The first set of the experimental results, as briefly summarized below, were published in the highly cited scientific journal: *Biophys J. 2017 Dec 5;113(11):2496-2507. doi: 10.1016/j.bpj.2017.09.023. [IF] 3.656.*

Mónika Sztretye, Nikolett Geyer, János Vincze, Dána Al-Gaadi, Tamás Oláh, Péter Szentesi, Gréta Kis†, Miklós Antal†, Ildikó Balatoni, László Csernoch, Beatrix Dienes‡ „Store-operated calcium entry is important for maintaining sarcoplasmic calcium content and release in mammalian skeletal muscle fibers"

When examining the key proteins of store operated calcium entry (SOCE) by Western blot, expression of Orai1 and both splice variants of STIM1 (STIM1L and STIM1S) were found to be reduced in myostatin Cmpt FDB muscles by 27% and 40 % respectively, when compared to wild type (WT) C57Bl6 mice. This reduction of SOCE proteins was paired with decreased SOCE activity in Cmpt fibers estimated based on the changes of the intracellular Ca^{2+} concentrations.

Analysis of depolarization-evoked calcium transients recorded under voltage-clamp conditions excluded the possibility of an altered excitation-contraction coupling (ECC) machinery that would manifest in the changes attributed to reduced SOCE function, since the reduced SOCE is not accompanied by altered V1/2 dependence of Ca2+ release (V1/2 for WT: -26.03±1.35 mV, n=6 vs -28.86±0.77 mV, n=7 for Cmpt). Further analysis of SR calcium-release fluxes and the net amount of Ca2+ released calculated from the global calcium transients in response to long depolarizing pulses revealed a 27% reduction in calcium released in the Cmpt fibers, in accordance with the reduced force. The most likely cause of the reduced calcium release is a reduction in SR content.

Application of a train of short (20 ms) depolarizing pulses to +30 mV revealed a reduction in the amount of calcium released from the SR in the consecutive stimuli, which was more pronounced in the case of mutant fibers. This finding reflects a greater depletion of Ca2+ from the SR in the mutant strain, where impaired SOCE activity was detected.

In our paper, we provided a comprehensive characterization of the SOCE mechanism in the Cmpt mouse model explaining the role of SOCE in refilling the SR Ca2+ stores in skeletal muscle. We found that SOCE has a role in maintaining and refilling SR Ca2+ stores not only in repetitive tetanic stimulation, as previously reported, but on an immediate basis, in agreement with the latest observations.

In the second set of experiments I was working towards completing specific aims 3 and 4 of the project. The results will be summarized in a paper that is in preparation and will be shortly submitted for revision.

I expressed a venus (modified YFP) tagged Orai1 plasmid via *in vivo* electroporation in the myostatin mutant muscles and studied the isolated single FDB fibers under whole cell voltage clamp. In these experiments I was interested to compare the magnitude of the calcium transients obtained under voltage clamp with the previously obtained results and detect

whether the re-expression of Orai1 can compensate somehow for the lack of endogenous Orai1 protein in Cmpt muscles. The V1/2 value was found -15.62±1.55 mV, n=7.

To complete these studies I used an Orai1-shRNA in order to silence the expression of endogenous Orai1 in control FDBs and see if this would affect the fatiguability of the muscle cells. I used a GFP tagged Orai1 silencing vector (purchased from Origene). The efficiency of silencing was verified on C2C12 myotubes and the best silencer (construct b) was used for the subsequent experiments. When examining the rate of Orai1 silencing by Western Blot I found a decrease of about 22% in case of the whole FDB muscle homogenates and a decrease of 21% in case of single fibers (protein samples were prepared from approximately 30 single FDBs that were identified as transfected by their 'green' fluorescence) when compared to untreated/non electroporated controls. The functional studies on the silenced FDBs revealed a slight rightward shift towards more positive voltages (-9.89±0.85 mV, n=7 for Orai1-shRNA vs -13.34±0.75 mV, n=9 for Orai1-scrambled) in the voltage activation by depolarization of the calcium transients. From these experiments, looking at the V1/2 values we could draw the conclusion that Orai1 most probably is not required for the skeletal ECC mechanism. An interesting feature observed is that the electroporation itself possibly affects the muscle function and alters its V1/2, but this is elusive and needs more research.

Lastly, I used a mitochondrial membrane potential dye (TMRE) to stain the mitochondria both in WT and Cmpt FDB fibers. Interestingly, in case of the mutant fibers I discovered regions with mitochondrial defects where the TMRE staining was lacking in patches. These regions averaged ~26% of the total fiber area. Simultaneously recorded confocal x-t line scan images of TMRE and fluo-8 fluorescence changes (F/F_0) in the region with normal and depolarized mitochondria during a maximal depolarizing pulse revealed a 28% decrease in the $\Delta F/F0$ values of the Ca^{2+} transients in the area with defective mitochondria.

In February 2018, I gave an oral platform presentation at the Biophysical Society Meeting in San Francisco, USA. I also gave an oral talk in June at the Hungarian Physiological Society Meeting in Szeged. Lastly, in September 2018 I presented a summary of the research (poster) at the European Calcium Society meeting in Hamburg.

Our results help resolve the long-standing question whether or not SOCE has any role during normal skeletal muscle activation. They favor the idea that *SOCE is immediately activated upon voltage-dependent SR calcium release*. By doing so it plays an important role in regulating SR calcium content both on the long run and also during a contraction-relaxation cycle. Reduced function and/or expression of SOCE partners (STIM1 and/or Orai1) could play an important role in muscle weakness associated to certain pathologies and aging. Tackling this calcium influx pathway could thus open novel and safe strategies to alleviate the symptoms of sarcopenia.