Synaptic Mechanisms Sustaining 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 the degeneration of motor neurons (MNs) in amyotrophic lateral sclerosis (ALS) and reduced astrocytic uptake was suggested as a cause for the increased synaptic availability of Glu (Rothstein et al., 1995). On the basis of our studies, we have proposed that abnormal release may represent another source for excessive extracellular Glu levels (Milanese et al., 2011; Giribaldi et al., 2013). Thus, targeting Glu release mechanisms that are altered in ALS may represent a possible strategy for new therapeutic approaches (Uccelli et al., 2012; Milanese et al., 2014). The main goal of this research was to investigate at the synaptic level which mechanisms support the excessive Glu exocytosis.
Synaptic nerve terminals (synaptosomes) were purified from the spinal cord of mice expressing high copy number of human SOD1 carrying the G93A point mutation (SOD1^{G93A}), the most studied experimental model for human ALS (Gurney et al., 1994), and of human WT SOD1-expressing control mice (SOD1). Studies were performed at two different stage of the disease, corresponding to the early and the late phase of pathology (4 and 17 weeks of life, respectively). As functional readouts we measured the release of Glu from superfused synaptosomes and the concentration of Ca^{2+} by fluorometric analysis at the pre-synaptic level. We also measured the expression/activation state of a number of pre-synaptic proteins involved in neurotransmitter release by confocal microscopy and western blot experiments.
The results showed that both the spontaneous and the stimulus-evoked exocytotic Glu release are increased in SOD1^{G93A} mice at 4 and 17 weeks of life. The expression of a number of pre-synaptic proteins (SNAP-25, stx-1A, VAMP-2, synaptophysin, munch-18, munch-13, rab2A, synaptotagmin, complexin 1/2, NSF, α/β snap, dynamin, actin and myosin) was analyzed. Few of them were found modified and only synaptotagmin resulted over-expressed in both 4 and 17 week old SOD1^{G93A} mice. Alteration of the cytoskeletal proteins, myosin and actin, was also observed. Increased pre-synaptic Ca^{2+} levels, over-activation of calcium/calmodulin-dependent kinase-II and ERK/MAP kinases correlates with hyper-phosphorylation of synapsin-I at both early and late stages of disease. In line with these findings, release experiments showed that the excessive Glu exocytosis was accompanied by the increase of the readily releasable pool of vesicles. Supporting the role of the above protein phosphorylation cascade, the excessive glutamate release was prevented by the selective block of synapsin-I phosphorylation, using specific antibodies.
Our results highlight that aberrant glutamate exocytosis is present in the spinal cord of late stage SOD1^{G93A} mice, an event accompanied by marked changes of specific pre-synaptic molecular mechanisms. The same synaptic alterations are also present in 4 weeks old SOD1^{G93A} mice and could represent a key feature in the early phase of experimental ALS, thus playing a pivotal role in the development of the disease.

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