mGluR-mediated calcium oscillations in the lamprey: a computational model

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Abstract

Slow Ca^2+ oscillations caused by release from intracellular stores have been observed in neurons in the lamprey spinal cord. These oscillations are triggered by activation of metabotropic glutamate receptors on the cell surface. The pathway leading from receptor activation to the IP3-mediated release of Ca^2+ from the endoplasmatic reticulum (ER) has been modelled in order to facilitate further understanding of the nature of these oscillations. The model generates Ca^2+ oscillations with a frequency range of 0.01-0.09 Hz. A prediction of the model is that the frequency will increase with a stronger extracellular glutamate signal.

Key words: mGluR5; Calcium oscillations; Lamprey; Spinal cord

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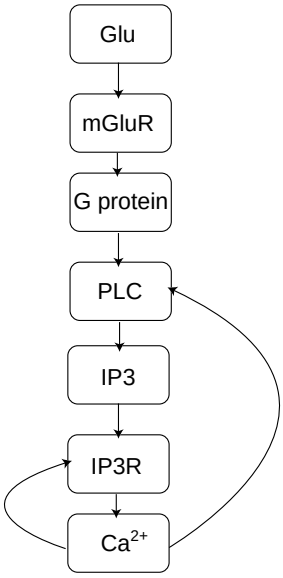

Fig. 1. The main components of the pathway considered in this model.

1 Introduction

Calcium is an important intracellular messenger molecule, both in neurons and other cells. Cells at rest have a cytoplasmic Ca^2+ level around 0.1 μM [3], but the concentration is dynamically regulated and can rise to approximately 1μM during oscillations [5]. The increase is mainly caused by release of calcium ions from intracellular stores, the most important of which is the endoplasmatic reticulum (ER). Gated channels such as the inositol triphosphate (IP3) receptor (IP3R) induce the release of calcium ions from the ER, and ionic pumps work in the other direction to bring Ca^2+ into the ER.

Intracellular, IP3-mediated calcium oscillations have been observed in the lamprey. These oscillations are dependent on activation of a certain type of metabotropic glutamate receptors (mGluR5, which belong to the group I metabotropic glutamate receptors) on the cell surface [7]. These receptors, in turn, activate G proteins that initiate a biochemical cascade which ends in the binding of IP3 to the IP3R and a subsequent release of calcium from the ER. Figure 1 shows the main components in the biochemical pathway leading from mGluR5 activation to IP3-induced release of calcium ions from the ER. The actual model contains many more intermediate and parallel reactions, but only the main steps are shown here.

2 Methods

The pathway was modelled using coupled differential equations based on standard biochemical kinetics and adapted from [1]. The equations were initially solved using the XPPAUT package [4]. Binding constants and other parameters were based on values found in the literature. Some of them were modified to improve the qualitative behaviour. For instance, the down-regulation of G protein activity is in itself rather slow, but the presence of GTPase-activating proteins (GAPs) increases the rate 1000 to 2000-fold [10]. The model takes into account that PLCβ is such a GAP for the G protein G_q [11].

As for the IP3 receptor (IP3R), several different models were tried. The best qualitative behaviour in the model, as judged by visual comparison against experimental traces of intracellular calcium concentration levels, was obtained using either the eight-state model described in [12] or the two-variable simplification of this model proposed in [9].

After the full biochemical model had been created using XPP, we implemented it in its entirety into a previously developed compartmental model of a lamprey spinal cord neuron [6]. This was accomplished using the CHEMESIS package [8] together with the GENESIS neural network simulator [2].

3 Results

Our model - depending on the parameter values - gives rise to calcium level oscillations with qualitatively (and sometimes quantitatively) similar behaviour to experimentally observed oscillations.

Figure 2 (from [7]) shows a typical experimental recording of intracellular Ca^2+ level oscillations in a lamprey spinal cord neuron. The group I mGluR agonist DHPG is applied in two distinct steps. When DHPG is removed, the amplitude of the oscillation decays. After re-application of DHPG, the first period of the oscillation has a larger amplitude than the following ones.

Figure 3 shows a simulation of a similar experimental protocol as the one shown above. Note that the time scales in the simulations are different. However, the oscillatory period in the simulation falls within the observed range for actual lamprey spinal neurons.

