Molecular mechanisms supporting abnormal glutamate release in the spinal cord of a mouse model of Amyotrophic Lateral Sclerosis.

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Amyotrophic Lateral Sclerosis (ALS) is a chronic, neuromuscular disorder characterized by muscle wasting, weakness and spasticity, reflecting a progressive neurodegeneration of upper and lower motor-neurons. Selective vulnerability of motor-neurons has been in turn ascribed as a consequence of multiple causes: protein misfolding, mitochondrial dysfunction, oxidative damage, insufficient growth factor signaling, inflammation and glutamate (Glu)-mediated excitotoxicity. High glutamate levels have been reported in ALS patients as well as in animal models of the disease and a reduced glutamate transport was suggested as a cause. Due to the complex interplay of multiple mechanisms in the aetiology of ALS, defects of glutamate transport may not be the only reason for excitotoxicity-based neurodegeneration and other causes should be considered for the augmented glutamate availability, including increase of glutamate release. To demonstrate this hypothesis, we here used mice expressing human SOD1 with the G93A mutation [$SOD1^{G93A}$], a transgenic animal model of human familial ALS, and studied the release of glutamate by labeling spinal cord synaptosomes with [^{3}H]D-aspartate, a non metabolizable analogue of glutamate. Exposure to 15 mM KCl or 0.3 µM ionomycin provoked Ca^{2+}-dependent glutamate release that was dramatically increased in $SOD1^{G93A}$ mice. Both Glycine and GABA release in spinal cord were not modified. The augmentation of basal and stimulated glutamate release was already present in asymptomatic (30-40 day-old) and early-symptomatic (60-70 day-old) mutant mice.
To investigate the molecular mechanisms at the basis of this phenomenon, we studied the expression of some synaptic proteins and found that only few of them were changed, namely synaptotagmin, actin, myosin and munc-18 in late-symptomatic $SOD1^{G93A}$ mice and synaptotagmin and actin only in asymptomatic $SOD1^{G93A}$ mice. Moreover, further studies revealed the increased resting and stimulated Ca^{2+} levels in spinal cord nerve terminals from late-symptomatic and asymptomatic $SOD1^{G93A}$ mice, accompanied by activation of Ca^{2+}/calmodulin-dependent kinase II (CaMKII) and increased phosphorylation of synapsin-I syte 2/3. Also synapsin-I syte 1 and 4/5 were more phosphorilated. Blocking synapsin-I phosphorylation normalized the excessive glutamate release in $SOD1^{G93A}$ mice. In line with this findings, release experiments supported the involvement of the readily releasable pool of vesicles and a greater capability of these vesicles to fuse upon stimulation.
We can conclude that glutamate exocytosis is abnormally elevated not only in late-symptomatic but also in asymptomatic $SOD1^{G93A}$ mutant mice, suggesting that this phenomenon could be a cause and not a conseguence of ALS. Moreover changes in cytosolic Ca^{2+}concentrations, auto-activation, synapsin I phosphorylation, augmentation of the pool of vesicle docked at the membrane and ready to fuse could represent important mechanisms at the basis of the augmented excitatory neurotransmitter release.

Keywords: Amyotrophic Lateral Sclerosis, glutamate release, exitotoxicity.