ENGLISH SUMMARY

Function of tomosyn-1 and -2 in the mammalian nervous system

Our brain is a highly complex organ containing billions of nerve cells - neurons - that communicate with each other through electrical signals in order to regulate for instance learning and memory, motor control, perception and body homeostasis. To facilitate long distance communication, neurons have a long axonal projection that conducts these signals away from the cell body. Highly branched dendritic projections receive such signals from other cells. These electrical signals are converted into chemical signals (neurotransmitters) within specialized contact points between cells, so called synapses. Upon short electrical stimulation of the presynaptic cell with an action potential, calcium can enter through voltage-sensitive channels. This triggers the activation of calcium-sensitive proteins that induce fusion of neurotransmitter containing vesicles with the plasma membrane. Thereby, vesicular content is released into the space between cells. Binding of neurotransmitter molecules to receptors on the signal-receiving cell causes the opening of ion channels. A flow of charged ions can subsequently evoke an electrical signal in the postsynaptic cell. Depression or potentiation of the electrical signals are forms of synaptic plasticity that can be induced by use-dependent changes in the amount of neurotransmitter released and the strength of postsynaptic responses.

Similar to neurotransmitter release from synaptic vesicles (SVs), neuropeptide proteins can be released from larger vesicles that have a denser core (large dense-core vesicles, LDCVs). These proteins function for example in the fine-tuning and adaptation of connectivity between nerve cells during learning and memory formation. A third type of neuronal vesicle is the plasmalemmal precursor vesicle, supplying membrane for neurite outgrowth. The fusion of such vesicles with the plasma membrane is induced by a specialized complex consisting of multiple proteins. In this thesis, research on tomosyn, a protein that inhibits the formation of this SNAP (Soluble NSF Attachment Protein) REceptor (SNARE) complex, is described. Most of our current understanding of this protein stems from in vitro experiments and research in simple organisms. In the more complex mammalian nervous system two tomosyn isoforms are expressed. This thesis therefore aimed to further examine the functions of tomosyn-1 and tomosyn-2 in the mammalian nervous system by using knockout mouse models.

Both tomosyn-1 (chapter 2) and tomosyn-2 (chapter 3) were shown to be important for embryonic survival, but not required for neurite outgrowth. Tomosyn-1 deficient cultured hippocampal neurons did show hampered sustained release from synaptic vesicles. This was correlated with reduced levels of Munc18, another regulator of SNARE complex formation. Interestingly, tomosyn-2 deficient mice showed a similar phenotype for transmission in the neuromuscular junction. In these animals...
spontaneous neurotransmitter release frequency was enhanced, while prolonged stimulation led to increased depression compared to wildtype mice. On the level of the whole organism this was marked by motor coordination and muscle strength impairments. Also prepulse inhibition of an acoustic startle reflex, a hallmark of schizophrenia, was decreased. Mechanistically, this data from both isoforms implies that tomosyn may function to inhibit basal release and that disinhibition may occur to support release upon higher demand. Functional differentiation between tomosyn-1 and tomosyn-2 may arise from differential spatial and temporal expression patterns.

Proteins required for vesicle secretion have to be localized to release sites. Most of these proteins are efficiently co-transported in transport precursor vesicles. In line with this, we showed in chapter 4 that tomosyn was expressed in a mixed cytosolic and punctate pattern in cultured neurons. These puncta were identified to be organelles containing SV and LDCV markers. Vesicular targeting of tomosyn could also function to capture vesicles at release sites and prevent off-site secretion. Tomosyn does not contain a membrane anchor; therefore it is likely to require another protein for vesicular targeting. Vesicular protein synaptotagmin-1 was excluded as a candidate protein.

In chapter 5 it was shown that small SUMO-2/3 units can bind to tomosyn-1. This post-translational modification may be important to refine or alter tomosyn's function at a given place or time. We identified the SUMO E3-ligase PIASγ as a tomosyn-interacting protein that could be involved in the transfer of SUMO onto tomosyn. Whether and how this might be involved in for example synaptic plasticity, as well as its functional implications related to other post-translational modifications, will have to be investigated in the future.

Chapter 6 discusses the main findings in view of the current literature, provides an updated model of tomosyn function and elaborates on standing issues and future prospects. Most importantly, a role for tomosyn in presynaptic plasticity by activity-dependent regulation of its inhibitory strength is proposed. Concerted actions of post-translational modifications and Munc18 functionality, affecting SNARE complex availability and stoichiometry, could be involved. Especially high demanding synapses, such as the neuromuscular junction, may rely on such mechanisms. Tomosyn additionally affects the release from other types of vesicles, such as LDCVs, for which regulation of inhibitory strength could have different implications. Furthermore, masked effects by functional redundancy between tomosyn-1 and tomosyn-2 may be an issue in single knockouts, requiring the assessment of double knockout mice. All in all, the current and future research improves the understanding of the mammalian brain and provides novel clues for its malfunctioning in diseased patients.