER,leak} = k_{ER,leak} Ca_{ER}, \quad (2e)$$

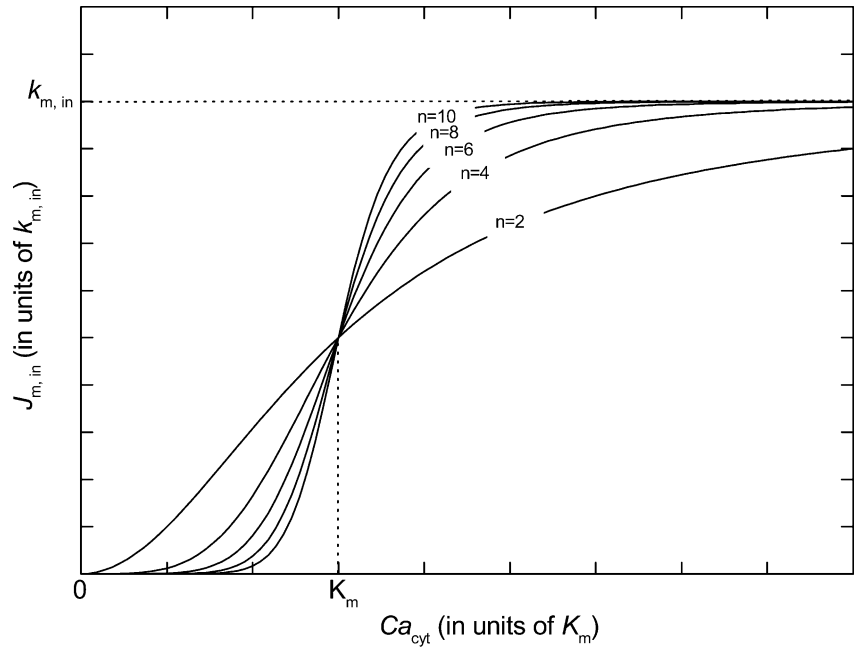

Fig. 2. The mitochondrial Ca^{2+} influx $J_{m,in}$ in dependence on the Hill coefficient n.

