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Resonance effects determine the frequency of bursting Ca^{2+} oscillations

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Abstract

A mathematical model for bursting Ca^{2+} oscillations is analysed from a physical point of view as a system of internally coupled fast and slow oscillators. We show that the fast subsystem determines the interburst frequency, whereas altering the kinetics of the slow processes changes the duration of the bursting phase in a resonant manner. The resonance effect appears between two oscillatory Ca^{2+}-buffering mechanisms. This may be biologically important for a highly selective Ca^{2+} signal transduction from cell receptors to target proteins.
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1. Introduction

The phenomenon of bursting oscillations appears in many chemical and biological systems. In chemical systems, bursting oscillations were observed experimentally in Belousov–Zhabotinskii [1] and peroxidase-oxidase [2,3] reactions, for example, and also studied theoretically [4,5]. Furthermore, bursting oscillations characterise changes of free cytosolic Ca^{2+} in excitable cf. [6] as well as in non-excitable cells [7–9]. The oscillatory changes of free cytosolic Ca^{2+}, known as Ca^{2+} oscillations, regulate many cellular processes from egg fertilization to cell death [10] and have recently been intensely investigated both from experimental and theoretical point of view (for review see [11]). In particular, trying to explain the mechanism of experimentally observed bursting Ca^{2+} oscillations [7–9], several mathematical models for complex Ca^{2+} oscillations in excitable [6] as well as in non-excitable cells [12–14] were proposed.

It has been shown that for generation of bursting oscillations the system has to incorporate fast and slow processes (see e.g. [15]). Therefore, special methods were developed to analyse bursting, taking benefit of the interplay between fast and slow processes in bursting systems. One of the most prominent methods for analysing bursting oscillations is the so-called fast–slow burster analysis proposed by Rinzel [16]. By this method, variables of the slow subsystem are considered as bifurcation parameters. Hence, the original system is mathematically reduced to a lower dimensional system in which the stability analysis can be carried out more efficiently.

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For a particular model that describes intracellular Ca^{2+} oscillations in non-excitable cells [13], we study bursters as internally coupled fast and slow oscillators. By separating the model system into fast and slow subsystems [16], we examine the influences of the fast and the slow subsystem on the interburst frequency and the duration of the bursting phase. In particular, a resonance dependency between the frequency of bursting oscillations and the kinetics of the slow subsystem is pointed out. A possible biological importance of the results is discussed.

2. Mathematical model

We use a mathematical model for bursting Ca^{2+} oscillations, originally proposed by Marhl et al. [13], which consists of three basic model compartments, i.e., the cytosol, the endoplasmic reticulum (ER), and the mitochondria. The Ca^{2+} exchange is considered between the cytosol and the ER (J_{ch}, J_{pump}, J_{leak}), and between the cytosol and the mitochondria (J_{in}, J_{out}). Calcium buffering is taken into account in the cytosol (explicitly by the J_{CaPr} and J_{Pr}), in the ER (β_{er}), and in the mitochondria (β_m). The volume ratios between the active parts of the ER and the cytosol, and between the mitochondria and the cytosol are taken into account by ρ_{er} and ρ_m, respectively.

The concentrations of free Ca^{2+} in the cytosol (Ca_{cyt}), in the ER (Ca_{er}), and in the mitochondria (Ca_m) are given by the following equations:

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

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

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

where

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

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

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

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

$$J_{CaPr} = k_{-}CaPr, \quad (8)$$

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

$$J_{out} = k_{out}Ca_m. \quad (10)$$

Concentrations of the free (Pr) and the occupied ($CaPr$) Ca^{2+} protein binding sites in the cytosol are given by two conservation relations (see [13]):

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

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

The parameter values are given in figure captions. The most important parameters are briefly discussed in the text, whereas a complete presentation of their meaning and biological relevance is given in [13].

3. Results

Bursting Ca^{2+} oscillations in the examined mathematical model (Eqs. (1)–(12)) are presented in Fig. 1. During phase I, after the Ca^{2+} release from the ER, a rapid Ca^{2+} uptake by mitochondria and partially by the Ca^{2+} binding proteins takes place. In Phase II Ca^{2+} is slowly transferred from the mitochondria to the cytosolic proteins. Concomitantly, during phase II a fast exchange of Ca^{2+} between the Ca^{2+} stores and the cytosol appears, which characterises the bursting phase between two main spikes (see Fig. 1). After the bursting phase (phase II), Ca^{2+} is released from the cytosolic proteins and the concentration of Ca^{2+} in the ER starts to rise rapidly (phase III), which starts a new oscillatory cycle.

