Localization of Activated Ca^{2+}/calmodulin-Dependent Protein kinase II within a spine: Modeling and computer simulation

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Abstract

Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) plays a crucial role in the induction of long-term potentiation (LTP). One form of LTP expression is thought to be the phosphorylation of glutamate receptor channels by CaMKII. Thus the localization of activated CaMKII within a spine will affect the expression of LTP. The translocation of activated CaMKII to PSD was reported. Here we investigated the localization of activated CaMKII in a model dendritic spine, and found that the diffusion of calmodulin and calcineurin but not CaMKII contribute to the localization of activated CaMKII near PSD. This will offer an additional mechanism for the localization.

Keywords: Synaptic plasticity; Spine; CaMKII; Calmodulin; Localization

1. Introduction

Long-term potentiation (LTP) and depression (LTD) of synaptic efficacy are thought to be controlled by the balance of activated kinases and phosphatases. The activation of kinases and phosphatases is triggered by the elevation of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) mainly thorough NMDA receptors (NMDARs) [1, 8]. By the induction of LTP, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) phosphorylates AMPA receptors (AMPARs) [5] leading to the alteration of AMPAR channel properties. The translocation of CaMKII to the postsynaptic density (PSD) by the activation of NMDA receptors (NMDARs) is reported [8].

The translocation of CaMKII is an important mechanism for the localization of CaMKII. To see the localization of CaMKII in a spine, we constructed a biochemical and biophysical model for CaMKII within a 3D shape mimicking the morphology of a spine, and the spatio-temporal characteristics of activated CaMKII ($CaMKII^*$) were investigated by the simulation using a modeling and simulation software, A-Cell-3D, which was developed for the construction and simulation of biochemical, electrical, and biophysical models within various morphologies including spines and whole neurons. The simulation results showed that the translocation of $CaMKII^*$

effectively localized its activity at PSD, and reduced its activity at the rest of the region within a spine. To our surprise, however, the diffusion of calmodulin and calcineurin without the translocation of CaMKII resulted in localization of $CaMKII^*$ near PSD region in a spine head.

2. Biochemical reaction model

The present biochemical reaction models were constructed using A-Cell, which was developed for the construction and simulation of biochemical reactions and/or electrical equivalent circuit models in a neuron [2].

NMDARs open upon the combined events of the binding of transmitter molecules released from the presynaptic terminal and the sufficient depolarization of postsynaptic membrane. Calcium ions flow into a spine through open NMDARs. In the present study, the increase in $[Ca^{2+}]_i$ was simulated using the following equation.

$$Ca(t) = Ca_0 (1 - \exp(-t/\tau_r)) \exp(-t/\tau_f) f(t) \qquad (1)$$

The value of Ca_0 was so determined that the single release of glutamate resulted in 1μM increase in $[Ca^{2+}]_i$. τ_r and τ_f are rising and falling time constant of $[Ca^{2+}]_i$. f(t) is a function simulating the desensitization of NMDARs. The stimulation frequency was 100Hz in the simulations.

Calmodulin (CaM) is known to be activated by the binding of four Ca^{2+} molecules, and the following scheme was employed in the present study.

$$CaM_0 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} CaM_1 \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} CaM_2 \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} CaM_3 \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} CaM_4 \qquad (2)$$

CaM_4, which is the four Ca^{2+}-bound CaM, is the activated state. Values for k_1, k_2, k_3, k_4, k_{-1}, k_{-2}, k_{-3} and k_{-4} were 2.22×10^7/M/s, 1.82×10^7/M/s, 9.3×10^6/M/s, 7.14×10^7/M/s, 20/s, 20/s, 200/s and 200/s, respectively.

CaMKII is known to be activated by the binding of activated CaM, and this leads to the autophosphorylation of CaMKII. The following scheme was employed.

$$K \underset{k_{-5}}{\overset{k_5}{\rightleftarrows}} K.CaM_4 \xrightarrow{k_6} K^*.CaM_4 \underset{k_7}{\overset{k_{-7}}{\rightleftarrows}} K^* \qquad (3)$$

K^* is the autophosphorylated CaMKII, and $K.CaM_4$, $K^*.CaM_4$ and K^* are the activated states. Values for k_5, k_{-5}, k_6, k_7 and k_{-7} were 1.5×10^8/M/s, 2.17/s, 0.1/s, 5×10^7/M/s and 0.001/s, respectively.