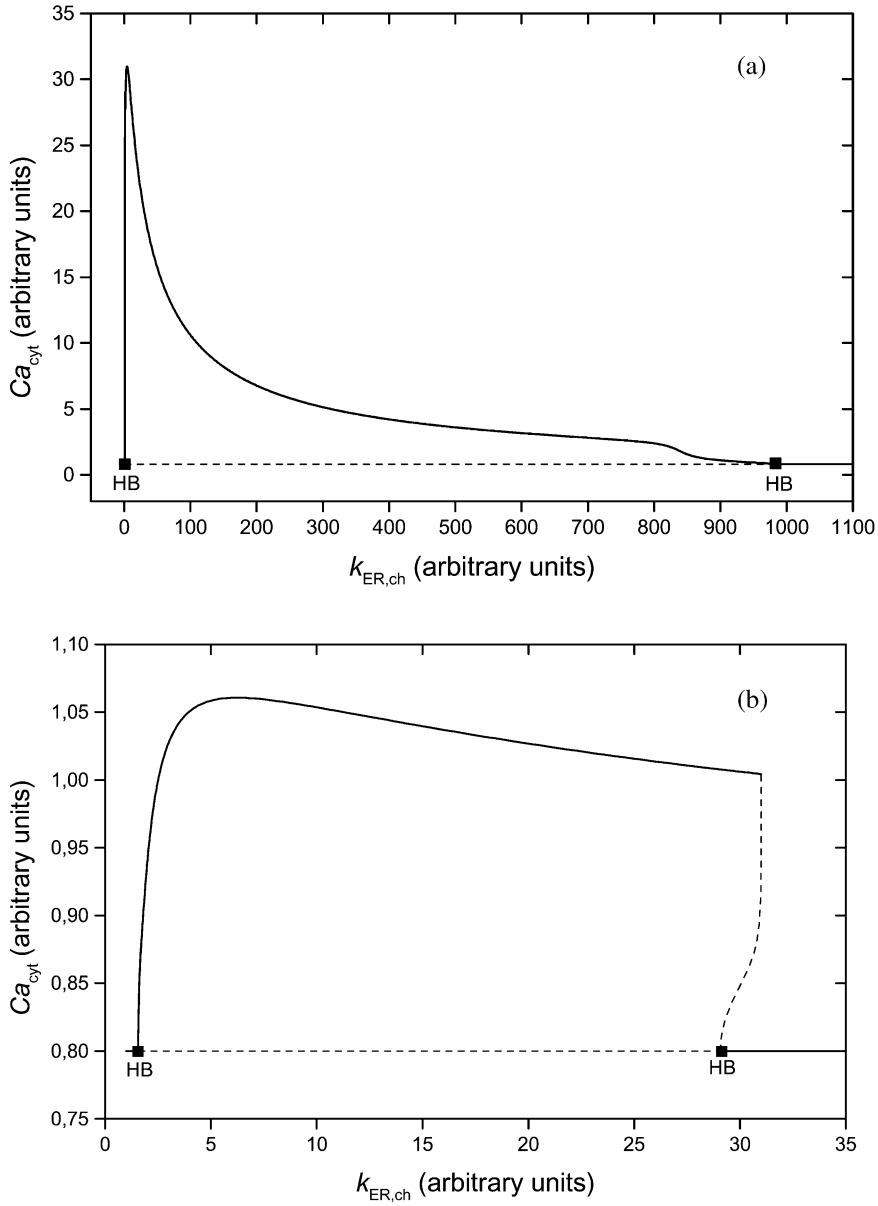

Fig. 3. Bifurcation diagram for the model [34] with $k_{ER,ch}$ as the bifurcation parameter. (a) Model without inclusion of mitochondria; (b) model with mitochondria. In addition to steady states (solid line for stable and dashed line for unstable steady states) the maximal amplitudes of stable (solid line) and unstable (dashed line) limit cycles are shown. The parameter values are $k_{in} = 0.8$, $k_{out} = 1$, $K_{ch} = 3$, $k_{ER,pump} = 2$, $k_{ER,leak} = 0.01$ for both cases (a and b), whereas in (b) the additional parameter values for mitochondrial Ca^{2+} fluxes are $k_{m,in} = 330$, $k_{m,out} = 0.5$, $K_m = 1.6$, $n = 8$.

$$J_{in} = k_{in}, \tag{2f}$$

$$J_{out} = k_{out} Ca_{cyt}. \tag{2g}$$

In this model, calcium induced calcium release is present ($J_{ER,ch}$) and a Hill coefficient of 4 is assumed for this process. Calcium transport through the calcium channels and by the leak flux is unidirectional. This assumption, however, is only valid, if the calcium concentration in the ER is always much higher than in the cytosol. This is not true under all physiological conditions [39]. All other terms are simple terms which describe calcium pumps and calcium in- and efflux from the extracellular space. The autocatalysis inherent to CICR is the mechanism responsible for oscillations here.

To study the oscillatory behavior of the model system, we take the rate constant of the Ca^{2+} release channel ($k_{ER,ch}$) in the ER membrane as bifurcation parameter. Changes in the value of this parameter correspond to changes in agonist stimulation of the cell. In Fig. 3a the bifurcation diagram for the original model is shown [Eqs. (2a), (2b), (2c), (2d), (2e), (2f) and (2g)]. The bifurcation diagram for the modified model, in which mitochondria are considered, is presented in Fig. 3b. Note that in this case, Eqs. (1a), (1b) and (1c) are added to the original set of model equations [Eqs. (2a), (2b), (2c), (2d), (2e), (2f) and (2g)]. Correspondingly, also Eq. (2a) has to be changed and it reads:

$$\frac{dCa_{cyt}}{dt} = J_{ER,ch} - J_{ER,pump} + J_{ER,leak} + J_{in} - J_{out} + J_{m,out} - J_{m,in}. \tag{3}$$

The main difference between the two bifurcation diagrams is that the amplitude of the oscillations in the model without mitochondria shows a strong dependency on the bifurcation parameter and decreases with increasing $k_{ER,ch}$, whereas the bifurcation diagram of the model which includes mitochondria shows an almost constant amplitude over the complete parameter range in which oscillatory behavior is displayed. Another major difference is that the amplitude is considerably smaller in the modified model. Also the oscillatory regime becomes smaller with the inclusion of mitochondria. However, this is only the case when using exactly the same set of model parameters in both cases (with and without mitochondria) and can be easily avoided by a slight change of only one parameter. For $K_{ch} = 7$, for example, the model with mitochondria nearly displays the same oscillatory regime compared to the model without mitochondria (data not shown). It is of special importance, that this change in K_{ch} does not affect the constancy and value of the amplitude in the complete parameter range of the oscillatory regime.

The second model examined is another one-pool model based on the CICR mechanism [35]. In addition to the processes described previously, a stimulus activated Ca^{2+} entry from extracellular medium is also considered. The model equations are:

$$\frac{dCa_{cyt}}{dt} = J_{ER,ch} - J_{ER,pump} + J_{ER,leak} + J_{in} - J_{out}, \tag{4a}$$

$$\frac{dCa_{ER}}{dt} = J_{ER,pump} - J_{ER,leak} - J_{ER,ch}, \tag{4b}$$

where

$$J_{ER,ch} = \beta k_{0,ch} \frac{Ca_{cyt}^4}{K_{ch,cyt}^4 + Ca_{cyt}^4} \frac{Ca_{ER}^2}{K_{ch,ER}^2 + Ca_{ER}^2}, \tag{4c}$$

$$J_{ER,pump} = k_{ER,pump} \frac{Ca_{cyt}^2}{K_{pump}^2 + Ca_{cyt}^2}, \tag{4d}$$

$$J_{ER,leak} = k_{ER,leak} Ca_{ER}, \tag{4e}$$

$$J_{in} = k_{0,in} + k_{\beta,in} \beta, \tag{4f}$$

$$J_{out} = k_{out} Ca_{cyt}. \tag{4g}$$

Similar to the previous model, calcium induced

calcium release is included with the same qualitative dependency on cytosolic calcium (Hill coefficient of 4). In the same term, a cooperative dependency on the calcium concentration in the ER is assumed. Again, calcium flux across the ER membrane (channel and leak) and the cell mem-

