Model of Intracellular Calcium Oscillations Activated by Inositol Trisphosphate

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Abstract. Transients or oscillations of intracellular free calcium concentration are general means for physiological intracellular signalling. Recently, it was shown that calcium ions modulate the $InsP_3$ receptor/releasing calcium channel of the endoplasmic reticulum in a concentration dependent manner. Calcium either enhances (at low concentrations) or decreases (at high concentrations) the permeability of the channel activated by $InsP_3$. A simple phenomenological model is proposed, based on these data. The model consists of two compartments. They are the cytoplasm and the endoplasmic reticulum with the calcium sequestering pump and the calcium release channel activated by $InsP_3$. Parameters of the model are related to the experimentally measurable quantities. A qualitative analysis has shown that the hypothesis, which includes calcium modulation of the calcium releasing channel activated by $InsP_3$, can explain all essential experimental findings. At least in cells with predominating calcium compartments with $InsP_3$ sensitivity, this type of channel is compatible with mechanisms which generate repetitive calcium transients.

Key words: Calcium oscillations — Calcium feedback — Inositol trisphosphate activated channel — Mathematical model — Qualitative analysis

Introduction

The initial phase of receptor-mediated calcium signalling, involving Ca release from intracellular stores by inositol 1,4,5- trisphosphate ($InsP_3$), is well characterized. $InsP_3$ is believed to act through a specific receptor that functions as an intracellular, ligand-gated Ca channel. This paper is focused on oscillating or spiking intracellular calcium responses in cells exposed to agonists acting at surface receptors. It seems that receptor-evoked intracellular calcium oscillations are based on cyclical $InsP_3$ evoked release of Ca from, and return of Ca to, an internal pool. Basal intracellular calcium is often remarkably stable. Sporadic rises of intracellular calcium can provide the signalling function without toxic effects.

Poledna (1991) developed a model of repetitive intracellular calcium transients. The model has been based on the dual effect of calcium to activate and to inactivate the calcium release channel. These processes are dependent on immediate calcium concentration at the channel receptor.

Recently, new data have appeared in this context. Calcium ions were shown to modulate the $InsP_3$ receptor/releasing channel in a concentration dependent manner. Calcium either enhances (at low concentrations) or decreases (at high concentrations) the permeability of the channel activated by $InsP_3$ (Bezprozvanny et al. 1991). This suggests that, at least in cells with predominating calcium compartments with $InsP_3$ sensitivity, this type of channel may be compatible with mechanisms that generate repetitive calcium transients.

The author has tried to reformulate the previous model to be able to describe the behavior of the $InsP_3$ receptor/releasing channel for conditions of calcium repetitive transients.

The proposed model gives a new form to the interpretation of the data reported by Bezprozvanny et al. (1991). It provides a much simpler description and interpretation for $InsP_3$ receptor/channel open probability at equilibrium.

Model and Results

Primarily, the calcium releasing channel is controlled by binding of $InsP_3$, which opens the channel. Permeability of the channel is further modulated by the receptor which binds calcium ions. There are either two receptors, one activating and the other inactivating, or only a single one which upon binding of calcium potentiates permeability and then slowly changes its conformation and subsequently inactivates the channel. The model is formulated for the first hypothesis, and the inactivation variable, w, is introduced. However, the second possibility can be expressed in a similar way. To obtain better discrimination for the modulation effect of calcium, binding of at least two calcium ions is supposed. Fast calcium binding to the receptor can be lumped into one reaction step and is described by

$$\frac{\mathrm{d}r}{\mathrm{d}t} = k_+ \cdot c^2 \cdot (1 - r) - k_- \cdot r \tag{1}$$

where k_+ and k_- are the forward and reverse calcium binding rate constants to the regulation site of the channel, and r is the ratio of the calcium occupied receptors out of the total number of receptors available on the open channels. For a steady state, when a fast binding rate is supposed

$$r_s = \frac{c^2}{c^2 + A^2} \quad \text{where} \quad A^2 = k_-/k_+ \tag{2}$$

Then, the channel permeability is

$$p_c = \frac{Q \cdot c^2}{c^2 + A^2} w + L \tag{3}$$

where w corresponds to channel inactivation, which is calcium dependent. Q is a maximal permeability, which is a function of cytoplasmic $InsP_3$ concentration. Leak permeability, L, is calcium independent but may depend on the channel activation by $InsP_3$.

