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A quantitative kinetic model for ATP-induced intracellular Ca^{2+} oscillations

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Abstract

A quantitative kinetic model is proposed to simulate the ATP-induced intracellular Ca^{2+} oscillations. The quantitative effect of ATP concentration upon the oscillations was successfully simulated. Our simulation results support previous experimental explanations that the Ca^{2+} oscillations are mainly due to interaction of Ca^{2+} release from the endoplasmic reticulum (ER) and the ATP-dependent Ca^{2+} pump back into the ER, and the oscillations are prolonged by extracellular Ca^{2+} entry that maintains the constant Ca^{2+} supplies to its intracellular stores. The model is also able to simulate the sudden disappearance phenomenon of the Ca^{2+} oscillations observed in some cell types by taking into account of the biphasic characteristic of the Ca^{2+} release from the endoplasmic reticulum (ER). Moreover, the model simulation results for the Ca^{2+} oscillations characteristics such as duration, peak $[Ca^{2+}]_{cyt}$, and average interval, etc., lead to prediction of some possible factors responsible for the variations of Ca^{2+} oscillations in different types of cells.

Keywords: Calcium oscillations; Sudden disappearance; Biphasic release; Endoplasmic reticulum; Quantitative model

1. Introduction

Calcium ion (Ca^{2+}) is an important second messenger that participates in many cellular signal transduction pathways and influence various physiological processes such as cell differentiation, growth, and death (Berridge et al., 1998; Rasmussen et al., 1990). The phenomenon of cellular Ca^{2+} oscillations has been first observed in non-exciting cells such as the hepatocytes as well as in periodically contracting muscle cells (Cuthbertson and Cobbold, 1985; Woods et al., 1986). Since then, it has also been found in many mammalian cells (Berridge et al., 1999; Goldbeter, 1996; Jones, 1998; Schulz et al., 1999; Soria and Martin, 1998) and in plant cells (Mcainsh et al., 1995).

It has also been shown that the characteristics of Ca^{2+} oscillations such as the frequency and peak value of cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) depend upon both the type and the concentration of agonists. Extracellular ATP acts via membrane-bound receptors as a neurotransmitter in the central and peripheral nervous systems and as a regulator of vascular and smooth muscle tone (Fredholm et al., 1994). Two pharmacologically distinct families of ATP receptors: the P_2X receptor class of ligand-gated ion channels and the P_2Y receptor class of GPCRs, have been described (Abbracchio and Burnstock, 1994; Ralevic and Burnstock, 1998). When prolonged ATP agonist is applied, the $[Ca^{2+}]_{cyt}$ initially increases sharply, and then oscillates at a plateau level for several minutes and then drops to the resting level with a sudden disappearance of Ca^{2+} oscillations observed in some cell types (DeSmedt et al., 1997; Tojyo et al., 2001). The patterns of the Ca^{2+} oscillations induced by ATP vary with ATP doses. Low concentrations of ATP (<1 mM) lead to ATP-induced Ca^{2+} oscillations with long periods. As the ATP dose increases, the interspike time intervals shorten

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and it comes with a sustained increase in $[Ca^{2+}]_{cyt}$. It has been concluded that the Ca^{2+} release from intracellular stores is the main driving force for this phenomenon and the extracellular Ca^{2+} influx is essential for the oscillatory actions by maintaining intracellular Ca^{2+} stores (Okuda et al., 2003; Sienaert et al., 1998; Tojyo et al., 2001).

Quite a few mathematical models have been developed to explain the phenomenon of Ca^{2+} oscillations since its discovery. Early models have successfully accounted for both Ca^{2+} release from intracellular stores and the ATP-dependent Ca^{2+} pumps (Goldbeter, 1989; Goldbeter et al., 1990; Meyer and Stryer, 1991; Somogyi and Stucki, 1991). Later, experimental data have shown that activation of phospholipase C (PLC) by agonists-induced receptor-coupled G-proteins results in the production of inositol 1,4,5-trisphosphate ($InsP_3$) (Cuthbertson and Chay, 1991) via the hydrolyzation of phosphatidylinositol (4,5)-bisphosphatethe (PIP_2), accompanying Ca^{2+} release from intracellular stores through the IP_3 receptors (IP_3R) in the ER membrane (Berridge, 1997; Watras et al., 1991).

Current models have covered many characteristics of Ca^{2+} oscillations rather successfully. For instance, the models constructed by Borghans (Borghans et al., 1997) and Houart (Houart et al., 1999) successfully simulate the dynamical behaviors of Ca^{2+} oscillations such as bursting, chaos, quasi-periodicity and birhythmicity; the models postulated by Kummer (Kummer et al., 2000) and Schuster (Schuster et al., 2002) have also explained different characteristics of simple and complex Ca^{2+} oscillations; the model posited by Cuthbertson and Chay (1991) has simulated probable effects of some complex biochemical processes such as receptor-coupled G-protein activation, and both PLC and protein kinase C (PKC) feedbacks upon the Ca^{2+} oscillations; the model given by Chay (Chay et al., 1995) has successfully simulated the characteristics of Ca^{2+} oscillations induced by periodic signals. The generation and termination of Ca^{2+} spikes have been explained by models as well (Hinch, 2004; Sobie et al., 2002). However, none of these models give explanation to the sudden disappearance of the Ca^{2+} oscillations while this phenomenon may be important for Ca^{2+} signal transduction. In particular, the dynamic behaviors within the ER such as the change of its free Ca^{2+} concentration and the chelation of calreticulin in response to the cytoplasmic Ca^{2+} oscillations are still unclear, although the interaction of calreticulin with the IP_3R (Camacho and Lechleiter, 1995) or the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) pump (John et al., 1998) has been simulated by Baker HL (Baker et al., 2002). In addition, until recently, there is little information available in literature about the variance of the Ca^{2+} oscillations in different types of cells.

Herein we offer a quantitative kinetic model that is posited to simulate the ATP-induced intracellular Ca^{2+} oscillations and the quantitative effect of ATP concentration on the oscillation characteristics such as the duration, peak concentration of intracellular Ca^{2+} and average interval. We also provided some reasonable explanations for the specific characteristic of the oscillation—the sudden disappearance of the Ca^{2+} oscillations observed in some cell types. The model was validated as simulation results were in good agreement with experimental data. Moreover, comparison of experimental data with simulation results of Ca^{2+} oscillations under different conditions revealed some probable factors responsible for the variation of the Ca^{2+} oscillations in different types of cells.

