Stretch-induced force enhancement studied with myosin inhibitors and by varying temperature

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Stretch of an activated muscle causes a transient increase in tension that decays, after the stretch, to a level greater than the steady state isometric tension at the corresponding muscle length. The mechanisms underlying this residual force enhancement (RFE) remain controversial (Edman, 2012; Rassier, 2012), but have been attributed to crossbridge and/or non-crossbridge components of the sarcomere. Insight into the crossbridge contribution to RFE may be achieved by perturbing crossbridge kinetics and the distribution of crossbridge states using myosin inhibitors such as N-benzyl-*p*-toluene sulphonamide (BTS) and blebbistatin (BBn) or by varying temperature. This study aimed to examine the crossbridge contribution to the development of RFE in intact mammalian muscle fibres by altering crossbridge kinetics and biasing the distribution of attached crossbridges to a pre-power stroke conformation.

Adult rats were killed with an IP injection of sodium pentobarbitone (Euthatal; > 200 mg kg^{-1}). Small bundles of intact fibres were isolated from the flexor hallucis brevis and mounted between a force transducer and a servomotor (initial sarcomere length = 2.5 μm; 20°C). The preparation was housed in a flow-through chamber perfused with physiological solution and continuously bubbled with 95% O_2 and 5% CO_2. Fibre bundles were tetanized and a ramp stretch of 5% optimal fibre length (L_0) applied on the tension plateau at a velocity of 1 L_0 s^{-1}. Stimulation was maintained for a further 500 ms after the ramp where RFE was measured as the increase in force above the reference isometric contraction. Experiments were repeated with either 10 μM BTS (n = 4), 10 μM BBn (n = 4) or in control solutions at 10, 20 & 30°C (n = 8). Tension records were analysed for the isometric tension before stretch (P_0), peak incremental tension during the stretch (P_k), and the residual force enhancement 500ms after the stretch (RFE). Bi-exponential functions fit to the tension records between P_k and RFE to quantify the amplitude and rate of tension decay after the stretch.

Exposure to 10 μM BTS reduced maximum tetanic tension to ~20% P_0 but increased RFE almost 2 fold compared to control. BBn exposure greatly inhibited maximum tetanic tension (to ~1% P_o) and attenuated the tension response to stretch. When ramp stretches were applied during washout of BBn the tension response to stretch resembled that for BTS; P_k remained high (~85% of control P_k tension) and RFE increased almost 2-fold. BTS and BBn significantly reduced the rate of tension decay after stretch ($p < 0.05$) and BTS also significantly reduced the amplitude of tension decay.

On cooling from 30 to 10°C, P_0 decreased by ~40% whereas P_k doubled in size and RFE increased by more than 5 fold. Whereas the amplitude of tension decay after stretch significantly increased on cooling from 30 to 10°C, the rate of tension decay decreased (by ~60-70%) over the same temperature range. To further examine the relationship between crossbridge cycling rate and RFE, we performed regression analyses on the rate of tension decay and RFE. There were significant, negative correlations between normalized RFE amplitude and the rate of tension decay for BTS and temperature ($P < 0.05$).

Three notable observations arise from these studies: 1) when crossbridge cycling was completely abolished (10 μM BBn), RFE was small, but still present which suggests the presence of a non-crossbridge contribution to RFE; 2) RFE was greatly enhanced when isometric tension was partly inhibited (10 μM BTS and washout of BBn) and crossbridges biased towards the pre-power stroke state (either by myosin inhibitors or at lower temperature); and 3) the rate of tension decay after stretch was inversely correlated with RFE amplitude which suggests that reduced crossbridge detachment rates contribute to RFE.

Together these data suggest that the development of RFE arises from multiple mechanisms which may include a non-crossbridge component such as a Ca^{2+}-dependent increase in stiffness of a parallel elastic element (*eg* titin; Herzog, 2013); and a crossbridge component which may include a transition from low force, pre-powerstroke crossbridges to high force states and/or strain induced decrease in the crossbridge detachment rate.

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