Stochastic contribution for the coding of agonist induced calcium oscillation in hepatocytes

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Stochastic contribution for the coding of agonist induced calcium oscillation in hepatocytes

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Abstract The influence of stochastic inositol-1,4,5-tris-phosphate receptor (IP$_3$R) dynamics and their clustering have been extensively investigated to explore the mechanism through which the stochastic molecular event finally shape the intracellular calcium signaling. Most of the previous works employed simplified models which take the concentration of IP$_3$ instead of that of the agonist as the stimulation intensity. However, the IP$_3$ level is not linearly dependent on the agonist concentration in stimulus induced signaling systems because there are feedback links in the transduction network. In this work, we include both the IP$_3$R dynamics and the typical agonist induced signaling transduction cascade in the model to investigate the essential influence of stochastic IP$_3$R dynamics on the coding of the stimulus induced calcium signal. Simulation results reveal two distinct oscillation areas under different stimulation levels. The signal is optimally modulate by the IP$_3$R cluster number in the weak stimulated area while affected by the stimulus intensity in the strong stimulated area. Different dependences of coefficient of variance (CV) on the number of clusters are obtained in these two areas, which explains the disagreement in the previous reported results. Besides, the transition between these areas explains the significant CV reduction observed in experiments.

Keywords IP$_3$R · Stochastic · Coding · Agonist induced calcium oscillation · Hepatocytes

Introduction

Calcium signaling had been modeled by deterministic methods for a long time on the assumption that enough molecules and channels are involved. However, recent investigations prove it is essentially stochastic. The standard deviation of the inter-spike intervals (ISI) in several types of cells is at the same level with the averaged ISI (Skupin et al. 2008). Statistical analysis of the regularity of Ca$^{2+}$ oscillations in agonist-stimulated hepatocytes also found that the fluctuations in the ISIs are far from negligible (Dupont et al. 2008; Dupont and Combettes 2009). It is shown that the stochastic opening of IP$_3$R and their clustering are the main random source for calcium signaling (Falcke 2003; Shuai and Jung 2002). However, different results are obtained in the IP$_3$R clustering effects investigations. Shuai and Jung (2002) prove the clustering of IP$_3$R may enhance the signal regularity at an appropriate clustering number, while Dupont et al.’s (2008) investigation in hepatocytes revealed that more clusters will lead to less of a stochastic signal. In their work, monotonous CV dependency on the number of clusters was found. In hepatocytes, Ca$^{2+}$ oscillations are stimulated by the binding of agonists to the G-protein coupled receptors, which then stimulates the hydrolysis of the membrane lipid phosphatidyl inositol-4,5-bisphosphate (PIP$_2$) through phospholipase C (PLC) to produce IP$_3$ and diacylglycerol (DAG). The release of Ca$^{2+}$ from the internal stores is ensured by the binding of IP$_3$ and Ca$^{2+}$ to the IP$_3$R. Most of the previous IP$_3$R dynamics related investigations took the IP$_3$ concentration as the stimulation strength (Dupont et al. 2008; Dupont and Combettes 2009; Shuai et al. 2008) and omitted the transmembrane receptor dynamics. But the IP$_3$ concentration is not a linear reflection of the extracellular stimuate, since G-protein coupled receptor dynamics...
is an important process in agonist triggered signals (Cuthbertson and Chay 1991; Kummer et al. 2000, 2005; Larsen et al. 2004; Wang et al. 2007), and it is regulated by the cytoplasm Ca\(^{2+}\) controlled negative feedback through protein kinase C (PKC) (Cuthbertson and Chay 1991; Wang et al. 2007; Bird et al. 1993). Therefore, it worth taking both the IP\(_3\)R and G-protein coupled receptor dynamics into consideration to investigate the agonist induced calcium signals. In this work, we discuss the essential contribution of stochastic IP\(_3\)R dynamics to the agonist induced calcium signal in a model which includes both the G-protein coupled receptor and the IP\(_3\)R dynamics.