2. Materials and methods

2.1. Mathematical model

Our model, which is based on the biochemical processes as shown in Fig. 1, is modified from receptor-controlled model (Cuthbertson and Chay, 1991).

Five variables were chosen in the model: (i) the concentration of Gα-GTP ([Gα-GTP]); (ii) the concentration of active PLC ([APLC]); (iii) the concentration of IP_3 ($[IP_3]$); (iv) the $[Ca^{2+}]_{cyt}$; and (v) the concentration of free Ca^{2+} in the ER ($[Ca^{2+}]_{ER}$).

The first variable in the model—[Gα-GTP], is the concentration for the complex of GTP and the active Gα subunits of G-proteins. The P_2Y receptors, which reside in the G-protein-coupled cell membrane, are sensitive to ATP's stimulation (Ralevic and Burnstock, 1998). The binding of ATP and P_2Y receptors activates the Gα subunits of G-proteins to form Gα-GTP complexes. The concentration of Gα-GTP decreases due to its hydrolyzation to Gα-GDP. The enzymatic kinetics of complex upon agonist binding can be explained by the autocatalytic formation of Gα-GTP (Biddlecome et al., 1996). This is denoted as an autocatalytic term (k_{1*}[Gα-GTP]) in the simulation together with a constant term (k_0) of representing the spontaneous formation of Gα-GTP which is in the Kummer's model (Kummer et al., 2000). It has been shown experimentally that the Gα-GTP hydrolyzation is accelerated by active PLC (Bourne and Stryer, 1992). Thus a kinetic term ($k_{2*}R_{APLC*}$[Gα-GTP]) in the simulation accounts for the PLC's acceleration effect. The Gα-GTP also decreases via phosphorylation of Gα-GTP or receptors activated by PKC (Cuthbertson and Chay, 1991; Woods et al., 1987). A kinetic term ($k_{3*}R_{PKC*}$[Gα-GTP]) is used in the simulation. The activity of PKC is controlled by the hydrolytic products of PIP_2—diacylglycerol (DG) and Ca^{2+} in the cytoplasm (Chay et al., 1995; Cuthbertson and Chay, 1991). The time-dependent [Gα-GTP] change is then represented by the differential equation

$$\frac{d[\text{G}\alpha\text{-GTP}]}{dt} = k_0 + k_1[\text{G}\alpha\text{-GTP}] - k_2 R_{APLC}[\text{G}\alpha\text{-GTP}] - k_3 R_{PKC}[\text{G}\alpha\text{-GTP}] \quad (1)$$

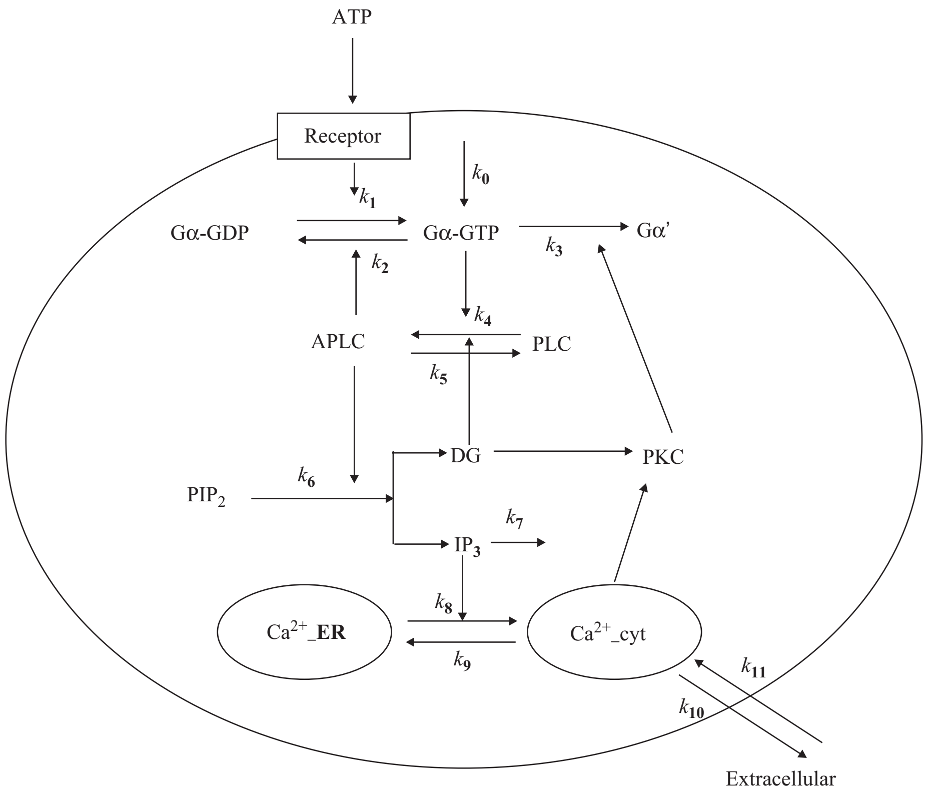

Fig. 1. Biochemical processes simulated in our proposed model.

where the fraction of active PLC and PKC,

$$R_{APLC} = \frac{[\mathrm{APLC}]}{K_P + [\mathrm{APLC}]},$$

$$R_{PKC} = \frac{[\mathrm{DG}]}{K_D + [\mathrm{DG}]}\frac{[\mathrm{Ca}^{2+}]_{cyt}}{K_R + [\mathrm{Ca}^{2+}]_{cyt}}.$$

The second variable in the model is the concentration of active PLC—[APLC]. The activation of PLC is mainly determined by active G-proteins and DG (Chay et al., 1995; Cuthbertson and Chay, 1991), a kinetic term ($k_{4^*}R_{G\alpha\text{-}GTP^*}R_{DG^*}$[PLC]) is applied in the model to represent the processes. In addition, enzymatic inactivation of APLC is represented by a term (k_{5^*}[APLC]). The time-dependent [APLC] change is then represented by the differential equation

$$\frac{\mathrm{d[APLC]}}{\mathrm{d}t} = k_4 R_{Ca\text{-}GTP} R_{DG}[\mathrm{PLC}] - k_5[\mathrm{APLC}], \qquad (2)$$

where the fraction of active Gα-GTP and DG is represented as follows:

$$R_{G\alpha\text{-}GTP} = \frac{[\mathrm{G}\alpha\text{-GTP}]^n}{K_G^n + [\mathrm{G}\alpha\text{-GTP}]^n}, \quad R_{DG} = \frac{[\mathrm{DG}]^m}{K_D^m + [\mathrm{DG}]^m}.$$

$[\mathrm{PLC}] + [\mathrm{APLC}] = C_{\mathrm{PLC,tot}}$ is used in the model.

