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Spatio-temporal modelling explains the effect of reduced plasma membrane Ca^{2+} efflux on intracellular Ca^{2+} oscillations in hepatocytes ☆

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Abstract

In many non-excitable eukaryotic cells, including hepatocytes, Ca^{2+} oscillations play a key role in intra- and intercellular signalling, thus regulating many cellular processes from fertilisation to death. Therefore, understanding the mechanisms underlying these oscillations, and consequently understanding how they may be regulated, is of great interest. In this paper, we study the influence of reduced Ca^{2+} plasma membrane efflux on Ca^{2+} oscillations in hepatocytes. Our previous experiments with carboxyeosin show that a reduced plasma membrane Ca^{2+} efflux increases the frequency of Ca^{2+} oscillations, but does not affect the duration of individual transients. This phenomenon can be best explained by taking into account not only the temporal, but also the spatial dynamics underlying the generation of Ca^{2+} oscillations in the cell. Here we divide the cell into a grid of elements and treat the Ca^{2+} dynamics as a spatio-temporal phenomenon. By converting an existing temporal model into a spatio-temporal one, we obtain theoretical predictions that are in much better agreement with the experimental observations.

Keywords: Cellular signalling; Calcium oscillations; Hepatocytes; Spatio-temporal dynamics; Intracellular oscillations

1. Introduction

Many non-excitable eukaryotic cell types, including hepatocytes, respond to extracellular agonists, such as hormones or neurotransmitters, by generating oscillatory changes in concentration of free Ca^{2+} in the cytoplasmic space (Ca^{2+} oscillations). Ca^{2+} oscillations play a vital role in intra- and intercellular signalling, thus regulating many cellular processes such as egg fertilisation, cell division, and exocytosis (for a review see Berridge et al., 1998). Indeed, Ca^{2+} oscillations are essential for regulating cell function. For example, in hepatocytes, oscillations in mitochondrial Ca^{2+} at a frequency of $0.5\,min^{-1}$ cause a sustained activation of mitochondrial dehydrogenases, whereas a sustained rise in mitochondrial Ca^{2+} evokes only a transient activation of these enzymes (Hajnoczky et al., 1995). Furthermore, the frequency of Ca^{2+} oscillations modulates the activity of Ca^{2+}-sensitive enzymes such as CaM kinase II (Oancea and Meyer, 1998) and protein kinase C (De Koninck and Schulman, 1998), as well as influencing the efficiency and specificity of gene expression (Li et al., 1998; Dolmetsch et al., 1998). Therefore, understanding the mechanisms underlying the generation and control of these oscillations is of great interest.

Since the 1980s, when self-sustained calcium oscillations in non-excitable cells were first observed (Cuthbertson and Cobbold, 1985; Woods et al., 1986), numerous experimental studies have been performed and their findings published (examples of reviews are: Goldbeter, 1996; Berridge et al., 1999; Green et al., 2003). Additionally,

☆Paper dedicated to the memory of Reinhart Heinrich. One of the authors (M.M.) worked in Reinhart's group for a long time and published several papers and conference contributions with him.

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many theoretical studies have been conducted to explain the mechanism underlying Ca^{2+} oscillations as well as the phenomenon of Ca^{2+} waves spreading across coupled cells (examples of reviews are: Schuster et al., 2002; Falcke, 2004). In the early studies, the importance of intracellular stores was recognised, in particular the role of the endoplasmic reticulum (ER) (Meyer and Stryer, 1988; Goldbeter et al., 1990; Somogyi and Stucki, 1991; Dupont and Goldbeter, 1993; cf. Heinrich and Schuster, 1996; Goldbeter, 1996). The possible role of ER transmembrane electric potential was also investigated (Jafri et al., 1992; Jafri and Gillo, 1994; Marhl et al., 1997). Several mathematical models were proposed that included, in addition to the ER as the main intracellular Ca^{2+} store, other intracellular calcium stores such as Ca^{2+}-binding proteins (Jafri et al., 1992; Jafri and Gillo, 1994; Marhl et al., 1997, 1998a, b, 2000; Haberichter et al., 2001). In addition to the role of proteins as determinants of models for Ca^{2+} oscillations, the binding of Ca^{2+} to proteins became increasingly important in the understanding of fundamental processes in the downstream decoding of intracellular Ca^{2+} signals (Goldbeter et al., 1990; Dupont and Goldbeter, 1993; De Koninck and Schulman, 1998; Dupont et al., 2003; Schuster et al., 2005a, b; Marhl et al., 2005, 2006a, b; Marhl and Grubelnik, 2007). As another intracellular Ca^{2+} store, mitochondria have also been recognised to play an important role in oscillatory Ca^{2+} signalling, and as such have been incorporated into several mathematical models (Meyer and Stryer, 1988; Magnus and Keizer, 1997; Selivanov et al., 1998; Marhl et al., 1998a, 2000, 2006b; Grubelnik et al., 2001; Roux and Marhl, 2004; Roux et al., 2006).