The stochastic simulation algorithm (SSA) proposed by Gillespie (1976) is a widely used method to simulate stochastic chemical kinetics in mesoscopic systems (Pahle 2009; Gillespie 2007). Since it provides an exact solution of the chemical master equation by sampling the trajectory of every reaction event, it is very time consuming when there are many reactants. In practical work, SSA is often used in combination with other accelerated approximate stochastic methods or deterministic algorithms under a hybrid framework (Vasudeva and Bhalta 2004; Samant et al. 2007; Haseltine and Rawlings 2002). Hybrid algorithms were also adopted in many calcium signaling investigations (Rüdiger et al. 2007; Tartakovsky et al. 2010; Keener 2006; Greenstein and Winslow 2002). It is widely accepted that the stochastic binding processes in IP\(_3\)R dynamics are the main random source in calcium signaling. Since the binding is much faster than other transduction processes, it is acceptable to apply the quasi-equilibrium assumption in the simulation, which assume that other transduction processes are in an equilibrium state and can be described by a deterministic method on the time scale of stochastic binding. In this work, we employ a hybrid method that use SSA on IP\(_3\)R dynamics and simulate other processes by ODE to investigate the influence of stochastic IP\(_3\)R dynamics on the agonist-induced calcium signal.

**Model and method**

The model used in this work is a modified vision of the receptor-controlled model (Cuthbertson and Chay 1991), which takes into consideration both the kinetics of the G-protein coupled receptor (Cuthbertson and Chay 1991; Kummer et al. 2000; Wang et al. 2007) and the detailed IP\(_3\)R dynamics (Dupont et al. 2008). The general biochemical processes involved are shown in Fig. 1. There are all together four variables in the model: the concentration of the complex of GTP (\([Ga\text{-GTP}]\)) and the active \(a\) subunit of the G-protein (\([Ga\text{-GTP}]\)), the active form of phospholipase C [PLC*], inositol 1,4,5-trisphosphate ([IP\(_3\))], and free Ca\(^{2+}\) in cytoplasm ([Ca\(^{2+}\)]), respectively.

The first variable \([Ga\text{-GTP}]\) is increased by the activation of this complex, which can be denoted as a constant term \((k_6)\) representing its spontaneous formation (Kummer et al. 2000) together with an autocatalytic term \((k_1)\) process (Chay et al. 1995). The intensity of external stimulation can be readily described by the value of \(k_1\). The deactivation of \(Ga\text{-GTP}\) is either due to the PLC* catalyzed hydrolyzation \((k_2)\) process or via PKC activated phosphorylation of \(Ga\text{-GTP}\) \((k_3)\) process (Cuthbertson and Chay 1991; Wang et al. 2007; Bird et al. 1993; Woods et al. 1987). The time dependent \([Ga\text{-GTP}]\) change is then represented by:

\[
\frac{d[ Ga\text{-GTP} ]}{dt} = k_0 + k_1[Ga\text{-GTP}] - k_2\frac{[PLC^*]}{K_P + [PLC^*]}[Ga\text{-GTP}] - k_3\frac{[DAG]}{K_D + [DAG]}\frac{[Ca^{2+}]}{K_R + [Ca^{2+}]}[Ga\text{-GTP}].
\]

(1)

The activation of PLC is mainly determined by the active G-proteins and DAG \((k_4)\) process (Cuthbertson and Chay 1991; Chay et al. 1995; Wang et al. 2007). Its enzymatic inactivation can be represent as a linear term \((k_5)\) process (Chay et al. 1995; Cuthbertson and Chay 1991). Therefore, the second equation takes the form:

\[
\frac{d[PLC]}{dt} = k_4\frac{[Ga\text{-GTP}]^4}{K_G + [Ga\text{-GTP}]^4}\frac{[DAG]^2}{K_D + [DAG]^2}[PLC] - k_5[PLC^*].
\]

(2)

\([IP_3]\) is increased by the PLC* controlled hydrolyzation of PIP\(_2\) \((k_6)\) process, and decreased by its metabolization \((k_7)\) process. Since DAG and IP\(_3\) are coming from the hydrolysis of PIP\(_2\) with the same stoichiometric coefficient, we assume that \([DAG]\) and \([IP_3]\) increase with the same rate (Cuthbertson and Chay 1991; Chay et al. 1995). Thus, the time dependence of \([IP_3]\) \(([DAG])\) is represent as:

\[
\frac{d[IP_3]}{dt} = k_6[PLC^*] - k_7[IP_3].
\]

(3)