Time courses in Fig. 1 indicate that bursting results from the interplay between fast and slow processes in the model. During the bursting phase (phase II), fast changing variables Ca_{cyt} and Ca_{er}

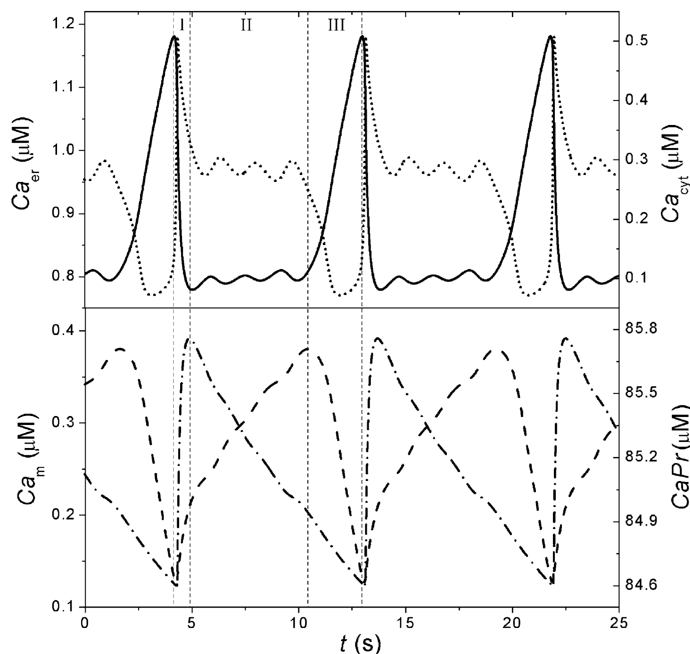

Fig. 1. Bursting Ca^{2+} oscillations for parameter values: $k_{leak} = 0.05\ s^{-1}$, $k_{pump} = 20.0\ s^{-1}$, $k_{in} = 300\ \mu M\ s^{-1}$, $k_{out} = 0.76\ s^{-1}$, $k_+ = 0.1\ \mu M^{-1}\ s^{-1}$, $k_- = 0.01\ s^{-1}$, $K_1 = 5.0\ \mu M$, $K_2 = 0.8\ \mu M$, $Ca_{tot} = 90\ \mu M$, $Pr_{tot} = 120\ \mu M$, $\rho_{er} = 0.01$, $\beta_{er} = 0.0025$, $\rho_m = 0.01$, $\beta_m = 0.0025$, and $k_{ch} = 3420\ s^{-1}$. Time courses of Ca_{er} (solid line), Ca_{cyt} (dotted line), Ca_m (dash-dotted line), and $CaPr$ (dashed line) are shown.

hint that the fast Ca^{2+} exchange between the ER and the cytosol determines the superimposed high-frequency oscillations, whereas the slow Ca^{2+} transfer from mitochondria to the cytosolic proteins is linked to the whole oscillation period. It can be shown mathematically that the high inter-burst frequency indeed depends exclusively on the kinetics of the fast Ca^{2+} exchange between the ER and the cytosol. We carry out the fast–slow burster analysis [16], taking Eqs. (1) and (2) as the fast subsystem, and considering Ca_m as the bifurcation parameter (see also [17,18]). The bifurcation diagram of the fast subsystem and the projection of the trajectory of the complete system are shown in Fig. 2. During the bursting phase, the trajectory runs through a domain of stable foci, passes the supercritical Hopf bifurcation (HB) and enters into a domain of unstable foci. Calculations of complex conjugate eigen values of the stable/unstable foci (e.g., $\lambda_{1,2} = \pm 3.96i$ at the HB) show that the frequency of the corresponding oscillations in