There should also be a binding site for calcium ions with lower affinity, which can inactivate the channel. This binding site can be described by the following simple kinetic equation:

$$\frac{dw}{dt} = k_{i-}(1-w) - k_{i+}w \cdot c \tag{4}$$

with the steady state

$$w_s = \frac{K_i}{c + K_i} \quad \text{where} \quad K_i = k_{i-}/k_{i+} \tag{5}$$

The steady state of this equation can be used to derive the parameters of the calcium release channel activation and inactivation from *in vitro* experiments with the channel incorporated into artificial membranes. The overall steady state channel permeability is

$$p_s = \frac{Q \cdot c^2}{c^2 + A^2} w_s + L = \frac{Q \cdot K_i \cdot c^2}{(c^2 + A^2) \cdot (c + K_i)} + L \tag{6}$$

The data of Bezprozvanny et al. (1991) can be approximated by this steady state equation (Fig. 1). Already the assumption of two activating and one inactivating binding sites for calcium ions have provided quite a good fit.

Let calcium concentration in the reticulum be c_r and that in the cytoplasm c. When the ratio of effective volumes of the cytoplasm and the reticulum is δ (taking into account low affinity binding sites), then

$$c_r = C + (C - c) \cdot \delta \quad \text{and} \quad c_r - c = (1 + \delta) \cdot (C - c) \tag{7}$$

where C is the average free calcium concentration in a cell as a whole. The parameter δ can change over a wide range according to morphological data. However, δ is determined rather functionally than morphologically.

The calcium flow between the endoplasmic reticulum and the cytoplasmic compartments is

$$F = -P\frac{c^2}{c^2 + B^2} + \left\{ L + Q\frac{c^2}{c^2 + A^2} w \right\} \cdot (C - c) \cdot (1 + \delta) \tag{8}$$

where the first term represents the calcium pump with a maximum flow P. According to experimental data, cooperativity of calcium binding to the pump needs two ions for one cycle.

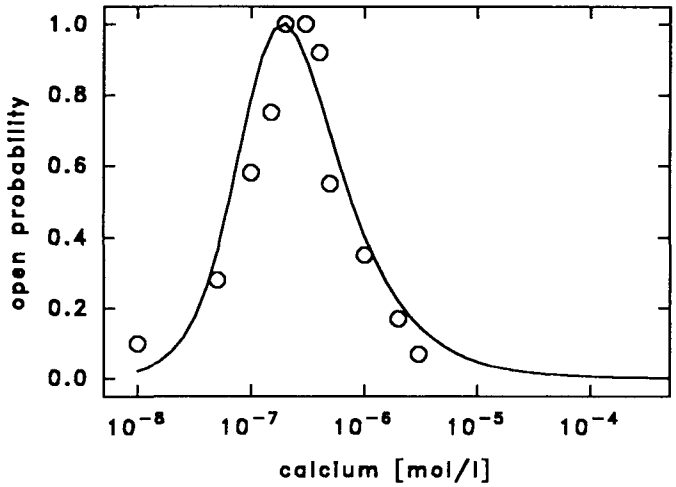

Figure 1. The data of Bezprozvanny et al. (1991) are approximated by the steady state permeability of the calcium releasing channel, which is described by equation (6). Already the assumption of two activating and one inactivating binding sites for calcium ions have provided quite a good fit, with parameters $A = 1.18 \times 10^{-7}$ mol.l^{-1}, and $K = 1.50 \times 10^{-7}$ mol.l^{-1}.

Changes of the cytoplasmic calcium concentration are determined only by the calcium flux, F. Therefore, the equation which describes the calcium concentration dynamics of the proposed model is given by

$$\frac{\mathrm{d}c}{\mathrm{d}t} = -P\frac{c^2}{c^2+B^2} + \{L + Q\frac{c^2}{c^2+A^2}w\} \cdot (C-c) \cdot (1+\delta) \tag{9}$$

It assumes that the only source or sink of calcium ions are intracellular compartments.

