Calpastatin and m-calpain have different cellular localizations in rat skeletal muscle: implications for function

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Calpastatin is the only known endogenous protein that specifically inhibits the proteolytic activity of the ubiquitously expressed calpains, μ- and m-calpain. There are widely varied reports on the subcellular localization of the calpains and calpastatin. Some reports concluded that the calpains and calpastatin are co-localized at sarcomeric structures within muscle fibres (Kumamoto *et al.*, 1992), while others concluded that both are widely dispersed within the cytoplasm of the cell and upon Ca^{2+} influx, calpain redistributes to plasma membrane whilst calpastatin localization is unaffected (Gil-Parrado *et al.*, 2003). The ambiguity in available data on the amount and localization of calpastatin raises the question of how and the degree to which it functions as a calpain inhibitor in skeletal muscle.

Extensor digitorum longus (EDL) and *soleus* (SOL) muscles were dissected from male Long-Evans hooded rats sacrificed by anaesthetic overdose (2% v:v isofluorane) with approval of the La Trobe University Animal Ethics Committee. Western blotting was used to compare the amount of full length calpastatin present in both muscle types, using purified full length human calpastatin with GST-tag as a point of reference due to the anomalous migration of calpastatin in SDS-PAGE. The expression of calpastatin was found to be 5-7 times higher in EDL than SOL and the absolute amount of calpastatin ~58 and ~9 nmol/kg muscle mass in EDL and SOL muscles, respectively. It should be noted, however, that these experiments used the human native calpastatin and it is not known whether the antibody binding efficacy was the same for rat and human calpastatin. Based on previous results (Murphy *et al.*, 2006; Mollica *et al.*, 2010), the absolute amount of calpastatin found in EDL and SOL muscle is 10-100 times less than the total amount of calpain present. Mechanically skinned fibres were used to assess protein diffusibility as previously described (Murphy *et al.*, 2006). Skinned fibre segments were bathed in a physiological-like intracellular solution with $[Ca^{2+}]$ buffered at the normal resting level, and the washed fibre segment and bathing solution were both collected and analysed by Western blotting. The results indicated that the majority of the total m-calpain is freely and rapidly diffusible within a quiescent fibre, but full-length calpastatin remains very tightly bound within the fibre for at least 30 min. Fractionation of muscle homogenates confirmed the observations made with the skinned fibres, with the majority of m-calpain appearing in the cytosolic fraction and very little associated with membranes or the cytoskeleton, and with calpastatin mostly associated with membranes and a small amount appearing in the cytosol.

In conclusion, these findings demonstrate that calpastatin and calpain exhibit considerable differences in their localization within resting muscle fibres and their amounts and relative expression in different muscle fibre types. This indicates that there may be considerable constraints and limitations on the ability of calpastatin to inhibit calpains in muscle fibres.

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