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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