The third variable in the model is the concentration of IP_3—[IP_3]. IP_3 is an important second messenger which is mainly formed via the PIP_2 hydrolyzation that is controlled by active PLC. It is modelled with a term (k_{6^*}[APLC]). And the term (k_{7^*}[IP_3]) represents metabolization of IP_3 into other products such as IP_2 and IP_4. The time-dependent [IP_3] change is thus represented by the differential equation

$$\frac{\mathrm{d}[\mathrm{IP}_3]}{\mathrm{d}t} = k_6[\mathrm{APLC}] - k_7[\mathrm{IP}_3]. \qquad (3)$$

The assumption that the [DG] = [IP_3] has been used in previous models (Chay et al., 1995; Cuthbertson and Chay, 1991).

The fourth variable in the model is free $[Ca^{2+}]_{cyt}$. It has two main sources: the release of intracellular Ca^{2+} stores induced by the IP_3; and the extracellular influx of Ca^{2+} due to the opening of Ca^{2+} channels in the cell membrane. Experimental data have indicated that ATP can bind P_2Y receptors in the cell membrane to induce the release of intracellular Ca^{2+} stores via IP_3R in the ER membrane (Ralevic and Burnstock, 1998) and that ryanodine receptors (RyR) seemed to have little contribution to the release process (Sienaert et al., 1998). These processes are represented by a term ($k_{8^*}R_{IP3^*}R_{ER}$) in our model. Free Ca^{2+} binds calmodulin (CaM) in the cytoplasm, following the activation of two ATP-dependent ion pumps which pump Ca^{2+} back into ER and the extracellular space (Pietrobon et al., 1990). They are denoted as simple Michaelis–Menten terms ($k_{9^*}R_{Cyt1}$ and $k_{10^*}R_{Cyt2}$) in the model. A slow Ca^{2+} leak entry from extracellular space provides constant supplies of the intracellular Ca^{2+} (Okuda et al., 2003; Sienaert et al., 1998), and this process is simulated with a constant term (k_{11}).

The endogenous buffering dynamics in the cytoplasm is derived from the reaction scheme:

$$\mathrm{BP} + \mathrm{Ca} \underset{k_-}{\overset{k_+}{\rightleftarrows}} \mathrm{CaBP},$$

where BP is the buffer protein, Ca is the free calcium and CaBP is buffered calcium. Then the kinetic equation is given by

$$\frac{\mathrm{d}[\mathrm{Ca}^{2+}]_{Cyt}}{\mathrm{d}t} = k_-[\mathrm{CaBP}] - k_+[\mathrm{Ca}^{2+}]_{Cyt}[\mathrm{BP}].$$

Calcium buffering can be modelled using a rapid-equilibrium approximation (Chay et al., 1995; Smith et al., 1996; Wagner and Keizer, 1994), in which case the buffer effect is modelled by a buffering factor as follows:

$$\beta = \frac{\mathrm{d}[\mathrm{Ca}^{2+}]_{cyt}}{\mathrm{d}[\mathrm{Ca}^{2+}]_{TC}} = \left\{1 + \frac{C_{TC}}{K_{BC}}\left(1 + \frac{[\mathrm{Ca}^{2+}]_{cyt}}{K_{BC}}\right)^{-2}\right\}^{-1},$$

where $[\mathrm{Ca}^{2+}]_{TC}$ is the total Ca^{2+} oscillations in the cytoplasm, C_{TC} is the total buffer concentration, and K_{BC} is the dissociation constant.

The time-dependent change of $[\mathrm{Ca}^{2+}]_{cyt}$ is then represented by the differential equation

$$\frac{\mathrm{d}[\mathrm{Ca}^{2+}]_{cyt}}{\mathrm{d}t} = \beta\{\rho(k_8 R_{IP_3} R_{ER} - k_9 R_{Cyt1}) - k_{10} R_{Cyt2} + k_{11}\}, \tag{4}$$

where ρ is the volume ratio between the ER and the cytoplasm ($0<\rho<1$), and

$$R_{IP_3} = \frac{\mathrm{IP}_3^3}{K_S^3 + \mathrm{IP}_3^3}, \quad R_{Cyti} = \frac{[\mathrm{Ca}^{2+}]_{cyt}}{K_{Ci} + [\mathrm{Ca}^{2+}]_{cyt}} \quad (i = 1, 2).$$

The fifth variable in the model is free $[\mathrm{Ca}^{2+}]_{ER}$. Except for the Ca^{2+} release from ER and the ATP-dependent Ca^{2+} pumps, it has also been shown that there exists Ca^{2+}-binding protein both in the ER as well as in the cytoplasm with two distinct types of Ca^{2+}-binding sites (Michalak et al., 1992, 2002): a P-domain with high affinity and low capacity and a C-domain with low affinity and high capacity, and they are called BP and SP, respectively. These Ca^{2+}-binding proteins play important roles in Ca^{2+}-signal transduction which directly affect the Ca^{2+} release (Groenendyk et al., 2004; Michalak et al., 1999). Thus, we considered the buffer proteins in the ER and the same assumption is applied to the Ca^{2+} and the buffer proteins as well as in the cytoplasm, and the buffering factor is given by

$$\lambda = \frac{\mathrm{d}[\mathrm{Ca}^{2+}]_{ER}}{\mathrm{d}[\mathrm{Ca}^{2+}]_{TE}} = \left\{1 + \frac{C_{TE}}{K_{BE}}\left(1 + \frac{[\mathrm{Ca}^{2+}]_{ER}}{K_{BE}}\right)^{-2}\right\}^{-1},$$

where $[\mathrm{Ca}^{2+}]_{TE}$ is the total Ca^{2+} oscillations in the ER, C_{TE} is the total buffer concentration, and K_{BE} is the dissociation constant.

