Properties of isolated skinned fast-twitch fibres from α-actinin-3 knockout mice

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α-Actinin-3 is found in the Z-disks of fast glycolytic skeletal muscle fibres, where it cross-links the actin filaments of the contractile apparatus. About 1 billion people worldwide are completely deficient in this protein. In this study we used individual skinned fibres from the EDL muscles of wild-type and *Actn3* knockout mice to examine possible mechanisms for the slowing of relaxation observed in α-actinin-3-deficient whole muscle. Animals aged 9 to 10 months were sacrificed with an overdose of halothane (ethics approval UNSW). Mechanically skinned fibres were first placed in K^+-HDTA solution containing low Mg^{2+} (0.25 mM) and 30 mM caffeine, to deplete the SR of endogenous Ca^{2+}, and 0.25 mM EGTA to chelate all released Ca^{2+} and prevent SR Ca^{2+} reaccumulation. The fibre was then reloaded with Ca^{2+} for predetermined periods of time by exposure to a highly buffered Ca^{2+} solution (pCa 6.57). Loading was rapidly terminated at the end of each loading period by a brief exposure to a relaxing solution, after which the fibre was washed in a K^+-HDTA solution to remove excess EGTA. The fibre was then reexposed to the caffeine solution and the force response was recorded. The area under the force response curve was used as a measure of the amount of Ca^{2+} released, and hence of the amount of Ca^{2+} loaded. For all loading periods, the amount of Ca^{2+} loaded by the SR, expressed as a percentage of the maximum amount it could load in our solution, was lower in knockout fibres than in wild-type fibres. This suggests that in knockout fibres the SR resequesters Ca^{2+} at a slower rate than in wild-type fibres. This result provides one possible reason for the slowing of relaxation observed in whole *Actn3* knockout muscle. Following the SR loading experiments the fibre was chemically skinned and the properties of the contractile filaments were examined. Force-pCa and force-pSr curves were obtained by exposing the fibres to a series of increasing [Ca^{2+}] and [Sr^{2+}]. No differences were found between wild-type and knockout fibres in their pSr_{50}-pCa_{50}, indicating that the slowing of relaxation was not due to any shift in myosin heavy chain isoforms from fast types to slow-type. However, the knockout fibres had significantly steeper force-pCa curves than wild-type fibres (Hill coefficient 3.31 ± 0.17 n=18 KO *vs* 2.68 ± 0.07 n=17 WT, p=0.002). The impact of this on whole muscle relaxation times is unclear, but it does indicate that loss of α-actinin-3 leads to subtle changes in the sensitivity of the contractile proteins to Ca^{2+}.