Effect of low ATP concentrations on action potential-induced Ca^{2+} release in mechanically-skinned EDL fibres of the rat

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During vigorous and/or prolonged activity, the average [ATP] within the cytoplasm may decrease from ~7 mM to ~1 mM (Karatzaferi *et al.*, 2001). It may decrease even lower in areas with high ATP utilisation and/or limited diffusion (e.g. triadic junction). Furthermore, ATP facilitates the opening of isolated Ca^{2+} release channels (RyRs) (Laver *et al.*, 2001), but it is currently unclear whether ATP is needed on the RyR for it to be activated by the voltage-sensor (VS) when the VS is activated in a potent and coordinated manner by an action potential (AP). By using adenosine (a competitive weak agonist for the ATP stimulatory site on the RyR) and examining force development of twitch and tetanic force responses, we sought to address whether ATP is crucial for normal AP-mediated Ca^{2+} release.

Male 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 solution (1mM free Mg^{2+}; 8 mM total ATP; 10 mM creatine phosphate (CP) at pH 7.10, containing 50 μM EGTA, pCa 7.0). Individual fibres were then electrically stimulated (75 V cm^{-1}, 2 ms pulse) to produce either twitch or tetanic (50 Hz) force responses at control (8 mM ATP) or at low [ATP] (0.1-2 mM, where ATP was replaced with CP) with or without adenosine present (2 or 4 mM). In parallel experiments, the response of the contractile apparatus to [Ca^{2+}] steps was examined by pre-equilibrating a fibre in a weakly Ca^{2+}-buffered K-HDTA solution (100 μM EGTA) at pCa 7.0 at a given [ATP] and/or adenosine condition, and then rapidly activating it by plunging it into a heavily Ca^{2+}-buffered solution (50 mM CaEGTA/EGTA, pCa 6.0 or 4.4) with the same [ATP] to produce either submaximal or maximal force. These fibres had been Triton X-100 treated so only the contractile apparatus was functional.

Compared to the bracketing control responses (8 mM ATP), the mean twitch peak amplitude was significantly ($P<0.05$) reduced under all low [ATP] conditions (to 71±4%, n=7; 66±3%, n=24; 56±3%, n=51 and 28±4%, n=8, in the presence of 2, 1, 0.5 and 0.1 mM ATP respectively). Peak tetanic force and the rate of tetanic force production was also reduced at low [ATP]. The slowing of the rise in tetanic force at ≤0.5 mM ATP was greater than that explicable by effects of low [ATP] on the rate of force development by the contractile apparatus. Therefore, it appears that the amount of AP-mediated Ca^{2+} release must have been reduced at ≤0.5 mM ATP. The reduction of twitch peak amplitude was exacerbated as the ratio of [adenosine]:[ATP] (mM:mM) was increased (2:8=96±2%, n=3; 2:2=67±4%, n=4; 2:1=41±4%, n=17; 4:1=36±3%, n=7, compared to the absence of adenosine). Since adenosine did not significantly hinder force development of the contractile apparatus, this finding indicates that adenosine competitively interfered with ATP binding to the RyR (Laver *et al.*, 2001), and hence caused reduced Ca^{2+} release. These experiments indicate that ATP must be bound to the stimulatory site on RyRs for the VS to trigger Ca^{2+} release in response to an AP, the normal *in vivo* stimulus.

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