Coupling and uncoupling of the DHPRs and Ca^{2+} release channels in skeletal muscle fibres

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This study focused on the importance of transverse tubular (T)-system potential on excitation-contraction (E-C) coupling and the consequences of disrupted dihydropyridine receptor (DHPR)-ryanodine receptor (RyR) coupling on sarcoplasmic reticulum (SR) Ca^{2+} handling. Male Long-Evans hooded rats were killed by anaesthetic overdose (4 % v:v isoflurane) and EDL muscles swiftly excised and immersed in paraffin oil at resting length. Individual fibres were mechanically skinned, connected to a force transducer (stretched to 120 %) and immersed in a standard K-HDTA-based solution. All solutions contained as follows (in mM); 1 free Mg^{2+}, 8 ATP; 10 creatine phosphate, 55, 66 or 126 K^+, at pH 7.1, and were equilibrated to room temperature (~23°C). Single fibres were electrically stimulated (75 V cm^{-1}, 1 ms pulse) to produce twitch or tetanic (50 and 100 Hz) force responses. Additionally, paired pulses with differing intervals (0-50 ms) were applied to determine the repriming period of sodium channels in the T-system membrane (Dutka & Lamb, 2007a).

The importance of T-system membrane potential. Partial long-lasting depolarization of the T-system membrane (achieved by lowering the cytoplasmic [K^+]) reduces tetanic force by impairing AP repriming and preventing the generation of APs in quick succession thus reducing DHPR-mediated Ca^{2+} release through RyR1. This reduced muscle excitability was not due to DHPR inactivation because lowering the frequency of stimulation partly ameliorated the effect. Furthermore, when fibres were chronically partially depolarized, PCr/CK ATP regeneration system was not optimal and slowed the repriming period of T-system Na^+ channels. Addition of Phospho(enol) pyruvate (PEP) hastened AP repriming and hence, improved T-system excitability (Dutka & Lamb, 2007b) implying Na^+/K^+-ATPases function better when ATP is produced glycolytically in the triad junction instead of by PCr/CK elsewhere.

The importance of strict DHPR-RyR1 coupling. Disruption of the interaction between DHPRs and RyR1 appears to cause an irreversible Ca^{2+}-leak from the SR through RyR1. When DHPR are in there *in situ* state they have been shown to suppress RyRs activity (*i.e.* Ca^{2+} spark) at rest in cultured mammalian muscle (Zhou *et al.*, 2006). Similarly, Weiss *et al.*, (2004) showed that a mutation in the DHPR cytoplasmic III-IV loop of alpha (1S) subunit (R1086H) greatly enhanced RyR1 sensitivity to activation by voltage/caffeine, indicating that DHPRs had a negative allosteric modulatory effect on RyR1. Furthermore, high Ca^{2+}-induced uncoupling of DHPRs from RyRs has also been shown to cause a similar irreversible SR Ca^{2+} leak in mechanically-skinned fibres (Lamb & Cellini, 1999). In the experiments described here, immediate application of S-nitrosoglutathione $GSNO_{imm}$ (a reactive oxygen and nitrogen species) to the fibres reduced twitch and tetanic force responses (15 and 10 % respectively) even though it caused an ~0.1 pCa unit increase in contractile Ca^{2+}-sensitivity. These reductions to AP-mediated force responses were due to impaired DHPR-RyR coupling and concomitantly $GSNO_{imm}$ treatment also caused Ca^{2+} leakage through RyRs, which was not reversible with DTT. The Ca^{2+} leak through RyRs was substantially blocked by raising the free [Mg^{2+}] from 1 to 10 mM. The irreversible Ca^{2+} leak caused by DHPR uncoupling observed by others (Lamb & Cellini, 1999; Weiss *et al*., 2004; Zhou *et al*., 2006) is strikingly similar to that observed here caused by exposure to $GSNO_{imm}$, which might help explain the ROS-mediated loss of signal transduction observed during prolonged low frequency fatigue (Bruton *et al*., 2008).

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