Calcium-phosphate precipitation in the sarcoplasmic reticulum reduces action potential-mediated Ca^{2+} release in mammalian skeletal muscle

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Rapid ATP buffering during vigorous activity is predominantly achieved by the enzyme creatine kinase and the substrate creatine phosphate (CrP), which is present at ~40 mM (Allen *et al.*, 1995). As ATP is hydrolysed to ADP and inorganic phosphate (P_i), CrP donates its phosphate to the ADP to resynthesize ATP, and the [P_i] within the cytoplasm of fast-twitch muscle fibres may reach ≥ 30 mM. Evidently P_i can enter the sarcoplasmic reticulum (SR) passively (Posterino & Fryer, 1998), via small conductance chloride channels that conduct P_i (Laver *et al.*, 2001). It has been proposed (Fryer *et al.*, 1995) that once inside the SR, P_i could bind to Ca^{2+} forming a calcium-phosphate (Ca-P) precipitate. We examined whether Ca-P precipitate formed in the SR and whether it reduced normal action potential (AP)-mediated Ca^{2+} release, and hence could contribute to the later stages of metabolic muscle fatigue that result from a failure of Ca^{2+} release (Allen *et al.*, 1995).

Long-Evans hooded rats were killed under deep anaesthesia (2% v:v halothane) and the extensor digitorum longus (EDL) muscles were excised. Single fibres were mechanically-skinned, connected to a force transducer and immersed in a standard K-HDTA 'control' solution (1mM free Mg^{2+}; 8 mM total ATP; 10 mM creatine phosphate (CP) at pH 7.10, containing 75 μM EGTA, pCa 6.9). Individual fibres were then stimulated: 1) electrically (75 V cm^{-1}, 20 pulses of 2 ms duration) to produce tetanic (50 Hz) force responses, or 2) by exposure to a 30 mM caffeine-0.05 mM Mg^{2+} solution with 0.5 mM EGTA present, which produced a submaximal longer-lasting force response (e.g. ~10 sec). 30 mM P_i solutions (replacing 23 mM HDTA with 30 mM P_i, and adjusting the total [Mg^{2+}]) were made similar to the standard K-HDTA solution (with or without 10 mM CrP present). The fibre was exposed to either no P_i (control), 10 or 30 mM P_i for 10 s, then immersed in paraffin oil (1 min), placed back into the same solution (10 s) as before and then transferred back into the oil (1 min). This procedure created a 'closed' system around the fibre and prevented any appreciable net Ca^{2+} uptake or loss by the SR from the weakly Ca^{2+}-buffered solution trapped inside the fibre. The fibre was then washed (30 s) in standard solution to remove any P_i in the cytoplasm before stimulating the fibre.

Total SR Ca^{2+} content was ascertained by pre-equilibrating the fibre for 20 s in standard solution with a known [BAPTA] present and then lysing all membranous compartments within the fibre by exposure to an emulsion of Triton-X100 (10% v:v) in paraffin oil (Owen *et al.*, 1998). All experiments were performed at 24 ±1 °C.

After a 2 min exposure to 30 mM P_i (with, n=4, or without, n=6, 10 mM CrP present) the total amount of Ca^{2+} released from the SR by caffeine-low [Mg^{2+}] stimulus was significantly (P<0.05) reduced by ~20%, and the initial rate of force development slowed (~55%). Peak tetanic (50 Hz) force was also significantly reduced by ~25% and ~45% after 10 and 30 mM P_i exposures respectively, n=4 for 10 mM P_i and n=14 for 30 mM P_i). Tetanic force responses produced after 30 mM P_i exposure were nearly identical to those seen in the same fibre following depletion of total SR Ca^{2+} by ~35% (using a tetanic stimulus in the presence of 2 mM BAPTA, the total Ca^{2+} remaining in the SR was 0.75 ± 0.03 mM, n=5). Ca^{2+} content assays revealed that the total amount of Ca^{2+} remaining in the SR was not detectably changed after 30 mM P_i exposure (initially 1.16 ± 0.04 mM, n=9 and 1.16 ± 0.07 mM, n=3 after 30 mM P_i exposure) thus indicating that Ca^{2+} had not leaked out of the SR but instead formed a precipitate with the P_i, thereby reducing the amount of available Ca^{2+} for rapid release.

These results suggest that Ca-P precipitation occurring within the SR may contribute to the failure of Ca^{2+} release observed in the later stages of metabolic muscle fatigue. They also demonstrate that a drop in the amount of total SR Ca^{2+} to a level substantially below the normal endogenous level will appreciably reduce tetanic force.

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