The model of glutamate-induced intracellular Ca^{2+} oscillation and intercellular Ca^{2+} wave in brain astrocytes.

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Abstract

An astrocyte has glutamate receptors as well as neurons do and it has been suggested to participate in the information processing of with neurons in brain. A cultured hippocampal astrocytes have spontaneous oscillation of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$). The application of Glu induces not only $[Ca^{2+}]i$ oscillation but also Ca^{2+} wave which is propagating among the astrocytes. In the present study, we proposed the PLCδ model which could induce some types of glutamate – induced $[Ca^{2+}]i$ responses and the intercellular Ca^{2+} wave observed in the experiment. Our simulation results suggested that PLCδ is a key molecule for $[Ca^{2+}]i$ oscillation and wave.

Keywords: $[Ca^{2+}]i$ oscillation; Astrocyte; Phospholipase C δ; IP_3; Ca^{2+} wave.

1. Introduction

One kind of glial cells, an astrocyte participates in the brain information processing with neurons. The astrocyte has some glutamate (Glu) receptors as a neuron does. The cultured hippocampal astrocytes have spontaneous oscillation of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) [2]. The application of Glu induces four patterns of $[Ca^{2+}]i$ responses in the astrocytes. They are a sustained $[Ca^{2+}]i$ oscillation, a damped oscillation, a step - rise response and a sustained oscillation whose frequency gradually decreases [2]. Glu activates the metabotropic Glu receptors (mGluR) and produces inositol 1,4,5-trisphosphate (IP_3) through G-protein and phospholipase C β (PLC β) system. IP_3 binds to IP_3 receptor/ Ca^{2+} channel (IP_3R) of the endoplasmic reticulum (ER) Ca^{2+} store and the store releases Ca^{2+} to the intracellular space. The application of Glu also induces Ca^{2+} wave propagating through some astrocytes [1]. The model, which simulates the above $[Ca^{2+}]i$ responses by Glu stimulation, has not yet been proposed. We propose our PLC δ model in the present paper.

2. Model

2.1 IP_3R dynamics (De Young and Keizer's model; YK model ([6]))

YK model was proposed for $[Ca^{2+}]i$ oscillation in the non-excitable cells [6]. We will explain the model briefly. IP_3R consists of four subunits to form a functional channel. It is assumed that a subunit has three binding sites, one is for IP_3, another is for Ca^{2+} and the last is for calmodulin (CaM). Hence one subunit has eight states dependent on the state of the occupation of the ligand binding sites. They are named as Sijk, where I, j and k equals 0 or

1. Binding site j is occupied if j = 1. Binding site 1 is the IP_3 binding site, site 2 is the Ca^{2+} activation site, and site 3 is the CaM binding site. The fraction of subunits in state Sijk is denoted by xijk. Only the state s110 contributes to the Ca^{2+} conductance of IP_3R and three subunits of four must be in this state for the channel to be open. Thus the open probability is proportional to $x110^3$. CaM is activated by Ca^{2+} and the activated CaM suppresses Ca^{2+} release from the store [3]. Hence we interpreted the CaM binding site as a Ca^{2+} inactivation site. Assuming the mass action kinetics, the equations of the x000 are as follows. The other valuables x001- x111 were all calculated in the same way.

$$dx000/dt = a4\ d4\ x001 - a4\ [Ca^{2+}]\ x000$$
$$+ a5\ d5\ x010 - a5\ [Ca^{2+}]\ x000$$
$$+ a1\ d1\ x100 - a1\ [IP_3]\ x000$$

2.2 Ca^{2+} dynamics

$[Ca^{2+}]i$ is calculated by the following equations,

$$d[Ca^{2+}]i/dt = c1\ (v1\ x110^3 + v2)([Ca^{2+}]_{ER} - [Ca^{2+}]i) + v3\ [Ca^{2+}]^2 / ([Ca^{2+}]^2 + k3^2)$$

where the first term is the outward flux of Ca^{2+} through IP_3R and the second term is the inward flux through ER Ca^{2+} pump. $[Ca^{2+}]$ influx from the extracellular space was not considered. That is, we assumed the total $[Ca^{2+}]i$ is constant. Total $[Ca^{2+}]i$, c0 is as follows:

$c0 = c1\ [Ca^{2+}]_{ER} + [Ca^{2+}]i$. (where c1 is the ratio of the volume of ER to that of cytosol)

2.3 IP_3 dynamics in the case of YK model

In YK model, the equations of $[IP_3]$ dynamics are as follows:

$$d[IP_3]/dt = vPLC\beta - Id\ [IP_3] \quad (1)$$

$$vPLC\beta = Ip\ f(t)$$

vPLCβ corresponds to the degree of Glu stimulation. Ip and f(t) are considered as the Glu concentration and the duration of Glu stimulation. Glu stimulation induces Glu - induced $[Ca^{2+}]i$ responses (GICR).

