Alterations of Synaptic Mechanisms and Excessive Glutamate Release in the Spinal Cord of SOD1^{G93A} Mice.

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INTRODUCTION: Glutamate-mediated excitotoxicity plays a pivotal role in the motoneuron degeneration in amyotrophic lateral sclerosis (ALS). Reduced astrocytic uptake and abnormal glutamate release play a pivotal role for excessive extracellular glutamate. We showed that both the spontaneous and the stimulus-evoked exocytotic release of glutamate were increased in pre-symptomatic and late-symptomatic SOD1^{G93A} mouse spinal cord.

AIMS: The aim of this research was to investigate the synaptic mechanisms supporting the excessive glutamate exocytosis in ALS.

METHODS: Synaptosomes were purified from SOD1 and SOD1^{G93A} mouse spinal cord and used for glutamate release, western blots, confocal microscopy and intracellular Ca^{2+} concentration experiments.

RESULTS: Measuring the expression/activation state of a large number of pre-synaptic proteins involved in neurotransmitter release, we showed that only few of them were modified and that synaptotagmin and actin resulted over-expressed both in pre-symptomatic and late-symptomatic SOD1^{G93A} mice. Moreover, increased pre-synaptic Ca^{2+} levels and over-activation of calcium/calmodulin-dependent kinase-II and ERK/MAP kinases supported the hyper-phosphorylation of synapsin-I. Release experiments demonstrated that the excessive glutamate exocytosis was sustained by the increase of the readily releasable pool of vesicles. In support of the role of Synapsin-I in the above phosphorylation cascade, the abnormal glutamate release in SOD1^{G93A} mice was prevented by blocking synapsin-I phosphorylation by specific antibodies.

CONCLUSION: Our results highlight that abnormal glutamate exocytosis, accompanied by marked changes of precise pre-synaptic molecular mechanisms, is present in pre-symptomatic and late-symptomatic SOD1^{G93A} mice. These mechanisms may support excessive glutamate release and play a role in the disease development.