Unraveling the function of Munc-18 in presynaptic vesicle release

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Synaptic vesicle release from neuronal processes is a complicated process, involving dozens of known proteins. Proteins involved in the release of vesicles are able to interact and thereby exert a specific role in the release mechanism. One protein that seems to be a key-player in the release of vesicles is Munc-18. Neurons from munc-18 knock-out mice do not show any synaptic vesicle release. Thus munc-18 is a necessary component of the release process.
A possible mechanism of controlling vesicle release by munc-18 is based on the interaction with syntaxin (SNARE protein). In addition to the interaction with syntaxin, munc18 also has other binding partners (e.g. mint, Doc2). Furthermore, munc18 has several PKC-phosphorylation sites. To investigate the (central) role of munc-18 in synaptic vesicle release we will use viral transfection as a tool to introduce mutated munc18 genes in munc-18 knock-out neurons. The expression of these mutated genes (e.g. syntaxin binding mutant, PKC phosphorylation site mutants) will help to unravel the presynaptic function(s) of munc18.
As a first step in studying the presynaptic function of munc-18 we investigated the concentration effect of munc18. We compared synaptic vesicle kinetics of wild-type neurons with munc18 heterozygous neurons and neurons overexpressing munc-18. Current data show that cells overexpressing munc-18 show faster refill kinetics of the ready releasable pool after electrical RRP depletion. Sucrose application (activity independent depletion of RRP) also showed an increase in refill kinetics in neurons overexpressing munc18, indicating that this effect is activity (Ca^{2+}) independent. In addition munc18 heterozygous neurons show slower refill kinetics after electrical RRP depletion. This makes munc18 the first presynaptic protein that increases synaptic efficacy in a concentration dependent manner. To further investigate the role of munc18 we will study the mutants of munc18 on munc18 knock-out background.

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