Department of Women's and Children's Health

Implications of localization and transport regulation of postsynaptic membrane proteins for synaptic function and psychiatric disorders

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Air & Fire conference room, SciLifeLab Gamma building, KI, Solna

Thursday the 12th of December, 09:00

av

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Stockholm 2013

ABSTRACT

A fundamental means of regulating protein function in cells is through modulation of protein abundance at the sites of action via controlled transport processes. The complexity of neurons makes them especially reliant on regulated delivery of proteins to specialized structures throughout the distant cell branches. Proteins destined for pre and postsynaptic membrane are synthesized within the cell then undergo regulated transport to the membrane and ultimately to the respective synaptic signaling domains. To date, details of membrane delivery for most types of postsynaptic proteins have been lacking. As moderators of many neuropsychological functions, signaling effects of G protein-coupled receptors (GPCRs) are of major interest to the scientific community. Yet few from this important class of synaptic membrane proteins have been thoroughly examined for trafficking regulation mechanisms.

In the included GPCR study, we have evaluated the postsynaptic transport routes for a number of GPCRs involved in affective and schizoaffective disorders to describe an alternative approach for therapeutic intervention. Bulk mobility measurements with fluorescence recovery after photobleaching (FRAP) in cultured hippocampal neurons describe the common GPCR trafficking pathway via energy-efficient lateral diffusion. One GPCR, the serotonin 1b receptor ($5\text{-}TH_{1b}$), deviated by transporting to dendritic compartments in secretory vesicles. Membrane delivery via exocytosis was detected at preferential sites throughout the dendrite branches before delivery to the synapses via diffusion. These results suggest that unique postsynaptic transport routes may provide novel approaches for selective therapeutic regulation of receptor abundance at the postsynaptic membrane.

The last decade of membrane trafficking research has deepened our understanding of the importance of diffusion regulation for synaptic restructuring and modulation of neurotransmission efficacy. An overlooked membrane protein that is essential for proper neuronal function is the sodium-potassium pump (Na,-ATPase). With the super-resolution methods of structured illumination microscopy (SIM) and photoactivated localization microscopy (PALM), we demonstrated that both neuron-specific alpha 3 (ATP1a3) and the ubiquitous alpha 1 (ATP1a1) isoforms can form nanoclusters throughout the dendrites and are enriched in excitatory spines. Using PALM for molecular quantification, we estimate many hundreds of pumps per spine.

To understand how dynamic this picture of pump abundance is in the living neuron, we performed a detailed trafficking study on postsynaptic ATP1a3. With quantum dot labeling of single molecules, we revealed high extrasynaptic diffusion and reduced mobility at excitatory synapses. Inducing or inhibiting synaptic activity by chemical methods revealed that the pump diffusion is highly regulated and differentially responsive to varying synaptic activation.

With the novel view of regulated pump diffusion at and around synaptic membrane, we assessed the potential for physiological regulation of pump mobility via extracellular interactions. We demonstrated that most membrane-bound ATP1a3 are maintained in an oligomer with the non-enzymatic beta subunit of the pump which contains a large extracellular domain. Furthermore, addition of a soluble peptide, known to bind with the beta subunit, significantly reduced the synaptic mobility of the pump. These studies describe regulation of postsynaptic membrane protein trafficking that adds to our fundamental knowledge of neuronal function and introduces new avenues for signal regulation.

ISBN 978-91-7549-387-9