Calcineurin (CaN) is important phosphatase for the induction of LTD [6]. CaN is also reported to bind with four Ca^{2+} and is known to be activated by the binding of CaM [3, 4]. This will affect the

activation of CaMKII, because there is a possibility of competitive binding of CaM to both CaMKII and CaN. The scheme for the activation of CaN is shown below.

$$\begin{aligned} &CaN_0 \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} CaN_1 \underset{k_{-9}}{\overset{k_9}{\rightleftharpoons}} CaN_2 \underset{k_{-10}}{\overset{k_{10}}{\rightleftharpoons}} CaN_3 \underset{k_{-11}}{\overset{k_{11}}{\rightleftharpoons}} CaN_4 \\ &CaN_4 \underset{k_{-12}}{\overset{k_{12}}{\rightleftharpoons}} CaN_4.CaM_4 \end{aligned} \qquad (4)$$

Values for k_8, k_9, k_{10}, k_{11}, k_{12}, k_{-8}, k_{-9}, k_{-10}, k_{-11} and k_{-12} were 2.5×10^8/M/s, 1.33×10^7/M/s, 1.33×10^7/M/s, 1.33×10^7/M/s, 5×10^8/M/s, 6/s, 200/s, 200/s, 200/s and 0.005/s, respectively.

3. Morphological model of a spine

Morphological model of a spine was constructed using A-Cell-3D. A-Cell-3D generates compartment models by the specification of small number of parameters.

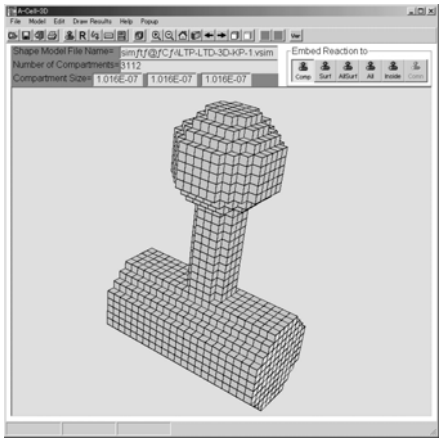

Figure1 Compartment model of a spine using A-Cell-3D

The biochemical and biophysical models constructed by A-Cell are embedded to the shape generated by A-Cell-3D. Fig.1 shows the compartment model of a spine. The diameter of spine head and dendrite is 1μm, and the length and the diameter of spine neck are 2 μm and 0.4 μm, respectively. The total number of automatically generated compartments is 3112, and the compartment size is $0.102\times0.102\times0.102\mu m^3$.

The diffusion of molecules within cytoplasmic space was calculated using Fick's low. The diffusion coefficients for Ca^{2+}, CaM, CaMKII and CaN were 10^{-10} m^2/sec, 10^{-10} m^2/sec, 3.4×10^{-11} m^2/sec, 10^{-10} m^2/sec, respectively.

4. Results

Fig.2 shows the simulation results of spatio-temporal characteristics of $[Ca^{2+}]_i$. The peak $[Ca^{2+}]_i$ was 6.5μM, and it decayed to basal level rapidly after the cessation of the stimulation (right most panel in Fig.2) as was suggested from experiments [7].

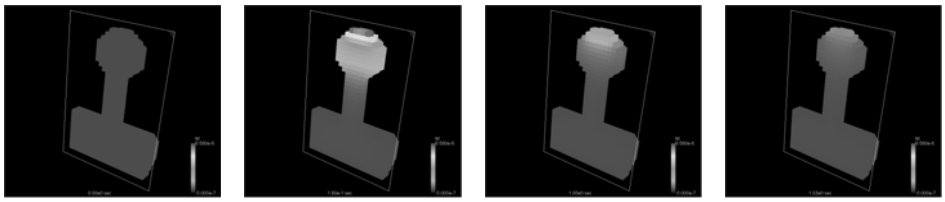

Figure 2 Spatio-temporal characteristics of $[Ca^{2+}]_i$. Distributions of Ca^{2+} before (0sec), during (0.18 and 1sec), and just after (1.03sec) stimulation are shown.

