TRPC1 is increased in *mdx* muscle, binds to caveolin-3 and is regulated by Src kinase: implications for Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is an X-linked genetic disease caused by the absence of dystrophin, a membrane anchoring protein. We have shown that calcium entry through stretch activated channels (SACs) contributes to muscle damage in the *mdx* mouse, an animal model of DMD (Yeung *et al.*, 2005). Transient receptor potential canonical 1 (TRPC1) forms SACs in mammalian cells (Maroto *et al.*, 2005) and interacts with caveolin-1 in smooth muscle cells (Lockwich *et al.*, 2000). Caveolin-3 (Cav-3), which is structurally homologous to caveolin-1, is increased in *mdx* muscle (Vaghy *et al.*, 1998). The aim of this study is to investigate the expression levels and interaction of Cav-3 and TRPC1 in *mdx* muscle. TRPC1 and Cav-3 co-localized, co-immunoprecipitated and had increased expression levels in *mdx* muscle (immunohistochemistry, Western blot). Fluorescence Energy Resonance Transfer (FRET) was used to confirm the interaction of the two proteins, in C2C12 myblasts co-transfected with TRPC1-CFP and Cav-3-YFP plasmids. Fluorescence Lifetime Imaging Microscopy (FLIM) showed a shortening of the donor lifetime (TRPC1-CFP) when cells were co-transfected with both plasmids (from 2.7ns to 2.1ns; $p < 0.001$), confirming the interaction between TRPC1 and Cav-3. As Src kinase can activate channels from the TRPC family (Kawasaki *et al.*, 2005), we investigated the role of this kinase on TRPC1 activity and binding properties with Cav-3. Incubation of C2C12 cells with hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS), increased the levels of both the active form of Src kinase (Western blot, $p < 0.01$) and the total tyrosine phosphorylation in those cells (immunohistochemistry, $p < 0.01$). Src activation and tyrosine phosphorylation were partially prevented when cells were incubated with PP2, a Src kinase inhibitor, prior to H_2O_2 treatment. Ratiometric calcium imaging (Fura Red) of C2C12 cells transfected with TRPC1-CFP and Cav-3-YFP revealed that calcium influx was increased upon H_2O_2 treatment only in cells expressing both TRPC1 and Cav-3 plasmids. Furthermore, the calcium influx was prevented when those cells were pre-incubated with PP2. These results suggest that Cav-3 is necessary for TRPC1 activity, which is triggered by activation of Src kinase by ROS. As ROS are known to be increased in *mdx*/DMD, we suggest that targeting the ROS-Src-TRPC1 pathway could lead to the development of new therapeutic approaches for the treatment of DMD.

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