In addition to the important role of Ca^{2+} release and sequestration in the intracellular stores, Ca^{2+} is also exchanged across the plasma membrane. In the 1990s, there was a vivid discussion about the importance of plasma membrane Ca^{2+} fluxes and their influence on the dynamics of intracellular Ca^{2+} signalling. Experimental and theoretical studies have shown that while, in many cell types, Ca^{2+} influx across the plasma membrane is essential for the maintenance of Ca^{2+} oscillations, some cell types can continue to generate Ca^{2+} oscillations in the absence of Ca^{2+} influx (as discussed by Shuttleworth, 1997). In hepatocytes, plasma membrane Ca^{2+} fluxes play an important role in maintaining (Jacob et al., 1988; Woods et al., 1990) and controlling Ca^{2+} oscillations. In particular, it has been shown that changes in plasma membrane Ca^{2+} fluxes modify the frequency of Ca^{2+} oscillations. For example, decreasing external Ca^{2+} (Woods et al., 1990) or inhibiting Ca^{2+} influx (Sanchez-Bueno et al., 1997) causes a decrease in Ca^{2+} oscillation frequency, whereas raising external Ca^{2+} causes an increase in oscillation frequency (Rooney et al., 1989a; Woods et al., 1990; Somogyi and Stucki, 1991). Stimulating net plasma membrane Ca^{2+} efflux decreases oscillation frequency (Green et al., 2002, 2003). In experiments applying glucagon-(19–29) (mini-glucagon) or carboxyeosin, inhibitors of hepatocyte Ca^{2+} efflux, to single rat hepatocytes microinjected with the bioluminescent Ca^{2+} indicator aequorin, it has been shown that both inhibitors enhance the frequency of Ca^{2+} oscillations induced by Ca^{2+}-mobilising agonists, but do not affect the duration of individual transients (Green et al., 1997, 2003).

The enhanced frequency of Ca^{2+} oscillations resulting from the inhibition of Ca^{2+} efflux can be explained by previous mathematical models for Ca^{2+} oscillations in non-excitable cells (for a review of the models see Schuster et al., 2002; Falcke, 2004). Importantly, however, none of these models are able to explain the experimental observation that while the frequency of Ca^{2+} oscillations increases, the duration of individual transients is not affected.

In this paper, we show that this problem can be solved by taking explicitly into account the spatial dynamics of Ca^{2+} inside the cell. We demonstrate this by modifying a previous model that originally considered only the temporal changes of Ca^{2+} (Marhl et al., 2000). In the modified model, the cell is partitioned into a grid of small elements, in which the dynamics of Ca^{2+} is governed by the complete set of original model equations that were previously applied for the temporal description of Ca^{2+} dynamics in the cell as a whole. This means that the elements incorporate complete cytoplasmic constituents, including intracellular organelles and proteins involved in the process of Ca^{2+} intracellular signalling. The elements are coupled via diffusion of Ca^{2+}. The experimental conditions of inhibited Ca^{2+} efflux (and simultaneously non-obviated Ca^{2+} influx into the cell) are simulated by introducing an additional net Ca^{2+} influx into the cell. Importantly, only 10% of the whole external cellular surface is amenable to the newly introduced influx. This accounts for the fact that the influx cannot affect the whole intracellular volume, and moreover, is in accordance with previous hypotheses indicating that Ca^{2+} fluxes might be modulated only within a localised subplasmalemmal region (Green et al., 2003).