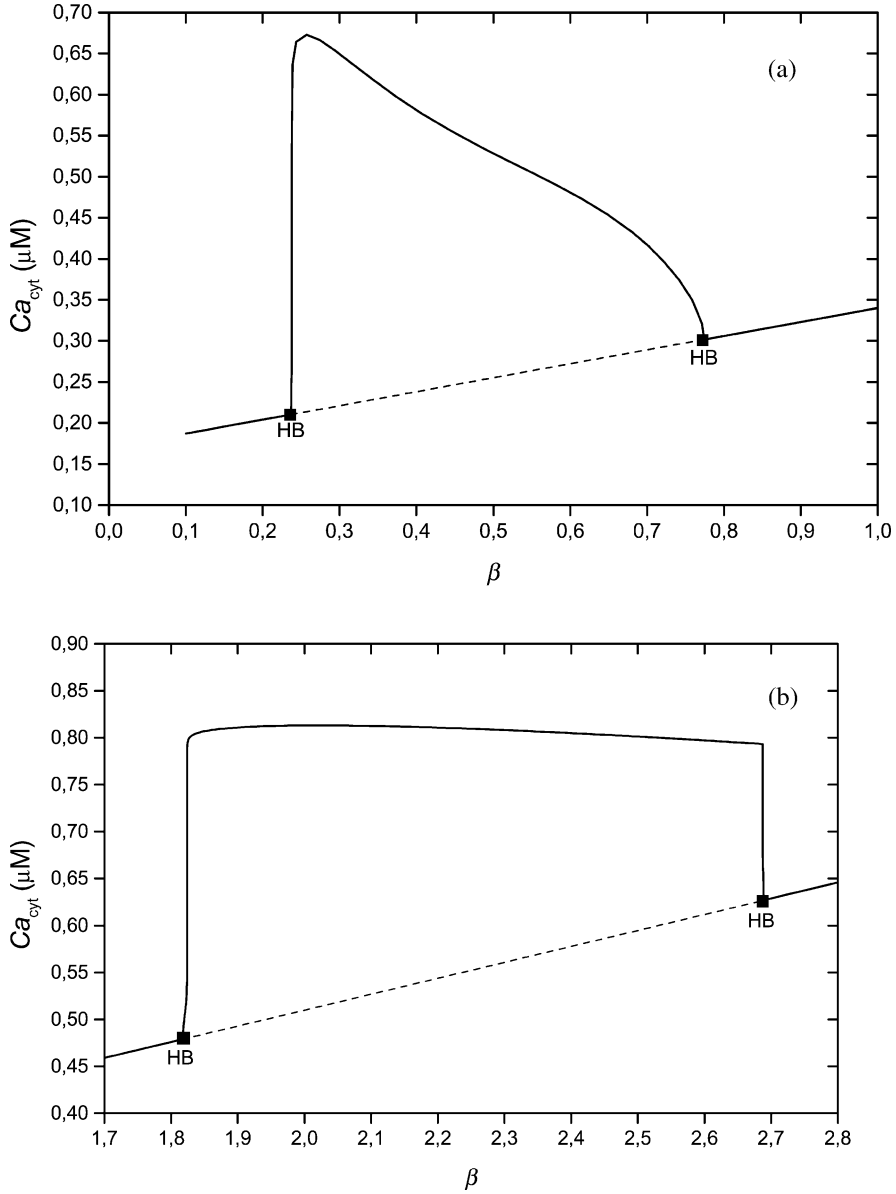

Fig. 4. Bifurcation diagram for the model [35] with β as the bifurcation parameter. (a) Model without inclusion of mitochondria; (b) model with mitochondria. In addition to steady states (solid line for stable and dashed line for unstable steady states) the maximal amplitudes of stable limit cycles are shown (solid line). The parameter values are $k_{0,in} = 1.7$ μM min^{-1}, $k_{\beta,in} = 1.7$ μM min^{-1}, $K_{out} = 10$ min^{-1}, $k_{0,ch} = 325$ μM min^{-1}, $K_{ch,ER} = 1$ μM, $K_{ER,pump} = 25$ μM min^{-1}, $k_{ER,leak} = 1$ min^{-1}, $K_{pump} = 0.5$ μM for both cases (a and b), whereas in (a) $K_{ch,cyt} = 0.45$ μM and in (b) $K_{ch,cyt} = 1.6$ μM, $k_{m,in} = 30\,000$ μM min^{-1}, $k_{m,out} = 50$ min^{-1}, $K_m = 1.5$ μM, $n = 8$.

brane (channel and leak) is unidirectional. The calcium pump across the ER membrane exhibits cooperativity for calcium with a Hill coefficient of 2 whereas the activity of the pump located in the cell membrane is linearly depending on calcium. Again, the autocatalysis of CICR is the origin of oscillations in this model.

The bifurcation diagrams for the original model and for the modified model with mitochondria included are presented in Fig. 4. The bifurcation parameter is β which represents the degree of stimulation by IP_3. In the model with mitochondria, Eqs. (1a), (1b) and (1c) are added to the original set of model equations [Eqs. (4a), (4b), (4c), (4d), (4e), (4f) and (4g)). Accordingly, Eq. (4a) is changed:

$$\frac{dCa_{cyt}}{dt} = J_{ER,ch} - J_{ER,pump} + J_{ER,leak} + J_{in} - J_{out} + J_{m,out} - J_{m,in}. \quad (5)$$

Again, the two bifurcation diagrams differ significantly in amplitude. The model without mitochondria displays decreasing amplitude whereas the model with mitochondria shows constant amplitude over almost the complete range of parameter space. The oscillatory regime in the latter model is again smaller if exactly the same set of parameters is used for both cases (with and without mitochondria). However, again it is sufficient to modify $K_{ch,cyt}$, e.g. to the value of 1.6 μM to obtain an even larger oscillatory regime compared to the model without mitochondria. Note, however, that the oscillatory regime is then shifted to higher values of β and further changes of parameter values are needed to avoid this.