It is unknown from experiments whether the intracellular IP3 levels also oscillate during Ca^2+ oscillations. In our model, the IP3 concentration is oscillatory because of a feedback loop between Ca^2+ and PLC. If, on the other hand, PLC

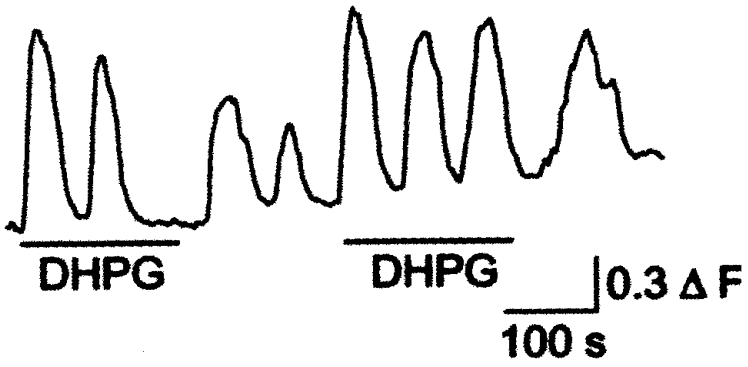

Fig. 2. Experimental response of a neuron to mGluR agonist application, withdrawal and re-application. x-axis, time in seconds; y-axis, relative intracellular calcium concentration.

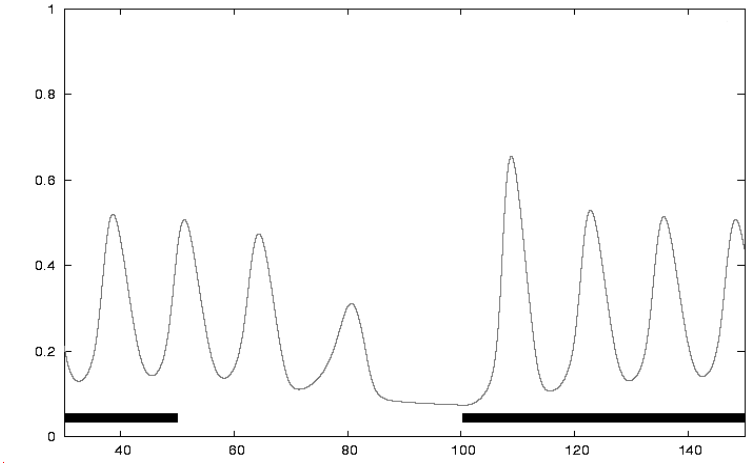

Fig. 3. Computational model's response of a neuron to mGluR agonist application, withdrawal and re-application. x-axis, time in seconds; y-axis, intracellular calcium concentration in μM. The black bars show the duration of DHPG application and re-application.

does not require Ca^2+ for activation, the feedback loop disappears and the IP3 concentration eventually reaches a constant level rather than oscillating. We simulated this scenario and found that the Ca^2+ oscillations are retained. Thus, the IP3 level does not need to be oscillatory for Ca^2+ oscillations to occur. Further experiments are needed to find out whether the IP3 levels are indeed oscillatory in lamprey spinal cord neurons.

We also made some exploratory attempts to connect the slow calcium oscillations to the ion channel machinery of the cell. It was shown in [7] that calcium influx through L-type calcium channels is necessary to obtain intracellular Ca^2+ oscillations. Some of these channels may be open at -60mV (close to the resting potential of these neurons) which would allow a small but steady influx of calcium ions. We introduced a calcium pool containing calcium that

enters through L-type calcium channels at low membrane voltages. The calcium in the pool is degraded with a characteristic time constant. This calcium pool, in the model, replenishes the intracellular calcium with a rate dependent on the concentration difference in the soma and the L-type calcium pool.

With such a model, slow intracellular calcium oscillations are obtained only when the L-type channels are working (results not shown). However, since no experimental evidence exists for the nature of the link between L-type calcium channels and somatic calcium level oscillations, these results must be considered for what they are: suggestions for possible mechanisms and nothing else.

It is known from experiments that intracellular Ca^2+ oscillations are correlated with a slowing down of the swimming rhythm in the lamprey. We examined if intracellular calcium oscillations could slow down the oscillation rhythm in a single neuron if its calcium-dependent potassium channels were sensitive to intracellular calcium concentration. Indeed, the oscillation frequency was seen to slow down somewhat in simulations using such a mechanism (results not shown). However, experimental evidence seems to indicate that calcium-dependent potassium channels are not involved in this phenomenon since activation of mGluR5 by DHPG does not induce any change in the resting membrane potential[7].

4 Conclusions

We have built a model to simulate intracellular, mGluR-mediated calcium level oscillations occurring in lamprey spinal cord neurons. It was found that a good qualitative agreement between the model and experimental results can be obtained. The model generates Ca^2+ oscillations with a frequency range of 0.01-0.09 Hz, as compared to 0.005-0.033 in the actual lamprey neurons. The model also predicts that an increased level of glutamate will increase the frequency of the oscillations.

The biochemical model has been incorporated into a previously developed electrophysiological model of a lamprey spinal neuron. This combined model can be used to probe possible ways that voltage-gated calcium inflow might be connected to intracellular calcium levels, and hypothetical mechanisms by which the intracellular calcium could control the frequency in a spinal interneuronal network.

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