AlteredMechanismsUnderlying the AbnormalGlutamate Release inAmyotrophicLateralSclerosisat a Pre-Symptomatic Stage of the Disease.

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AmyotrophicLateralSclerosis (ALS) is a fatal neurodegenerative disease, characterized by degeneration of upper and lowermotorneurons (MNs) (Brown, 1995). The mechanisms of neuronaldeath in ALS are stilllargelyobscure and theyhavebeenascribed to severalcellular and molecularalterationsthatalsoinvolved non neuronalcellssuchasastrocytes and microglia (Ilieva et al., 2009).In line with this, itiswellknownthatGlutamate (Glu)-mediatedexcitotoxicityplays a major role in the degeneration of motorneurons (Vucic et al., 2014).Severalevidenceshavealreadydemonstratedthatbothexcessiveneuronal Glu release and defectiveglial Glu uptakecontribute to increment the extracellular Glulevel (Rothstein et al., 1995; Milanese et al., 2011; Giribaldi et al., 2013), contributing to motorneurondeath.
In this scenario, wehavepreviouslystudied the molecularmechanismssustaining the excessiveexocytoticGlu release from the spinalcordnerveendings (synaptosomes) in SOD1^{G93A} mice, a transgenic mouse model of human ALS (Gurney et al., 1994),at the late stage of the disease (Milanese te al., 2011). Thus, herein,westudied the mechanismsunderlying Glu release in spinalcordsynaptosomes of SOD1^{G93A} mice at a presymptomaticdisease stage (30 days) in order to definehowthisphenomenonoccursduringALSprogression.
Firstly, by release experiments, wefoundthat the basal release of Glu was more elevated in the spinalcord of SOD1^{G93A}mice with respect to age-matchedSOD1 control mice, and that the surplus of release relied on synapticvesicleexocytosis. Exposure tohigh KCl or ionomycinprovoked Ca^{2+}-dependent Glu release thatwaslikewiseaugmented in SOD1^{G93A}mice.Equally, the Ca^{2+}-independenthypertonicsucroseinducedhigher Glu release in SOD1^{G93A}micethan in age-matchedcontrols. Also in this case, the surplus of Glu releasewasexocytotic in nature. Studyingwhichmolecularmechanismsare able to sustain the abovedescribedabnormal Glu release, wefoundelevatedcytosolicCa^{2+}levelsaswellasincreasedphosphorylation of Synapsin-I, whichwascausallyrelated to the abnormal Glu releasemeasured in spinalcordsynaptosomes of pre-symptomatic SOD1^{G93A} mice, and increasedphosphorylation ofglycogensynthase kinase-3 at the inhibitorysites, an eventthatfavours SNARE proteinassembly, by using FURA dye and

Western blotting, respectively. Moreover, Western blotexperimentsrevealed an increasednumber of SNARE proteincomplexesat the nerve terminal membrane,with no changes of the three SNARE proteins (VAMP, SNAP 25 and Syntaxin) and increasedexpression of synaptotagmin-1 and β-Actin, butnot of an array of other release-relatedpresynapticproteins (synaptophysin, munch-18, munch-13, rab2A, complexin 1/2, NSF, α/β snap, dynamin, synapsin-I, and myosin). Finally, the abnormal Glu release wasnormalized by entrappingsynaptosomes with specificantobodies for Synapsin-I phosphorilationsites, confirming the massiverole of thisprotein in the excessive Glu releaseat the pre-syntomatic stage of the disease.

In conclusion, theseresults indicate that the abnormalexocytoticGlu release occurs inthe spinalcord of pre-symptomatic $SOD1^{G93A}$ mice and itismainlybased on the increasedsize of the readilyreleasable pool of vesicles and release facilitation, supported by plasticchanges of specificpre-synapticmechanisms.Hence, thesemechanismsmightrepresent a keyfeature and play a pivotalrole in the development of the disease.

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