Tytuł:
„Biochemical and functional assessment of the spectrin alpha II chain Arg1098Gln point mutation effects in *in vitro* and *in vivo* models”

Streszczenie:
Spectrins, as membrane-associated periodic skeleton proteins, perform a number of functions. First of all, due to their characteristic elastic properties resulting from structure of spectrin repeat, they act as shock absorbers, protecting the cell membrane against mechanical stresses. They are particularly important in elongated axons, being necessary for the development and stability of nerve cells. Additionally, through numerous interactions with other proteins, they participate in the organization of membrane channels, adhesion processes, regulation of actin dynamics and neurotransmission. Mutations of spectrin proteins cause numerous disease symptoms, such as: myoclony, microcephaly, cerebellar atrophy, hypomyelination, motor neuropathy, spastic paraplegia and cerebellar ataxia. These symptoms are recognized in patients as well as in mouse models in a diverse spectrum and variable severity. Spectrins are also a substrate for calpains and caspases during cytoskeletal remodelling and apoptosis. The SH3 and CCC domains located within 9-10 repeats of an αII-spectrin pose an important crossing point between the regulatory pathways based on the calmodulin and concentration of calcium ions and those of phosphorylation based signaling. It constitutes a methabolic link between calpain-based proteolysis and caspases. Numerous products of spectrin proteolysis have been identified after neurological injuries and in neurodegenerative diseases such as Parkinson's and Alzheimer's disease. In this study, a survey based on the R1098Q mouse model with a point mutation in αII-spectrin gene and on transgenic hCAST mice with overexpression of human calpastatin (a natural calpain inhibitor) was conducted. Also recombinant proteins containing two 9-10 spectrin repeats were used. A reduced stability of the 10th repeat structure and its reduced affinity for calmodulin was observed based on a series of instrumental analyzes of α-II spectrin. By using immunochemical methods it was demonstrated, that αII-spectrin carrying the R1098Q mutation increased its susceptibility to specific proteolysis by calpain. This fact has been also visualised by immunostaining the primary cell cultures from the brain of R1098Q mice for the presence of a specific proteolysis product of αII-spectrin by calpain (SNTF). Due to the increased level of αII-spectrin proteolysis in R1098Q mice, it was decided to use hCAST mice to create a new mouse line, useful for assessing the effect of reduced calpain activity on the phenotype of these animals. Motor tests revealed an improvement of muscle strength in R1098Q+hCAST mice and better step control without a significant improvement in other tests of motor ataxia severity.