2.4 IP_3 dynamics in the case of PLCδ model

We added the term of the reaction rate of PLCδ (vPLCδ) to the above equation (1) in YK model. $[IP_3]$ dynamics in our model is as follows;

$$d[IP_3]/dt = vPLC\beta + vPLC\delta - Id\ [IP_3]\ ,\ vPLC\delta = vca\ [Ca^{2+}]^2 / ([Ca^{2+}]^2 + kca^2)$$

Vca corresponds to the concentration of the expressed PLCδ in an astrocyte. Kca is the dissociation constant of PLCδ. It is found PLCδ is in astrocytes [5]. Ca^{2+} activates PLCδ and PLCδ produces IP_3. Vca and kca have not yet been measured in an astrocyte. Hence the values in the rat lever [4] were used in our simulation.

2.5 The simulation of Ca^{2+} wave

We connected two hundred astrocytes of YK or PLCδ models with the diffusion of Ca^{2+} and IP_3 in one dimension. Diffusion coefficients of IP_3 and Ca^{2+} are 280 and 20 $\mu m\ sec^{-2}$, respectively.

3. Results

3.1 YK model

Astrocytic $[Ca^{2+}]$ did not oscillate without Glu stimulation. When the astrocyte is

stimulated, astrocytic [Ca^{2+}] began to oscillate (Fig. 1A). YK model could reproduce three patterns of GICR, while it could not simulate the sustained oscillation whose frequency gradually decreases. The model did not have the spontaneous [Ca^{2+}]i oscillation (SCO), either. In YK model, [Ca^{2+}]i oscillated and [IP_3] did not. Moreover we could not observe Ca^{2+} wave in the model, either (Fig. 3).

3.2 PLCδ model

This model shows that when the concentration of Glu stimulation, which is referred as Ip, increased, the steady [Ca^{2+}]i was unstable and [Ca^{2+}]i began to oscillate (Fig. 1B). The oscillation region shifted to the left when vca value was increased. Vca reflects the concentration of the expressed PLCδ in an astrocyte. Hence, the more PLCδ is expressed in an astrocyte, the easier it is for the cell to begin [Ca^{2+}]i oscillation. As observed by YK model, three patterns of GICR were also observed in this model (Fig. 1C - E). PLCδ model could also reproduce sustained oscillation (Fig. 1C), a damped oscillation (Fig. 1D) and a step - rise response (Fig. 1E). Note that the wave form of the sustained oscillation is similar to that of sine wave.

(Figure 1 is here.)

In PLCδ model, [IP_3] could oscillate synchronously with [Ca^{2+}]i (Fig. 1C – E).

PLCδ model could reproduce SCO, while YK model could not (Fig. 2A). Without Glu stimulation, we haven't observed SCO if vca was below 0.99. With the increase in vca value, SCO appeared. SCO was observed in a range between the two vca values from 0.99 to 1.51

(Fig. 2B). When vca increased above 1.51, $[Ca^{2+}]i$ reached to another steady state. Therefore, the proper conc. of PLCδ in an astrocyte is necessary for SCO to be induced. In SCO, $[Ca^{2+}]i$ gradually increased at first and increased rapidly after then. The forms of $[Ca^{2+}]i$ oscillation is like that of the relaxation oscillation (Fig. 2A). In addition to that, we compared the period of Glu – induced oscillation with that of SCO. Fig. 2C shows that the periods of SCO are longer than those of Glu - induced oscillation at both vca = 0.4 and 0.8. Vca value corresponds to the concentration of PLC δ expressed in an astrocyte. It is suggested that the periods and the wave form of $[Ca^{2+}]i$ oscillation are modulated by the conc. of the expressed PLC δ .

Our PLC δ model could induce intercellular Ca^{2+} wave, too, although YK model could not (Fig. 3). For the generation of Ca^{2+} wave PLC δ is also necessary. As far as we know, our PLC δ model can simulate both astrocytic $[Ca^{2+}]i$ oscillation and intracellular Ca^{2+} wave for the first time.

(Figure 2 is here.)

(Figure 3 is here.)

4. Discussion

When vca increased, SCO appeared. This result suggested that the emergence of an astrocytic SCO is controlled by the conc. of the expressed PLC δ ([PLC δ]). In physiological studies, some astrocytes have SCO while the others have not. The astrocytes, which have SCO, can have the optimum [PLC δ].