Then, the time-dependent change of free $[\mathrm{Ca}^{2+}]_{ER}$ is represented by the differential equation

$$\frac{\mathrm{d}[\mathrm{Ca}^{2+}]_{ER}}{\mathrm{d}t} = \lambda(-k_8 R_{IP_3} R_{ER} + k_9 R_{Cyt1}). \tag{5}$$

Notice that if K_{BC} (K_{BE}) is much greater than $[\mathrm{Ca}^{2+}]_{cyt}$ ($[\mathrm{Ca}^{2+}]_{ER}$), $\beta(\lambda)$ will be constant. In this paper, we assume $\beta(\lambda)$ to be constant.

Experimental evidence has shown that IP_3R require a certain level of $[\mathrm{Ca}^{2+}]_{ER}$ for maximal activity (Barrero et al., 1997), indicating that when $[\mathrm{Ca}^{2+}]_{ER}$ drops to some extent, the Ca^{2+} release from the intracellular stores would be inhibited. This is formulated with a Michaelis–Menten equation with a high coefficient (w) as follows:

$$R_{ER} = \frac{[\mathrm{Ca}^{2+}]_{ER}^w}{K_{ER}^w + [\mathrm{Ca}^{2+}]_{ER}^w}.$$

In the model, k_1 in Eq. (1) is related to the ATP concentration proportionally. Numeric values of main parameters used in the simulations (unless otherwise stated) are given in Table 1.

Some of these parameters have the following significance:

(1) k_3 represents the inhibition of active PKC which is controlled by [DG] and $[\mathrm{Ca}^{2+}]_{cyt}$.
(2) k_4 together with k_5 represents the PLC activation, which is also controlled by activated G-proteins and DG.
(3) k_6 together with k_7 represents the formation of IP_3, which is controlled by activated PLC.

The five differential equations were processed numerically by the XPPAUT software (http://www.math.pitt.edu/~bard/xpp/xpp.html) and the maximum time step (Δt) was set to 0.01 s.

Table 1
Parameters and values used in our model

Parameter	Value (unit)	Parameter	Value (unit)
k_0	0.1 ((nmol/L Cyt) s^{-1})	K_P	4 (nmol/L Cyt)
k_2	4 (s^{-1})	K_R	200 (nmol/L Cyt)
k_3	4.5 (s^{-1})	K_G	25 (nmol/L Cyt)
k_4	1.2 (s^{-1})	K_S	25 (nmol/L Cyt)
k_5	0.12 (s^{-1})	K_{ER}	75 (nmol/L ER)
k_6	14 (s^{-1})	K_{C1}	1000 (nmol/L Cyt)
k_7	2 (s^{-1})	K_{C2}	2000 (nmol/L Cyt)
k_8	10.5 ((μmol/L ER) s^{-1})	β	0.05
k_9	0.6 ((μmol/L ER) s^{-1})	λ	0.001
k_{10}	3.0 ((μmol/L Cyt) s^{-1})	ρ	0.2
k_{11}	0.26 ((μmol/L Cyt) s^{-1})	n	4
$C_{\mathrm{PLC,tot}}$	10 (nmol/L Cyt)	m	2
K_D	10 (nmol/L Cyt)	w	3

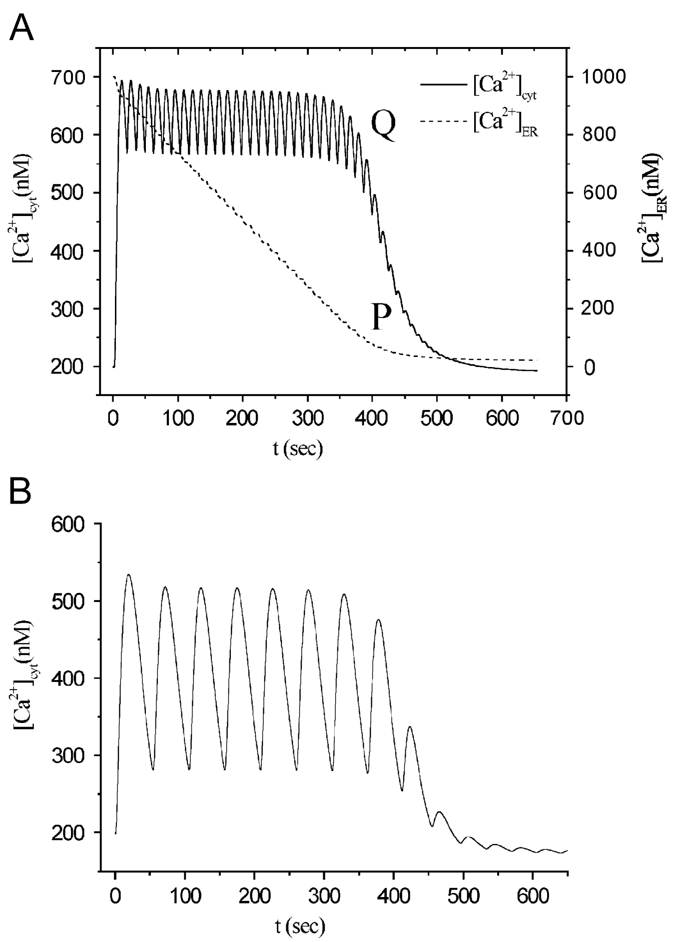

Fig. 2. Typical simulated ATP-induced intracellular Ca^{2+} oscillations in Eqs. (1)–(5). (A) Responses of $[Ca^{2+}]_{ER}$ and $[Ca^{2+}]_{cyt}$ due to the exogenous ATP stimulant. The biphasic characteristic of the release process (P) is consistent with the sudden disappearance (Q) of intracellular Ca^{2+} oscillations. Parameters: $k_1 = 3.4\,s^{-1}$, the others are shown in Table 1. (B) Response of $[Ca^{2+}]_{cyt}$ in another typical ATP-induced Ca^{2+} oscillations which is similar to the Tojyo's observations (Tojyo et al., 2001). Parameters: $k_1 = 2.0\,s^{-1}$, $k_5 = 0.02\,s^{-1}$, $k_7 = 0.5\,s^{-1}$, $k_{10} = 5.0\,\mu M\,s^{-1}$, $k_{11} = 0.4\,\mu M\,s^{-1}$, and the others are shown in Table 1. Initial conditions: [Gα-GTP] = 1 nM, [PLC*] = 1 nM, $[IP_3]$ = 1 nM, $[Ca^{2+}]_{cyt}$ = 200 nM, $[Ca^{2+}]_{ER}$ = 1000 nM.

