Group I Metabotropic Glutamate Receptors Potentiate Glutamate Release in a Mouse Model of Amytrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disease characterized by muscle wasting, weakness and spasticity, reflecting a degeneration of upper and lower motor-neurons (MNs). The mechanisms of neuronal death in ALS are still largely obscure. It is well known that Glutamate (Glu)-mediated excitotoxicity plays a major role in the degeneration of motor neurons (Vucic *et al.,* 2014). According to our studies, we suggested that the high levels of synaptic Glu are not only due to a impaired glial re-uptake (Rothstein *et al.,*1995), but also to an excessive and precocious release of that excitatory neurotransmitter (Milanese *et al.,* 2011). Recently, pre-synaptic mGlu1 and mGlu5 receptors were described in rat cerebral cortex nerve terminals, where their activation produced a positive modulation of Glu release (Musante *et al.,* 2008). Several studies, performed in experimental ALS models, show that Group I metabotropic glutamate receptors (mGluR1 and mGluR5) are over-expressed in spinal cord astrocytes, microglia and neuron (Aronica *et al.,* 2001; Berger *et al.,* 2012). These receptors are actively involved in the regulation of important cellular processes altered in ALS such as synaptic glutamate homeostasis, intracellular calcium currents, astrocytes proliferation and reactivity, microglia activation and neuroinflammation. Converging data show that blocking mGluR5 in ALS beneficially modulate several processes that contribute to glial cell activation and degeneration of the surrounding neurons, supporting the idea that Group I mGluRs play a key role in the complex scenario of the disease.

In the present work we investigated on the presence and functionality of Group I metabotropic Glu receptors in the spinal cord of $SOD1^{G93A}$ mice, a widely used animal model of human ALS (Gurney *et al.,* 1994). Exposure of spinal cord synaptosomes to increasing concentrations of the non-selective mGluR1/5 agonist 3,5-DHPG, produced distinct effects in $SOD1^{G93A}$ mice and controls. The concentration higher than 0.3 μM stimulated the basal release of Glu, measured as [^{3}H]D-Aspartate to label the endogenous pools of glutamatergic vescicles, both in control and $SOD1^{G93A}$ mice. Interestingly, concentrations of 3,5-DHPG equal to or lower than 0.3 μM increased Glu release in $SOD1^{G93A}$ mice only. Experiments with selective mGluR1 or mGluR5 antagonists indicated that the effects of either high or low concentration of 3,5-DHPG implicated the activation of both mGluR1 and mGluR5. High 3,5-DHPG concentrations induced IP3 formation in both mouse strains, whereas, low concentrations of 3,5-DHPG induced increase of IP3 in $SOD1^{G93A}$ mice, but not in control mice. Release experiments confirmed that 3,5-DHPG produced exocytotic release of Glu, involving intra-terminal Ca^{2+} release through IP3-sensitive channels. Protein and mRNA determination pointed towards a more elevated expression of mGluR5 in $SOD1^{G93A}$ mice.

Overall our results demonstrate the existence of abnormal mGluR1/5-mediated release of Glu in the spinal cord of $SOD1^{G93A}$ mice, which contributes *in-vivo* to the excitotoxic and compromise scenario characterized by the presence of excessive Glu levels in ALS. This phenomenon can be modulated by selective antagonists, confirming an interplay between the two receptors and providing a rationale for new pharmacological approaches in ALS, based on the selective block of Group I mGluRs.

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