Altered Mechanisms Underlying the Abnormal Glutamate Release in Amyotrophic Lateral Sclerosis at a Pre-Symptomatic Stage of the Disease.

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease, characterized by degeneration of upper and lower motor neurons (MNs) (Brown, 1995). The mechanisms of neuronal death in ALS are still largely obscure and they have been ascribed to several cellular and molecular alterations that also involve non-neuronal cells such as astrocytes and microglia (Ilieva et al., 2009). In line with this, it is well known that glutamate (Glu)-mediated excitotoxicity plays a major role in the degeneration of motor neurons (Vucic et al., 2014). Several evidences have already demonstrated that both excessive neuronal Glu release and defective glial Glu uptake contribute to increment the extracellular Glu level (Rothstein et al., 1995; Milanese et al., 2011; Giribaldi et al., 2013), contributing to motor neuron death.

In this scenario, we have previously studied the molecular mechanisms sustaining the excessive exocytotic Glu release from the spinal cord nerve endings (synaptosomes) in SOD1⁵⁹³A mice, a transgenic mouse model of human ALS (Gurney et al., 1994), at the late stage of the disease (Milanese et al., 2011). Thus, herein, we studied the mechanisms underlying Glu release in spinal cord synaptosomes of SOD1⁵⁹³A mice at a pre-symptomatic disease stage (30 days) in order to define how this phenomenon occurs during ALS progression.

Firstly, by release experiments, we found that the basal release of Glu was more elevated in the spinal cord of SOD1⁵⁹³A mice with respect to age-matched SOD1 control mice, and that the surplus of release relied on synaptic vesicle exocytosis. Exposure to high KCl or ionomycin provoked Ca²⁺-dependent Glu release that was likewise augmented in SOD1⁵⁹³A mice. Equally, the Ca²⁺-independent hypertonic sucrose-induced higher Glu release in SOD1⁵⁹³A mice than in age-matched controls. Also in this case, the surplus of Glu release was exocytotic in nature. Studying which molecular mechanisms are able to sustain the above described abnormal Glu release, we found that elevated cytosolic Ca²⁺ levels as well as increased phosphorylation of Synapsin-I, which was causally related to the abnormal Glu release measured in spinal cord synaptosomes of pre-symptomatic SOD1⁵⁹³A mice, and increased phosphorylation of glycogen synthase kinase-3 at the inhibitory sites, an event that favours SNARE protein assembly, by using FURA dye and...
Western blotting, respectively. Moreover, Western blot experiments revealed an increased number of SNARE protein complexes at the nerve terminal membrane, with no changes of the three SNARE proteins (VAMP, SNAP 25 and Syntaxin) and increased expression of synaptotagmin-1 and β-Actin, but not of an array of other release-related presynaptic proteins (synaptophysin, munch-18, munch-13, rab2A, complexin 1/2, NSF, α/β snap, dynamin, synapsin-I, and myosin). Finally, the abnormal Glu release was normalized by entrapping synaptosomes with specific antibodies for Synapsin-I phosphorilationsites, confirming the massive role of this protein in the excessive Glu release at the pre-syntomatic stage of the disease.

In conclusion, these results indicate that the abnormal exocytotic Glu release occurs in the spinal cord of pre-symptomatic SOD1<sup>G93A</sup> mice and it is mainly based on the increased size of the readily releasable pool of vesicles and release facilitation, supported by plastic changes of specific pre-synaptic mechanisms. Hence, these mechanisms might represent a key feature and play a pivotal role in the development of the disease.

Vucic et al. (2014). *Trends Neurosci*. 37(8), 433-442