Effects of calmodulin on protein synthesis in mechanically skinned skeletal muscle fibres of the rat

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The rate of protein synthesis in mechanically skinned skeletal muscle fibres of the rat has been shown to depend markedly on the level of ionised Ca^{2+} present (Jame *et al.*, 2006). Protein synthesis was completely abolished when the [Ca^{2+}] was raised from 280 nM to 1 μM; however, the mechanism behind this is not known. Recent studies (Rose *et al.*, 2005) have revealed that during dynamic exercise a Ca^{2+}-calmodulin dependent process activates eukaryotic elongation factor2 (eEF2) kinase causing increased phosphorylation of eEF2 thereby decreasing its activity. This inhibits mRNA translation indicating that the depression in muscle protein synthesis during exercise is regulated by Ca^{2+}-calmodulin. As the skinned fibre preparation enables control over the intracellular environment, the aim of this study was to determine whether the Ca^{2+}-sensitive rate of protein synthesis was also calmodulin dependent.

Experiments were conducted using our novel technique in accordance with procedures described previously (Jame *et al.*, 2006). In brief, fibres were mechanically skinned from freshly dissected soleus muscles of rats (3-5 months old Long-Evans, hooded) killed by isoflurane overdose. The skinned fibre segments were then incubated at 30ºC 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. Protein synthesis rates were measured as the cycloheximide-sensitive incorporation of ^{3}H-leucine in the presence and absence of 8 μM calmodulin in the incubation medium.

In media containing 280 nM Ca^{2+} strongly buffered with EGTA, the presence of 8 μM calmodulin markedly reduced the rate of protein synthesis. However, reducing the calmodulin concentration in the medium to 0.8 μM had no effect on the synthesis rate. There was also no significant difference between the rates of synthesis in the presence and absence of 8 μM calmodulin when the [Ca^{2+}] in the incubating media was less than 100 nM. These findings reveal that calmodulin does have an inhibitory action on protein synthesis in muscle and that this inhibition is dependent on the concentration of both calmodulin and Ca^{2+}. In conclusion, Ca^{2+}-calmodulin dependent processes play a critical role in the regulation of protein synthesis in skeletal muscle.

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