The location of nascent proteins in mechanically skinned skeletal muscle fibres of the rat

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A novel technique for measuring protein synthesis in segments of single mechanically skinned muscle fibres was recently developed by us to investigate the cellular and molecular events underpinning protein synthesis in muscle (Jame, Jois & Stephenson, 2006 & 2007). Given the absence of a surface membrane, which is removed in this preparation by microdissection, it was important to determine where in the preparation the newly synthesised proteins are located.

As previously described, mechanically skinned fibre segments were prepared from freshly dissected soleus muscles of rats (3-5 months old Long-Evans, hooded) killed by isoflurane overdose in accordance with animal ethics procedures approved at La Trobe University. The skinned fibre segments were then incubated at 30°C for 2 hours in a medium mimicking the myoplasmic ionic environment and containing ^{3}H-leucine and a mixture of all the 20 amino-acids required for protein synthesis (Jame, Jois & Stephenson, 2007). Protein synthesis was determined by measuring the difference between ^{3}H-leucine incorporation in paired segments of the same skinned fibre, one incubated in the absence and the other in the presence of the well established protein synthesis inhibitor, cycloheximide (CHX). Following incubation, the two fibre segments were washed under identical conditions and then the membranous compartments of the single skinned muscle fibre segments were lysed by osmotic shock, in double distilled H_2O followed by freezing and thawing and finally by exposure to 1% Triton X-100 in a relaxing solution heavily buffered for very low free [Ca^{2+}].

Effectively all CHX-sensitive ^{3}H-leucine incorporation in the mechanically skinned fibres could be released when the intracellular membrane compartments were subjected to the lysing procedure (103 ± 30 % total CHX-sensitive ^{3}H-leucine incorporation, n = 7), indicating that the newly synthesised proteins were accumulating in a membraneous compartment. As any protein synthesis occurring on free ribosomes would be expected to freely diffuse out in the absence of a surface membrane, the results provide strong evidence that the major site for protein synthesis measured in the skinned fibre preparation is the rough endoplasmic (sarcoplasmic) reticulum. Considering that the sarcoplasmic reticulum remains structurally and functionally intact after the skinning procedure (Lamb, Junankar & Stephenson, 1995), this study shows that it is now possible to measure protein synthesis at single muscle fibre level associated with the intact rough endoplasmic (sarcoplasmic) reticulum under controlled conditions.

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