Ca^{2+} uptake by the tubular (t-) system membrane of rat fast-twitch muscle

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The tubular (t-) system of skeletal muscle is an internalization of the plasma membrane. The t-system forms a junction with the terminal cisternae of the sarcoplasmic reticulum (SR) at every sarcomere of skeletal muscle. At any given moment the $[Ca^{2+}]$ within the small volume bound by the junctional membranes will be critically determined by the leak of Ca^{2+} through ryanodine receptors and the net Ca^{2+} handling ability of the t-system. The Ca^{2+} in this microdomain can change rapidly on a very large scale during excitation-contraction coupling and also under situations where ryanodine receptor leak alters or the Ca^{2+} handling ability of the t-system may change, as during metabolic fatigue. In this study we aimed to assess the basic Ca^{2+} handling ability of the t-system of rat fast-twitch muscle fibres.

Wistar rats were euthanized under CO_2 asphyxiation and the extensor digitorum longus (EDL) muscles were rapidly dissected under protocols approved by the Animal Ethics Committee of The University of Queensland. Isolated EDL muscles were pinned in a Petri dish above a layer of Sylgard under a layer of paraffin oil. A bundle of fibres were then isolated and exposed to a physiological solution containing (mM): fluo-5N or rhod-5N, 1-10; NaCl, 145; KCl, 3; $MgCl_2$, 2 and HEPES, 10 (pH adjusted to 7.4 in NaOH). In some solutions all Na^+ was replaced with K^+. After waiting 10 – 15min, isolated fibres were mechanically skinned and transferred to an experimental chamber and bathed in an internal solution containing (mM): EGTA, 50; Na^+, 36; K^+, 126; Mg^{2+}, 1; total ATP, 8; creatine phosphate, 10 and HEPES, 90 (pH adjusted to 7.1 in KOH). Free Ca^{2+} was adjusted in the range 0-800 nM. Ca^{2+}-dependent fluorescence was continuously imaged on an Olympus FV1000 confocal microscope in *xyt* mode, with the dyes excited by laser lines 488 or 543 nm.

In situ calibration determined the half signal of fluo-5N and rhod-5N in the t-system to be close to 335 and 872 µM, respectively. Further experiments were conducted with rhod-5N as mM levels of Ca^{2+} were expected to be achieved in the t-system ($[Ca^{2+}]_{t\text{-sys}}$). Chronic depletion of $[Ca^{2+}]_{SR}$ with caffeine reduced $[Ca^{2+}]_{t\text{-sys}}$ to 0.1 mM *via* chronic activation of store-operated Ca^{2+} entry. We then exposed Ca^{2+}-depleted preparations to 0, 50, 100 and 800 nM (n = 4, 17, 10, 16 respectively) $[Ca^{2+}]_{cyto}$ in 50 mM EGTA. At $[Ca^{2+}]_{cyto}$ > 100 nM the $[Ca^{2+}]_{t\text{-sys}}$ reached a plateau at 1.8-1.9 mM after 3-5 s. At $[Ca^{2+}]_{cyto}$ < 100 nM the $[Ca^{2+}]_{t\text{-sys}}$ did not always reach this plateau and showed a biphasic uptake of Ca^{2+}. At the plateau $[Ca^{2+}]_{t\text{-sys}}$ lowering $[Ca^{2+}]_{cyto}$ to < 1 nM did not cause a significant loss of $[Ca^{2+}]_{t\text{-sys}}$. There was an apparent absence of effect of removing $[Na^+]_{cyto}$ on these results. Mathematical modeling of these results suggests that the plasma membrane CaATPase (PMCA) with its low K_m for Ca^{2+} is the major protein responsible for t-system Ca^{2+} uptake in the resting muscle, despite the higher transport capacity of the Na-Ca exchanger. Furthermore, these results show that the t-system membrane is able to establish the "physiological" Ca^{2+} gradient from within the cytoplasm without a requirement for Ca^{2+} to enter the t-system from the extracellular fluid surrounding the fibre.