In order to examine very different model types, we have also studied a two-pool model (cf. [35,36]). We used the same procedure as in the previous examples and the results were qualitatively the same, i.e. the amplitudes of Ca^{2+} oscillations became nearly constant over the whole range of the bifurcation parameter if additional equations [Eqs. (1a), (1b) and (1c)] were added to the original set of model equations. The reader can check this for parameter values $k_{m,in} = 500\ \mu M\ min^{-1}$, $k_{m,out} = 0.5\ min^{-1}$ and $K_m = 1\ \mu M$.

Apart from models for spiking calcium oscillations, we also studied models for simple and complex bursting calcium oscillations. One of these models, which was proposed by Kummer et al., takes the kinetics of the G_α subunit explicitly into account. This was based on the experimental observation that different types of stimuli result in qualitatively different types of calcium oscillations which can only be explained if there is a dynamic mechanism for differentiation at the site of the receptor. This again leads to different types of dynamics of calcium concentration. The corresponding equations are:

$$\frac{dCa_{cyt}}{dt} = J_{ER,ch} - J_{ER,pump} + J_{in} - J_{out}, \quad (6a)$$

$$\frac{dCa_{ER}}{dt} = J_{ER,pump} - J_{ER,ch}, \quad (6b)$$

$$\frac{dPLC}{dt} = J_{PLC,act} - J_{PLC,inact}, \quad (6c)$$

$$\frac{dG_\alpha}{dt} = J_{G_\alpha,act} - J_{G_\alpha,inact}, \quad (6d)$$

where

$$J_{ER,ch} = k_{10} Ca_{cyt} PLC \frac{Ca_{ER}}{K_{11} + Ca_{ER}}, \quad (6e)$$

$$J_{ER,pump} = k_{16} \frac{Ca_{cyt}}{K_{17} + Ca_{cyt}}, \quad (6f)$$

$$J_{in} = k_{12} PLC + k_{13} G_\alpha, \quad (6g)$$

$$J_{out} = k_{14} \frac{Ca_{cyt}}{K_{15} + Ca_{cyt}}, \quad (6h)$$

$$J_{PLC,act} = k_7 G_\alpha, \quad (6i)$$

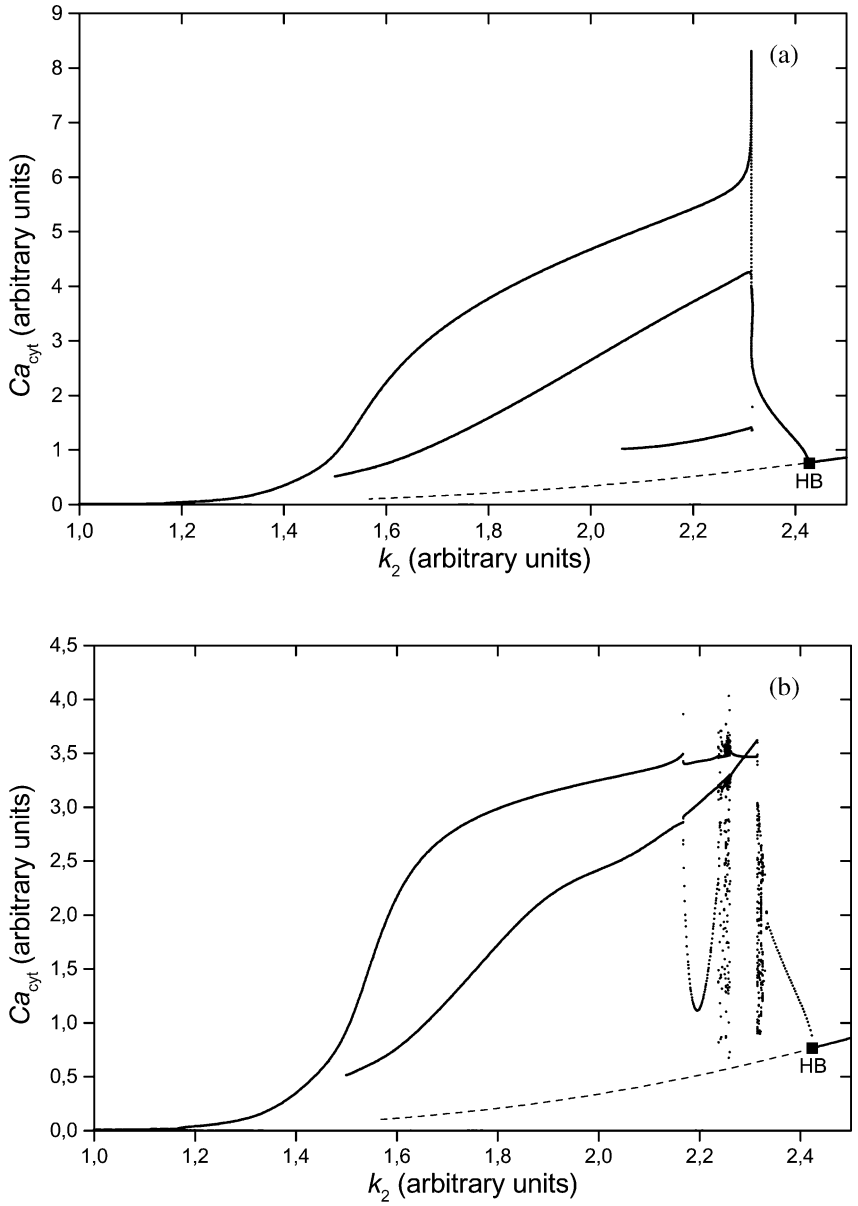

Fig. 5. Bifurcation diagram for the model [37] with k_2 as the bifurcation parameter. (a) Model without inclusion of mitochondria; (b) model with mitochondria. In addition to steady states (solid line for stable and dashed line for unstable steady states) the maximal amplitudes of stable limit cycles are shown (filled points). The parameter values are $k_1 = 0.01$, $k_3 = 0.64$, $K_4 = 0.09$, $k_5 = 4.88$, $K_6 = 1.18$, $k_7 = 2.08$,$k_8 = 32.24$, $K_9 = 29.09$, $k_{10} = 5$, $K_{11} = 3$, $k_{12} = 2.8$, $k_{13} = 13.4$, $k_{14} = 153$, $K_{15} = 0.16$, $k_{16} = 5.37$, $K_{17} = 0.05$ for both cases (a and b), whereas in (b) the additional parameters for mitochondria are $k_{m,in} = 80$, $k_{m,out} = 2.58$, $K_m = 3.5$, $n = 8$.

$$J_{\mathrm{PLC,inact}} = k_8 \frac{PLC}{K_9 + PLC}, \tag{6j}$$

$$J_{\mathrm{G_\alpha act}} = k_1 + k_2 G_\alpha, \tag{6k}$$

$$J_{\mathrm{G_\alpha inact}} = k_3 \mathrm{PLC} \frac{G_\alpha}{K_4 + G_\alpha} + k_5 \mathrm{Ca_{cyt}} \frac{G_\alpha}{K_6 + G_\alpha}. \tag{6l}$$