When numerically investigating the newly proposed mathematical model, we focus on the mean concentration of Ca^{2+} in all cellular grid elements, which directly reflects the Ca^{2+}-dependent luminescence of the signal emitted by the entire aequorin-injected cell as it was measured in the performed experiments. We show that in the presence of an increased net Ca^{2+} influx into the cell, the spiking frequency of the observed mean-field signal also increases, whereas, most importantly, the width of the spikes remains constant. We show that due to the newly introduced extension of the mathematical model, taking explicitly into account the spatial dynamics of Ca^{2+} inside the cell, our theoretical results are in much better agreement with the experimental observations than those predicted by previous mono-compartmental models considering only the temporal dynamics of Ca^{2+} inside the cell as a whole.

The paper is organised as follows. First, materials and methods used in the experiments are presented, and next, the mathematical model is described. The main results are partitioned into two sub-sections, separately describing

experimental evidences and model predictions respectively, thus enabling direct comparisons of both. Finally, the good agreement of model predictions with the experimental results is discussed in view of possible generalisations, implying that presented results may also be valid for other mathematical models of Ca^{2+} oscillations.

2. Experimental set-up, materials and methods

Single hepatocytes were isolated from fed 150 to 250 g male Wistar rats by collagenase digestion as described previously (Woods et al., 1987) and microinjected with the Ca^{2+}-indicator aequorin, a Ca^{2+}-sensitive photoprotein. Microdialysis and microinjection of aequorin, data acquisition and analysis have been described previously (Dixon and Green, 2001). The experimental medium, Williams medium E (Gibco) gassed with CO_2/air (1:19), was superfused over single hepatocytes at 37 °C. Phenylephrine (Sigma) was added to this medium. 5-(and 6-)carboxyeosin diacetate, succinimidyl ester (carboxyeosin) and 5-(and 6-)carboxyfluorescein diacetate, succinimidyl ester (carboxyfluorescein) (Molecular Probes) were dissolved in DMSO and stored as 10 mM stocks at −20 °C. Aliquots were added to the experimental medium to give the required concentration (20 μM). Carboxyeosin is a potent inhibitor of the plasma membrane Ca^{2+} ATPase (PMCA) (Gatto and Milanick, 1993; Bassani et al., 1995) that inhibits Ca^{2+} efflux from rat hepatocytes (Green et al., 1997) and other cell types, including rat ventricular myocytes (Choi et al., 2000). It does not alter Ca^{2+} influx into rat ventricular myocytes (Choi et al., 2000). Carboxyfluorescein is a closely structurally-related compound to carboxyeosin, but, unlike carboxyeosin, it does not inhibit either human red cell PMCA (Gatto and Milanick, 1993) or hepatocyte Ca^{2+} efflux (Green et al., 1997).

3. Mathematical model

The mathematical model used to describe the temporal dynamics of Ca^{2+} inside each of the newly introduced grid elements of the cell is determined by the theoretical framework proposed by Marhl et al. (2000). As shown in Fig. 1(a), the model considers changes of free Ca^{2+} concentration in the cytoplasmic space (Ca_{cyt}), in the endoplasmic reticulum (Ca_{ER}), and in the mitochondria (Ca_m). Here the model equations are presented only briefly. For details we refer to the original work of Marhl et al. (2000). The free Ca^{2+} concentrations in the cytoplasmic space (Ca_{cyt}), in the ER (Ca_{ER}), and in mitochondria (Ca_m) are calculated by the following differential equations:

$$\frac{dCa_{cyt}}{dt} = J_{ER,ch} - J_{ER,pump} + J_{ER,leak} + J_{m,out} - J_{m,in} + J_{CaPr} - J_{Pr}, \quad (1)$$