3. Results

3.1. Characteristics of ATP-induced Ca^{2+} oscillations

ATP-induced Ca^{2+} oscillations were successfully simulated by the present model as shown in Fig. 2. It can be seen from both of the two curves that the $[Ca^{2+}]_{cyt}$ initially increases sharply, then oscillates at a plateau level, lasting for several minutes, and then falls off drastically. The simulation results are similar to previous experimental observations (DeSmedt et al., 1997; Tojyo et al., 2001). The sudden disappearance of the oscillations is successfully obtained due to the biphasic characteristic of the Ca^{2+} release process. Fig. 2A shows the change of free Ca^{2+} concentration in the ER together with the Ca^{2+} concentration in the cytoplasm. The time of the reverse phase point (P) is consistent with that of the sudden disappearance (Q). Accordingly, we can conclude that our hypothesis of the biphasic release process is adaptable to explain the sudden disappearance of ATP-induced intracellular Ca^{2+} oscillations.

3.2. Effect of ATP concentration on the Ca^{2+} oscillations

The Ca^{2+} oscillations in response to increasing degrees of agonist stimulation were also successfully simulated by adjusting the value of parameter k_1 while keeping other parameters constant. Experiments have shown that ATP is a strong ligand for P_2Y receptors (Ralevic and Burnstock, 1998). For computational convenience, we presumed that each P_2Y receptor protein has one binding site for each ATP molecule. So the ATP concentration can be related to the parameter k_1 with a simple Michaelis–Menten equation as follows:

$$k_1 = k_{\max} \frac{C_{ATP}}{K_{ATP} + C_{ATP}},$$

where $k_{\max}$ is the maximal activity of ATP stimulant and K_{ATP} is the Michaelis constant.

Ratio between peak $[Ca^{2+}]_{cyt}$ and initial $[Ca^{2+}]_{cyt}$ ($[Ca^{2+}]_{cyt\_p}/[Ca^{2+}]_{cyt\_i}$) and average interval of the Ca^{2+} oscillations were applied to study the ATP dependence, and the simulation results are shown in Fig. 3. As ATP concentration increases from 0.5 to 10 μM, the peak value of $[Ca^{2+}]_{cyt}$ increases notably (Fig. 3A) and the average interval is shortened (Fig. 3B). However, when ATP concentration is higher than 10 μM, both indexes vary to a certain degree. Compared with experimental data by Tojyo (Tojyo et al., 2001) and other researchers (Okuda et al., 2003; Sienaert et al., 1998), the simulation results generated by the current model agree well with experimental observations.

3.3. Effect of extracellular Ca^{2+} entry on ATP-induced Ca^{2+} oscillations

It has been concluded from previous studies (Okuda et al., 2003; Tojyo et al., 2001) that these oscillations are primarily due to the Ca^{2+} mobilization from intracellular stores, and extracellular Ca^{2+} is the Ca^{2+} source for intracellular Ca^{2+} stores that maintain the oscillations. These conclusions are also demonstrated here by adjusting the value of k_{11} which represents slow Ca^{2+} entry from extracellular space, while keeping other parameters constant. The results are shown in Fig. 4. It can be concluded that when there is no Ca^{2+} entry from extracellular space ($k_{11} = 0$), the oscillations can still happen (Fig. 4A). The relationship between duration of the oscillations and extracellular Ca^{2+} entry was also simulated (Fig. 4B). It is shown that as k_{11} increases, the duration of the oscillations increases. Our model also predicts that peak $[Ca^{2+}]_{cyt}$ increases linearly while the average interval nearly