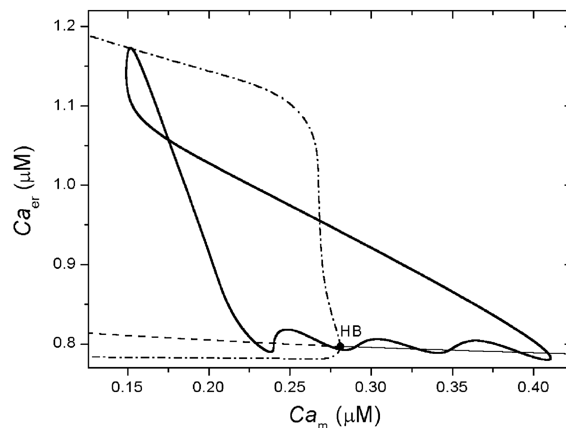

Fig. 2. Bifurcation diagram of the fast subsystem (only Ca_{er} is depicted), whereas the slow variable (Ca_m) is used as the bifurcation parameter. Solid (dashed) lines represent stable (unstable) steady states. Dash-dotted lines represent stable periodic solutions. Circle represents the supercritical Hopf bifurcation. The thick solid line represents the 2D projection of the trajectory in the whole phase space. Parameter values are the same as in Fig. 1.

the fast subsystem exactly matches the interburst frequency ($v = 0.63$ Hz) of the complete system. This means that the interburst frequency is indeed determined exclusively by the fast Ca^{2+} exchange between the ER and the cytosol.

To determine the role of the slow subsystem, we alter the kinetic constants that influence the slow Ca^{2+} transfer from the mitochondria to the cytosolic proteins. If we enlarge k_{out} from the reference value $k_{out} = 0.76\ s^{-1}$ (see Fig. 1) for 50%, 100%, and 150%, for example, the interburst frequency remains unchanged (i.e., $v = 0.63$ Hz), which even further confirms the above result showing that the interburst frequency is determined exclusively by the fast Ca^{2+} exchange between the ER and the cytosol. However, changing the parameter k_{out} influences the length of the bursting phase and herewith the main frequency of the bursting oscillations. To examine this dependency, we vary the parameter k_{out} and measure the period of Ca^{2+} oscillations. The results are presented in Fig. 3. We obtain a resonance dependency with the maximal duration of the bursting phase at $k_{out} \cong 1.58\ s^{-1}$. Furthermore, bursting is well expressed for values $0.23\ s^{-1} < k_{out} < 4.32\ s^{-1}$, whereas for values $k_{out} < 0.23\ s^{-1}$ and $k_{out} > 4.32\ s^{-1}$ bursting degenerates to spike-like oscillations.

To explain the obtained resonance dependency in Fig. 3, we study Ca^{2+} net fluxes for the ER ($J_{net,\,er}$), mitochondria ($J_{net,\,m}$) and the cytosolic proteins ($J_{net,\,Pr}$):

$$J_{net,\,er} = J_{pump} - J_{ch} - J_{leak}, \tag{13}$$

$$J_{net,\,m} = J_{out} - J_{in}, \tag{14}$$

$$J_{net,\,Pr} = J_{Pr} - J_{CaPr}, \tag{15}$$

We calculate the net fluxes (Eqs. (13)–(15)) for $k_{out} = 1.58\ s^{-1}$ and $k_{out} = 4.25\ s^{-1}$ (marked by dashed vertical lines in Fig. 3). The results are presented in Fig. 4. By comparing Fig. 4a and b, the crucial difference in time courses of the net fluxes can be observed during the bursting phase (phase II). When the net Ca^{2+} efflux from the mitochondria exactly matches the net Ca^{2+} uptake by the proteins (see Fig. 4a), the resonance peak in Fig. 3 takes place. By this fine-tuning of the mitochondrial kinetics with the kinetics of the cytosolic proteins, the slow transferring of Ca^{2+} from the

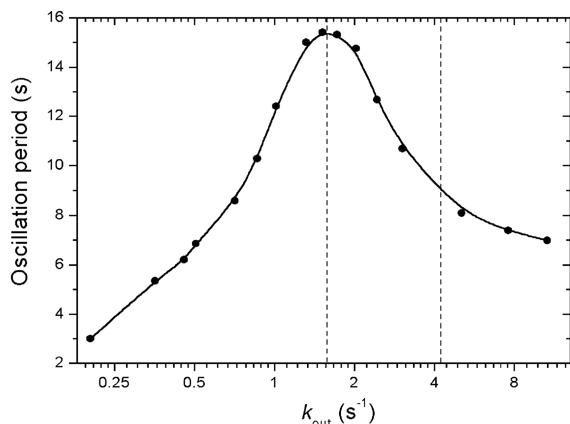

Fig. 3. Resonance dependency of the oscillation period of Ca^{2+} oscillations on the Ca^{2+} efflux rate from the mitochondria (k_{out}).

