Movements of calcium in skeletal muscle fibres in the absence of calsequestrin

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Calsequestrin (CSQ) is the major Ca^{2+}-buffer in skeletal muscle fibres, existing inside the internal Ca^{2+} store of the fibre, the sarcoplasmic reticulum (SR). Therefore, it was surprising that when a CSQ knock out mouse was engineered it remained viable with only relatively minor deficiencies in excitation-contraction coupling and total fibre calcium levels (Lamboley *et al.*, 2016). Interestingly, though, the mouse showed significant susceptibility to environmental heat stroke (EHS) and agonists of malignant hyperthermia (Dianese *et al.*, 2009). Therefore this mouse model must develop modifications in its Ca^{2+} handling to remain viable and able to respond to stimulation with normal force responses but also provides a model of EHS that can be examined. This study aimed to assess the Ca^{2+} movements and steady state localization of Ca^{2+} in these mice to gain a better understanding of muscle adaptations under compromised Ca^{2+} storage and EHS susceptibility.

CSQ isoform 1 (CSQ1) knock out mice colony were established at The University of Queensland. Wild type (WT) and CSQ1 KO mice were euthanized *via* CO2 overdose and EDL muscles were rapidly excised. Individual fibre segments from those muscles were mechanically skinned under paraffin oil so that they still contained their endogenous Ca^{2+} content. The total amount of endogenous Ca^{2+} contained in each fibre could be quantified by pre-equilibrating the fibre in a solution with a known concentration of the very fast calcium-buffer BAPTA and then transferring the fibre to an emulsion of 1% Triton X-100 and paraffin oil (TX-oil) in order to lyse all membranous compartments and release any Ca^{2+} from within the fibre (Fryer & Stephenson, 1996). The total amount of Ca^{2+} present in the fibre can be calculated from the known BAPTA concentration in the equilibration solution and the magnitude of the force response upon the lyses. Furthermore, other fibre segments, prior to the TX-oil lysing, were (1) totally depleted from their endogenous SR Ca^{2+} content by a 2 minute exposure to a solution containing 30 mM caffeine, 0.05 mM Mg^{2+} and with or without 25 uM FCCP, or (2) loaded to their maximal SR Ca^{2+} capacity by a 4 minute exposure to a solution containing 0.2 μM free Ca^{2+} (buffered with 1 mM CaEGTA – EGTA). Ryanodine receptor (RyR) Ca^{2+} leak and t-system Ca^{2+} uptake properties of the tubular (t-) system were determined using a recently developed technique that utilizes measuring basal Ca^{2+} movements into the t-system with t-system-trapped rhod-5N as imaged on the confocal microscope (Cully *et al.*, 2018).

Total calcium assays showed a reduction of ~40% in both maximal and resting calcium content in CSQ1 KO mice compared to WT. SR calcium content in CSQ1 KO fibres was also reduced by ~70% compared to WT, but a ~5-fold increase in mitochondrial calcium content was measured in CSQ1 KO mice compared to WT. Assays of RyR Ca^{2+} leak showed that CSQ1 KO fibres were significantly leaky compared to WT and that this leak could be reduced following 5 min treatment with a reducing agent like DTT. A rapid rate of t-system Ca^{2+} uptake was also observed in CSQ1 KO fibres, consistent with the RyR leak saturating the t-system PMCA with Ca^{2+}.

These results are consistent with a significant Ca^{2+} storage capacity of the SR being reduced in the absence of CSQ1. Our assessment of the compartmentalization of calcium in the muscle fibres of CSQ1 KO mice shows that ˜half of the total calcium at rest is not stored in the SR. The leaky RyR of the CSQ1 KO fibres was due to some oxidation of the RyR, which may be due to overload of the mitochondria with Ca^{2+}. A model has been developed demonstrating how these changes in Ca^{2+} handling may increase the susceptibility of the animal to EHS.

Cully TR, Choi RH, Bjorksten AR, Stephenson DG, Murphy RM, Launikonis BS (2018). Junctional membrane Ca^{2+} dynamics in human muscle fibres are altered by malignant hyperthermia causative RyR mutation. *Proc Natl Acad Sci USA* doi: 10.1073/pnas.1800490115

Dainese M, Quarta M, Lyfenko AD, Paolini C, Canato M, Reggiani C, Dirksen RT, Protasi F (2009). Anesthetic- and heat-induced sudden death in calsequestrin-1-knockout mice. *Faseb J* **23**, 1710-1720.

Lamboley CR, Kake Guena SA, Touré F, Hébert C, Yaddaden L, Nadeau S, Bouchard P, Wei-LaPierre L, Lainé J, Rousseau EC, Frenette J, Protasi F, Dirksen RT, Pape PC. (2015) *J Gen Physiol* **145**, 127–153.

Fryer MW, Stephenson DG. (1996). *J Physiol* **493**, 357-370.

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