Quantification of endogenous and maximal sarcoplasmic reticulum calcium content in human *vastus lateralis* muscle

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The ability of skeletal muscle to produce strong and repeated contractions depends on a coordinated sequence of molecular processes known as excitation-contraction coupling. Briefly, contraction in skeletal muscle is initiated when action potentials propagate into the transverse tubular (T) system, causing rapid depolarization, which in turn triggers Ca^{2+} release from the sarcoplasmic reticulum (SR). The subsequent force production is closely related to the amount of Ca^{2+} released from the SR. Since the latter depends strongly on the content of total Ca^{2+} in the SR ($[Ca_T]_{SR}$) it would be important to have a reliable measurement of $[Ca_T]_{SR}$ in human skeletal muscle fibres under physiological resting conditions.

The present study examined for the first time, in individual fibres from human skeletal muscle biopsies, whether endogenous SR Ca^{2+} content and maximal SR Ca^{2+} capacity are different between fast-twitch (FT) and slow-twitch (ST) fibres.

The study was approved by the Human Ethics Committees at Victoria University and La Trobe University. A muscle biopsy was taken from the *vastus lateralis* muscle from eleven healthy young adults. After injection of a local anaesthetic into the skin and fascia (1% lidocaine (Xylocaine)), a small incision was made and a muscle sample taken (~150 mg) using a Bergstrom biopsy needle. Individual fibre segments, obtained from the biopsy, 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 for 20 s and then transferring the fibre to an emulsion of 10% 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). If the preequilibrating [BAPTA] was chosen such that the fibre produced a finite, non-maximal force response upon lysis, then the total amount of Ca^{2+} present in the fibre can be calculated from the BAPTA concentration in the equilibration solution and the magnitude of the force response. Furthermore, other fibre segments, prior to the TX-oil lysing, were (1) totally depleted of their endogenous SR Ca^{2+} content by a 1 minute exposure to a solution containing 30 mM caffeine and 0.05 Mg^{2+} 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). Finally, using Western blotting, each muscle fibre was classified as FT or ST according to the myosin heavy chain isoform present.

When fibres with an endogenous Ca^{2+} content were assayed, the endogenous $[Ca_T]_{SR}$ obtained was 0.71±0.03 (n=8) and 0.80±0.02 (n=12) mmol.l^{-1} (expressed relative to intact fibre volume) for ST and FT fibres, respectively (P<0.05). By loading the SR of the fibres maximally, the study also revealed that the maximal SR Ca^{2+} capacity for ST and FT fibres was 1.35±0.04 (n=13) and 1.70±0.03 (n=17) mmol.l^{-1}, respectively (P<0.01).

In conclusion, the present results show that the SR properties of the FT human fibres were significantly different from those of the ST fibres. The FT fibres had a larger SR capacity and the endogenous Ca^{2+} content was a relatively lower percentage of the maximum compared with ST fibres (47 and 53%, respectively). Future studies, using the same technique could reveal whether these SR properties alter with the diverse changes that can occur with ageing, inactivity or chronic diseases.

Fryer M.W. & Stephenson D.G. (1996). Total and sarcoplasmic reticulum calcium contents of skinned fibres from rat skeletal muscle. *Journal of Physiology*, **493**, 357-370.