SENSITIVITY OF AMPA RECEPTOR CHANNEL TO CALCIUM OSCILLATIONS: A COMPUTATIONAL STUDY

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Abstract

We used a computational model of biochemical pathways that are involved in the phosphorylation/dephosphorylation of AMPA receptor to study the responses of the receptor to calcium oscillations. In the model, the biochemical pathways are assumed to be located immediately under the postsynaptic membrane, and we included three states of AMPA receptor (dephosphorylated, and phosphorylated in one or in two sites). To characterize the effects of calcium oscillation on the AMPA receptor, we exposed the model to stimuli with three varying parameters, namely frequency, number of pulses and calcium spike duration. Our model showed sensitivity to all of these three parameters.

Keywords: AMPA; Biochemical networks; Plasticity; Calcium Oscillation

1. Introduction

Calcium spike trains are usual responses of cells to stimuli [9] and have been implicated with activity of some kinases, such as CaMKII [3, 5] and protein kinase C [8],

which are sensitive to calcium oscillations. Protein kinases are involved in several intracellular processes, including the phosphorylation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor channels [7], which are implicated in excitatory synaptic transmission in the central nervous system [12]. The AMPA receptor is a heteromer, composed of four subunits (GluR1-4) [4]. The functions of this receptor channel can be regulated by the phosphorylation and dephosphorylation of individual subunit proteins [2]. The AMPA receptor is phosphorylated by cAMP-dependent protein kinase (PKA), protein kinase C (PKC), calmodulin kinase II (CaMKII) and other unspecified kinases, and dephosphorylated by some phosphatases like protein phosphatase 1 (PP1), calcineurin and PP2A [4]. AMPA receptor channels mediate fast synaptic transmission in response to presynaptic glutamate release [11] and play an important role in synaptic plasticity [10, 13].

Recently, Lee et al. [6] have proposed a model for bidirectional synaptic plasticity regulated by intracellular calcium concentration in which CaMKII, PKA, PP1 and PP2A act on AMPA receptor channels. In that model (Fig. 1A) the phosphorylation and dephosphorylation of AMPA receptors occurs in two sites (Serine 831- CaMKII site; and Serine 845 - PKA site).

In this work, we constructed a computational model incorporating the bidirectional model of Lee et al. [6] and the biochemical pathways of Bhalla and Iyengar [1] to study the responses of these receptors to calcium oscillations.

2. Methods

Bhalla and Iyengar [1] model of biochemical pathways includes CaM KII, calmodulin (CaM), adenylate cyclase (AC), phosphodiesterase (PDE), protein phosphatase 1 (PP1), calcineriun, and cAMP-dependent protein kinase (PKA) (Fig. 1B). In our computational model, these pathways were assumed to be located immediately under the postsynaptic membrane.

We also included three states of AMPA receptor channel (dephosphorylated, phosphorylated in one site, and phosphorylated in two sites) based on the work of Lee et al. [6] to study the behavior of the receptor channel exposed to different calcium stimuli.

To characterize the effects of calcium oscillations on the AMPA receptor channel, we exposed our model to stimuli with different values of i) frequency (measured by the inverse of the constant interspike interval), viz. 0 Hz (no frequency), 0.1 Hz, 0.5 Hz, 0.8Hz, 2.5 Hz, 5.0 Hz, and 10 Hz; ii) number of pulses during the exposition time, viz. 6 or 75; and iii) duration of calcium spike, viz. 80 ms or 1 s. The stimulation protocol was adapted from De Koninck and Schulman [3]. All stimuli have the same calcium concentration (1μM) and total time of exposition to calcium (6s). The simulation was done in the GENESIS 2.2 neural simulator.

3 Results

To observe the effects of calcium oscillation on the AMPA receptor channel, we measured the activity of CaMKII, PKA and PP1 (Fig. 2) and the phosphorylation and dephosphorylation of AMPA receptor channel consequently to the effects of calcium oscillation on the biochemical pathways (Fig. 3). The results indicate that the

phosphorylation and dephosphorylation of the AMPA receptor channel are affected by the oscillations.

