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 may involve also non neuronal cells such as astrocytes and microglia (Ilieva et al., 2009). In line, 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 levels (Rothstein et al., 1995; Milanese et al., 2011; Giribaldi et al., 2013), contributing to motor neuron death.

In this scenario, we have previously shown that the spontaneous and the stimulus-evoked exocytotic Glu release from spinal cord nerve endings (synaptosomes) was excessive in SOD1G93A mice, a transgenic mouse model of human ALS (Gurney et al., 1994), at a late stage of the disease (120 days of life) and we studied the molecular mechanisms sustaining the release modifications (Milanese et al., 2011). We investigated here the release of Glu and its underlying mechanisms in spinal cord synaptosomes of SOD1G93A mice at a pre-symptomatic disease stage (30 days of life) in order to define whether this phenomenon occurs and, if this was the case, by similar or different mechanisms.

We found that the basal release of Glu was more elevated in the spinal cord of pre-symptomatic SOD1G93A mice with respect to age-matched SOD1WT control mice. Exposure to high KCl or ionomycin provoked Ca2+-dependent exocytotic Glu release that was likewise augmented in SOD1G93A mice. Noticeable, also the surplus of Glu release was exocytotic in nature. Equally, the Ca2+-independent hypertonic sucrose-induced higher exocytotic Glu release, which selectively involves the readily releasable pool of vesicles, was augmented in pre-symtomatic SOD1G93A mice. When studying the molecular mechanisms sustaining the above described abnormal Glu release, we found elevated cytosolic Ca2+ levels, measured by the Fura-2 dye in spinal cord synaptosomes, as well as increased phosphorylation of synapsin-I. Phosphorylation of synapsin-I was causally related to the abnormal Glu release, since the latter was normalized by entrapping synaptosomes with specific antibodies for synapsin-I phosphorylation sites, supporting the prominent role of this protein in the excessive Glu release measured in SOD1G93A mice at the pre-symtomatic stage of the disease. We also found increased the phosphorylation of glycogen synthase kinase-3 at the inhibitory sites, an event that favours SNARE protein assembly. Moreover, Western blot experiments revealed increased number of SNARE 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, out of an array of release-

related presynaptic proteins (synaptophysin, munch-18, munch-13, rab2A, complexin 1/2, NSF, α/β snap, dynamin, synapsin-I, and myosin), whose expressions were unmodified.

In conclusion, abnormal exocytotic Glu release occurs in the spinal cord of pre-symptomatic SOD1G93A mice and it mainly bases on an increased size of the readily releasable pool of vesicles and on release facilitation; two events supported by plastic changes of specific pre-synaptic protein expression and function. Our results suggest that similar mechanisms can support the abnormal Glu release at pre- and late symptomatic stages of the disease. The precociousness of the phenomenon may imply that it represents a cause of the disease rather than a consequence of the neuronal damage during disease progression.

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