Different Capacity for Store-Operated Ca\textsuperscript{2+} Entry and Ca\textsuperscript{2+} Extrusion Across the Plasma Membrane of Wild-Type and Dystrophic mdx Mouse Muscle

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Store-operated Ca\textsuperscript{2+} entry (SOCE) is a ubiquitously expressed signalling system that is highly specialized in skeletal muscle. "Deregulated" SOCE has been proposed as a pathway for Ca\textsuperscript{2+} entry into dystrophic muscle that leads to fibre degradation. We recently showed that this mechanism remains tightly regulated in mdx mouse muscle but the intact SOCE proteins, STIM1 and Orai1, are upregulated 3-fold (Edwards et al., 2010). We now report that the newly identified isoform STIM1L (Darbellay et al., 2011) is upregulated 1.8-fold in intact SOCE muscle of mdx mice in skinned fibres (2-fold greater in mdx compared with WT for the same SR Ca\textsuperscript{2+} release amplitude. However cytoplasmic fluo-4 transients in depleted intact fibres showed SOCE in the absence of a sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} pump blocker to be of reduced influx rate in mdx compared to WT fibres. A similar level of SR Ca\textsuperscript{2+} re-loading was determined in both muscle types following SOCE deactivation. Fura-2 imaging in intact fibres in the presence of 50 \(\mu\)M cyclopiazonic acid (CPA) (of the intracellular muscles in COPD). Supported by NIH R01-AR055099, T32-AR007592 and T32-HL072751.

Enriching Satellite Cells with x261 Promotes Differentiation

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Cell transplants into skeletal muscle of patients with muscular dystrophy are limited by donor cell attachment, migration, and survival in the host tissue. In animal models, despite HLA matching and a reduction of the host's immune response, few donor cells are retained in the host muscle. Enriching cells for a surface marker that enhances ability of the cell to attach, migrate, and survive will result in improved cell retention. The purpose of this study was to determine whether x261 markers primary satellite cells were better candidates for cell transplant than satellite cells without this surface marker. The x261 subunit is part of the L-type calcium channel but appears earlier than the other subunits. We isolated satellite cells from the hind limb muscles of neonatal mice and separated four subpopulations of cells based on the presence or absence of x261 and a marker of quiescence, CD34, by fluorescence activated cell sort (FACS). Satellite cells enriched with x261 survived in heat deactivated media past day 19, while there was no evidence of attachment or survival of cells without x261. In addition, cells that were positive for x261 and negative for CD34 demonstrated the most robust myogenesis out of all subpopulations. Enhanced myogenesis of this subpopulation was determined by morphology, the pattern of expression of myogenic transcription factors, and the development of excitation-contraction coupling as demonstrated by the presence of L-type calcium currents and calcium transients. As the data relate to differentiation and survival, these results suggest that cells enriched with x261 and without CD34 will demonstrate greater cell retention and force generation after satellite cell transplant, which is a strong candidate for therapy in muscular dystrophy. Supported by MDA.