In Fig. 2A, the activities of PKA, CaMKII and PP1 show different responses to stimuli with the same number of pulses (6) and duration (1s) but varying frequencies (0 Hz, 0.1 Hz, 0.5 Hz and 0.8 Hz). The increase in the stimulus frequency causes an increase in the activity of CaMKII, a decrease in the activity of PP1 and a reduction in the latency time of the first PKA spike. The increase in the PKA activity together with the decrease in the PP1 activity changes the AMPA receptor state (according to the model of Fig. 1A) from the dephosphorylated state (AMPA-0P) to the phosphorylated in one site state (AMPA-1P) (Fig. 3A). On the other hand, the increase in the CaMKII activity together with the decrease in the PP1 activity changes the AMPA receptor state (according to the model of Fig. 1A) from the phosphorylated in one site state (AMPA-1P) to the phosphorylated in two sites state (AMPA-2P) (Fig. 3A).

In Fig. 2B, PKA, CaMKII and PP1 responded to another stimulus protocol in which stimuli with constant number of pulses (75) and duration (80 ms) were applied with different frequencies (0 Hz, 2.5 Hz, 5 Hz and 10 Hz). In this second protocol, the increase in the frequency of stimulus increases the activity of CaMKII, decreases the activity of PP1 and the PKA spike occurs only for the highest frequency stimulus (10.0 Hz). Consequently, the AMPA receptor channel does not change its state for lower frequencies (0.0 Hz, 2.5 Hz, 5.0 Hz) but only for the highest frequency stimulus (10.0 Hz) (Fig 3B).

4. Discussion

All stimulation protocols had the same calcium concentration (1μM) and total time of exposition to calcium (6s), suggesting that the different responses observed in the model come from a sensitivity of the system to frequency, number of pulses and duration of calcium spikes.

Our results indicate that the AMPA receptor channel can be influenced by the calcium oscillation through the variation in the activity of some kinases and phosphatases involved in the phosphorylation/dephosphorylation of the receptor.

Acknowledgments

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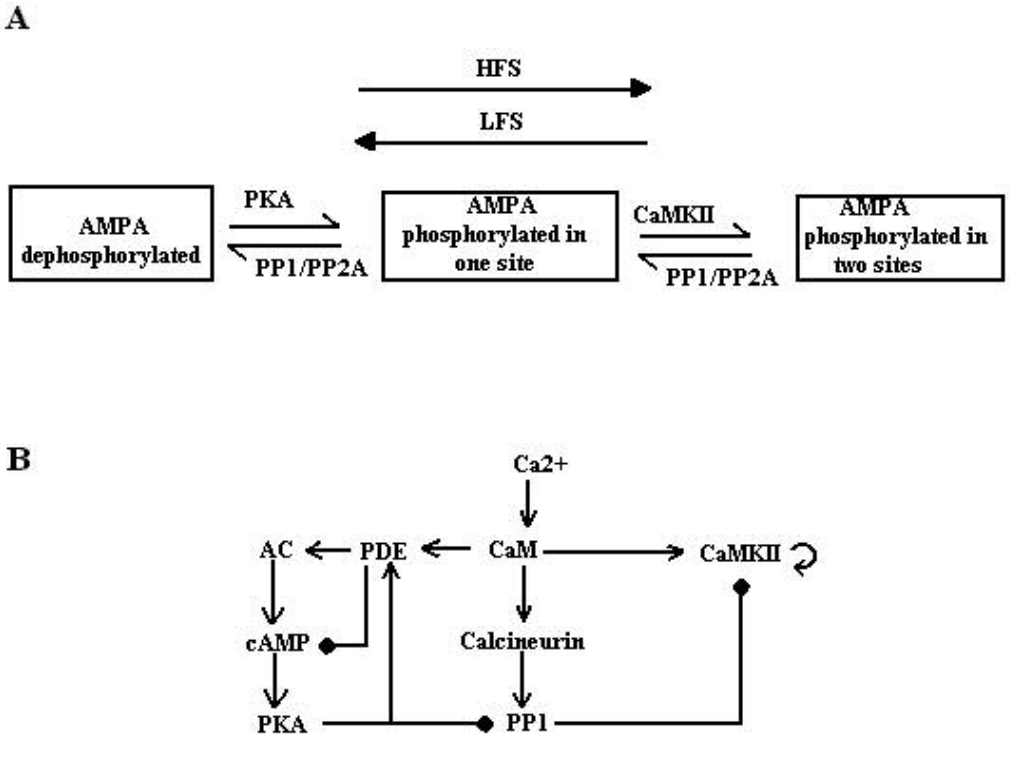
A
HFS
LFS
AMPA dephosphorylated
PKA
PP1/PP2A
AMPA phosphorylated in one site
CaMKII
PP1/PP2A
AMPA phosphorylated in two sites
B
Ca2+
AC
PDE
CaM
CaMKII
cAMP
Calcineurin
PKA
PP1