To be able to analyze this system of equations (4) and (9), and to be able to find characteristics of cytoplasmic calcium changes, we should simplify the system. We exclude time and represent the system in a state space, which spans the variables c and w (Fig. 2). These variables fully characterize the system. Cytoplasmic calcium concentration, c, gives the calcium concentration in the endoplasmic reticulum, c_r, via the parameters C and δ. Moreover, c describes, using A, activation of the channel, and it represents, through the parameters P and B, the calcium pump activity. Inactivation of the channel is expressed by the variable w. The time behavior of the system is reflected by a curve in the state space, the trajectory. The ratio of the right sides of equations (4) and (9) is a slope of tangent to the trajectory, and it defines evolution of the system, i.e. direction and speed in the state space. The structure of a field of tangents characterizes possible trajectories,

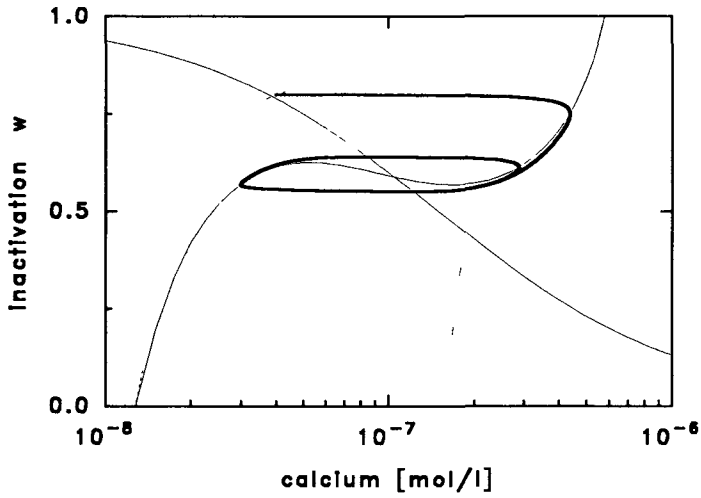

Figure 2. The representation of the system in the state space, which spans the variables c and w. The time behavior of the system reflects a curve in a state space, the trajectory. Important points of the state plane are represented by nullclines, which are the steady states of eqs. (8) and (6). The parameters used are in Table 1.

Table 1

Parameters

P	$[\text{mol.l}^{-1}.\text{s}^{-1}]$	2.50×10^{-6}
L	$[\text{s}^{-1}]$	2.50×10^{-3}
δ	[1]	19.0
A	$[\text{mol.l}^{-1}]$	1.18×10^{-7}
B	$[\text{mol.l}^{-1}]$	0.90×10^{-7}
K	$[\text{mol.l}^{-1}]$	1.50×10^{-7}
C	$[\text{mol.l}^{-1}]$	1.00×10^{-6}
k_{1+}	$[\text{mol}^{-1}.\text{l.s}^{-1}]$	$2.50 \times 10^{+5}$
Q	$[\text{s}^{-1}]$	3.00×10^{-1}

and thus the system behavior. Important points of the state plane are represented by nullclines, which are the steady states of eqs. (8) and (6). These nullclines are

$$w = \frac{1}{Q} \cdot \left\{ P \frac{c^2}{(1+\delta) \cdot (c^2 + B^2) \cdot (C - c)} - L \right\} \frac{c^2 + A^2}{c^2} \tag{10}$$

$$w = \frac{K}{c + K} \tag{11}$$

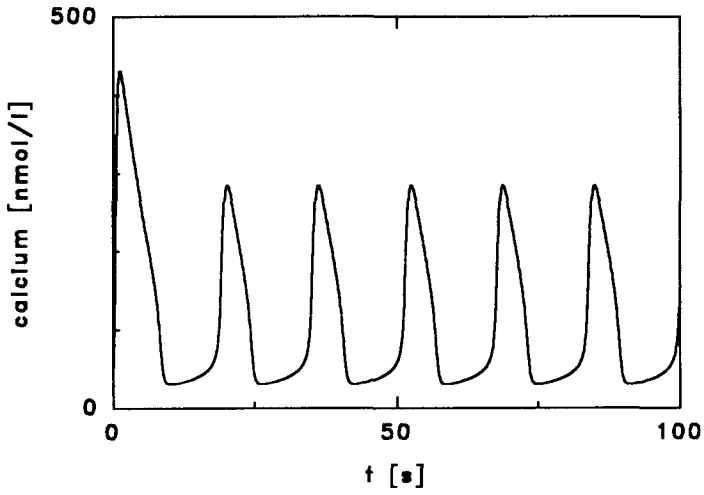

Figure 3. The time representation of calcium oscillations, which correspond to the trajectory in Fig. 2. For the parameters used see Table 1.