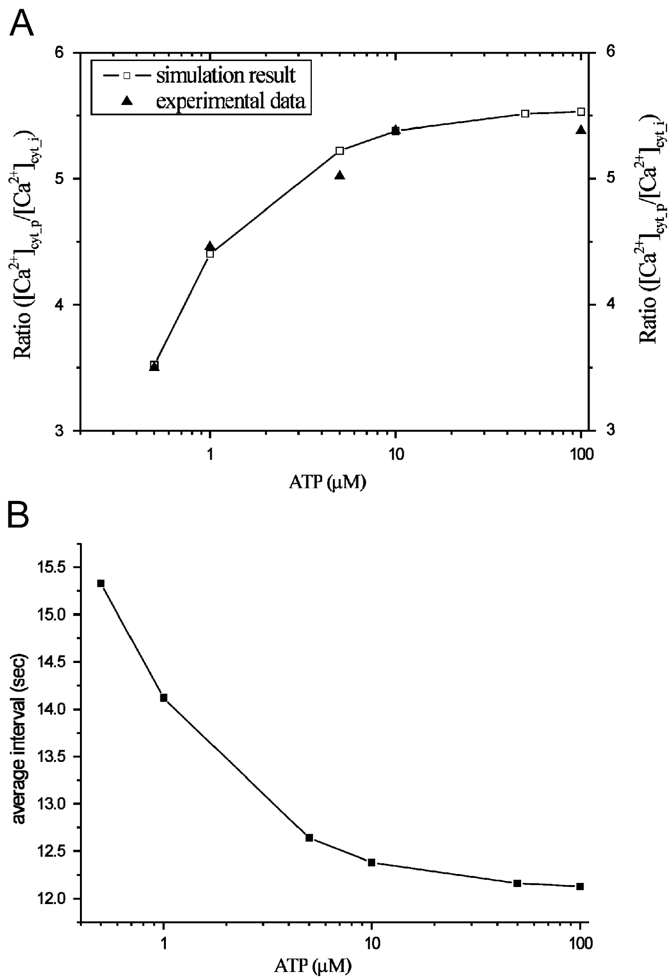

Fig. 3. Simulated oscillations in response to increasing degrees of agonist stimulation in Eqs. (1)–(5). (A) Relationship between $[Ca^{2+}]_{cyt\_p}/[Ca^{2+}]_{cyt\_i}$ and ATP concentration compared with experimental observation by Tojyo; (B) relationship between average interval and ATP concentration. Six values of ATP concentration are simulated: 0.5, 1, 5, 10, 50, 100 μM. Parameters: $k_{max} = 3.8\,s^{-1}$, $K_{ATP} = 0.18\,\mu M$, and the others are shown in Table 1. Initial conditions: [Gα-GTP] = 1 nM, [PLC*] = 1 nM, $[IP_3]$ = 1 nM, $[Ca^{2+}]_{cyt}$ = 150 nM, $[Ca^{2+}]_{ER}$ = 1000 nM.

maintains constant (Fig. 4C). This indicates that extracellular Ca^{2+} entry can result in some increase of intracellular Ca^{2+} concentration but has little effect on the frequency of the oscillations.

3.4. *Effect of intracellular* Ca^{2+} *stores on ATP-induced* Ca^{2+} *oscillations*

In the model, it is assumed that the sudden cessation of these oscillations is due to the mobilization of intracellular stores on the basis of previous experimental data, suggesting that IP_3R require a certain level of $[Ca^{2+}]_{ER}$ for maximal activity (Barrero et al., 1997). Moreover, it is presumed in the current model that the buffer proteins in the ER also determine the duration of these oscillations which was also successfully simulated by adjusting the value of parameter λ. As shown in Fig. 5, as λ increases, the duration of oscillations decreases sharply at first and slowly later. The peak $[Ca^{2+}]_{cyt}$ and average interval nearly keep constant (not shown). As λ represents the buffering ability of the C-domain proteins, these results indicate that the character of C-domain proteins in the ER is one possible factor accounts for experimental duration variations of these oscillations in different types of cells and/or in different cells of the same type (Okuda et al., 2003; Sienaert et al., 1998; Tojyo et al., 2001).

3.5. *Effect of activation of PKC and PLC on ATP-induced* Ca^{2+} *oscillations*

The effect of activation of PKC on ATP-induced Ca^{2+} oscillations was also simulated by adjusting the value of parameter k_3 while keeping other parameters constant. The result is shown in Fig. 6. The simulation predicts that as k_3 increases, the peak $[Ca^{2+}]_{cyt}$ decreases and average interval increases, indicating that PKC activation, probably via the phosphorylation of G-proteins (Woods et al., 1987), may inhibit ATP-induced Ca^{2+} oscillations as demonstrated by their degree and the frequency.

The value of parameter k_5, related to the activation of PLC, affects the shape of the Ca^{2+} oscillations significantly as shown in Fig. 7, indicating that the PLC's activation is another possible factor responsible for variations of ATP-induced Ca^{2+} oscillations in different types of cells.

4. Discussion

Based on the biological process scheme (Fig. 1), we construct a quantitative kinetic model to simulate ATP-induced intracellular Ca^{2+} oscillations. The present model has successfully simulated typical ATP-induced intracellular Ca^{2+} oscillations, especially the termination characteristics—a sudden disappearance of the oscillations (Fig. 2). The quantitative effect of ATP concentration upon the Ca^{2+} oscillations were successfully simulated as well (Fig. 3A), indicating that the present model can predict ATP-induced intracellular Ca^{2+} oscillations rather well. The mechanism responsible for the Ca^{2+} oscillations, which is mainly due to the interaction of Ca^{2+} release from the ER and the ATP-dependent Ca^{2+} pump back into the ER, was also demonstrated in our model (Fig. 4). Moreover, it can be further concluded that the variations of these oscillations in different types of cells are probably due to the varied Ca^{2+}-binding capability of calreticulin within the ER (Fig. 5) and different enzymatic actions by PKC (Fig. 6) and PLC (Fig. 7) via their activation or inhibition.

It has been demonstrated that most exogenous stimulants transmit signals into cells via binding with specific receptors in the cell membrane and ATP is a strong ligand for P_2Y receptors (Ralevic and Burnstock, 1998). Herein we related the ATP concentration to the stimulative strength, parameter k_1, by a simple Michaelis–Menten equation. Therefore, the quantitative effect of ATP

