Mechanisms of Ca^{2+} release in human and toad skeletal muscle in response to halothane

D.P. Singh, L. Pearce, C.R. Lamboley and B.S. Launikonis, School of Biomedical Sciences, The University of Queensland, St Lucia, QLD 4072, Australia.

Ca^{2+} release through the ryanodine receptor (RyR) can be directly activated by agonists such as volatile anaesthetics (*e.g.* halothane). In humans with a RyR mutation, typically used concentrations of halothane can cause Ca^{2+} release, making them susceptible to malignant hyperthermia (MH). The mechanism of Ca^{2+} release through the RyR of people who are MH susceptible is not well understood. While it is known that direct activation of RyR opening is dependent on local Ca^{2+} levels, how Ca^{2+} is interacting with the RyR, and adjacent RyRs, to open these channels and cause propagating Ca^{2+} that probably underlie an MH episode require examination.

In skeletal muscle, RyRs can be opened by Ca^{2+} induced Ca^{2+} release (CICR) or activated by luminally high Ca^{2+}. The latter mechanism has been referred to as store overload-induced Ca^{2+} release (SOICR). In amphibian skeletal muscle, two RyR isoforms exist and a prominent CICR mechanism is active when cytoplasmic Ca^{2+} is raised. In mammalian skeletal muscle CICR is either weak, not present at all or completely inhibited by an interaction with adjacent voltage sensors. We suspected that comparing how halothane induced Ca^{2+} release in toad and human muscle susceptible to MH would assist in distinguishing between the mechanisms that work during an MH episode. Therefore we aimed to compare halothane-induced Ca^{2+} release in toad and MH susceptible human muscle fibres under identical conditions.

All experiments performed were approved by The University of Queensland Human Ethics & Animal Ethics Committees. Human muscle biopsies were collected under local anaesthesia from the Vastus Lateralis (VL) muscle. Cane toads (*Bufo Marinus*) were euthanized by double pithing and the Iliofibularis (IL) muscle was extracted. Single fibres were isolated and mechanically skinned under paraffin oil.

We hypothesized that by using mechanically skinned fibres from toad and MHS humans in the same experimental chamber that any differences in Ca^{2+} release properties under 1 mM halothane would be observed by rapidly imaging cytoplasmic Ca^{2+} in a K^+-based cytoplasmic solution containing rhod-2 and 0.1 mM EGTA (0.1 - 0.2 μM [Ca^{2+}]) on a Zeiss LSM 5 live microscope (Cully *et al.*, 2016). To do this we positioned two fibres perpendicularly to each other, that is, they crossed over to form a junction between to the two preparations. Ca^{2+} waves reaching the junction allowed the effect of locally increased cytoplasmic Ca^{2+} to be observed as a new wave was established on the adjacent fibre. The two fibres placed in the chamber were placed in the combinations: toad v toad; human v human; and toad v human.

In toad *vs* toad experiments, Ca^{2+} wave propagation into the quiescent fibre from the active fibre occurred rapidly (1.02 ± 0.08 s; n = 10). In human *vs* human (MHS muscle), a delay of 4.17 ± 0.51 s (n = 7) in the propagation of Ca^{2+} release from the active to quiescent fibres was observed. In toad *vs* human fibre experiments (n=11), the characteristics of wave initiation in the quiescent fibres were maintained in each taxa. These Ca^{2+} wave propagation rates differed significantly between toad (1.02 ± 0.08 secs) *vs* human MHS (4.17 ± 0.51 s, *T*-test, $P < 0.05$). The delay in initiation of Ca^{2+} waves in the quiescent fibre from the local Ca^{2+} rise in the active fibre indicate that the cytoplasmic Ca^{2+} immediately causes Ca^{2+} release in the toad (CICR) whereas the delay in human fibres indicates that the SR needs to load Ca^{2+} to reach the threshold for luminal activation of Ca^{2+} release.

Cully TR, Edwards JN, & Launikonis BS (2014). Activation and propagation of Ca^{2+} release from inside the sarcoplasmic reticulum network of mammalian skeletal muscle. *J Physiol*, ***592***, 3727-3746.