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Functional Consequences of Gephyrin Phosphorylation on Inhibitory Synapses

Promotionsfach: Anatomie und Zellbiologie
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Proper function of synapses depends on the appropriate targeting and localization of effector proteins in presynaptic terminal and postsynaptic sites. The scaffolding protein gephyrin has been postulated to form a hexagonal scaffold underneath the postsynaptic membrane, which provides binding sites for the glycine and $GABA_A$ receptors, and elements of the cytoskeleton, but up to now, the molecular mechanisms underlying the formation of gephyrin and/or the inhibitory receptors clusters are only poorly understood.

Due to early postnatal mortality of the gephyrin knockout mouse, a conditional gephyrin knockout mouse was generated by Hong-Xing Chen (MPI for Brain Research, Frankfurt, Germany), in which the expression of gephyrin within principal neurons of the mouse forebrain was ablated. γ2-$GABA_A$R immunostaining revealed a drastic reduction of γ2-$GABA_A$R cluster numbers in all sub-layers of hippocampus, except in the pyramidal layer of CA1 region.

Many recent data suggest that phosphorylation of gephyrin influences the scaffold formation at GABAergic synapses. Previous work of Dr. Kuhse's research group suggests a possible involvement of cyclin-dependent kinases 1, 2 and/or 5 in regulating gephyrin phosphorylation detected with a phosphospecific antibody (mAb7a). In order to identify the specific kinases involved in gephyrin phosphorylation, and due to the fact that Cdk5 is highly expressed in post-mitotic neurons, a lentivirus system with fairly high specificity to recognize their targets was used to deliver Cdk5-specific sh-RNAs to hippocampal cells and the functional consequences on gephyrin and $GABA_A$Rγ2 clustering were analyzed. Cdk5 knockdown results in a decrease of the gephyrin mAb7a immunoreactivity and clustering. The clustering of γ2-$GABA_A$ receptors is affected as well without a significant effect on the presynaptic components, suggesting that gephyrin phosphorylation might regulate $GABA_A$ receptor binding and their postsynaptic localization or trafficking. Furthermore, co-immunoprecipitation experiments showed

that Cdk5 associates with gephyrin in neuronal cell extracts and double immunofluorescence staining showed a cellular colocalization in a minor neuronal subpopulation. Finally, recombinant Cdk5/p25 readily phosphorylates gephyrin *in vitro*. Combined Alanine mutations of residues S188-S194-S200, reduced gephyrin phosphorylation *in vitro*, suggesting that all of the fore-mentioned residues or one of them is/are (a) putative target site(s) for Cdk5.

Generally, the results presented in this thesis suggest that gephyrin scaffolds provide a platform for Cdk5/p35 kinase activity, which allows the clustering and/or maintenance of gephyrin and $GABA_A$Rs at inhibitory postsynapses to be controlled by phosphorylation reactions.