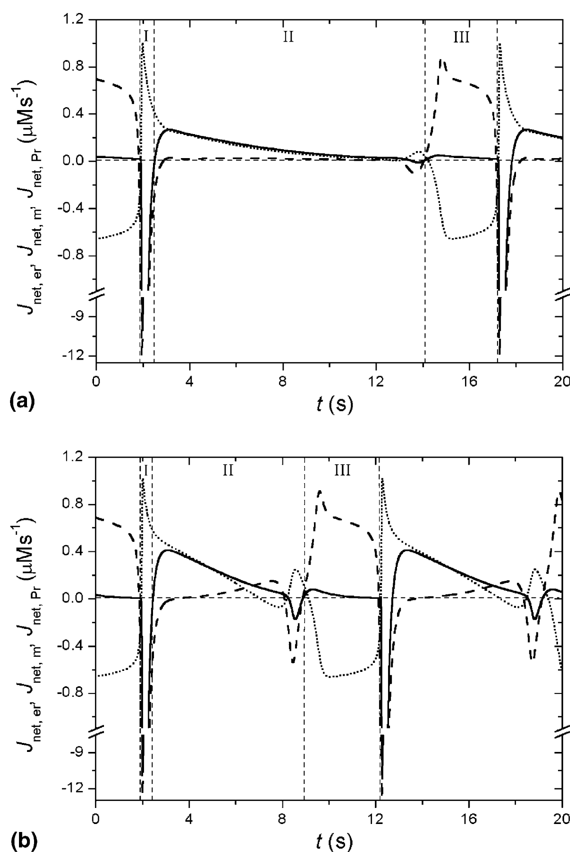

Fig. 4. Analysis of Ca^{2+} net fluxes for the ER (dashed line), the mitochondria (solid line), and Ca^{2+} binding proteins (dotted line) at: (a) $k_{out} = 1.58\ s^{-1}$ and (b) $k_{out} = 4.25\ s^{-1}$.

mitochondria to the proteins is maximized in time, whereas the transfer of Ca^{2+} to the ER, and consequently the emergence of a new main Ca^{2+} spikes, is maximally delayed. This results in the longest bursting phase and thus the longest oscillations period of bursting oscillation (see Fig. 3).

Contrary, in Fig. 4b the mitochondrial release is too fast for a direct Ca^{2+} uptake by the proteins. Therefore, already during phase II the ER starts to take up the Ca^{2+} that the proteins were unable to bind. This facilitates the emergence of a new main spike and thus shortens the oscillation period. By even further increasing of k_{out}, the mitochondrial kinetics becomes too fast with respect to other processes, so that the interrelation of slow and fast processes essential for the emergence of bursting oscillations disappears. Consequently, spike-like oscillations, characterized by even smaller oscillation periods, come into existence.

Also for $k_{out} < 1.58\ s^{-1}$, the period of bursting Ca^{2+} oscillations becomes smaller. This appears because the Ca^{2+} efflux decreases at the constant Ca^{2+} influx. In consequence, more Ca^{2+} is buffered in the mitochondria and the amount of Ca^{2+} involved in the inter-compartmental exchange becomes smaller. Due to the smaller Ca^{2+} transfer from mitochondria to the cytosolic proteins, the transfer becomes faster and the period of Ca^{2+} oscillations decreases.

Taken together, the two oscillatory Ca^{2+}-buffering mechanisms, i.e., the mitochondria and the cytosolic proteins, determine the frequency of bursting oscillations. If they are in resonance, the period of bursting oscillations is maximised, whereas for both cases of uncorrelated kinetics, i.e., for faster or slower mitochondrial net efflux compared to the net Ca^{2+} uptake by the cytosolic proteins, the period is smaller.

4. Discussion

In this Letter, we investigated a mathematical model for bursting Ca^{2+} oscillations [13] from a physical point of view as a system of internally coupled fast and slow oscillators. Using the fast–slow burster analysis [16], we showed that the interburst frequency is determined by the fast subsystem, whereas the slow Ca^{2+} transfer from the mitochondria to the cytosolic proteins determines the period of bursting Ca^{2+} oscillations in a resonant manner. For the resonance effect, a fine-tuning of the net Ca^{2+} release from the mitochondria with the net Ca^{2+} uptake by the cytosolic proteins is necessary. If both oscillatory Ca^{2+}-buffering mechanisms are in resonance, the period of bursting oscillations and the amount of the transferred Ca^{2+} to the cytosolic proteins is maximised, whereas for faster or slower mitochondrial net efflux compared to the net Ca^{2+} uptake by the cytosolic proteins, the period and the amount of the transferred Ca^{2+} is smaller.

