Real time imaging of trans-sarcolemma Ca^{2+}-fluxes in mammalian skeletal muscle

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Intracellular calcium (Ca^{2+})-release provides the basis for skeletal muscle contraction. Large amounts of Ca^{2+} are stored in the sarcoplasmic reticulum (SR) to be released into the cytosol during excitation–contraction (EC) coupling.

Besides the predominant Ca^{2+}-release from the SR, a Ca^{2+}-influx across the skeletal muscle t-system membrane has been reported to contribute to the rise in intracellular Ca^{2+} during excitation. Two entry pathways have been described. (i) Store-operated Ca^{2+}-entry (SOCE), an SR dependent process activated by a drop of intraluminal Ca^{2+}-levels, (ii) excitation-coupled Ca^{2+}-entry (ECCE), a Ca^{2+}-flux that is most likely carried by the DHPR. Despite their minor contribution to Ca^{2+}- inside the fibre during EC coupling both pathways are related to a series of muscle diseases and are most likely involved in the long term homeostasis of Ca^{2+}. Unfortunately, the currently available experimental techniques and model systems to study these processes are still very limited.

Here, we report the simultaneous measurements of calibrated t-system and cytosolic Ca^{2+}-signals during single action potentials and high frequency stimulation in skinned fast-twitch skeletal muscle fibres of the rat. Using fast laser scanning confocal microscopy in xyt mode we achieve a time resolution of 18 ms for the dual recording of rhod-5N and fluo-4 dye intensity, trapped in the t-system and loaded into the cytosol, respectively. Further, we greatly increase our signal to noise ratio by averaging the spatial information of the acquired image series.

We found that in electrically stimulated fibres each individual AP triggered a fast transient trans-sarcolemma Ca^{2+}-flux that resulted in a stepwise depletion of the t-system upon repetitive stimulation. The amount of t-system Ca^{2+} depletion as well as the rate was dependent on the stimulation frequency. The size of the individual depletion steps was proportional to the Ca^{2+} concentration within the t-system and occurred within one acquisition time frame, *i.e.* faster than 18 ms. Inhibition of the ryanodine receptor by 10 μM tetracaine substantially inhibited SR Ca^{2+}-release and concurrently reduced t-system Ca^{2+}-depletion in a highly correlated manner providing strong evidence for an underlying store-dependent mechanism. Interestingly, further raising the concentration of tetracaine completely abolished the SR Ca^{2+}-release while a reduced t-system Ca^{2+}-depletion could still be observed indicating a second, store-independent mechanism. The latter was responsible for the depletion of about 0.14 mM Ca^{2+} (with respect to t-system volume) upon each action potential. Store dependent depletion was only observed above a certain threshold of SR Ca^{2+} being released and rose gradually with increasing amounts of SR Ca^{2+}-released to reach a maximum of about 0.16 mM.

Taken together, we provide strong experimental evidence for the concerted action of SOCE as well as ECCE to underlie t-system Ca^{2+}-flux during single APs in mammalian skeletal muscle. These new insights will help to understand the role of SOCE and ECCE in skeletal muscle physiology under conditions of health and disease.