Calcium induced calcium release is present, but is described by first order dependency on calcium. However, it was shown that the model's behavior remains basically unchanged, if more complicated terms (e.g. Hill coefficients between 2 and 4) are considered [40]. PLC is activated by G_α with linear dependency. The model assumes a quasi steady state of IP_3 with respect to the concentration of activated PLC and therefore, does not consider IP_3 as an independent variable. Calcium flux across the cell membrane is unidirectional (due to the enormous concentration of calcium outside the cell). The same is true for calcium flux across the ER membrane. There is no leak flux across ER or cell membrane. There is positive feedback regulation of active G_α by itself and a small spontaneous activation of G_α. Two negative feedback loops of activated PLC and calcium on active G_α are considered and modelled by the enhanced inactivation. Calcium influx from extracellular space is considered to be stimulated by the stimulated receptor (receptor operated calcium channels) and by IP_3. Calcium pumps that pump Ca^{2+} across ER and cell membrane follow Michaelis Menten kinetics. In contrast to the previous model, here, the main responsible mechanism for oscillations is the autocatalysis in G_α and the two negative feedbacks on G_α.

In Fig. 5 the bifurcation diagrams of the original model and the modified model are presented. The bifurcation parameter is k_2 which corresponds to increasing agonist concentration. In the model with mitochondria, Eqs. (1a), (1b) and (1c) are added to the original set of model equations [Eqs. (6a), (6b), (6c), (6d), (6e), (6f), (6g), (6h), (6i), (6j), (6k) and (6l)]. Accordingly, Eq. (6a) is changed:

$$\frac{\mathrm{d}(Ca_{\mathrm{cyt}})}{\mathrm{d}t} = J_{\mathrm{ER,ch}} - J_{\mathrm{ER,pump}} + J_{\mathrm{in}} - J_{\mathrm{out}} + J_{\mathrm{m,out}} - J_{\mathrm{m,in}}. \tag{7}$$

The two bifurcation diagrams again differ in the amplitude. The model without mitochondria shows increasing amplitude with increasing agonist concentration whereas the variation in amplitude is significantly reduced in the model with mitochondria. The amplitude in the model with mitochondria is somewhat smaller than the maximum amplitude of the original model. Another major difference between the two models is that the bifurcation scenario differ.

In the last model examined [38], representing a store-operated model for bursting and complex Ca^{2+} oscillations, mitochondria are already included. For this model, it has been shown that mitochondria participate in the modulation of Ca^{2+} oscillations and in particular also in the generation of complex forms of Ca^{2+} oscillations like regular and chaotic bursting (see also [41]). Here, we are presenting the role of mitochondria for the regulation of the maximal amplitude of Ca^{2+} oscillations. Therefore, in this case, the corresponding model without mitochondria has to be constructed. We simply simulate this by setting the corresponding flux rate constants ($k_{\mathrm{m,in}}$ and $k_{\mathrm{m,out}}$) in the original model equal to zero. The following model equations represent this case:

$$\frac{\mathrm{d}Ca_{\mathrm{cyt}}}{\mathrm{d}t} = J_{\mathrm{ER,ch}} - J_{\mathrm{ER,pump}} + J_{\mathrm{ER,leak}} + J_{\mathrm{CaPr}} - J_{\mathrm{Pr}}, \tag{8a}$$

$$\frac{\mathrm{d}Ca_{\mathrm{ER}}}{\mathrm{d}t} = \frac{\beta_{\mathrm{ER}}}{\rho_{\mathrm{ER}}} (J_{\mathrm{ER,pump}} - J_{\mathrm{ER,leak}} - J_{\mathrm{ER,ch}}), \tag{8b}$$

where

$$J_{\mathrm{ER,ch}} = k_{\mathrm{Er,ch}} \frac{Ca_{\mathrm{cyt}}^2}{K_{\mathrm{ch}}^2 + Ca_{\mathrm{cyt}}^2} (Ca_{\mathrm{ER}} - Ca_{\mathrm{cyt}}), \tag{8c}$$