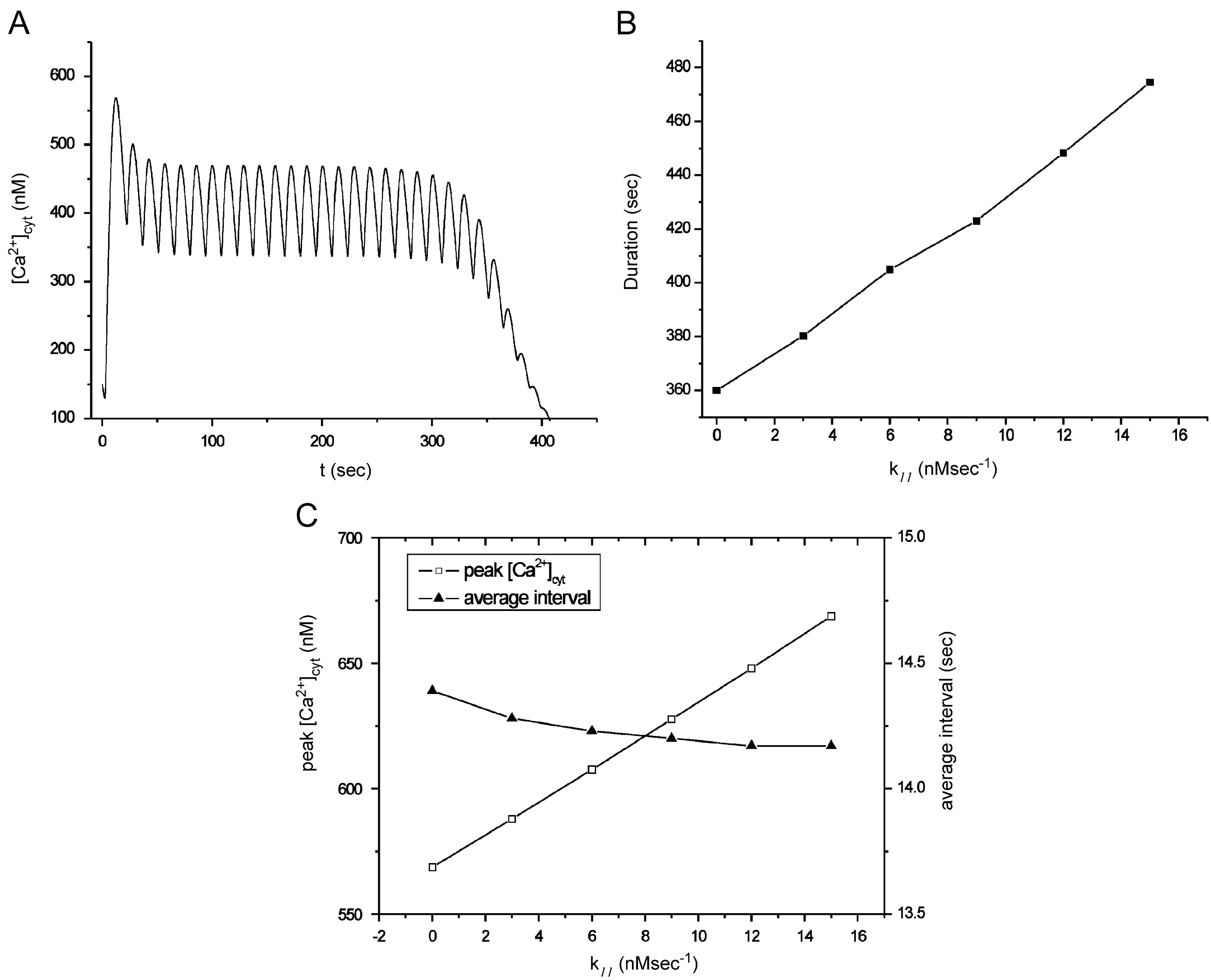

Fig. 4. Oscillations in response to Ca^{2+} entry from extracellular space simulated in Eqs. (1)–(5). (A) Without extracellular Ca^{2+} entry ($k_{11} = 0$); (B) simulated relationship between duration of ATP-induced Ca^{2+} oscillations and the extracellular Ca^{2+} entry; (C) peak $[Ca^{2+}]_{cyt}$ and average interval in response to extracellular Ca^{2+} entry. Parameters: $k_1 = 3.2\,s^{-1}$, the others are shown in Table 1. Initial conditions: [Gα-GTP] = 1 nM, [PLC*] = 1 nM, $[IP_3] = 1$ nM, $[Ca^{2+}]_{cyt} = 150$ nM, $[Ca^{2+}]_{ER} = 1000$ nM.

concentration on the Ca^{2+} oscillations can be simulated by the present model although the detailed kinetics for agonist binding to the specific receptors and the consequential activation of G-proteins are very complicate. It has been shown that desensitization of P_2Y receptors would occur when other G-protein-coupled receptors are phosphorylated (Ralevic and Burnstock, 1998). This implies that the value of k_1 would probably change over time. However, P_2Y receptors are generally thought not to be readily desensitized, especially in ATP-induced Ca^{2+} oscillations which always last for only a few minutes (Ralevic and Burnstock, 1998). Consequently, we hypothesized that the effect of desensitization of P_2Y receptors is insignificant in our model, and we can thus predict the dose-dependent intracellular Ca^{2+} oscillations rather well.

There are some plausible mechanisms that can be used to explain the termination of ATP-induced Ca^{2+} oscillations. Hinch's and Sobie's models (Hinch, 2004; Sobie et al., 2002) provided an explanation for the termination of Ca^{2+} sparks which related to ryanodine receptors. However, it seems that ryanodine receptors have little contribution to the releasing process in ATP-induced Ca^{2+} oscillation experiments (Sienaert et al., 1998). There has been increasing evidence that ATP-induced cytoplasmic calcium oscillations are accompanied by a sudden disappearance in some non-exciting cells (DeSmedt et al., 1997; Tojyo et al., 2001). Tojyo et al. attributed this disappearance to the desensitization of receptors. However, as discussed above, P_2Y receptors are generally thought not to be readily desensitized in ATP-induced Ca^{2+} oscillations (Ralevic and Burnstock, 1998). Consequently, the experimental findings call for some other mechanistic understandings of cytoplasmic Ca^{2+} oscillation termination. It has been shown that IP_3R require a certain level of $[Ca^{2+}]_{ER}$ for maximal

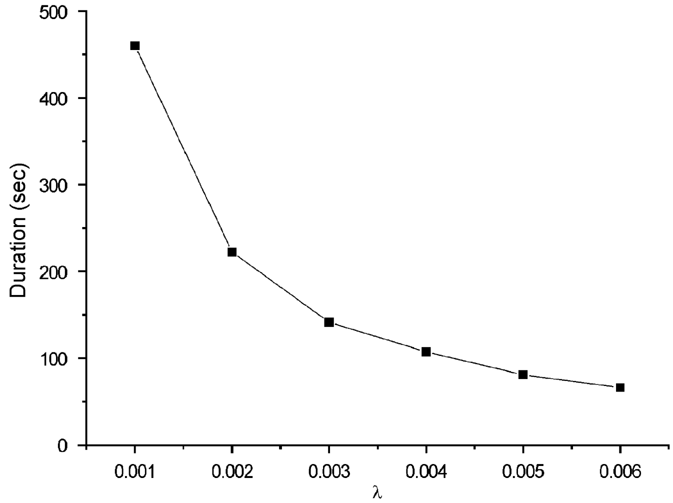

Fig. 5. Simulated relationship between duration of ATP-induced Ca^{2+} oscillations and the ability of C-domain proteins. Parameters: $k_1 = 3.2\,s^{-1}$, $[Ca^{2+}]_{crit} = 150\,nM$, the others are shown in Table 1. Initial conditions: [Gα-GTP] = 1 nM, [PLC*] = 1 nM, $[IP_3] = 1\,nM$, $[Ca^{2+}]_{cyt} = 200\,nM$, $[Ca^{2+}]_{ER} = 1000\,nM$.

