Protein synthesis measurements in mechanically skinned skeletal muscle fibres of the rat

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Maintenance of muscle mass is dependent on the balance of synthesis and breakdown of proteins in the muscle. Understanding the regulation of cellular and molecular events underpinning protein synthesis in muscle is critical for developing effective treatment and management strategies of pathophysiological conditions to prevent muscle wasting. All studies on the regulation of protein synthesis to date have used intact cells/organs/whole body. Here we report the development of a novel technique to measure protein synthesis in mechanically skinned muscle fibres from which the surface membrane is removed by microdissection allowing direct access to the myoplasm. Rats were killed by halothane overdose in accordance with procedures approved by Animal Ethics Committee. Mechanically skinned single fibres from soleus were prepared under a dissection microscope as previously described (Fink *et al.*, 1986). The skinned fibres were then placed into a solution (1μL) mimicking the resting environment and containing in addition 0.1mM of each of the 20AAs. The overall rate of protein synthesis was measured from the rate of ^{3}H-leucine incorporation. The net rate of ^{3}H-leucine incorporation in mechanically skinned fibres (volume 3.8-7.5nL) from the rat soleus muscle at 30°C remained constant for at least two hours (2.23±0.14pmol ^{3}H-leucine/μL fibre volume/h or 8.9±0.6pmol/mg muscle protein/h considering that 1μL fibre contains about 0.25mg protein). The results show that the entire machinery for protein synthesis remains fully functional and coupled in the mechanically skinned fibre preparation exposed for several hours to aqueous solutions opening up the possibility of investigating the regulation of protein synthesis in single muscle fibres of known type under controlled myoplasmic conditions.

Fink RH, Stephenson DG & Williams DA (1986) *Journal of Physiology*, **370**: 317-337.