$$J_{\mathrm{ER,pump}} = k_{\mathrm{Er,pump}} Ca_{\mathrm{cyt}}, \tag{8d}$$

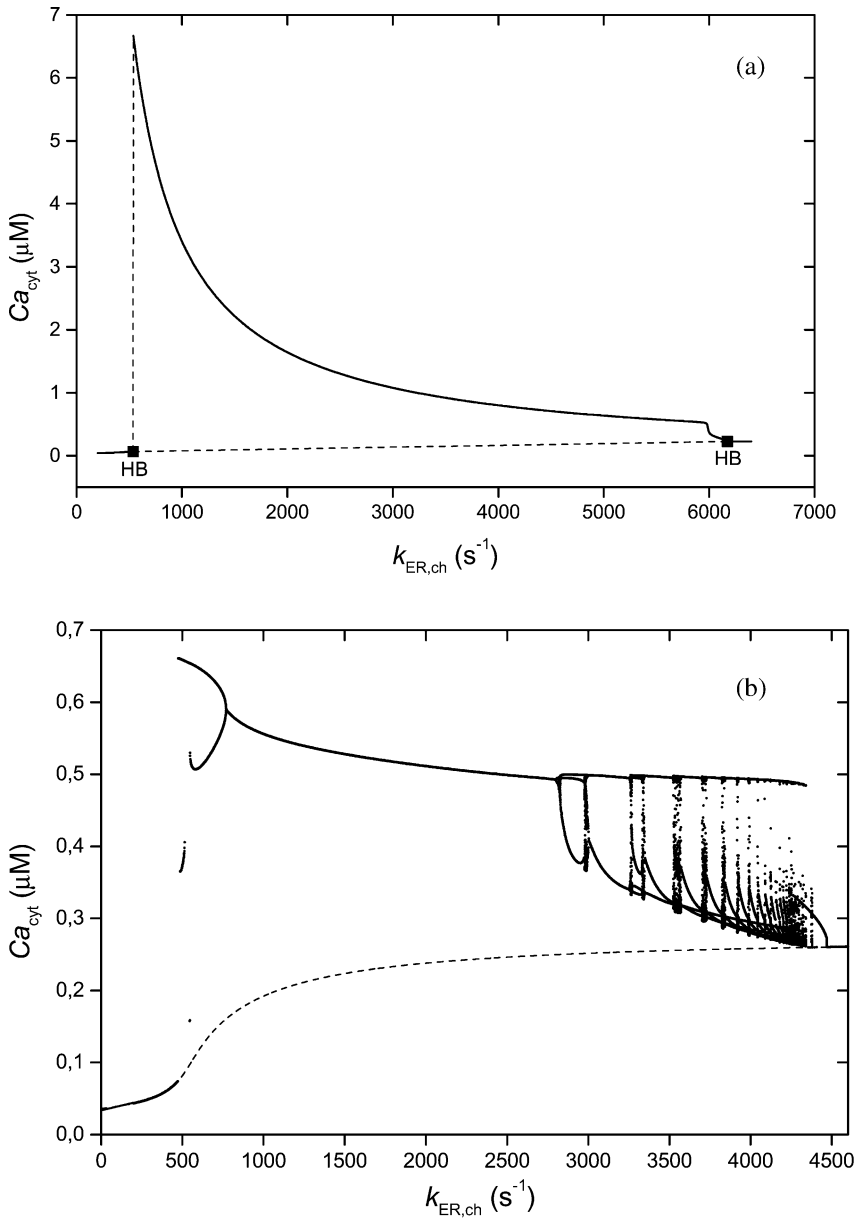

Fig. 6. Bifurcation diagram for the model [38] with $k_{ER,ch}$ as the bifurcation parameter. (a) Model without inclusion of mitochondria; (b) model with mitochondria. In addition to steady states (solid line for stable and dashed line for unstable steady states) the maximal amplitudes of stable (filled points) and unstable (dashed line) limit cycles are shown. The parameter values are $K_{ch} = 5$ μM, $k_{ER,pump} = 20$ s^{-1}, $k_{ER,leak} = 0.05$ s^{-1}, $\rho_{ER} = 0.01$, $\beta_{ER} = 0.0025$, $k_{-} = 0.01$ s^{-1}, $k_{+} = 0.1$ μM^{-1} s^{-1}, $Ca_{tot} = 90$ μM, $Pr_{tot} = 120$ μM for both cases (a and b), whereas in (b) the additional parameter values for mitochondrial Ca^{2+} handling are $\rho_m = 0.01$, $\beta_m = 0.0025$, $k_{m,in} = 75$ μM s^{-1}, $k_{m,out} = 0.1265625$ s^{-1}, $K_m = 0.8$ μM.

$$J_{\mathrm{ER,leak}} = k_{\mathrm{ER,leak}}(Ca_{\mathrm{Er}} - Ca_{\mathrm{cyt}}), \tag{8e}$$

$$J_{\mathrm{CaPr}} = k_{-}CaPr, \tag{8f}$$

$$J_{\mathrm{Pr}} = k_{+}Ca_{\mathrm{cyt}}Pr, \tag{8g}$$

$$Ca_{\mathrm{tot}} = Ca_{\mathrm{cyt}} + \frac{\rho_{\mathrm{ER}}}{\beta_{\mathrm{ER}}}Ca_{\mathrm{ER}} + CaPr, \tag{8h}$$

$$Pr_{\mathrm{tot}} = Pr + CaPr, \tag{8i}$$

This model takes calcium induced calcium release with a Hill coefficient of 2 into account. The leak flux of calcium across the membrane of the ER and the flux through the calcium channels are reversible. Calcium binding to proteins is also considered and calcium pumping across the ER membrane is linearly dependent on calcium. Similar to the first two models, the necessary strong non-linearity, which causes oscillations in this model, is CICR.

