Examining the access to and handling of calcium by the t-tubular system in skinned rat fibres

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The t-tubular (t-) system of skeletal muscle is an internalization of the plasma membrane that allows action potentials to penetrate deeply into the muscle. Upon electrical stimulation dihydropyridine receptors activate and transmit the signal to the adjacent ryanodine receptors (RyRs) on the sarcoplasmic reticulum (SR) to trigger the release of calcium (Ca^{2+}) and initiate contraction. Additionally, depletion of the SR leads to activation of store-operated calcium entry across the plasma membrane with as yet undetermined mechanism and physiological function. Importantly, in the resting fibre leakage of Ca^{2+} through the RyR into the triadic cleft generates Ca^{2+}-levels well above the bulk cytosol in this spatially restricted domain (Cully *et al.* 2015). The current knowledge of t-system Ca^{2+}-handling remains poor and is mostly derived from in vitro studies. Here we aimed to gain a better understanding of this important process *in situ* particularly investigating the impact of the t-system potential, the changes in cytosolic sodium (Na^+) and adenosine triphosphate (ATP) concentrations, and the influence of RyR function.

The skinned muscle fibre technique was employed to load the t-system of male Wistar rat fast-twitch *extensor digitorum longus* muscle fibres with the Ca^{2+}-sensitive dye Rhod-5N and to enable experimental access to the fibre's cytosol (Cully et al. 2013). Real time confocal microscopy tracked the changes in dye fluorescence upon the respective experimental conditions.

The uptake of Ca^{2+} into the t-system at different cytosolic Ca^{2+}-concentrations ($[Ca^{2+}]_{cyto}$; 10nM−1μM) exhibited multiple kinetics, presumably reflecting the involvement of different compartments and/or function of distinct proteins. At 28nM $[Ca^{2+}]_{cyto}$ a fast and a delayed slow component was discernible. The steady state levels of $[Ca^{2+}]_{t\text{-}sys}$ could be well described by a Hill equation with a maximal value of about 1.5mM and a half point of about 28nM. Steady state values were not changed when the cytosolic potassium concentration ($[K^+]_{cyto}$) was reduced from 126 to 0mM (replacement with choline or Na^+) to depolarize the cell, but were reduced significantly upon removal of ATP. Increasing $[Na^+]_{cyto}$ slowed the uptake rate at low $[Ca^{2+}]_{cyto}$ but left steady state values unaltered. The dependence on ATP and different $[Na^+]$ suggests ATPases and Na^+-dependent exchangers to be involved in t-system uptake.

Blocking the RyR with 1μM ryanodine resulted in a reduction of steady state $[Ca^{2+}]_{t\text{-}sys}$ at all tested $[Ca^{2+}]_{cyto}$, most likely caused by an inhibition of the slow uptake component as became apparent at low $[Ca^{2+}]_{cyto}$. The respective Hill curve was not shifted. In contrast, under 0mM $[K^+]_{cyto}$ and in the presence of ryanodine the curve was shifted to higher $[Ca^{2+}]_{cyto}$ and reached the same maximal values as under drug free conditions. Similar results were obtained with 1mM tetracaine. These data show a fundamental link of the RyR, the triadic cleft, and subsequently the SR, in determining t-system Ca^{2+}-handling and further point to the fact that Ca^{2+} in the bulk cytoplasm may be restricted from re-entering the triadic cleft, presumably involving a mechanism dependent on the membrane potential of the t-system.

Cully TR, Edwards JN, Sannon TR, Launikonis, B.S.(2013). *Proc Aust Physiol Soc* **44**, 61P.
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