Focus of the PhD-project (Jun.-Prof. Dr. Ralf Mohrmann)

SNAP-25 mediated release mechanisms at postsynaptic sites

Synaptosomal-associated protein (SNAP)-25 is an essential component of the *solubleN-ethylmaleimide-sensitive-factor attachment protein receptor* (SNARE) complexwhose formation represents a key step during fast Ca^{2+}-regulated exocytosis at chemical synapses.*Snap-25*$^{-/-}$deficient miceare embryonic lethal andsuffer from vastly diminished synaptic transmission (Washbourne et al., 2001), very similar to the phenotype of knockout mice for the cognate R-SNARE synaptobrevin-2 (Schochet al., 2001).However, cultivated *Snap-25*$^{-/-}$neurons also exhibit unique defects in form ofdecreasedcell viability and reduced dendritebranching(Delgado-Martinez et al., 2007).Furthermore, the amplitudes of excitatory and inhibitory miniature currents are reducedin *Snap-25*$^{-/-}$ neurons (Tafoya et al., 2006; Delgado-Martinez et al., 2007). These phenotypic features suggest that SNAP-25 does not only act in presynaptic transmitter release, but also participates in yet unidentified secretory pathwaysrequired for postsynaptic differentiation.An attractive hypothesis to explain these observations would bethat SNAP-25 mediatesthe delivery of cargo towardspostsynaptic sites in conjunction with unknown Q_a and R-SNARE proteins. Toprovide evidence for such role of SNAP-25 we plan toco-cultivate*Snap-25*$^{-/-}$ neurons and labeled wildtype neurons in order to establish a defined situation, in which we can specificallycharacterizethe properties of synaptic contacts formed by wildtype presynaptic boutons onpostsynaptic *Snap-25*$^{-/-}$ neurons. Since SNAP-25 retains its functionality even after N-terminal fusion to GFP or other small-sized proteins, we plan to specifically target SNAP-25 to axonal or dendritic sites by constructing SNAP-25 fusion proteins containing appropriate targeting motifs or domains. At least on a short time-scale,this approachshould allow for a site-specific rescue, whichmight also permit theidentificationof the corresponding SNARE partners by biochemical methods. As neurotransmitter receptors likely constitute the cargo of such postsynaptic transportroutes, we willalso investigate the involvement of SNAP-25 in postsynaptic receptor cycling using electrophysiological methods and imaging of fluorophor-tagged receptor-subunits (Ashby et al., 2004).

Literature

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