SYNAPTIC MECHANISMS UNDERLYING THE EXCESSIVE AND PRECOCIOUS GLUTAMATE RELEASE IN THE SPINAL CORD OF SOD1^{G93A} MICE.

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Glutamate (Glu)-mediated excitotoxicity plays a major role in motor neuron (MNs) degeneration in amyotrophic lateral sclerosis (ALS). Reduced astrocytic uptake was suggested as a cause. On the basis of our studies, we have proposed that abnormal release may represent another source for excessive extracellular Glu levels.
The aim of this study was to investigate which mechanisms support the excessive Glu exocytosis. Synaptic nerve terminals were purified from the spinal cord of SOD1^{G93A} mice, the most used experimental model for human ALS, and of SOD1 control mice. Studies were performed at two different stages of the disease, the early and the late one (4 and 17 weeks of life, respectively). As functional readouts, we measured the release of Glu, pre-synaptic $[Ca^{2+}]i$ and the expression/activation state of a number of pre-synaptic proteins involved in neurotransmitter release.
The results showed that both the spontaneous and the stimulus-evoked exocytotic Glu release were increased in SOD1^{G93A} mice, at 4 and 17 weeks of life. The expression of the pre-synaptic proteins analyzed did not show significant differences except for synaptotagmin and some cytoskeletal proteins. Increased pre-synaptic $[Ca^{2+}]i$, over-activation of CaM kinase-II and ERK/MAP kinases, correlated with hyper-phosphorylation of synapsin-I, were found at both early and late phases of disease. In line with these findings, release experiments highlighted that the excessive Glu exocytosis was supported by the increase of the readily releasable pool of vesicles (RRP), which was prevented by selectively blocking synapsin-I phosphorylation.
Our results show an aberrant Glu exocytosis in the spinal cord of SOD1^{G93A} mice. This event is accompanied by pre-synaptic altered mechanisms that lead to a significant augmentation of the RRP and determine a higher probability of these vesicles to fuse. These changes are also present in 4 weeks old SOD1^{G93A} mice, thus playing a pivotal role in the development of ALS.