In Fig. 6 the bifurcation diagrams for the model with and without mitochondria are presented. The bifurcation parameter is $k_{\mathrm{ER,ch}}$ which should increase with increasing agonist concentration. In the model with mitochondria, Eqs. (1a), (1b) and (1c) are added to Eqs. (8a), (8b), (8c), (8d), (8e), (8f), (8h) and (8i). We should note that Eq. (1c) corresponds to a simplified definition of J_{out} taken from the original model (see Discussion in [38]]). Thus, the differential equation for Ca_{cyt} reads:

$$\frac{\mathrm{d}(Ca_{\mathrm{cyt}})}{\mathrm{d}t} = J_{\mathrm{Er,ch}} - J_{\mathrm{Er,pump}} + J_{\mathrm{Er,leak}} + J_{\mathrm{CAPr}} - J_{\mathrm{Pr}} + \frac{\rho_{\mathrm{m}}}{\beta_{\mathrm{m}}}(J_{\mathrm{m,out}} - J_{\mathrm{m,in}}), \tag{9}$$

and the total Ca^{2+} concentration is given by:

$$Ca_{\mathrm{tot}} = Ca_{\mathrm{cyt}} + \frac{\rho_{\mathrm{ER}}}{\beta_{\mathrm{ER}}}Ca_{\mathrm{ER}} + \frac{\rho_{\mathrm{m}}}{\beta_{\mathrm{m}}}Ca_{\mathrm{m}} + CaPr. \tag{10}$$

In this case the bifurcation diagram without mitochondria qualitatively resembles the original bifurcation diagram of Somogyi and Stucki [34]. It displays decreasing amplitude. The bifurcation diagram of the model with mitochondria shows

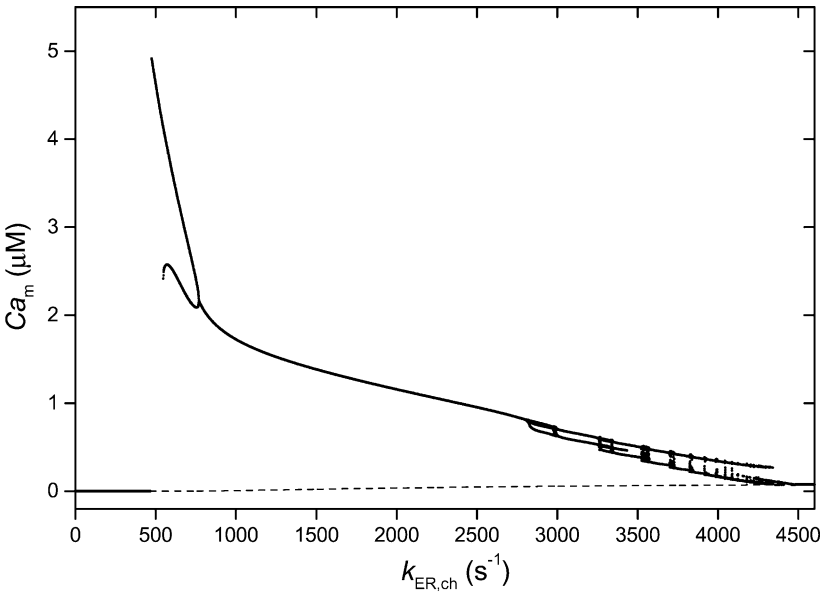

Fig. 7. Maximal concentrations of free mitochondrial calcium vs. bifurcational parameter $k_{\mathrm{ER,ch}}$ for the model [38]. Steady states are also shown (solid line for stable and dashed line for unstable steady states). Parameter values are the same as in Fig. 6b.

fairly constant amplitude. The amplitude in this case is much lower and the oscillatory regime is somewhat smaller.

All models studied here show fairly constant amplitude upon inclusion of mitochondria into the modelling of intracellular calcium oscillations. In order to understand the underlying mechanism for this phenomenon we also analyzed the calcium concentration in the mitochondria themselves. Again, these results are very similar and qualitatively identical for all models considered in the paper. Therefore, we only present calculations for the last model. In Fig. 7 the maximum concentration of free calcium in mitochondria is plotted vs. the bifurcation parameter used before. Comparing Fig. 7 with both Fig. 6a,b, it is obvious that mitochondria sequester larger amounts of calcium in those cases where the difference between the calcium concentrations of Fig. 6a,b is big. This means that mitochondria efficiently take up any excess calcium released from the ER, thereby regulating the free calcium concentration in the cytosol. It is noteworthy that mitochondria are able to accumulate very large amounts of calcium. Since the majority of mitochondrial calcium is bound to macromolecules, the total calcium concentration usually exceeds the free calcium concentration by orders of magnitude.

Moreover, to demonstrate the efficiency of mitochondrial Ca^{2+} uptake we changed the mitochondrial activity gradually from 0 to 100% (Fig. 8). This is done by varying a factor which is multiplied with all Ca^{2+} fluxes across the inner mitochondrial membrane from 0 to 1 ($k_{ER,ch} = 1000\ s^{-1}$). A sharp decrease of the cytosolic Ca^{2+} amplitude is evident. The final value is almost reached with only approximately 20% of the mitochondrial activity which underlines the efficiency of this mechanism.

