Regulation of neurotransmitter release by C-domain Ca²-sensors

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Abstract

This thesis focuses on two different aspects of presynaptic mechanisms related to the regulation of neurotransmission. On one hand, it deals with presynaptic Ca^{2+} signalling and on the other hand with various Ca^{2+}-sensing proteins, known as C2 domain proteins. Altogether, the general aim is to provide new insight in the presynaptic mechanisms regulating neurotransmitter release, from the regulation of Ca^{2+} event generation to the control of synaptic vesicle fusion by various Ca^{2+} sensors. The general aim was to elucidate the role of several Ca^{2+} sensors in evoked and spontaneous neurotransmitter release and their implication in synaptic plasticity.

The first part of this thesis deal with the role of presynaptic fast Ca^{2+}-transients and global $[Ca^{2+}]_i$ rises in resting neurons. An algorithm was developed to accurately and automatically identify rapid Ca^{2+} events arising in small cellular compartments, such as the presynaptic element. Two main classes of Ca^{2+} events were defined: spontaneous Ca^{2+} elevations (SCEs) which comprise all types of Ca^{2+}-events detected under resting conditions, and a subclass of SCEs termed spontaneous Ca^{2+}-transients (SCTs) discernible by their single peak and fast kinetics. Previous evidence indicated that spontaneous release is affected by blockade of voltage-gated Ca^{2+}-channels but also correlated to Ca^{2+} signals originating from intracellular stores, depending on the neuronal type. Using different imaging methods we studied the regulation of mEPSCs by extracellular and intracellular $[Ca^{2+}]$. We found no strong evidence for a tight coupling of stochastic Ca^{2+} elevations to mEPSCs in this neuronal preparation, suggesting that spontaneous neurotransmitter release events are governed by global $[Ca^{2+}]_i$ levels or, alternatively, are coupled to slow spontaneous Ca^{2+} elevations.

The second part, provides insight in the functional involvement of several Ca^{2+} sensors and C_2 domain proteins. We focused on the phenotypic effect of Doc2b Ca^{2+}-binding site mutations on spontaneous and evoked neurotransmitter release in primary cultures of hippocampal neurons. We revealed a potential function for the protein in short-term plasticity release during and after high frequency stimulation and reconciled conflicting data concerning its effect for spontaneous release. We then extended extends our study on spontaneous release, aiming to elucidate additional C_2 domain proteins responsible for the remaining spontaneous release in absence of the main sensors Doc2a/b. Using transgenic mouse strategy we found that Doc2c and Synaptotagmin-7 do not regulate mEPSCs, while a potential effect for Rabphilin3A appeared in glutamatergic spontaneous release specifically in network cultures of hippocampal neurons.