Differential oxidation of ryanodine receptors in male and female calsequestrin knock-out mice

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Ca^{2+} is stored inside the sarcoplasmic reticulum (SR) of skeletal muscle fibres and provides the massive flux of Ca^{2+} to the cytoplasm following depolarization and the release of Ca^{2+} through the ryanodine receptor (RyR) on the SR that regulates contraction. Ca^{2+} inside the SR is strongly buffered by calsequestrin (CSQ). The properties of this Ca^{2+} buffer are unique. It is able to change its aggregation state with the concentration of calcium to significantly increase the buffering power of the SR. CSQ is densely packed in the terminal cisternae of the SR, immediately behind the RyRs. Mutations in CSQ have recently been linked to muscle myopathies and may make one susceptible to malignant hyperthermia (MH), a condition that arises under a general anaesthesia as a reaction to the anaesthetic that causes over-active Ca^{2+} release and heat generation as the SR uses ATP to constantly clear Ca^{2+} from the cytoplasm. Mice with CSQ knocked out (CSQ-KO) are known to be susceptible to MH under exposure to the volatile anaesthetic halothane. Interestingly this susceptibility has a gender bias towards male mice. We aimed to determine whether the RyRs of male and female CSQ-KO mice had different responses to halothane by directly imaging cytoplasmic Ca^{2+} transients in skinned muscle fibres from these mice challenged with halothane.

All experiments were approved by The University of Queensland Animal Ethics Committee. Wild-type and CSQ-KO mice were euthanized with an overdose of CO_2. The *extensor digitorum longus* (EDL) muscles with excised and pinned to a layer of Sylgard in a Petri dish with the muscle immersed under a layer of Paraffin Oil. A bundle of fibres was isolated with fine forceps and then individual fibres were isolated. The sarcolemma of single fibres was mechanically removed to make the cytoplasmic environment of the fibre open to experimental manipulation. Skinned fibres were moved to an experimental chamber that used a coverslip as a base with the fibre bathed in an cytoplasmic solution containing (in mM): K^+, 136; Na+, 36, Mg^{2+}, 1; Ca^{2+}, 0.0001; ATP, 8; rhod-2, 0.01; HDTA, 49.9; creatine phosphate, 10; and EGTA, 0.1; with pH adjusted to 7.1 ± 0.1 with KOH. 0.1 to 1 mM halothane was added to the internal solution. The reducing agent Dithiothreitol (DTT) was also added to internal solution. Rhod-2 fluorescence signal from the cytoplasm of skinned fibres was continuously recorded on a confocal microscope in xyt mode.

The addition of halothane to the cytoplasmic solution induced regenerative Ca^{2+} waves in all mice, with an order of sensitivity, male CSQ-KO > female CSQ-KO > wild type mice. The average frequency of Ca^{2+} waves was the most variable amongst female CSQ-KO mice. To test whether the RyR of CSQ-KO showing greater sensitivity to halothane was due to the oxidised state of the RyR, we treated the fibres with 10 mM DTT for 5 min to reduce the RyR oxidation state. Treatment with DTT significantly reduced the frequency of halothane-induced Ca^{2+} waves in male CSQ-KO mice and in the fibres from female CSQ-KO mice that displayed initial high Ca^{2+} wave frequency. These results suggest that the RyRs in a greater proportion of fibres from male CSQ-KO mice succumb to oxidative stress than the RyRs in muscle fibres from female CSQ-KO mice. It appears the increased leakiness of the RyR induced by oxidative stress make the muscle fibres susceptible to halothane-induced opening.