Fig. 1

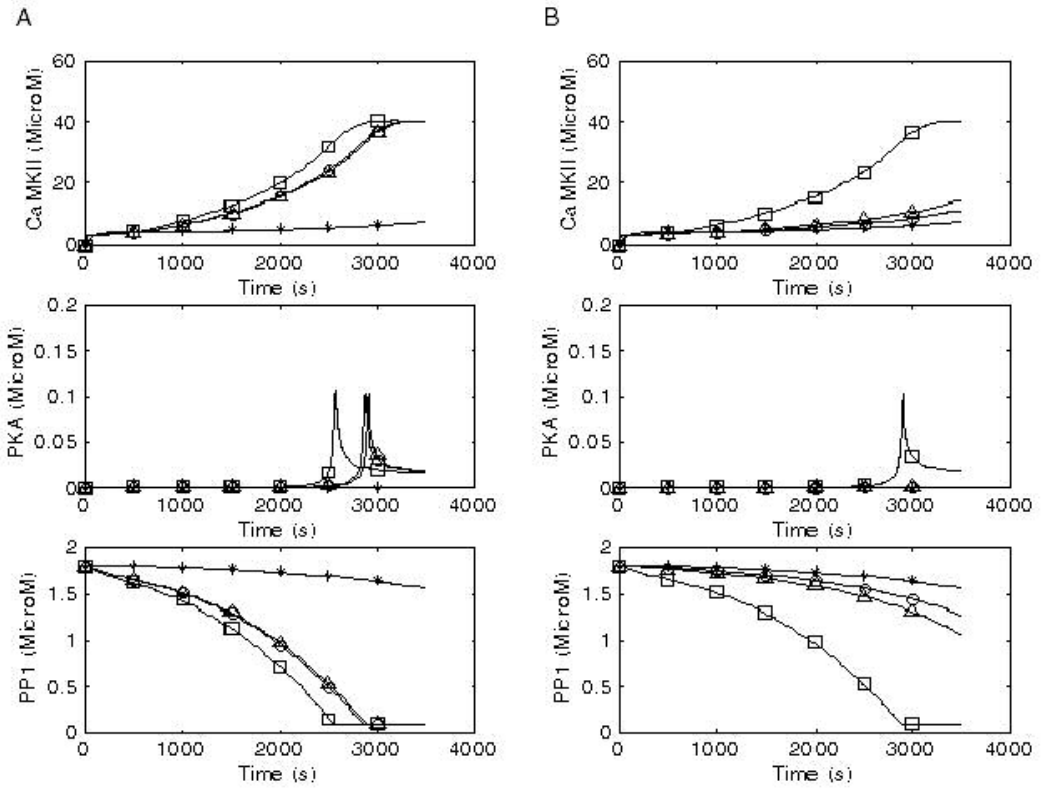
A
B
Ca MKII (MicroM)
PKA (MicroM)
PP1 (MicroM)
Time (s)
0
1000
2000
3000
4000
0
20
40
60
0.05
0.1
0.15
0.2
0.5
1
1.5
2

