Ruprecht-Karls-Universität Heidelberg

Medizinische Fakultät Mannheim

Dissertations-Kurzfassung

Regulation of Synaptic Plasticity in Skeletal Muscle

Autor: Kyeong Rok CHOI

Institut / Klinik: Institut für Molekular- und Zellbiologie der Hochschule Mannheim
(Institut für Toxikologie und Genetik, Karlsruher Institut für Technologie)

Doktorvater: Prof. Dr. M. Hafner

The pentameric nicotinic acetylcholine receptor (AChR) is the primary postsynaptic component of vertebrate nerve-muscle synapses (neuromuscular junctions, NMJs). Basal AChR turnover is mediated by exocytic delivery and endocytic retrieval of AChRs, and activity-dependent synaptic plasticity is achieved by regulated recycling of previously surface-exposed receptors. Impairment of this recycling leads to reduced AChR loads typical for various types of myasthenic syndromes, which are characterized by severe, activity-dependent muscle weakness. The mechanisms underlying AChR recycling are still not well-understood, but functional evidence points towards an involvement of second messenger signaling (primarily cAMP) and of the motor protein, myosin Va. In this study, a series of biochemical and imaging approaches was used to investigate the functional interaction of AChR with candidate proteins presumably playing a role in receptor recycling.

First, the establishment of a novel pull-down assay allowed me to detect myosin Va and PKA type I but not PKA type II in a complex with synaptic, endocytic or recycling AChRs. Conversely, upon isolation of the total population of AChRs, containing primarily intracellular and unassembled receptors, PKA type I and PKA type II but not myosin Va were found to co-precipitate. This suggests that myosin Va and PKA type I specifically interact with surface-exposed or endocytic/recycling receptors, while PKA type II might exert other functions in AChR biology. Furthermore, these data nicely complemented previously obtained *in vivo* imaging results allowing now to propose a model where myosin Va is crucial to tether recycling AChRs together with PKA type I in proximity to the postsynaptic membrane.

Second, to consolidate this assumption and to clarify, how PKA type I might be anchored to recycling carriers containing AChR and myosin Va, putative A-kinase anchoring proteins (AKAPs) were tested. This study concentrated on rapsyn as a candidate molecule, because it is known to be tightly linked to AChR and to exhibit typical molecular features of AKAPs including a presumptive α-helical domain for interaction with the well-characterized dimerization/docking domain on PKA regulatory subunits. Bimolecular fluorescence complementation (BiFC) as a read-out for protein-protein interaction was nicely achieved between PKA type I and rapsyn in culture cells as well as in live mouse muscle. Notably, in the latter, BiFC signals were highly concentrated in the NMJ region, showing the specificity of this approach. Furthermore, BiFC signals in cells strongly depended on the presence of the dimerization/docking domain and the α-helical domain of PKA type I and rapsyn, respectively. Next, co-localization of co-transfected PKA type I and rapsyn constructs in culture cells was much higher in the presence than in the absence of the protein domains mentioned before. Finally, over-expressed rapsyn was found to co-immunoprecipitate with endogenous PKA type I in the presence but not in the absence of the α-helical domain of rapsyn. Altogether these data revealed that rapsyn is very likely to act as an AKAP for PKA type I. Furthermore, due to this thesis and further studies a picture emerges in which rapsyn- and myosin Va-mediated tethering of PKA type I in proximity to the postsynaptic membrane might help to allow for appropriate activity-dependent recycling of AChR.