Properties of Heat Shock Protein 25 and 72 in rat skeletal muscle

N.T. Larkins, R.M. Murphy and G.D. Lamb, Department of Zoology, La Trobe University, Melbourne, VIC 3086, Australia.

Skeletal muscle has a remarkable ability to adapt to physiological stresses. Stresses such as glycogen depletion, myoplasmic free Ca^{2+} accumulation, ischemia, heat and exercise can all induce a family of proteins known as heat shock proteins (Hsp). These proteins are considered to play an essential role in maintaining cellular homeostasis through their role as molecular chaperones. These proteins are categorized by their molecular weights and show a high degree of homology among various species. The function and properties of Hsp 25 and Hsp 72 in non-stressed muscle are not well understood. In the present study we have assessed the relative and absolute amounts of Hsp 25 and Hsp 72 as well as their diffusibility and fibre type dependency in resting rat skeletal muscle.

Male Long-Evans hooded rats (6-8 months old) were sacrificed using a lethal overdose of Fluothane in accordance with the La Trobe University Ethics Committee and the extensor digitorum longus (EDL) and soleus (SOL) muscles were excised. Portions of muscle were homogenized and whole muscle homogenates were analysed using a sensitive Western blotting technique. To compare fibre type differences EDL (exclusively type II) and SOL (predominantly type I) muscles homogenates were run side by side. To determine the absolute amounts of Hsp 25 and 72 in muscle, known amounts of pure Hsp 25 and Hsp 72 were run on Western blots alongside the muscle homogenates samples (Murphy *et al.,* 2008). To measure protein diffusibility individual fibres were dissected from muscles that had been immersed in paraffin oil and then mechanically-skinned and exposed to physiological K^+-based solution (pCa < 10) for various times (2-120 mins). The wash solution and their matched fibres were analysed by Western blotting (Murphy *et al.*, 2006).

When equal amounts of whole EDL and SOL homogenates were compared, there was 2-3 times more Hsp 25 and Hsp 72 in SOL compare to EDL muscle homogenate. The absolute amounts of Hsp 25 and Hsp72 in SOL muscle were found to be ~5μM and ~2.6 μM, respectively. After mechanically-skinning SOL and EDL fibres, ~two-thirds of Hsp 25 and Hsp 72 were found in the wash solutions, indicating these proteins are essentially free to diffuse within the cytoplasm.

Murphy RM, Larkins NT, Mollica JP, Beard NA, Lamb GD. (2008) *Journal of Physiology*, submitted.
Murphy RM, Verburg E, Lamb GD. (2006) *Journal of Physiology*, **576:** 595-612.