In addition, our results showed that [PLC δ] modulated the range of Glu concentration to induce $[Ca^{2+}]i$ oscillation (Fig. 1B), the period (Fig. 2C) and the shape of $[Ca^{2+}]i$ oscillation (Fig. 1A and 2A). It can also determine whether Ca^{2+} wave is generated or not (Fig. 3). It is suggested that the positive feedback loop of PLC δ is important to form spatio – temporal pattern of $[Ca^{2+}]i$.

According to YK model, Glu couldn't induce $[IP_3]$ oscillation in an astrocyte, while PLC δ model predicts that $[IP_3]$ oscillates synchronously with $[Ca^{2+}]i$. So far, $[IP_3]$ has not yet been measured with Glu stimulation. If you can check whether $[IP_3]$ oscillates or not with Glu stimulation, you can see the validity between YK and PLC δ model. Further studies will be necessary.

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Figure Legends

Fig. 1. (A) The hopf bifurcation diagram of YK model. High concentration of IP_3 bifurcated the steady state of $[Ca^{2+}]i$. **(B)** The hopf bifurcation diagram of PLC δ model. The solid and dashed lines indicate the bifurcation curves at vca = 0.4 and 0.8 $\mu M^{-1}s^{-1}$. **C, D, E.** Three patterns of GICR in PLC δ model. An astrocyte was stimulated at the various strength of Ip from 200 sec for 400 sec. **(C)** a sustained oscillation (Ip = 0.4). **(D)** a damped oscillation. **(E)** a step rise response (Ip = 0.18).

Fig.2. (A) SCO induced by PLC δ model (when vca is 1.00). **(B)** The hopf bifurcation diagram of PLC δ model. **(C)** Comparison of the periods of GICR in left and center columns and the period of SCO in the right column by boxplot. The horizontal lines in boxes show the medians of the periods.

Fig. 3. Astrocytic Ca^{2+} wave in YK and PLC δ models. The number 100 astrocyte in the figure was stimulated by Glu.

Figures

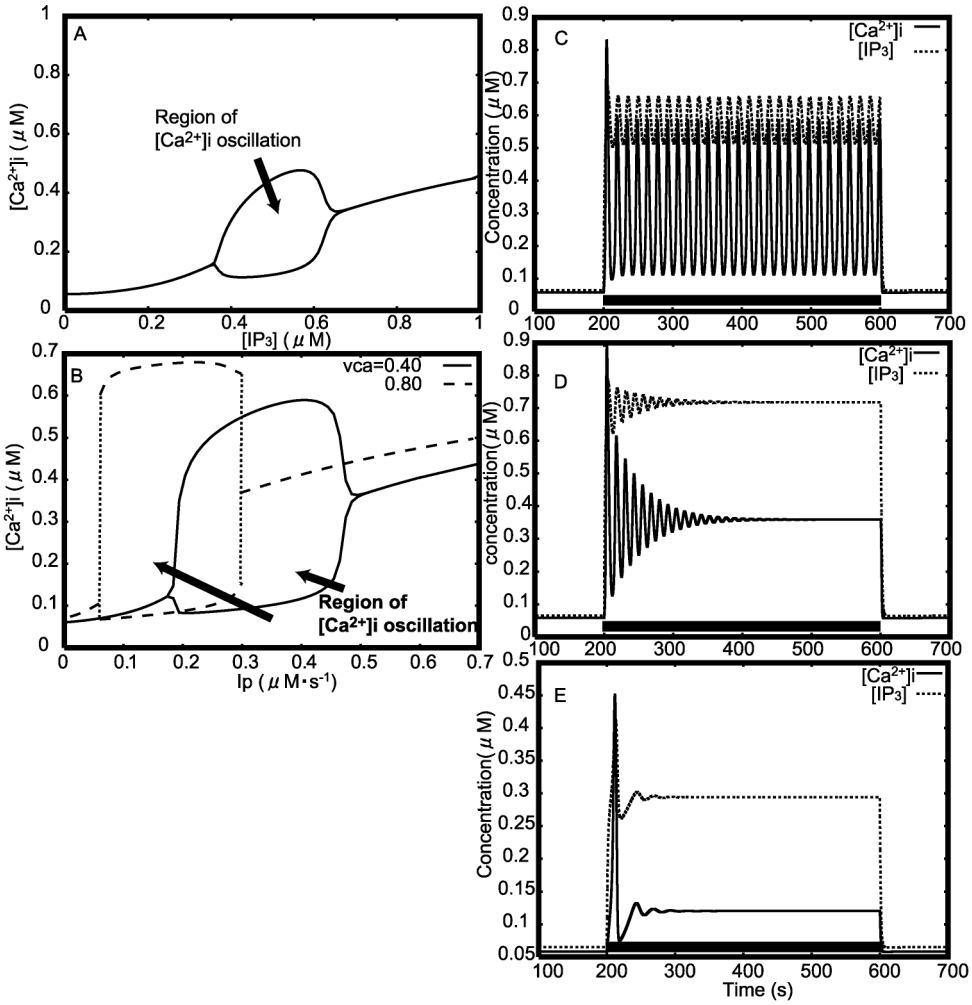

Figure 1.