The last variable \([Ca^{2+}])\) is determined by both the intracellular Ca\(^{2+}\) release and the transmembrane Ca\(^{2+}\) fluxes. The release of Ca\(^{2+}\) from endoplasmic reticulum (ER) via IP\(_3\)R is represented by the \(k_8\) term with \(f_{\text{open}}\) and indicates the percentage of opened IP\(_3\)Rs among its total number and \(K_S\) the half-saturation constant of IP\(_3\) for its receptor. Pumping from the cytosol back into ER is modeled by a Hill function, with the maximal velocity and the half-saturation constant represented by \(k_8\) and \(K_{e1}\), respectively. The unregulated Ca\(^{2+}\) leak from ER to cytoplasm is assumed to be constant and is represented.
The pump of Ca\(^{2+}\) into extracellular space through Ca\(^{2+}\) pump is also denoted as a Hill function (\(k_{11}\) process). A slow Ca\(^{2+}\) leak entry from the extracellular space is simulated with a constant term \(k_{12}\). The \(\beta\) stands for the effective buffering capacity of the cytoplasm. Then, the change of [Ca\(^{2+}\)] can be described as:

\[
\frac{d[Ca^{2+}]}{dt} = \beta \left[ k_{8\text{open}} \frac{[IP_3]}{K_P + [IP_3]} - k_9 \frac{[Ca^{2+}]^2}{K_{C1}^2 + [Ca^{2+}]^2} + k_{10} - k_{11} \frac{[Ca^{2+}]^2}{K_{C2}^2 + [Ca^{2+}]^2} + k_{12} \right].
\]

Parameters used in this work are listed in Table 1.

The stochastic binding of IP\(_3\) and Ca\(^{2+}\) on IP\(_3\)R has been proved to be crucial in the calcium signaling. Instead of the previous modeling method using a quasi-steady state assumption (Chay et al. 1995), various dynamical models have been proposed to describe the binding processes. The Li-Rinzel model (Li and Rinzel 1994) had been adopted in many coarse grained simulations use the fast process assumption on the binding processes. The De Young-Keizer model (1992) gives the detailed binding dynamics. It is widely employed in the investigations of IP\(_3\)R dynamics, and several variant models had been proposed based on it to give a different simplified form (Dupont et al. 2008; Jung et al. 2010). In this work, we employ its nine variable variants proposed by Dupont et al. (2008). Generally, it is accepted that the IP\(_3\)R has four equal and independent subunits. Each subunit has three binding sites: an IP\(_3\) binding site, a Ca\(^{2+}\) activation binding site, and a Ca\(^{2+}\) inhibition binding site, each of which is either occupied or unoccupied. In the current model, the IP\(_3\) (un)binding processes is assumed to be always in a quasi-equilibrium state since it occurs on a time scale much faster than other processes. The channel is opened when two Ca\(^{2+}\) ions are

![Diagram of biochemical processes](image)

**Table 1** Parameter used in this work

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
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<td>(k_0)</td>
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<td>(k_{10})</td>
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<td>(\beta)</td>
<td>0.05</td>
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</table>
bound at the activation site and no Ca$^{2+}$ ion is bound at the inhibitory site. The detailed binding scheme is as shown in the inset of Fig. 1. The $R_{20}$ state stands for the opened IP$_3$R. $f_{\text{open}}$ is the ratio of $R_{20}$ to the total number of IP$_3$R in the cell, which is assumed to be 5,400 here (Dupont et al. 2008). A $N$-channel cluster is modeled as one “mega-channel” whose conductance is $N$ times that of a single IP$_3$R. The total number of the cluster will be 5,400/$N$. For detailed IP$_3$R channel model description and parameter values, see Ref. (Dupont et al. 2008). The whole system is simulated by a hybrid method (Keener 2006; Haseltine and Rawlings 2002) with the IP$_3$R dynamic realized by SSA and other process described by deterministic ODE.

Results and discussion

With the increase of stimulate intensity $k_1$, regular signals are found in two distinct regions (Fig. 2). Oscillation occurs with frequency similar to other simulation works (Dupont et al. 2008; Dupont and Combettes 2009) present in the higher $k_1$ area (O2 region). In a very narrow scope below this level (O1 region), the calcium signal with higher amplitude is found, whose frequency is much lower and close to the experimental results (Dupont et al. 2008). This may related to the incorporation of the G-protein coupled receptor dynamics in our model (Dupont et al. 2008; Kummer et al. 2000). Between these oscillation areas, there’s a transition area with weak, unregulated signals.

