NEW BIOMARKERS IN ALS ETIOLOGY: ANALYSIS OF THE BIOPHYSICAL PROPERTIES OF SKELETAL MUSCLE IN SOD-1 MOUSE MODELS AND REPURPOSING OF ACETAZOLAMIDE AS A NEW THERAPEUTIC APPROACH

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Amyotrophic lateral sclerosis (ALS) is a progressive disease characterized by degeneration of motor neurons, muscle weakness, fasciculation, muscle atrophy, progressive paralysis and death caused by respiratory failure. Many of the familial 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 an increase in sarcolemma excitability (Pierno et al., 2002; Desaphy et al., 2013). 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 gCl and the potassium conductance (gK), 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 µS/cm2 (19 fibers) and 2410±79 µS/cm2 (22 fibers), respectively. Resting gK was increased in SOD-1 animals by 67±27%. Preliminary patch clamp studies showed different activity of the KATP channels and an altered sensitivity to ATP in accord with the increase of gK. Also sarcolemma excitability, evaluated as the maximum number of action potentials, was accordingly increased from 7.1±0.8 (16 fibers) in WT to 12.6±1.5 (10 fibers) in SOD-1 muscle fibers. Resting intracellular calcium level was increased in these animals and an altered response to caffeine was found. In order to evaluate the muscular involvement in the pathology we also examined an animal model in which the mutated 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 to 1694±146 µS/cm2 (19 fibers). In this animal model we tested the in vitro effect of acetazolamide, previously found to beneficially improve ClC-1 function in Myotonia Congenita by regulating its voltage-dependence (Desaphy et al., 2013). Interestingly, in the presence of acetazolamide, the resting gCl was increased toward the control value, being 2097±119 µS/cm2 (19 fibers). Accordingly, sarcolemma hyperexcitability was improved. Indeed, the maximum number of spikes was 7.0±1.4 (11 fibers) in the presence of the drug. In conclusion, we found that chloride channel function is modified in skeletal muscle of the SOD-1 transgenic animals

suggesting their contribution to muscle damage in ALS and opens the possibility to investigate on acetazolamide as a promising therapy.

Dobrowolny et al. (2008). Cell Metabolism. 8, 425–36

Pierno et al. (2002). Brain. 125, 1510–21

Desaphy et al. (2013). Exp Neurol. 248: 530-540.