

Glycinergic Regulation of Motoneurons is Abnormal in amyotrophic lateral sclerosis Mice

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Abstract

Altered motoneuron excitability is involved in amyotrophic lateral sclerosis (ALS) pathobiology. Using immunohistochemistry and quantitative confocal microscopy analysis, we examined glycinergic and GABAergic innervations of spinal motoneurons in an ALS mouse model expressing a mutant form of human superoxide dismutase-1 with a Gly93→Ala substitution (G93A-SOD1). Glycine and GABA are the major inhibitory neurotransmitters of motoneurons. Glycinergic innervation of motoneurons was significantly decreased in asymptomatic 8-week-old G93A-SOD1 mice compared to controls. No significant differences in GAD-bouton densities were found in G93A-SOD1 mice. Reduction of glycinergic innervation preceded mitochondrial swelling and vacuolization. Calbindin-positive Renshaw cell number was decreased significantly at 12 weeks of age in G93A-SOD1 mice. We next examined the function of glycine receptors in cultured spinal motoneurons prepared from ALS mouse embryos. We developed a dissociated spinal cord culture system using transgenic mice expressing eGFP driven by the Hb9 motoneuron promoter. Motoneurons were identified as large (> 20 μm), Hb9-eGFP⁺ neurons with a specific morphology. Strychnine-sensitive, Cl⁻-dependent responses were detected in cultured GFP⁺ motoneurons by whole-cell patch clamp recordings after brief pulses of glycine (1 mM). Glycine-evoked currents and glycinergic miniature inhibitory post-synaptic currents (mIPSCs) were studied in cultured Hb9-eGFP⁺ motoneurons prepared from control and G93A-SOD1 embryos. The current densities of glycine-evoked currents and glycinergic mIPSCs were significantly smaller, and the decay phase of mIPSC events was faster in G93A-SOD1 cultures than in controls. Our results suggest that selective deficiency of inhibitory glycinergic regulation of motoneuron function or glycinergic interneuron degeneration could contribute to motoneuron degeneration in ALS.