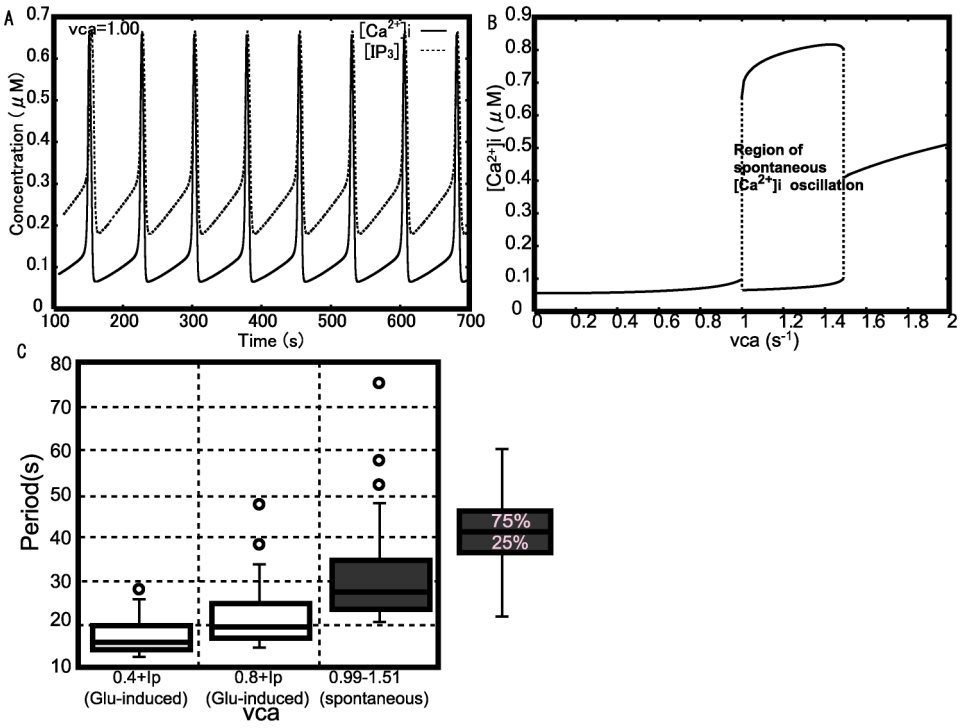
A
vca=1.00
[Ca2+]i
[IP3]
Concentration (μM)
Time (s)
B
[Ca2+]i (μM)
Region of
spontaneous
[Ca2+]i oscillation
vca (s-1)
C
Period(s)
75%
25%
0.4+Ip
(Glu-induced)
0.8+Ip
(Glu-induced)
0.99-1.51
(spontaneous)
vca

Figure 2.

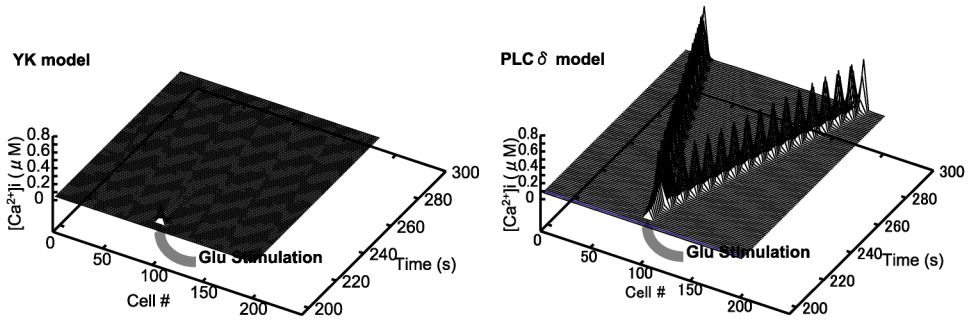
YK model
PLC δ model
[Ca2+]i (μM)
0.8
0.6
0.4
0.2
0
Glu Stimulation
Cell #
0
50
100
150
200
Time (s)
200
220
240
260
280
300

Figure 3.