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

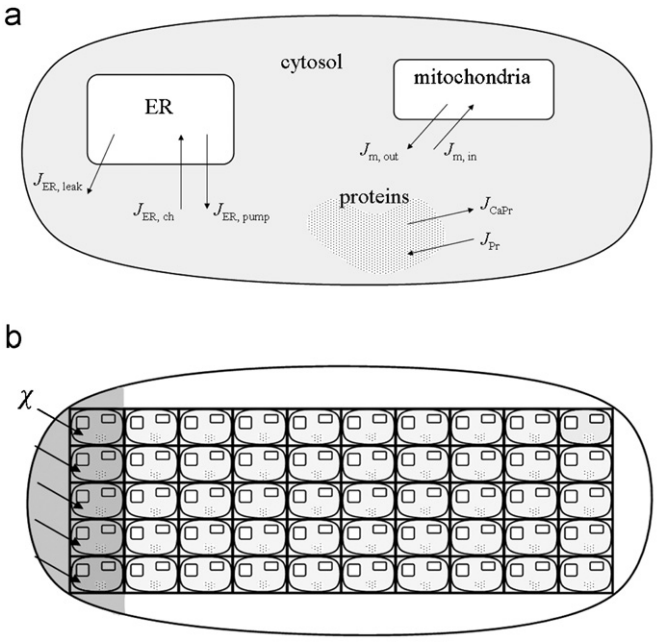

Fig. 1. Schematic presentation of the model representing the basic components and processes considered in each grid element of the cell (a) and the scheme for the spatial-extended model (b), where the shaded region represents the localised plasma membrane region that is affected by the additional net influx χ.

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

where

$$J_{ER,ch} = k_{ER,ch}\frac{Ca_{cyt}^2}{K_1^2 + Ca_{cyt}^2}(Ca_{ER} - Ca_{cyt}), \quad (4)$$

$$J_{ER,pump} = k_{ER,pump}Ca_{cyt}, \quad (5)$$

$$J_{ER,leak} = k_{ER,leak}(Ca_{ER} - Ca_{cyt}), \quad (6)$$

$$J_{Pr} = k_+ Ca_{cyt} Pr, \quad (7)$$

$$J_{CaPr} = k\_CaPr, \quad (8)$$

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

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

The concentrations of free and occupied protein binding sites of proteins (Pr and $CaPr$, respectively) are calculated by the conservation relations for the total protein binding sites (Pr_{tot}) and the total Ca^{2+} concentration in the cell (Ca_{tot}):

$$Pr_{tot} = Pr + CaPr, \quad (11)$$

$$Ca_{tot} = Ca_{cyt} + \frac{\rho_{ER}}{\beta_{ER}}Ca_{ER} + \frac{\rho_m}{\beta_m}Ca_m + CaPr. \quad (12)$$

Grid elements, which, when combined, form the whole cell, are arranged on a two-dimensional lattice of size $L_x \times L_y$ ($i \in [1,L_x]$, $j \in [1,L_y]$). Moreover, each element of the cell is diffusively coupled with its nearest neighbours via Ca_{cyt}, which is modelled by an additional diffusive flux of the form $D\nabla^2 Ca_{cyt}^{i,j}$ that is added to each differential equation describing changes of the free Ca^{2+}. The Laplacian $D\nabla^2 Ca_{cyt}^{i,j}$ is incorporated into the numerical scheme via a first-order numerical approximation as described by Barkley (1991) with no-flux boundary conditions. Thus, the described spatio-temporal model warrants that Ca^{2+} ions can be exchanged between nearest neighbour elements constituting the cell.

The experimental conditions of inhibited Ca^{2+} efflux (and allowed Ca^{2+} influx into the cell) are simulated by introducing an additional flux χ, representing a net flux of Ca^{2+} into the cell. The net influx χ is added as an additional term in the model equation describing changes of the free Ca^{2+} concentration. Due to the additional Ca^{2+} flux into the cell, the Ca^{2+} concentration in the cytoplasmic space increases. In accordance with the changes of the free Ca^{2+} concentration, the total concentration of Ca^{2+} in the cell also varies. Therefore, an additional differential equation describing changes of the total Ca^{2+} concentration, Ca_{tot}, is needed:

$$\frac{dCa_{tot}}{dt} = \chi. \tag{13}$$

It is reasonable to assume that the plasma membrane influx directly affects the Ca^{2+} concentration only in localised regions close to the plasma membrane (Green et al., 2003). Therefore, the introduced influx, equalling $\chi = 0.03\,\mu M\,s^{-1}$, is applied only to the 10% of the exterior cellular grid elements. The scheme of the spatially extended model is presented in Fig. 1(b). The parameter values used in all subsequent calculations are those we derived previously (Marhl et al., 2000) and are quoted in the caption of Fig. 3.

