

Higher expression of caveolin-3 in mechanically-skinned single fibres from slow-twitch muscle compared with fast-twitch muscle

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

Caveolin-3 is a muscle specific membrane protein which is important in the formation of caveolae (caveolin-containing lipid rafts) and which has been linked to membrane trafficking and signal transduction events. Mutations in caveolin-3 cause limb girdle muscular dystrophy type 1C (LGMD1C) and rippling muscle disease. Caveolin-3 is transiently associated with the t-tubular membrane during muscle development and it is widely reported to be localised exclusively at the sarcolemma in mature muscle fibres. Using microscopy, caveolin-3 was localized to the t-tubule in adult mouse soleus muscle fibres. 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. From these muscles, segments of fibres were collected with either their sarcolemma present (intact) or with their sarcolemma removed by mechanically-skipping the fibres (skinned). We found a high expression of caveolin-3 in skinned fibres, supporting an intracellular pool of caveolin-3, likely localised to the t-tubular membrane. A higher expression of caveolin-3 was seen in both skinned and intact fibres from the predominantly type I *soleus* muscle compared to the fibres from the predominantly type II EDL muscle (eg., for intact fibres there was $200 \pm 25\%$ caveolin-3 in SOL fibres relative to that in EDL fibres, $p < 0.05$, $n = 12$, unpaired, two-tailed *t*-test). The relative amount of caveolin-3 in skinned and intact segments was quantified by comparing skinned and intact segments from the same fibre. For EDL and SOL fibres the amount of caveolin-3 in a skinned segment was 70-80% of that in the intact segment. The amount of caveolin-3 present in EDL skinned fibres was also analysed alongside their corresponding sarcolemmal portions and when expressed as a percentage of the total caveolin-3 present, $29 \pm 9\%$ ($n = 6$) of the caveolin-3 was found in the sarcolemma. As expected, caveolin-3 was found to be tightly bound in skeletal muscle fibres with none of the protein being present in various physiologically-based wash solutions (including very low and physiologically high Ca^{2+} concentrations) following washes of skinned fibres for up to 60 min. However, 80% or more of the detectable caveolin-3 became diffusible following a 30 min treatment with 1% Triton X-100 solution. These findings show that there is a greater expression of caveolin-3 in slow twitch muscle fibres compared with fast-twitch muscle fibres and that a greater proportion of the caveolin-3 is found inside muscle fibres than in the sarcolemma.

Parton RG, Way M, Zorzi N & Stang E. (1997) *Journal of Cell Biology*, **136**: 137-54.

Ralston E & Ploug T. (1999) *Experimental Cell Research* **246**: 510-5.