Molecular mechanisms of neuronal dense core vesicle release

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ENGLISH SUMMARY

Chapter 1 outlines the importance of dense core vesicle (DCV) release in the neuronal network and describes the current knowledge of the proteins involved in the fusion machinery of DCVs. In **Chapter 2** we investigated the location of DCV release. We found that DCVs are released from synaptic and extra-synaptic sites. DCV release occurred preferentially at synapses and synaptic release required less robust stimulation than extra-synaptic release. The synaptically localized priming protein Munc13-1 regulated this synaptic specificity and increased release probability at the synapse. In addition, Munc13-1 is sufficient to make extra-synaptic release as efficient as synaptic release. In **Chapter 3** we investigated how CAPS proteins regulate DCV release. CAPS and Munc13-1 share an important priming domain, and CAPS deletion resulted in a similar reduction of DCV release efficiency as observed in munc13-1/2 null mutant neurons. In contrast to Munc13 deletion however, CAPS deletion did not affect the location of release. **Chapter 4** focused on the involvement of the SM protein Munc18-1 in DCV release. We obtained unexpected results that stand in contrast to the regulation of DCV release from adrenal chromaffin cells, as deletion of Munc18-1 in immature neurons did not affect DCV release. Furthermore, Munc18-1 overexpression in mature neurons failed to increase DCV release efficiency, again different from DCV release in chromaffin cells. This suggests that both cell types use different release machineries to drive DCV release during different stages of development. **Chapter 5** focused on the proteins that assemble into the SNARE complex: VAMP2, syntaxin-1 and SNAP25. We showed that a Botulinum toxin C-sensitive syntaxin (syntaxin-1, -2 or -3) is essential for DCV release, but that other VAMP isoforms than the canonical VAMP2 isoform are involved. Especially during development release was insensitive to Tetanus toxin suggesting a role for TI-VAMP (VAMP7) in DCV release at this developmental stage. Furthermore, SNAP25 null mutant neurons showed almost normal DCV release, suggesting that other SNAP isoforms can replace SNAP25 in the SNARE complex that drives DCV release. In addition to the temporal changes in the use of different SNARE complexes, the different sub cellular locations of SNARE protein isoforms imply that different SNARE complexes could be responsible for fusion at different target membranes. In **Chapter 6** we investigated which Ca2+-sensors regulate DCV secretion. Deletion of the low-affinity calcium sensor synaptotagmin-I did not affect DCV release. But, deletion of the high-affinity calcium sensing protein Doc2 strongly reduced DCV release, suggesting that DCV release is triggered by a rise in global calcium rather than the local high concentrations of calcium that trigger synchronous synaptic vesicle release. Like deletion of Munc13-1, deletion of Doc2 affected the synaptic preference of DCV release. This suggests that a complex of both interacting proteins is involved in synaptic targeting of DCV release. **Chapter 7** discusses the results of the different chapters in respect to the literature. Two models of neuronal dense core vesicle release are described: one that hypothesizes the use of different fusion machinery during different developmental stages and one that hypothesizes how vesicles are released from the microtubule tracks in the cell and diffuse to the plasma membrane.