Further characterization of the signal regularity reveal that the signal in the two oscillation areas show different dependency of CV on the number of the cluster (Fig. 3). In O1, there’s always an intermediate number of clusters that show the optimal CV, while in O2, CV generally decrease with the number of the cluster. This kind of difference is also reported in other works. In Shuai and Jung’s work (2002), an optimal amount of channel in a cluster is found to show coherent responses. In Dupont et al.’s (2008) investigation, however, monotone dependency similar to the O2 area is reported. It is shown here that this difference is actually the response in a different dynamic area. The channel opening character vs calcium concentration diagram in these two areas (Fig. 4) reveals that in the O1 area, the percentage of the opened channel changes in a wider range and can arrive at almost 100 % while the Ca$^{2+}$ concentration changes in a wider scope, which resembles the excitable system character. In O2, however, only part of the channels are involved. This is similar with the responding behavior in the oscillatory area. Therefore, clustering in O1 is the compromise between the weak stimulation and cell sensitivity, which guarantees the robustness of oscillation, while in O2, the number of clusters mainly reflects the extent of internal fluctuation.

The change of CV with external stimulate intensity $k_1$ is also investigated. As shown in Fig. 5, it linearly increases with $k_1$ in O1, while in the O2 area, CV first decreases dramatically and then increases. This counterintuitive difference may be connected with the experimental observation that an increased agonist dose may induce oscillation with reduced CV (Dupont et al. 2008). From Fig. 2 we can figure out that the special dependency in O2 area happens in the scope of $k_1$ when the system transforms from an excitable-like area to an oscillatory-like area. Thus, the optimal stimulation amount essentially indicates the selective signal response to the medium character. Note that the amplitude of IP$_3$ concentration linearly increases with $k_1$ in this area, it is not strange that a similar tendency is obtained if it is taken as the stimulate intensity (Dupont et al. 2008).

These results suggest an interesting “modulation origin shift” phenomenon. The CV of O1 oscillations increases monotonously with external stimulation, but have an
optimal value upon the adjusting of the number of clusters. On the contrary, the CV of O2 oscillations decreases monotonously with the number of clusters but the external stimulation can adjust it to have the lowest value. This indicates in different dynamic regions, that the signal can respond to different regulatory conditions. Excitable signals under weak stimulation respond to the modulation of detailed clustering properties, while for oscillatory signals obtained at stronger stimulation, the global stimulation level takes the clustering properties’ place to control the signal.

One drawback of this model is that spatial information is not included. We still choose to use it for the following reasons. For one thing, including spatial distribution is too time consuming for stochastic simulations and the IP$_3$R dynamic model employed here is too simplified for the detailed description. For another thing, the main task of this work is to discuss the whole cell coding response, which is less related to spatial character, homogenous assumption is still acceptable and effective. Besides, following some previous work that modeled the agonist induced calcium signal, we include the DAG catalyzed PLC formation process in the model, which has less experimental evidence support. The O1 oscillations are proved to be sensitive to the presence of this item since they exist in a quite narrow scope. But these upstream regulations generally have less influence on the dependence of signal on stochastic IP$_3$R dynamics, which is the main topic of this work. The results obtained here are helpful to analysis the modulation respond in signaling and understand the essential relationship between the stochastic nature and the coding response.

Summary

In this work, we explore the influence of the stochastic IP$_3$R dynamic on the coding of agonist induced calcium signal by hybrid simulations. Two separate oscillation areas are revealed in different stimulation levels. The clustering of IP$_3$R is shown to be capable of optimizing the signal when the stimulation is weak, but it gradually lost the modulation ability with the increase of stimulation level. The stimulation intensity plays the role of signal control instead. Detailed channel opening characters reveal
the system in the low stimulation area may be corresponding to the excitable region while the high stimulation part resembles the oscillatory system. These results provide an explanation for the disagreement in the previous reports that show different CV dependency on the cluster numbers. It is indeed the different signaling response in different dynamic regions. The results obtained here are helpful to analysis calcium coding from the systems biology aspect and to understand the essential contribution of the calcium signal’s stochastic nature on its whole cell response.

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References


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