For living cells, resonance effects between two Ca^{2+}-buffering mechanisms could be important for a highly selective Ca^{2+} signal transduction from cell receptors to target proteins. Since the target proteins differ in their kinetic constants, only specific protein classes can resonantly response. Consequently, only to the specific classes of proteins, the amount of the transferred Ca^{2+} is enlarged and a higher stage of the protein phosphorylation can be achieved. It seems that temporal buffers, like mitochondria for example, may function as intermediate servers for the Ca^{2+} distribution to different classes of target proteins.

The real physiological role of the temporal buffers is poorly understood; however, it is well known that they exist. In many cells, like in chromaffin cells for example, it has been shown that after the Ca^{2+} release from the ER, the majority of the Ca^{2+} (up to 80% [19]) is first sequestered by mitochondria, and after that, the Ca^{2+} is slowly shifted to the cytosolic proteins. Also for skeletal muscle cells, it is known that Ca^{2+} released into the cytosol is first very rapidly sequestered by intracellular organelles and fast-binding domains of troponin C, and after a brief lag phase, the bound Ca^{2+} population shifts to the slow-binding protein parvalbumin [20].

Additional experimental studies will be necessary to verify the biological relevance of our results. In further studies, it would also be interesting to investigate the influences of resonance effects on bursting oscillations in other fields of research, where such resonance effects may also be of importance.

References

[1] F. Argoul, A. Arneodo, P. Richetti, in: G. Baier, M. Klein (Eds.), A Chaotic Hierarchy, World Scientific, Singapore, 1991, p. 79.
[2] L.F. Olsen, H. Degn, Nature 267 (1977) 177.
[3] B.D. Aguda, L.L.H. Frisch, L.F. Olsen, J. Am. Chem. Soc. 112 (1990) 6652.
[4] T.V. Bronnikova, M.W. Schaffer, L.F. Olsen, J. Phys. Chem. B 105 (2001) 310.
[5] M.W. Schaffer, T.V. Bronnikova, L.F. Olsen, J. Phys. Chem. B 105 (2001) 5331.
[6] T.R. Chay, Biol. Cybern. 75 (1996) 419.
[7] N.M. Woods, K.S.R. Cuthbertson, P.H. Cobbold, Nature 319 (1986) 600.
[8] A.K. Green, C.J. Dixon, A.G. MacLennan, P.H. Cobbold, M.J. Fisher, FEBS Lett. 322 (1993) 197.
[9] A.K. Green, P.H. Cobbold, C.J. Dixon, Biochem. J. 302 (1994) 949.
[10] M.J. Berridge, M.D. Bootman, P. Lipp, Nature 395 (1998) 645.
[11] S. Schuster, M. Marhl, T. Höfer, Eur. J. Biochem. 269 (2002) 1333.
[12] P. Shen, R. Larter, Cell Calcium 17 (1995) 225.
[13] M. Marhl, T. Haberichter, M. Brumen, R. Heinrich, BioSystems 57 (2000) 75.
[14] U. Kummer, L.F. Olsen, C.J. Dixon, A.K. Green, E. Bornberg-Bauer, G. Baier, Biophys. J. 79 (2000) 1188.
[15] A. Goldbeter, Biochemical Oscillations and Cellular Rhythms, Cambridge University Press, Cambridge, 1996.
[16] J. Rinzel, in: B.D. Sleeman, R.J. Jarvis (Eds.), Ordinary and Partial Differential Equations, Lecture Notes in Mathematics, Springer, Berlin, 1985, p. 304.
[17] T. Haberichter, M. Marhl, R. Heinrich, Biophys. Chem. 90 (2001) 17.
[18] M. Perc, M. Marhl, Chaos, Solitons and Fractals 18 (2003) 759.
[19] D.F. Babcock, B. Hille, Curr. Opin. Neurobiol. 8 (1998) 398.
[20] J.J. Falke, S.K. Drake, A.L. Hazard, O.B. Peersen, Q. Rev. Biophys. 27 (1994) 219.