Poster presentation

Modeling TRPC1 mediated slow EPSPs in cerebellar Purkinje cells

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Background

Synaptic plasticity in granule cell (GC) inputs to Purkinje cells (PCs) has been a major and ongoing subject of experimental investigation ever since the publication of the Marr/Albus hypothesis of cerebellar learning. It is known that this synaptic plasticity may very well be associated with a slow EPSP through metabotropic glutamate receptors (mGluR) which have been demonstrated to be central to mechanisms for synaptic change in the cerebellum. In PCs, mGluR1 has been studied more thoroughly than any other mGluRs. This excitatory action is through a mixed channel which has inward and outward components. This cation channel has also recently been identified as a transient receptor potential channel 1 (TRPC1, [5]) which can apparently be involved in the prolonged PC responses known as slow EPSPs. This report describes the first effort to model the kenetic effects of these interactions at cellular, channel and subcellular biochemical levels in PCs. This effort is the first stage in constructing an eventual kinetic model of long term plasticity including LTD based on experimental data.

Methods
Experiment

Sprague-Dawley rats (14–31 days old, Charles River) were used to prepare cerebellar cortical slices cut 350 μm thick in coronal sections. Slices were incubated at either 30°C or room temperature in oxygenated ACSF following standard procedures. Stimulation was delivered using glass electrodes prepared from theta tubes, using a stimulus isolater set to provide stimulation intensity between 5–100 μA and with a 2 ms duration. To generate slow EPSPs, five pulses of train stimulation at 100 Hz were given in the molecular layer (ML). In order to study the slow EPSPs that have been shown to result from stimulating GC axons, in some experiments, the ionotropic glutamate receptor antagonist, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydro-benzoquinoxaline-7-sulfonamide (NBQX, 5 μM) was used to block fast EPSPs. After the fast EPSP is blocked, the amplitude of sEPSPs is stimulation intensity dependent and saturated after it reaches 30 μA.

Computational simulations

Genesis 2.3 was used to simulate the TRPC1 mediated sEPSP. The model was implemented using two distinct parts. The first involved modeling one dendritic segment, a spine neck, and a spine head. Averaged values from electron microscopic data were used for the dimension of each compartment [4]. The second part was created by Kinetikit under the path/kinetics to model the chemical reactions between glutamate binding, mGluR receptors and the $G\alpha_q$-activated TRPC1 channels. The/kinetics model was modified from Bhalla and Iyengar [1]. The activation of TRPC1 channels is simulated in the spine head. Upon stimulation, there is an increase in synaptic glutamate concentration. This increase in glutamate concentration activates mGluR receptors which, in turn, increases the amount of GTP-bound G protein. These activated G proteins will activate TRPC1 receptors. In the second modeling step, local model was applied to the whole PC dendritic tree using the whole cell model [2,3].

Results

Voltage clamp data suggested that the current through the TRPC1 channel can result in a somatic response as large as 80 pA. The value can be used together with the time course to guide the simulation in both local and full models. In the local model, about 10 mV voltage response can be generated in the dendritic compartment as a consequence of a 1.5 pA current through the channel of TRPC1. In the full model, the membrane potential is hyperpolarized before the synaptic inputs were delivered at 200 ms. However, a sEPSP similar with the one in the local model was evoked. Since the base line shift was not in the local model, this change must result from the inclusion of the mGluR related processes in spines. Surprisingly, the hyperpolarization continues even after the sEPSP ended around 2 sec. This unexpected phenomenon requires further study. Once the base line is stabilized, the channel kinetics of TRPC1 as tuned so that the somal peak current and time course matched experimental data.

Conclusion

The TRPC1 mediated sEPSP is successfully simulated in the local model. An interaction was observed between the kinetic biochemical models and the electrical response of the full PC model that was unexpected and is now under further study. It is likely that a more realistic model is needed to accurately simulate this sEPSP. The current work is the first step towards modeling the long term plasticity in PC synapses which have important implications for cerebellar function.

References

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