Fig. 2

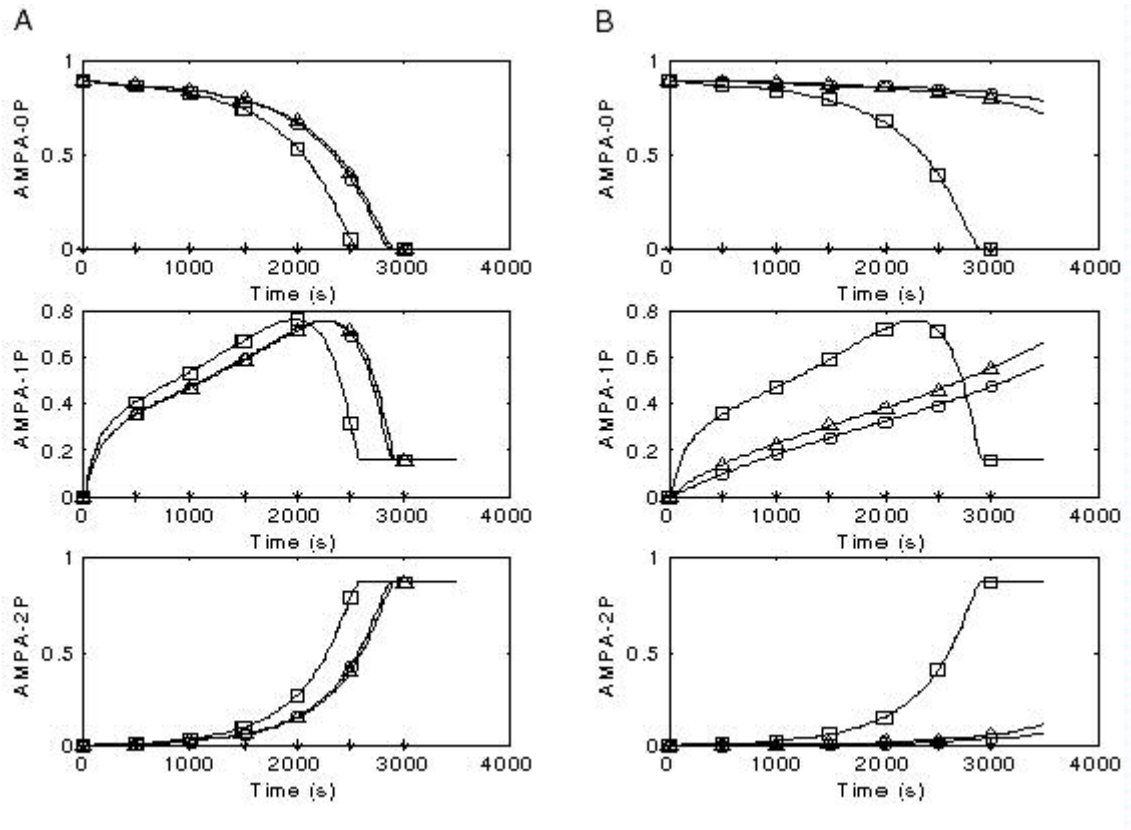
A
B
AMPA-0P
AMPA-1P
AMPA-2P
Time (s)
0
1000
2000
3000
4000
0.2
0.4
0.5
0.6
0.8
1

Fig. 3

Fig. 1. A) Bidirectional model of synaptic plasticity, modified from Lee et al [6]. In this model, the AMPA receptor can be phosphorylated in two sites (Serine 831- CaMKII site; and Serine 845 - PKA site) and dephosphorylated by PP1 and PP2A. High frequency stimulation (HFS) and low frequency stimulation (LFS) shift the AMPA receptor state to the right or left, respectively. B) Scheme of the biochemical pathways located immediately under the postsynaptic membrane. The interactions between these biochemical pathways suggest they can form a complex biochemical network, with feedback loops and cross-talks between the signaling pathways, and may have properties that are nonintuitive (modified from [1]).

Fig. 2. Responses of PKA, PP1 and CaMKII to stimuli with different numbers of pulses, frequencies and duration of calcium spikes. A) PKA, PP1 and CaMKII responses to 6 pulses of 1s duration with 0Hz(asterisk), 0.1Hz(circle), 0.5Hz(triangle) and 0.8Hz(square). B) PKA, PP1 and CaMKII responses to 75 pulses of 80ms duration with 0Hz(asterisk), 2.5Hz(circle), 5Hz(triangle) and 10Hz(square).

Fig. 3. Responses of AMPA receptors to stimuli with different numbers of pulses, frequencies and duration of calcium spikes. A) Dephosphorylated AMPA (AMPA-0P), phosphorylated in one site AMPA (AMPA-1P) and phosphorylated in two sites AMPA (AMPA-2P) responses to 6 pulses of 1s duration with 0Hz(asterisk), 0.1Hz(circle), 0.5Hz(triangle) and 0.8Hz(square). B) Dephosphorylated AMPA (AMPA-0P), phosphorylated in one site AMPA (AMPA-1P) and phosphorylated in two sites AMPA (AMPA-2P) responses to 75 pulses of 80ms duration with 0Hz(asterisk), 2.5Hz(circle), 5Hz(triangle) and 10Hz(square).