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 receptor responses 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 oscillations 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 receptor; Biochemical networks; Plasticity; Calcium oscillations

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 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] whose functions 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].

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-Top Panel) the phosphorylation and dephosphorylation of AMPA receptors occur 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's [1] model of biochemical pathways includes CaMKII, calmodulin (CaM), adenylate cyclase (AC), phosphodiesterase (PDE), protein phosphatase 1 (PP1), calcineriun, and cAMP-dependent protein kinase (PKA) (Fig. 1B-Top Panel). In our computational model, these pathways are assumed to be located immediately under the postsynaptic membrane.

Based on the work of Lee et al. [6] we also included three states of AMPA receptor channel (dephosphorylated, phosphorylated in one site, and phosphorylated in two sites) 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. 0.1 Hz, 0.5 Hz, 0.8Hz, 2.5 Hz, 5.0 Hz, 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. 1-Middle Panel) and the phosphorylation and dephosphorylation of AMPA receptor channel consequently to the effects of calcium oscillation on the biochemical pathways (Fig. 1-Bottom Panel). The results indicate that the phosphorylation and dephosphorylation of the AMPA receptor channel are affected by the oscillations.

In Fig. 1A-Middle Panel, the activities of PKA, CaMKII and PP1 show different responses to stimuli with the same number of pulses (6) and duration (1 s) but varying frequencies (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-Top Panel) from the dephosphorylated state (AMPA-0P) to the phosphorylated in one site state (AMPA-1P) (Fig. 1A-Bottom Panel). 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-Top Panel) from the phosphorylated in one site state (AMPA-1P) to the phosphorylated in two sites state (AMPA-2P) (Fig. 1A-Bottom Panel).

In Fig. 1B-Middle Panel, 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 (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 (2.5 Hz, 5.0 Hz) but only for the highest frequency stimulus (10.0 Hz) (Fig 1B-Bottom Panel).

4. Discussion and Conclusion

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 channels can be influenced by the calcium oscillations through the variation in the activity of some kinases and phosphatases involved in the phosphorylation/dephosphorylation of the receptor.

Acknowledgments

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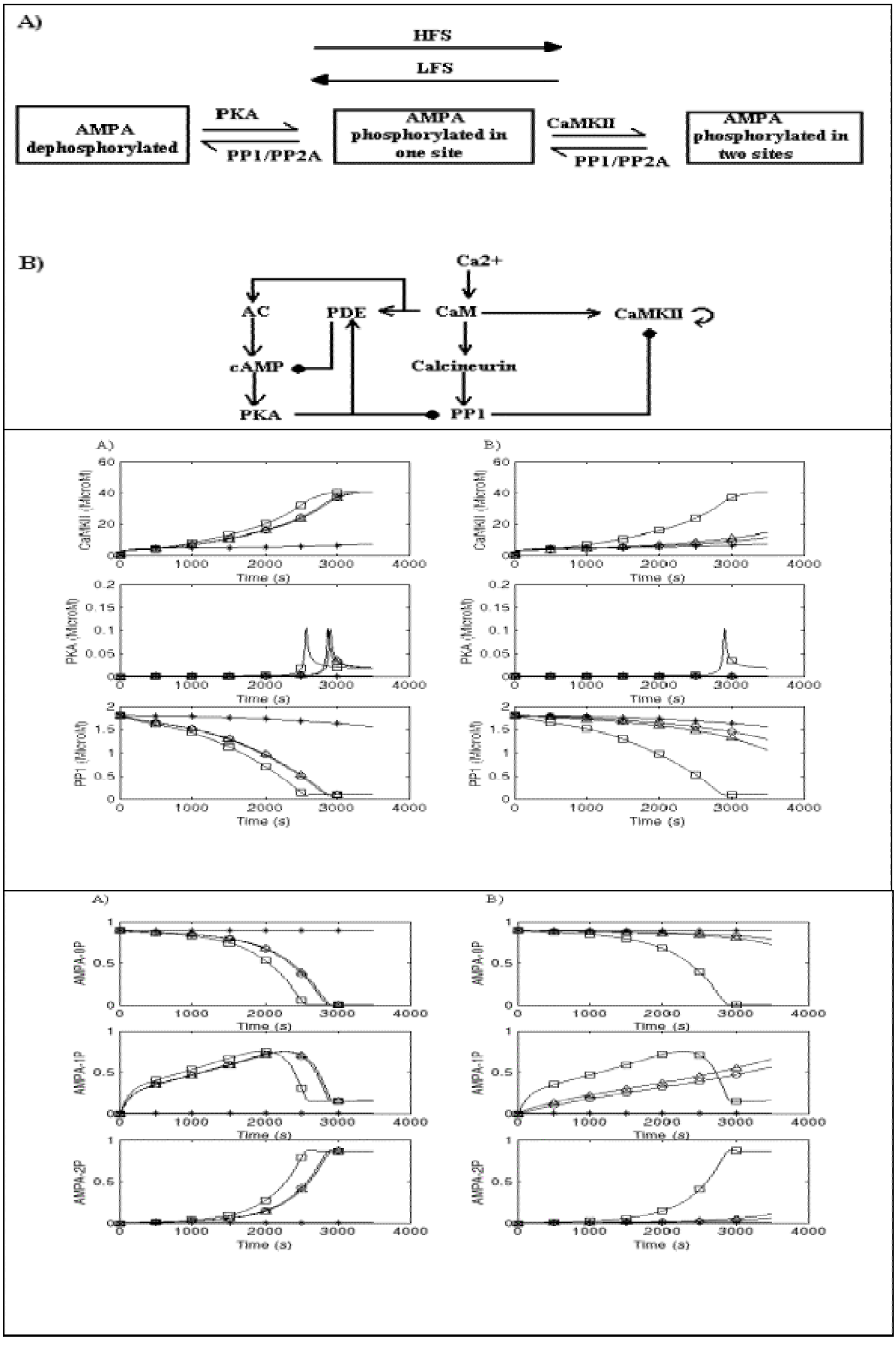
A)
HFS
LFS
AMPA dephosphorylated
PKA
PP1/PP2A
AMPA phosphorylated in one site
CaMKII
PP1/PP2A
AMPA phosphorylated in two sites
B)
Ca2+
AC
PDE
CaM
CaMKII
cAMP
Calcineurin
PKA
PP1
A)
B)
CaMKII (Microlt)
PKA (Microlt)
PP1 (Microlt)
Time (s)
AMPA-0P
AMPA-1P
AMPA-2P

Figure Caption

Fig. 1. Top Panel: 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- CaM KII 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 crosstalks between the signaling pathways, and may have properties that are non-intuitive (modified from [1]).

Middle Panel: 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 0.1Hz(○), 0.5Hz(Δ) and 0.8Hz(□).B) PKA, PP1 and CaMKII responses to 75 pulses of 80ms duration with 2.5Hz(○), 5Hz(Δ) and 10Hz(□). Responses for constant stimulation (*) are also indicated for comparison.

Bottom Panel: 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 0.1Hz(○), 0.5Hz(Δ) and 0.8Hz(□). 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 2.5Hz(○), 5Hz(Δ) and 10Hz(□). Responses for constant stimulation (*) are also indicated for comparison.