4. Results

4.1. Experimental results

As shown previously by Green et al. (1997), the application of 20 μM carboxyeosin to single rat hepatocytes responding to the α_1-adrenergic agonist phenylephrine by the generation of repetitive Ca^{2+} oscillations, resulted in a progressive increase in the frequency of the oscillations but no alteration in the duration of individual spikes (Fig. 2; representative of 15/15 hepatocytes). In 10/15 hepatocytes, the increase in frequency was followed by a gradual decrease in the amplitude of the oscillations. The extent of the decrease in amplitude varied between cells; after 10 min exposure to carboxyeosin, the mean percent decrease in amplitude in this group of 10 cells was $17 \pm 4\%$. As a control, 20 μM carboxyfluorescein had no effect on phenylephrine-induced oscillations (9/9 cells; results not shown). Application of 20 μM carboxyeosin alone (in the absence of Ca^{2+}-mobilising agonists) for at least 15 min had no effect on $[Ca^{2+}]_i$ (3/3 cells; results not shown).

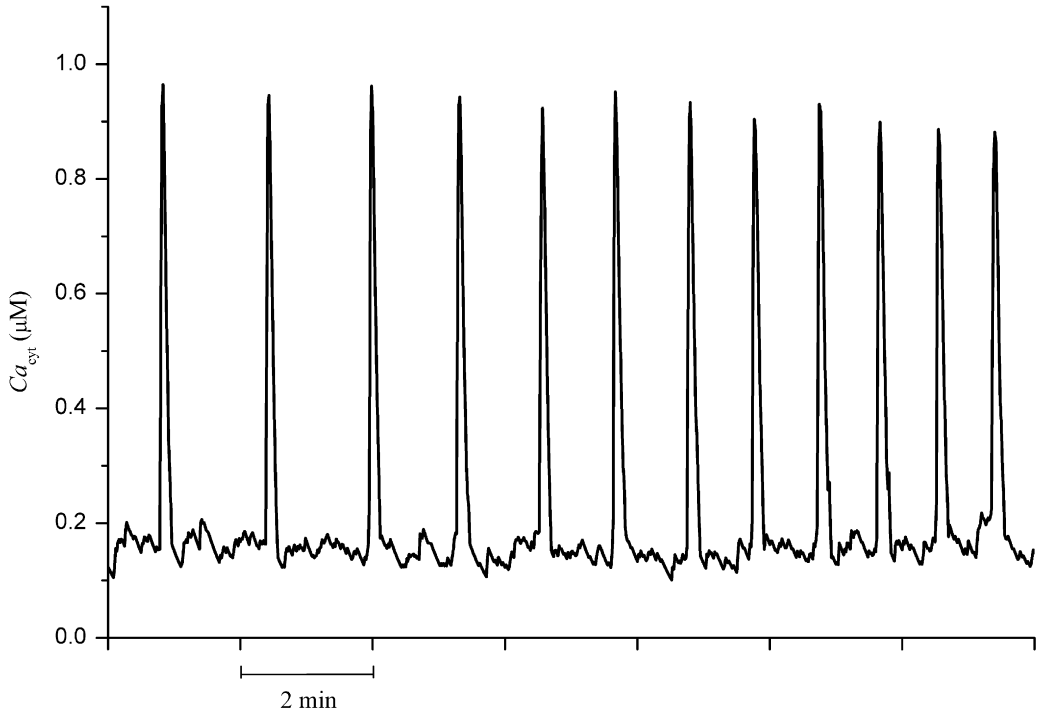

Fig. 2. A single rat hepatocyte responding to 5 μM phenylephrine by the generation of $[Ca^{2+}]_i$ oscillations was co-supplied with 20 μM carboxyeosin.