4. Discussion

In a number of different cells, experiments show that in dependence on the agonist type and its concentration the frequency of Ca^{2+} oscillations changes much more significantly than the amplitude which remains almost constant [2]. We conclude from our computational results that the amplitude of Ca^{2+} oscillations is regulated by the

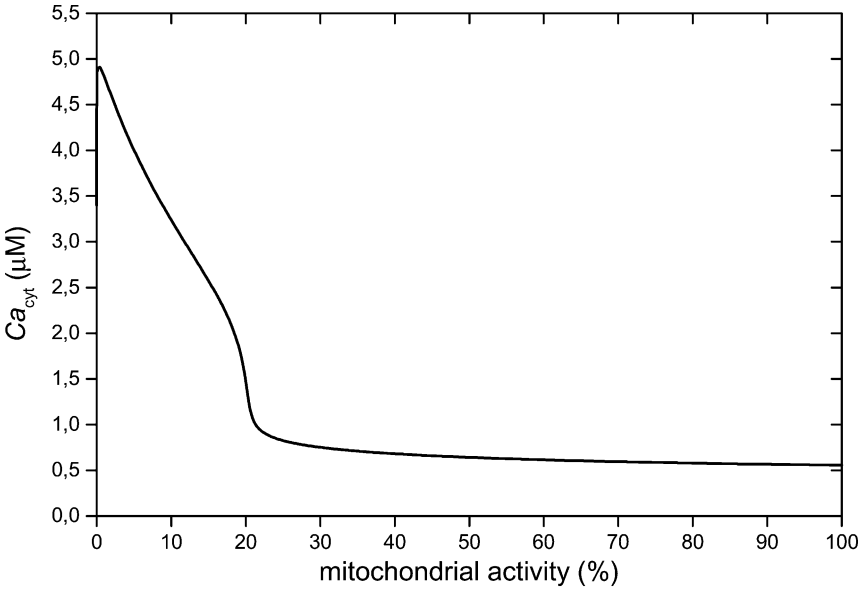

Fig. 8. Demonstration of the efficiency of mitochondrial Ca^{2+} uptake by changing the mitochondrial activity gradually from 0 to 100% for the model [38] at $k_{ER,ch} = 1000\ s^{-1}$.

sequestration of Ca^{2+} in the mitochondria, at least for those cells in which mitochondrial calcium uptake follows the RAM mechanism. This mechanism effectively cuts off Ca^{2+} peaks at about the threshold level of the Ca^{2+} uptake. Mitochondrial Ca^{2+} uptake corresponding to this mechanism involves fast step-like kinetics as shown in Fig. 2. This mechanism ensures that the calcium uptake is slow below a certain threshold and very fast above this threshold (cf. [28–32]). Moreover, mitochondria contain a lot of calcium binding molecules like phospholipids, Ca^{2+}-binding proteins, and phosphate compounds. Therefore calcium, once entering the mitochondrial matrix, is buffered very rapidly in a reversible manner with a very high effective Ca^{2+} binding ratio (cf. [31]). The Ca^{2+} binding ratio (bound/free) is estimated to be up to 5000 [27]. Under these circumstances it is evident that mitochondria can sequester very large amounts of calcium and some experiments even show that Ca^{2+} uptake by mitochondria is very hard to saturate [32]. This is in correspondence with our computational results which indicate that mitochondria can sequester a very large amount of excess cytosolic calcium (see Fig. 7). Consequently, the amplitude of calcium oscillations is regulated for the whole parameter range of agonist stimulation. This was demonstrated on various, very different models which gives a strong indication that mitochondria have an influence on the constancy of the amplitude of Ca^{2+} oscillations no matter which exact mechanism for calcium regulation is otherwise present in a specific cell type.

The models vary not only in the variables considered and in some qualitative terms, but also in the mechanisms which are responsible for the non-linear dynamics they exhibit. This is very important, since this means that they strongly differ in their essence. Thus, three models consider CICR to be the only responsible non-linearity whereas the model by Kummer et al. [37] features feedback loops on the production of G_α and an autocatalysis in this production as important components. Still, the inclusion of mitochondria results in similar effects on the model's behavior.

Due to the varying mechanisms the bifurcation scenario of the different models also vary. There are bifurcation diagrams which show an increasing amplitude with increasing agonist concentration whereas others show a decreasing amplitude. Still, adding mitochondria stabilizes the amplitude in all these cases. However, two of the models show a dramatic decrease in calcium amplitude with mitochondria [34,38] which is not observable when mitochondrial calcium uptake is inhibited in cells (e.g. [18]). Even though this shows that not all features of the models are highly realistic the result that the mitochondrial uptake characteristics stabilizes the amplitude of the calcium oscillations remains valid.

Constant amplitudes of Ca^{2+} oscillations are likely to allow the cell to encode information almost exclusively in the frequency. However, it is worth pointing out that other possibilities for encoding information, e.g. in a complex way both in the frequency and amplitude (cf. [42]) have been suggested as well. Furthermore, it was shown for some cell types that the amplitude of calcium oscillations does not stay constant for the complete range of stimulus concentration (e.g. [43]). In any case, experiments should be performed with the aim of studying those cells which feature the RAM mechanism compared to those which do not. According to our calculations, the first ones should show a clear tendency to maintain a constant amplitude of calcium oscillation.

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