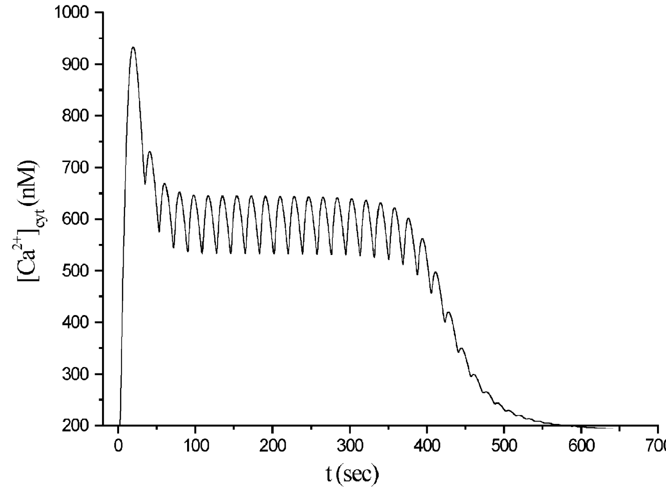

Fig. 7. Response of the oscillation shape to the PLC's activation simulated in Eqs. (1)–(5). Parameters: $k_1 = 3.4\,s^{-1}$, $k_5 = 0.06\,s^{-1}$, the others are shown in Table 1. Initial conditions: [Gα-GTP] = 1 nM, [PLC*] = 1 nM, $[IP_3] = 1\,nM$, $[Ca^{2+}]_{cyt} = 200\,nM$, $[Ca^{2+}]_{ER} = 1000\,nM$.

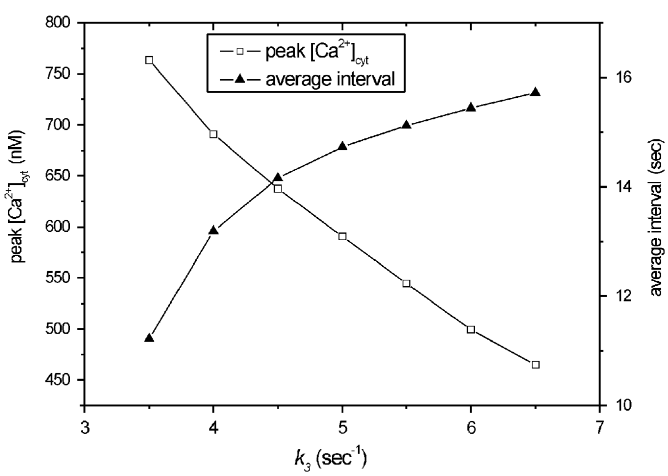

Fig. 6. Effects of activation of PKC and PLC on ATP-induced Ca^{2+} oscillations simulated in Eqs. (1)–(5). Parameters: $k_1 = 3.2\,s^{-1}$, the others are shown in Table 1. Initial conditions: [Gα-GTP] = 1 nM, [PLC*] = 1 nM, $[IP_3] = 1\,nM$, $[Ca^{2+}]_{cyt} = 200\,nM$, $[Ca^{2+}]_{ER} = 1000\,nM$.

activity (Barrero et al., 1997) of Ca^{2+} release from the ER, and the Ca^{2+} release flux is driven by the Ca^{2+} gradient across the ER membrane (Berridge, 1997; Watras et al., 1991), indicating that the release process is $[Ca^{2+}]_{ER}$-dependent. Accordingly, in our model the release flux is assumed to be biphasic with $[Ca^{2+}]_{ER}$. Based on our model analysis, we thought that the biphasic characteristic may be directly responsible for the sudden disappearance of the Ca^{2+} oscillations.

Calcium buffering is another hot issue in current research. The binding stoichiometry between the Ca^{2+} buffering proteins and $[Ca^{2+}]_{ER}$ is very variable and about 20–30 mol Ca^{2+}/mol protein (Gelebart et al., 2005). That may be the reason why the calcium oscillations can last even over 1 h (Okuda et al., 2003). As such, a mathematic model for such complicated buffering process needs a large set of parameters determined by experiments. Calcium buffering proteins in the cytoplasm have been investigated in many models (Chay et al., 1995; Haberichter et al., 2001; Li et al., 2005; Marhl et al., 1998), and a buffering SERCA pump has recently been proposed (Higgins et al., 2006). We have also included buffering proteins in the ER in our model to determine the relationship between the concentrations of free Ca^{2+} and total Ca^{2+} in the ER. The buffering proteins are assumed to bind free Ca^{2+} with a ratio of 1:1 for computation simplicity, and the Ca^{2+} binding is treated as a rapid-equilibrium approximation, the same treatment for the cytoplasm (Chay et al., 1995; Smith et al., 1996; Wagner and Keizer, 1994). The Ca^{2+} release flux from the ER is unequal to the Ca^{2+} flux pumped back from the cytoplasm. In fact, the total calcium in the ER decreases gradually due to the extra stimulants. Thus, the free Ca^{2+} concentration decreases with the time. However, the decrease rate of free $[Ca^{2+}]_{ER}$ is actually less than that of the total $[Ca^{2+}]_{ER}$ due to the buffering effect of calreticulin in the ER. This can be demonstrated by the marginal decrease of the concentration in each Ca^{2+} spike observed in previous experiments (Okuda et al., 2003; Sienaert et al., 1998; Tojyo et al., 2001).

Taken together, our modified model is able to simulate ATP-induced Ca^{2+} oscillations. The quantitative effect of ATP concentration on the Ca^{2+} oscillations can be successfully modelled. Moreover, no other model has so far explained the sudden disappearance of these oscillations which is actually a significant phenomenon in Ca^{2+} signalling. This is because that most models do not consider the detailed dependence between the Ca^{2+} release flux and the free Ca^{2+} concentration in the ER. By simulating the effects of main processes on the oscillations, the present model demonstrates the experimental conclusion that these oscillations are mainly due to the Ca^{2+} release from the ER and also predicts some possible factors on the variance of these oscillations in different types of cells. Our simulation results warrant further studies on the dynamic behaviors of the initial receptor complex and the interaction of calreticulin and calcium ions in the ER.

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