Next we investigated the localization of activated CaMKII by changing simulation conditions. These were combination of diffusion of molecules and translocation of CaMKII*. The set of experiments are shown in Table 1.

Table 1 Experimental set for CaMKII* localization

experiment	diffusion			translocation of CaMKII*
#	CaM	CaN	CaMKII	
Exp.1	no	no	no	no
Exp.2	yes	no	no	no
Exp.3	yes	yes	no	no
Exp.4	yes	yes	yes	no
Exp.5	yes	yes	yes	yes

Simulation results at 1sec after the cessation of stimulation are shown in Fig.3.

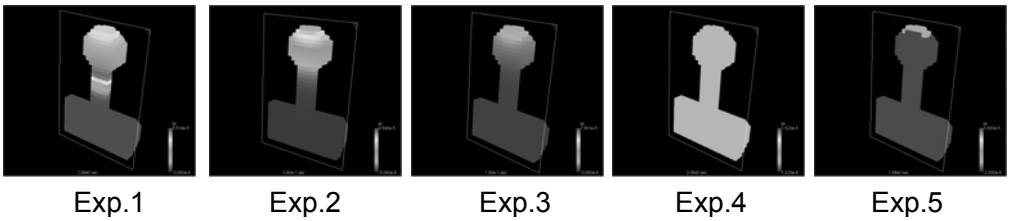

Figure 3 Spatial localization of activated CaMKII

In Exp.1, where there is no diffusible molecules except Ca^{2+}, the max [$CaMKII^*$] is located in the spine neck but not in the spine head. $CaMKII^*$ is not localized within the spine head. In the condition where CaM diffuses (Exp.2), $CaMKII^*$ is localized within the spine head, and its peak is appeared at PSD. When both CaM and CaN diffuse (Exp.3), $CaMKII^*$ localizes within much narrower region than in Exp.2. When CaM, CaN and CaMKII diffuse (Exp.4), however, the localization of $CaMKII^*$ is completely abolished, and its concentration is almost uniform through the spine and the dendrite. When translocation of $CaMKII^*$ is introduced into the model (Exp.5), $CaMKII^*$ localized at PSD.

It was our surprise that the diffusion of CaM and CaN increased localization of $CaMKII^*$ (Exp.3), which is clearly seen in Fig.4 where normalized [CaMKII*] as a function of relative distance from PSD to dendrite is shown.

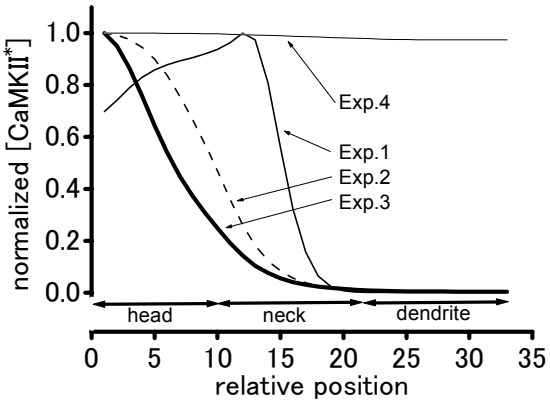

Figure 4 Spatial profile of activated CaMKII

It is natural that the translocation of $CaMKII^*$ increased its localization (Exp.5). In the present study, we found that the diffusion of CaM and CaN localizes $CaMKII^*$ near PSD in a spine without its translocation to the membrane.

5. Conclusion

The localization of activated CaMKII was investigated in the model spine using modeling and simulation tool A-Cell and A-Cell-3D. We found that the diffusion of CaM and CaN localized activated CaMKII within the spine head near PSD, even if the translocation of $CaMKII^*$ is not involved in the model. This suggests the existence of additional mechanisms for the localization of $CaMKII^*$ other than the translocation. Diffusion of CaM and CaN increased the localization when only CaM diffuses suggesting the competitive binding of CaM to CaMKII and CaN as was discussed in Biochemical reaction model. section.

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Kazuhisa Ichikawa received his Ph.D. from Kyoto University for his work on "A model for open-close control of cation channels in the plasma membrane of retinal rod outer segments". His current research topic is modeling on the signal transduction in nerve cells, especially on the change in the long-term synaptic efficacy.