For $B < A$, the nullcline (10) can be nonmonotonous. There exists an interval where three different values of c correspond to each value of w (Fig. 2). The intersection of nullclines is a steady state point. It is stable when it lies on the ascending limb of the nullcline (10), and represents the resting state. It attracts all trajectories appearing in its neighborhood. If the intersection lies on the descending limb of the nullcline (10), the steady state point is unstable and a stable circle appears, which corresponds to sustained oscillations (Fig. 3). The position of the intersection depends on the mutual position of the two nullclines (10) and (11). The parameter $1/Q$ multiplies nullcline (10) and shifts the intersection along it. This shift changes stability status of the steady state point. Therefore, Q is a principal bifurcation parameter. However, the average calcium concentration in the cell, C, can also influence the position of the steady state. The bifurcation diagram is shown in Fig. 4. This bifurcation diagram shows dependence of the steady state calcium concentration on the channel permeability, Q. The dashed line corresponds to the unstable steady state point at the intersection, and the amplitude of calcium oscillations is represented by the dotted lines.

From the numerical simulations some estimations can be made concerning the effects of varying certain parameters. Upon increasing the calcium independent permeability, L , the basal level of calcium cytoplasmic concentration also increases. The figures presented have been drawn for a constant value of L. However, L should be a function of the channel activation by $InsP_3$, i.e. of Q. Qualitative simulations with L linearly dependent on Q have given similar results as those with a constant

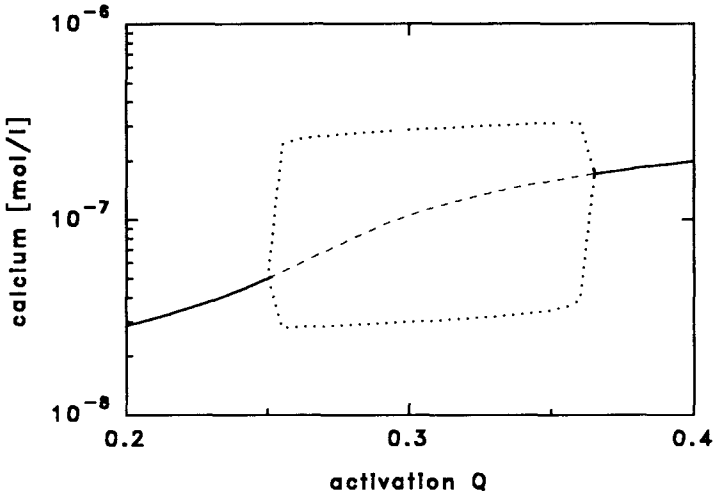

Figure 4. This bifurcation diagram shows dependence of the steady state concentration of calcium on the channel permeability Q. The dashed line corresponds to the unstable steady state point at intersection, and the amplitude of calcium oscillations is represented by the dotted lines.

L. Variations of the parameter B changed the shape of nullcline (10), particularly of its nonmonotonous part. Upon decreasing B, when $B < A$, the negative slope of the unstable part of nullcline (10) also decreases. The binding rate constant, k_{i+}, modifies the shape of transients (more precisely, the ratio of the speed coefficients Q and k_{i+}). Increasing either Q or C also increases the frequency of calcium oscillations.

