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# Regulacja ekspresji neuroligin w synapsie w warunkach fizjologicznych i w zespole łamliwego chromosomu X

**Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne**

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**Streszczenie w języku angielskim**

Synapses are highly specialized sites of interaction between two nerve cells. Proper synaptic connection is possible due to the presence of cell adhesion proteins on the pre- and postsynaptic side. The interaction of postsynaptic neuroligins with presynaptic neurexins plays a key role in this process. Studies in recent years indicate a significant role of neuroligins not only in maintaining the physical bridge that ensures the stability of the synapse, but above all in the regulation of proper synaptic transmission.

Disturbances in the regulation of synaptic protein level can lead to synaptic dysfunction. It has been revealed that mutations in neuroligin genes correlate with the occurrence of autism spectrum disorders. Further research at the molecular level has shown that the discovered mutations disrupt the synaptic localization of neuroligins and result in impaired signal transduction. It has recently been suggested that the proteolytic cleavage process may be a factor regulating the level of neuroligins at the synapse. NLGN1 has been shown to be cleaved by two synaptic proteases: MMP-9 and ADAM-10. Moreover, the cleaved NLGN3 fragment proved to be a mitogen stimulating the growth of brain tumors. Nevertheless, the exact mechanism of the proteolytic shedding of neuroligins remained unknown, as did the proteases involved in cleaving other isoforms of neuroligins.

Fragile X syndrome (FXS) is a monogenic disease classified as syndromic autism spectrum disorder and the second cause of intellectual disability in the world after Down's syndrome. The direct cause of the disease is a dynamic mutation in the *fmr1* gene that results in the lack of the fragile X mental retardation protein (FMRP) expression. FMRP is an RNA-binding protein highly expressed in neurons. FMRP’s primary function is to inhibit synaptic translation of mRNA. The failure to regulate translation by FMRP leads to the impaired expression of many important synaptic proteins essential to neuronal function, proper synaptic transmission and plasticity. Despite many years of research on the basis of the fragile X syndrome, the mechanism of changes taking place at the cellular level has not yet been fully understood, not allowing for the implementation of an appropriate therapeutic strategy and effective treatment.

The main goal of this research was to investigate the molecular mechanisms regulating the level of neuroligins – genes associated with both autism and cancer – in the synapse under physiological conditions and in the fragile X syndrome.

The first aim of the study was to assess the level of neuroligins expression (mRNA and protein) in the synaptoneurosomes isolated from wild type and *Fmr1* KO mice. Assessment of

neuroligins protein level was also performed in primary cultures of hippocampal neuronal cells. It has been proved that the level of NLGN1 and NLGN3 protein is increased in the synapse of *Fmr1* KO mice, while the level of *Nlgn1*, *Nlgn2* and *Nlgn3* mRNA does not differ between the genotypes of wild type and *Fmr1* KO mice. This task was crucial as it led to the research hypothesis that the increased level of neuroligins was the result of increased synaptic translation of these proteins in synapses of the *Fmr1* KO mice. Another goal was to study the interaction of FMRP with *Nlgn1*, *Nlgn2* and *Nlgn3* mRNA. Using RNA co-immunoprecipitation and fluorescent *in situ* hybridization methods, it was confirmed that FMRP interacts with *Nlgn1, Nlgn2* and *Nlgn3* mRNAs in neurons. Then, using the method of chemical *in situ* crosslinking of surface proteins, the characterization of the activity-dependent synaptic distribution of neuroligin 1, 2 and 3 in the intracellular part and at the surface of postsynaptic membrane of the synapse was performed. These studies showed that the lack of FMRP in the fragile X syndrome leads to increased incorporation of neuroligin 1 and 3 into the postsynaptic membrane. Moreover, it has been proved that all neuroligin isoforms - NLGN1, NLGN2, NLGN3 undergo proteolytic cleavage at the synapse under physiological conditions. The process of proteolytic processing of neuroligins depends on synaptic activity and occurs as early as 2.5 minutes after neuronal stimulation. Moreover, it has been shown that proteolytic cleavage of neuroligin proteins also occurs at synapses of *Fmr1* KO mice, what suggests that this process is not fully impaired in the fragile X syndrome. At the final stage of the research, an attempt was made to explain this mechanism regulating the level of neuroligins in the synapse in more detail. For this purpose, the preliminary identification of proteases responsible for cleavage of neuroligins in synaptoneurosomes was performed. The results of these experiments indicate the role of the MMP-13 protease in the proteolytic shedding of the neuroligin proteins.

In summary, the results gathered in this dissertation indicate that the expression level of neuroligins in the synapse depends on two factors: the control of neuroligin mRNA translation by the FMRP and regulation by the proteolytic cleavage of neuroligin proteins. The presented results broaden our understanding of synapse function under physiological conditions at the molecular level. Moreover, they present the mechanism by which the lack of FMRP may contribute to abnormal levels of proteins associated with autism spectrum disorders. Moreover, the obtained data may indicate pathways for the development of new therapeutic strategies in the future.