Amyotrophic Lateral Sclerosis: Modification of Biophysical Properties and Gene Expression in Skeletal Muscle of a SOD-1 Related Mouse Model

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by degeneration of motor neurons, muscle weakness, fasciculations, muscle atrophy, progressive paralysis and death caused by respiratory and heart failure. ALS is epidemiologically classified into two general forms: sporadic (90–95%) and familial (5–10%), with a positive family history. Many of the familiar cases are due to mutations within the gene encoding for the superoxide dismutase 1 (SOD-1) protein, an enzyme involved in the detoxification of reactive oxygen species. Transgenic animals carrying mutations in the SOD-1 gene develop similar symptoms than those observed in clinic. In this animal model skeletal muscle has been demonstrated to be primarily involved in SOD-1-mediated toxicity (Dobrowolny et al., 2008). In this context, sarcolemma ion channels play a crucial role for muscle function. Resting chloride conductance (gCl), sustained by the ClC-1 channel, controls the resting membrane potential and excitability, indeed a large reduction of gCl produces myotonic-like symptoms and increase in excitability (Pierno et al., 2002). At the moment, there are no data describing the involvement of skeletal muscle ion channels functions in this pathology, thus, in our study we measured the resting potassium conductance (gK) and gCl, as well as muscle excitability parameters in extensor digitorum longus muscle of 4-months old transgenic SOD-1 mice, at the onset of the symptoms, by using the two-intracellular microelectrodes technique (Pierno et al., 2002). We found that resting gCl was strongly reduced in 4 month-old SOD-1 mice as compared to wild-type (WT), being it 1593±100 $\mu S/cm^2$ (19 fibers) and 2410±79 $\mu S/cm^2$ (22 fibers), respectively. Resting gK was increased in SOD-1 animals by 67±27%. Preliminary patch clamp studies showed a different sensitivity of the KATP channels to ATP in accord with the increase of gK. Also sarcolemma excitability, evaluated as the maximum number of action potentials, was accordingly increased from 7.08±0.8 (16 fibers) in WT to 12.6±1.5 (10 fibers) in SOD-1 muscle fibers. Interestingly, we found the same modification in gCl and excitability also in skeletal muscle of 2-3 months old pre-symptomatic transgenic SOD-1 mice. In order to evaluate the muscular involvement in the pathology we also examined an animal model in which the SOD1 G93A gene is selectively overexpressed in skeletal muscle under the control of the MLC promoter (Dobrowolny et al., 2008). Similar modifications were found in skeletal muscle of these animals, since resting gCl was reduced from 2432±136 $\mu S/cm^2$ (5 fibers) to 1906±61 $\mu S/cm^2$ (28 fibers). Because it is known that the ClC-1 channel is modulated by the protein kinase C (PKC), we tested the *in vitro* effect of chelerythrine, a PKC inhibitor, on skeletal muscle fibers of ALS animals. We found that this PKC inhibitor was able to restore the gCl value of transgenic animal muscle fibers toward the normal control values suggesting the involvement of PKC in the reduction of gCl in this condition. To note, calcium channels are important for proper contractility of skeletal muscle, so we analyzed calcium homeostasis and we found in these animals a significant increase of resting intracellular calcium level and an altered response to caffeine. We are currently evaluating the mRNA expression of ion channels by Real Time-PCR analysis. In conclusion, ion channel function is modified in skeletal muscle of the SOD-1 transgenic animals suggesting their contribution to muscle damage and potential pharmacological modulation.

Dobrowolny et al. (2008). *Cell Metabolism.* 8, 425–36.
Pierno et al. (2002). *Brain*. 125, 1510–21.