4.2. Model predictions

In the experiment, the concentration of Ca^{2+} is measured on the basis of the aequorin luminescence of the entire cell (see Fig. 2). Thus, in order to assure direct comparability of experimental results with the predictions of the mathematical model, we have to consider the collective dynamics of all the grid elements, which can be determined via a simple mean-field approximation $\overline{Ca}_{cyt} = (1/(L_x L_y))\sum_{i=1}^{L_x}\sum_{j=1}^{L_y} Ca_{cyt}^{i,j}$. Eqs. (1)–(13) are integrated numerically using the fourth-order Runge–Kutta procedure. The resulting temporal trace of the mean-field is presented in Fig. 3. By comparing the theoretical result with the experimental measurements presented in Fig. 2, it is evident that the two are in best agreement. In particular, experimental and theoretical results show a typical increase in the frequency of Ca^{2+} oscillations, a slight decrease in the amplitude of Ca^{2+} oscillations and, what should be particularly emphasised, a fairly constant width of individual Ca^{2+} spikes.

It should be emphasised that the dynamics of the average Ca^{2+} concentration in the cell presented in Fig. 3 is not the same as the temporal dynamics of Ca^{2+} inside each of the grid elements. To demonstrate this, Fig. 4 shows the mean-field (solid line) result together with the temporal trace of Ca_{cyt} for a single grid element adjacent to the plasma membrane (dashed line) where the influence of net Ca^{2+} influx on transients is most noticeable. Evidently, Fig. 4 reveals an important difference between the dynamics of the two signals. While the frequencies of both signals are the same (vary by the same amount in a given period of time), the form of the Ca^{2+} spikes is different. In particular, while the mean-field oscillations are characterised by a fairly constant spike width, the width of the Ca_{cyt} spikes inside the grid element adjacent to the plasma membrane increases steadily. We argue that this difference between the dynamics of $\overline{Ca}_{cyt}$ and Ca_{cyt} appears because the additive influx χ considerably increases the total concentration of Ca^{2+} only in the exterior parts of the cell, i.e. close to the cellular surface, whereas the mean-field concentration of Ca^{2+} in the cell remains nearly constant. In addition, due to the higher concentration in the exterior parts of the cell, the transients are triggered more frequently and thus result in an ever-increasing frequency of Ca^{2+} oscillations. Moreover, it is proposed that exterior parts of the cell act as pacemakers for the Ca^{2+} waves. The latter originate near the surface and then spread throughout the whole cell (Rooney et al., 1989b). When the wave is activated in the plasma membrane region, the inner parts are activated due to the diffusive coupling of the grid elements, even though their innate Ca^{2+} concentration is in fact too low to enable self-sustained firings. Evidently, the diffusion driven Ca^{2+} wave (starting at the exterior, i.e. close-to-surface parts of the cell) is responsible for the activation of all elements in the cell. It should be emphasised that even if the spike duration in the exterior parts of the cell increases, there is no mentionable effect on signals of inner grid elements or on the mean-field signal. In this manner the predicted theoretical signal is in perfect agreement with the experimental measurements. It should

