Ion channels as biomarkers and therapeutic targets in dystrophic myopathies

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Some forms of muscular dystrophy are due to disorganization of the dystrophin-glycoprotein (DGC) complex linking intracellular cytoskeletal and contractile machinery to the extracellular matrix. The susceptibility of dystrophin-deficient myofibres, as in Duchenne muscular dystrophy (DMD), to contractile stress, both *in vivo* and *ex vivo*, supports a biophysical and signaling role of DGC in mechanotransduction, *i.e.* the events linking electro-chemical signals to contraction. For some time I have been interested in the possible outcome of the absence of dystrophin on ion channel function and/or expression. The model of the chronically-exercised dystrophic mdx mice, in use for many years in our laboratories, along with a multidisciplinary approach, help to investigate the link between channel alteration and modified mechanotransduction and to better understand how observed alterations are related to the primary defect, so to evaluate the role of channels as biomarkers and potential drug targets. We first focused on chloride channels sustaining the macroscopic chloride conductance (gCl) of fast twitch myofibres; gCl accounts for 80% of the resting ionic conductance and plays a pivotal role in sarcolemmal electrical stability. Therefore, changes in gCl in dystrophic muscle, as in myotonic dystrophy, may actively contribute to excitability pattern and in turn to the amount of contractile stress faced by the myofibre. Although recordings of chloride currents in adult native fibres are hindered by technical issues, several findings support a main role of ClC-1 channel. These include the parallel increase of muscle ClC-1 expression and gCl during postnatal development, the decrease in both gCl and ClC-1 expression in aged rodent muscle and the role of ClC-1 and gCl in myofibre phenotype. A decrease in gCl is a typical feature of progressively degenerating diaphragm muscle in mdx mice. In EDL muscle, gCl undergoes a transient increase in the period of mdx life span when active muscle regeneration occurs. Interestingly this latter phenomenon is blunted in EDL myofibres from chronically-exercised mdx mice (De Luca *et al.*, 2003). Parallel observations disclosed that gCl is a sensitive-biomarker of disease-related inflammation; in fact the decrease in gCl is counteracted by treatment of mdx mice with anti-inflammatory drugs or with drugs able to enhance dystrophin expression, such as gentamicin (De Luca *et al.*, 2005; 2008). Preliminary results from currently ongoing qPCR experiments show that ClC-1 channel is rather similarly expressed in muscles of mdx mice, irrespective of exercise regimen, corroborating an important role of other mechanisms in modulating gCl. In fact *in vitro* pharmacological studies favour possible role of cytokines and pro-inflammatory mediators, in relation to exercise and pathology, on the pathways controlling channel phosphorylation state (Pierno *et al.*, 2007; personal unpublished results). The possible different level of a functional ClC-1 protein or the involvement of other chloride channel types are currently under investigation. Exercise protocol also allowed to investigate the enhanced calcium entry *via* mechanosensitive channels, which may account for alterations of calcium homeostasis and excitation-contraction coupling during eccentric contraction in dystrophic muscle (Fraysse *et al.*, 2004; De Luca *et al.*, 2003). These channels are interesting as potential drug targets in muscular dystrophy and may include members of the TRP channel family and/or of the store-operated calcium entry mechanism. Cell attached patch clamp experiments on native myofibres identified a voltage insensitive calcium-permeable current with higher occurrence and open probability in mdx *vs* wildtype and in exercised *vs* sedentary animals. This provided a novel evidence about the presence of a channel physiologically involved in calcium adaptation to muscle work whose activity is aberrant in dystrophic fibers (Rolland *et al.*, 2006). The pharmacology and biophysical profile of the channel reminded the TRPV2 proposed by others (Iwata *et al.*, 2009). The channel dysfunction also appears to critically depend on the dystrophin presence (De Luca *et al.*, 2008). Interestingly, channel overactivity in mdx myofibres can be contrasted by drugs enhancing cyclic nucleotides, such as pentoxifylline, which also exerts positive effects on pathology signs in the animal model (Rolland *et al.*, 2006; Burdi *et al.*, 2009). Further investigations are needed to unequivocally define the molecular entity of the channel involved in the pathology; meanwhile protocols for drugs screening on hypothesized channel type, followed by validation in the animal model, may help to identify novel therapeutics.

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