PERSPECTIVES

Between channels and tears: aim at ROS to save the membrane of dystrophic fibres

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Lengthening during contraction allows muscles to develop force well above the levels reached in isometric conditions. Such enhanced performance has, however, a negative counterpart: damage which is revealed by pain (DOMS), structural and ultra-structural alterations and reduced ability to develop force (Asmussen, 1952). The phenomenon has been demonstrated *in vitro* and *in vivo*, in amphibian as well as in mammalian muscles and two main factors have been identified as determinants of eccentric contraction-induced damage: myofibrillar disorganization (Z line streaming and sarcomere length heterogeneity) (Allen, 2001) and calcium homeostasis alteration, possibly related to T tubule disruption (Warren *et al.* 1993).

About 15 years ago Petrof and coworkers showed that *mdx* dystrophic muscles are highly sensitive to eccentric contraction damage as they show greater loss of force and cellular damage, indicated by sarcolemmal disruption and procion orange penetration, than wild-type muscles (Petrof *et al.* 1993). Although only sparse evidence is available, the phenomenon seems to be specific to *mdx* mice and not present in other murine models of muscular dystrophy (Head *et al.* 2004).

Since Petrof's paper was published, many studies aimed to identify the mechanism responsible for the extra damage of dystrophin-lacking fibres exposed to eccentric contractions. It was assumed that the sarcolemma, made fragile by the lack of dystrophin, was easily broken by the intense mechanical strain generated by eccentric contraction. This led to the 'delta lesions' early observed in DMD fibres (Pestronk *et al.* 1982), which were sufficient to give access to large extracellular molecules. Calcium influx and subsequent increase of intracellular free calcium were considered the trigger for degeneration and death of the muscle fibres.

The scenario was considerably shifted by more recent studies which suggested that calcium entry could follow a more physiological pathway. The involvement of channels of the TRPC family, which support both stretch activated and store operated calcium influx, was demonstrated (Vandebrouck *et al.* 2002) and inhibitors of the stretch-activated channels were proved sufficient to control the calcium influx (Yeung *et al.* 2005). Thus, calcium influx might be a cause and not a direct effect of the membrane tears, and the activation of a relatively slow intracellular mechanism is needed to make the connection (Yeung *et al.* 2005).

The paper published by Whitehead *et al.* (2008) in this issue of *The Journal of Physiology* unveils such a connection. Some years ago it was shown that *mdx* muscle fibres are more susceptible to ROS damage (Rando *et al.* 1998) and show signs of increased lipid and protein oxidation in spite of the enhanced expression of antioxidant enzymes (Hauser *et al.* 1995). Whitehead and coworkers demonstrate that an active antioxidant treatment based on *N*-acetylcysteine (NAC), which is membrane permeant and known to be active in muscle fibres, significantly reduces the impact of eccentric contraction on *mdx* muscle fibres when given either *in vivo* or *in vitro*. Thus, ROS may represent the missing intracellular link between calcium entrance and membrane tears.

Two important questions follow: where are ROS produced and what are their targets? Whitehead *et al.* (2008) suggest that the two main sites of ROS production are mitochondria, whose activity is stimulated in response to increase of intracellular calcium, and NADPH oxidase. The targets of ROS are, obviously, many, but one is of special interest for the fate of dystrophic fibres. Sarcolemma can be destabilized by the lack of β-dystroglycan (BDG), which is essential for the structure of costamere, the skeleton of the muscle membrane. Src kinase activated by ROS (Chen *et al.* 2005) phosphorylates BDG leading to its internalization. This process is counteracted by dystrophin and utrophin and enhanced by caveolin 3. Besides, lipid peroxidation and membrane protein oxidation might be involved. The proposed interpretation receives strong support from the results of the NAC treatment, which prevents the eccentric contraction damage in *mdx*, but not in wild-type, muscles, restores the presence of BDG and decreases the amount of caveolin 3. All together, this represents a substantial contribution to maintaining the structural and functional integrity of the membrane.

Whether the mechanism outlined above is the only one responsible for the triggering of degenerative processes in muscles lacking dystrophin is still to be defined, but it may represent, in any case, a significant component of the worsening of the pathological phenotype. As suggested by the experiments reported by Whitehead and coworkers the control of oxidative stress may also become an important avenue to study therapies to improve the dystrophic muscle conditions.

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