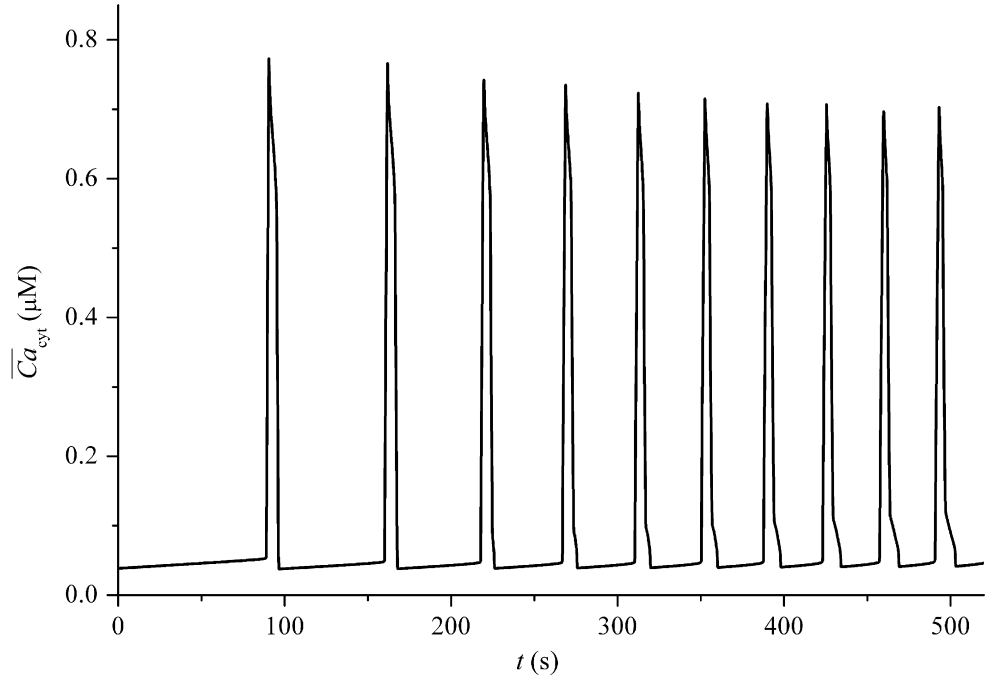

Fig. 3. The mean-field temporal dynamics of cytosolic Ca^{2+}. The system parameters employed are the same as used in Marhl et al. (2000): $Pr_{tot} = 120\,\mu M$, $\rho_{ER} = 0.01$, $\rho_m = 0.01$, $\beta_{ER} = 0.0025$, $\beta_m = 0.0025$, $k_{ER,ch} = 400\,s^{-1}$, $k_{ER,pump} = 20\,s^{-1}$, $k_{ER,leak} = 0.05\,s^{-1}$, $k_{m,in} = 800\,\mu M\,s^{-1}$, $k_{m,out} = 125\,s^{-1}$, $k_{mit} = 0.00625\,s^{-1}$, $k_+ = 0.1\,\mu M\,s^{-1}$, $k_- = 0.01\,s^{-1}$, $K_1 = 5\,\mu M$, $K_2 = 1.2\,\mu M$, $K_3 = 5\,\mu M$, $j_{io} = 0.03\,\mu M\,s^{-1}$, $D = 30\,s^{-1}$, $L_x = 100$, $L_y = 10$. Initial conditions: $Ca_{tot}(t=0) = 90\,\mu M$, $Ca_{cyt}(t=0) = 0.056\,\mu M$, $Ca_{ER}(t=0) = 1.98\,\mu M$, $Ca_m(t=0) = 1.39\,\mu M$.

