The role of presynaptic proteins in maintaining neuronal viability

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Summary

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Presynaptic proteins have been identified as central components in synaptic transmission, but some presynaptic proteins appear to be also required for neuronal survival and to be dysregulated in neurodegenerative diseases. Understanding the mechanism(s) of neuronal cell loss is a central aim in neuroscience. The research described in this thesis makes use of different presynaptic protein mutant mouse lines to increase our knowledge about neuronal degeneration and its links to neurodegenerative diseases, such as Alzheimer's disease.

When presynaptic proteins syntaxin-1, SNAP-25 or Munc18-1 were depleted from neurons, neuronal cell loss occurred before synaptogenesis. However, loss of synaptobrevins/VAMP1/2/3 did not lead to cell loss. In **chapter 2**, we showed a condensed Golgi apparatus in Munc18-1 KO (knockout) and SNAP-25 depleted neurons before cell death occurred. Furthermore, depletion of Munc18-1 from neurons led to defects in syntaxin-1 targeting to the plasma membrane and accumulation at the Golgi. Our data show that the atypical cell death upon Munc18-1 loss occurs via a degenerative pathway unrelated to the known synapse function of this protein, since Munc18-3 isoform restored cell viability and Golgi morphology but not syntaxin-1 targeting or synaptic transmission.

In **chapter 3**, we further investigated the condensed *cis*-Golgi in Munc18-1 KO neurons. Depletion of vacuolar protein sorting 45 (VPS45) or Golgi Phosphoprotein 3 (GOLPH3) is known to lead to a condensed Golgi (phenocopy Munc18-1 KO). However, overexpression of either of these proteins did not rescue neuronal survival or *cis*-Golgi morphology in Munc18-1 KO neurons. We found that the Golgi pool of phosphatidylinositol 4-phosphate (PI(4)P) and the cellular levels of phosphatidylinositol 4-kinase IIIα (PI4KIIIα) were reduced by 20-30% in Munc18-1 KO. However, neuronal survival and *cis*-Golgi morphology were not rescued by PI4KIIIα overexpression. Taken together, our data exclude VPS45-, GOLPH3- and PI4KIIIα-dysfunction as single causes of the *cis*-Golgi abnormalities in Munc18-1 KO neurons and suggest that changes in phosphoinositides metabolism might contribute to the condensed *cis*-Golgi in Munc18-1 KO neurons.

Dysregulation of the phosphatidylinositol 4,5-biphosphate (PI(4,5)P2) or phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P3) and phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB or Akt) (PI3K/Akt) pathway can trigger cell death. In **chapter 4** we showed that PI(4)P and PI(4,5)P2 levels were reduced in Munc18-1 KO neurons. However, increasing PI(4,5)P2, by overexpressing phosphatidylinositol 4-phosphate 5-kinase (PIP5K), did not rescue Munc18-1 KO cell death. PI(3,4,5)P3 levels were increased in Munc18-1 KO neurons, contrasting with a decrease in activation/phosphorylation of Akt at Thr308. Together, our data suggest that the decrease in PI(4)P and PI(4,5)P2 are not the single causes of Munc18-1 KO cell death. Our data implicate the reduced activation of the PI3K/Akt pathway in the atypical neurodegeneration observed in Munc18-1 KO neurons.

Spatial separation of amyloid precursor protein (APP) and beta-site APP cleaving

enzyme 1 (BACE1) is critical in preventing amyloidogenic processing of APP by BACE1. In **chapter 5**, we investigated the role of Munc18-1 in the spatial separation of APP and BACE1. In addition to the previously published Munc18-1-APP interaction, we showed that Munc18-1 also binds BACE1 when co-expressed in HEK293 cells. This interaction was confirmed by co-immunoprecipitation in human brain lysate. Cyclin-dependent kinase 5 (cdk5) phospho-mimicking and non-phosphorylated forms of Munc18-1 both interacted with APP and BACE1. Furthermore, APP levels were increased in Munc18-1 KO mouse brain. Together, these findings suggest that Munc18-1 plays a role in the amyloidogenic processing of APP, which may impact the development of pathology related to Alzheimer's disease.

In **chapter 6**, we discuss the main findings in view of the current literature. We discuss the functional cooperation between Munc18-1 and syntaxin-1 and exclude it as critical for neuronal survival. We further discuss the loss of synaptic transmission and conclude it cannot explain the neuronal cell loss observed in neurons lacking presynaptic proteins Munc18-1, SNAP-25 or syntaxin-1. We discuss the involvement of the PI3K/Akt pathway in Munc18-1 KO neuronal cell loss. Finally, we discuss how the research presented in this thesis provides new evidence for the role of Munc18-1 in neurodegenerative diseases, such as Alzheimer's disease.