Discussion

Transients or oscillations of free intracellular Ca^{2+} concentration are known to occur in many cell types during physiological cell signalling (Kristian et al. 1991; Poledna 1991; Poledna and Šimurdová 1992a,b). There is an enormous variety of oscillatory patterns between the cells and also within an individual cell, responding to different agonists. A ryanodine receptor-based Ca release system, therefore analogous to the sarcoplasmic reticulum, has been shown to coexist with the Ca release system activated by $InsP_3$, which is thought to be ubiquitous. The relative importance of these types of the calcium release channels for a specific cell is also given by the diffusion coefficients for calcium and $InsP_3$ measured by Allbritton et al. (1992). The slow diffusion of Ca^{2+} in the physiological concentration range results from its binding to slowly mobile or immobile buffers. The calculated ef-

fective ranges of free Ca^{2+} before being buffered, for buffered Ca^{2+}, and for $InsP_3$ as determined from their diffusion coefficients and lifetimes, were 0.1 μm, 5 μm, and 24 μm, respectively. Therefore, $InsP_3$ transmits signal spatially, whereas Ca^{2+} acts in restricted domains.

Most frequently, transient calcium oscillations are induced by activating those receptors which act through phosphoinositide pathway (Berridge 1990).

The presented model is based on data of Bezprozvanny et al. (1991). These authors proposed two models of calcium binding to the channel/receptor. The first one

$$P = \frac{P_{\max} \cdot [Ca]^n \cdot k^n}{([Ca]^n + K^n)([Ca]^n + k^n)} \quad \text{with} \quad n = 1.8, \quad K = k = 200 \text{ nmol/l}$$

is interpreted so that calcium binds cooperatively to at least two sites to open the channel, and to two other sites to close the channel, and that the affinities for calcium to the activation and inhibitory sites are equivalent. With a modification of the model,

$$P = \left\{ \frac{P_{\max} \cdot [Ca] \cdot k}{([Ca] + K)([Ca] + k)} \right\}^m \quad \text{with} \quad m = 2.7, \quad K = k = 200 \text{ nmol/l},$$

it is assumed that every $InsP_3$ channel complex is composed of three independent subunits, and that all subunits affect the channel open probability equally. The model of DeYoung and Keizer (1992) is based on this last interpretation, which however is in contradiction with the four subunits the ryanodine receptor is composed of. In such a case it is better to use the simplest hypothesis compatible with our knowledge of the system behavior to explain experimental data.

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New Books

Role of Free Radicals in Biological Systems

Editors: J. Fehér, A. Blázsovics, B. Matkovics, M. Mézes (77 contributors)
258 pages, hard cover, Akadémiai Kiadó, Budapest, 1993

The book contains 29 selected papers presented at the IVth Hungarian Conference with International Participation (Balatonaliga, Hungary, May 11–13, 1991) on the "Role of Free Radicals in Biological Systems".

It gives a balanced portrayal of the current scientific knowledge in the field of generation, detection, physiological and pathophysiological significance of reactive oxygen species. Numerous research teams studying the role of reactive oxygen species in different fields of medicine have shown that reactive oxygen species formed in the metabolism of arachidonic acid and during microsomal drug metabolism, as well as in mitochondrial electron transport chain play an important role not only in the function and defense mechanism of the living cells but also in pathophysiological processes. The presented results give evidence on the importance of changes in lipid peroxidation, oxidative status, the defense mechanisms of the living systems and the antioxidant therapy in health. The book draws on accounts from observations, experience, and evaluations from various fields (biochemistry, physiology, pathophysiology, pharmacology).

The papers included give further evidence in support of the importance of imbalance between production and elimination of reactive oxygen species in organ injury and disease, e.g. diabetes, inflammation, occlusion/reperfusion (open heart surgery, hemodialysis), arrhythmia, hyperlipidemia, liver cirrhosis, malignancy, immune reactions, aging, as well as food degradation. Some papers describe methods which allow measurement overproduction of reactive oxygen species from the intensity of lipid peroxidation, reduction in glutathion level, etc. in blood samples. The use of human lymphocyte micronucleus assay and direct measurement of reactive oxygen species by spin trapping are also described.

Some of the papers demonstrate the protective or therapeutic effects of natural antioxidants (vitamin E, vitamin C), chronic urea supplementation, immunologically released reactive nitrogen intermediates, flavonoids and other extracts isolated from medicinal plants (e.g. Sempervirum Tectosum), 4-hydroxynonenal, phenothiazines, Cu^{2+} and its complexes.

The described findings successfully cover the complexity of the problem and give insight into the physiological and pathophysiological role of reactive oxygen species, and into the possible pharmacological intervention. Practical advice is provided for experimental and clinical research.

V. Bauer