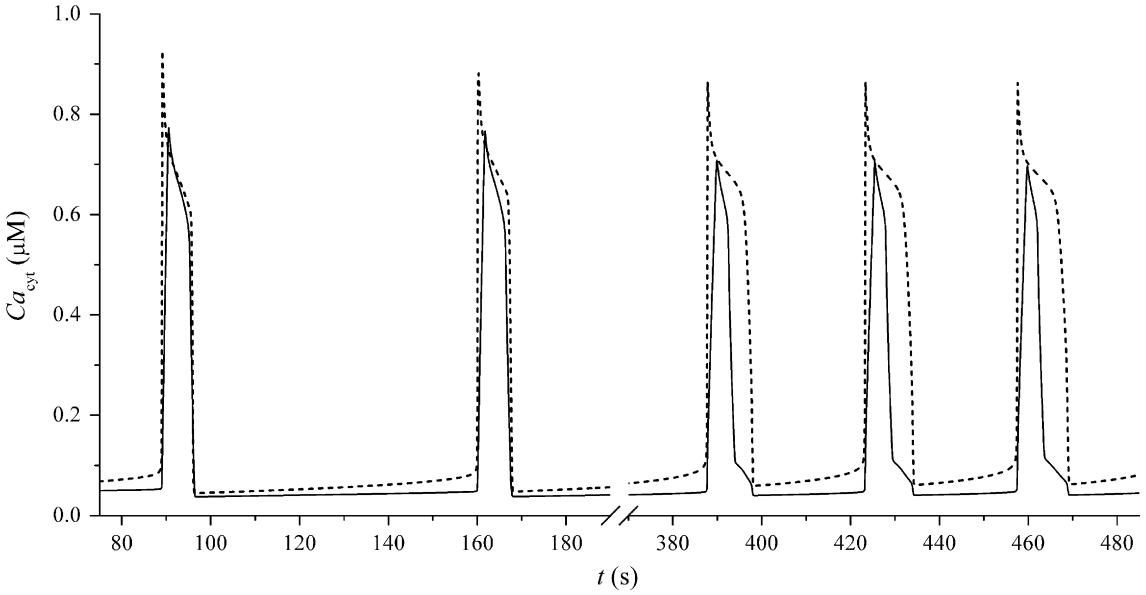

Fig. 4. Temporal dynamics of the average (mean-field) cytosolic concentration of Ca^{2+} in the cell (solid line) and that of a single exterior grid element located near the plasma membrane (dashed line). See text for details.

be noted that if the Ca^{2+} dynamics is modelled by the non-spatial model (the original model by Marhl et al., 2000), the results are practically, up to the effects of small diffusive fluxes (much smaller than the Ca^{2+} influx), the same as obtained for the grid element in Fig. 4, and hence do not fit the experimental data. Moreover, not only the model system under consideration, but most previous models, fail to explain the nearly constant width of the Ca^{2+} spikes. This comparison with other models will be discussed more precisely in the next section.

5. Discussion

An explanation of the effect of reduced plasma membrane Ca^{2+} efflux on intracellular Ca^{2+} oscillations in hepatocytes is given. The experiments in hepatocytes show that when net plasma membrane Ca^{2+} efflux is reduced, and hence the total concentration of Ca^{2+} in the cell increases (Green et al., 1997), the frequency of Ca^{2+} oscillations increases as well, but importantly, the width of the spikes remains constant.

The first part of these observations, evidencing that the frequency of Ca^{2+} oscillations increases alongside with the increasing total Ca^{2+} concentration, can be explained by the majority of (if not all) previous mathematical models of Ca^{2+} oscillations. However, most previous models fail to explain the nearly constant width of the Ca^{2+} spikes. In particular, this is true for all previous models in which the model system is considered to be a single well-stirred compartment with no differences in Ca^{2+} concentration (see Schuster et al., 2002; Falcke, 2004). If, in these models, a net Ca^{2+} flux into the cell is introduced, so that the total concentration of Ca^{2+} increases, both the frequency of Ca^{2+} oscillations and the width of the spikes increase. Here, we show that this is actually the situation in the outer 10% of the grid elements of our model where the Ca^{2+} net influx is directly applied (see dashed line in Fig. 4), whereas the whole mean-field dynamics, which is directly observed experimentally, behaves differently. It is noteworthy that this discrepancy has already been put forward by Green et al. (1997), for example for the mathematical model of Goldbeter et al. (1990). Now, by explicitly considering the spatial dynamics of Ca^{2+} inside the cell, we show that indeed only the frequency of Ca^{2+} oscillations increases with the increasing total concentration, whereas in accordance with the experimental results, the width of the spikes remains constant. We have verified the validity of the newly introduced extension from considering solely the temporal dynamics of Ca^{2+} inside the cell towards considering explicitly also the spatial dynamics for several other mathematical models (e.g. Somogyi and Stucki, 1991; Höfer, 1999; Marhl et al., 2000), and found that the present results are valid beyond particularities of individual systems describing the temporal dynamics of Ca^{2+} inside each cellular grid element.

As already hypothesised by Green et al. (2003), the experimentally observed increase in the frequency of Ca^{2+} oscillations, and the accompanying constant width of the spikes when a Ca^{2+} net flux into the cell is applied, demand the consideration of the spatial extensity and heterogeneity of the cell. In our model, the outer 10% of the cell, positioned adjacent to the plasma membrane as depicted in Fig. 1(b), act as Ca^{2+} wave initiation sites. As evidence in Fig. 4, the Ca^{2+} spikes are wider in this pacemaker-like subplasmalemmal region, while the mean-field Ca^{2+} dynamics in the cell does not show any considerable changes with respect to the width of the spikes, and thus the mean-field Ca^{2+} dynamics presented in Fig. 3 is fully in agreement with the experimental measurements depicted in Fig. 2.

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