Characterisation of SERCA, phospholamban and sarcolipin proteins in human skeletal muscle

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Sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) proteins are ATP-dependent Ca^{2+}-pumps located within the membrane of sarcoplasmic reticulum (SR), and together with phospholamban (PLBN) and sarcolipin (SLN), are associated with maintenance of intramuscular Ca^{2+} homeostasis. Skeletal muscle is heterogeneous, comprised of slow-twitch (MHC I) and fast-twitch (MHC IIA and IIX) fibres, which are distinct in their metabolic and contractile properties. In skeletal muscle there are fibre-specific isoforms of SERCA (SERCA1 and SERCA2a), whilst PLBN and SLN are differentially expressed between fibre types. Additionally, PLBN is present in interchangeable monomeric (mon-PLBN, active) and pentameric (pen-PLBN, inactive) forms. In human single muscle fibres, MHC II fibres predominantly express SERCA1, and in contrast, MHC I fibres express predominantly SERCA2a (Lamboley *et al.*, 2014). Further, the relative amount of PLBN protein in human single fibres is ~two-fold greater in MHC I than MHC IIA fibres, whereas SLN is ~four-fold greater in MHC IIA than MHC I fibres (Fajardo *et al.*, 2013). However, despite these MHC I and IIA fibre differences in protein abundances, only minor fibre-type differences in maximum Ca^{2+}-uptake rate and SR ATPase rate have been shown (Lamboley *et al.*, 2014, Szentesi *et al.*, 2001). Interestingly, Szentesi *et al.*, (2001) did find that the maximum SR ATPase rate was ~3-fold greater in MHC IIA-X and IIX fibres than MHC IIA fibres. Importantly, the relative amounts of SERCA, PLBN and SLN proteins in fibres expressing MHC IIX fibres are not known.

In this study, segments of individual muscle fibres (n= 341) were collected from *vastus lateralis* muscle biopsies taken from 19 participants (19-73 years old), taken using the Bergstrom biopsy technique following injection of 1% Lidocaine (Xylocaine) into the skin and fascia. Individual fibre segments were prepared for Western blotting analysis of SERCA1, PLBN and SLN proteins. Further to this, subcellular localisation of SERCA, PLBN and SLN protein was determined using both crude whole muscle fractionation and single fibre diffusibility followed by Western blotting analysis (Murphy *et al.*, 2012).

SERCA1 in both MHC IIA-X and MHC IIX fibres was ~1.3-fold greater than in MHC IIA fibres, and ~2.5-fold higher than in MHC I-II fibres, whilst SERCA1 was not detectable in any MHC I fibres. Both pen-PLBN and mon-PLBN were detected in all MHC fibre types characterized, where compared with MHC I fibres, pen-PLBN was ~2.5, 10 and 3-fold less abundant in MHC IIA, IIA-X and IIX fibres, respectively, and mon-PLBN was ~1.4, 3 and 5-fold less abundant in MHC IIA, IIA-X and IIX fibres, respectively. Interestingly, pen-PLBN was not present in detectable amounts in some MHC IIA-X fibres. SLN was also detected in all MHC fibre types, and was on average ~two-fold higher in all MHC II fibre types than in MHC I fibres.

As expected, very little SERCA, mon-PLBN and SLN protein pools were located in the cytosolic fractions of skeletal muscle using both techniques, and crude fractionation isolated them in membrane fractions. However this was not the case for pen-PLBN, which was found equally in the cytosolic and membrane fractions using crude fractionation but not seen in the cytosolic fraction using single fibre diffusiblity experiments. We believe the former is artefactual, indicating the necessity for rigorous experimental design and analyses of all fractions whenever muscle is fractionated.

This work provides novel insight into the ratios of pen-PLBN: mon-PLBN: SLN protein amounts in the different MHC fibre types within human skeletal muscle, giving plausible explanation for the higher SR ATPase rates seen in fibres expressing MHC IIX isoforms, being that those fibres have a decreasing abundance of the inhibitory protein, PLBN, in both the pentameric and monomeric forms.

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