Force enhancement and relaxation rates after stretch of activated muscle fibres

Dilson E. Rassier* and Walter Herzog

Human Performance Laboratory, Faculty of Kinesiology, University of Calgary, 2500 University Drive, Calgary, AB, Canada T2N 1N4

The residual force enhancement following muscle stretch might be associated with an increase in the proportion of attached cross-bridges, as supported by stiffness measurements. In this case, it could be caused by an increase in the attachment or a decrease in the detachment rate of cross-bridges, or a combination of the two. The purpose of this study was to investigate if the stretch-induced force enhancement is related to cross-bridge attachment/detachment kinetics. Single muscle fibres dissected from the lumbrical muscle of frog were placed at a length $\sim 20\%$ longer than the plateau of the force–length relationship; they were maximally activated, and after full isometric force was reached, ramp stretches were imposed with amplitudes of 5 and 10% fibre length, at a speed of 40% fibre length s^{-1} . Experiments were performed in Ringer's solution, and with the addition of 2, 5 and 10 mM of 2,3-butanedione monoxime (BDM), a drug that places cross-bridges in a pre-power-stroke state, inhibiting force production. The total force following stretch was higher than the corresponding force measured after isometric contraction at the corresponding length. This residual force enhancement was accompanied by an increased relaxation time. BDM, which decreases force production during isometric contractions, considerably increased the relative levels of force enhancement. BDM also increased relaxation times after stretch, beyond the levels observed during reference contractions in Ringer's solution, and beyond isometric control tests at the corresponding BDM concentrations. Together, these results support the idea that force enhancement is caused, at least in part, by a decrease in cross-bridge detachment rates, as manifested by the increased relaxation times following fibre stretch.

Keywords: cross-bridges; BDM; detachment rate; myosin; weak binding; strong binding

1. INTRODUCTION

The steady-state isometric force produced by activated muscles after stretch is higher than that produced during purely isometric contractions at the corresponding length (Julian & Morgan 1979; Edman *et al.* 1982; Sugi & Tsuchiya 1988; Morgan *et al.* 2000; Herzog & Leonard 2002; Herzog *et al.* 2003; Rassier *et al.* 2003). This phenomenon, called residual force enhancement, has been observed for over 50 years (Abbott & Aubert 1952), but its precise mechanisms are not understood. In a previous study, we observed rather incidentally that force enhancement was accompanied by an overall increase in relaxation times (Rassier *et al.* 2003). Relaxation rates are typically associated with the kinetics of cross-bridge detachment (Huxley & Simmons 1970, 1971), and an increase in relaxation time during the force-enhanced state would suggest that force enhancement may be associated with a decrease in cross-bridge detachment rate, and a corresponding increase in the proportion of attached cross-bridges.

A mechanism of force enhancement that includes an increased proportion of attached cross-bridges would be consistent with findings of increased stiffness in the force-enhanced state (Herzog & Leonard 2000), but inconsistent with experiments that failed to detect such changes (Julian & Morgan 1981; Sugi & Tsuchiya 1988). Thus,

stiffness results are inconclusive, presumably because stiffness does not only reflect the proportion of attached cross-bridges, but also depends on the cross-bridge attachment distribution, and other compliant structures in the muscle (Forcinito *et al.* 1998).

Although cross-bridge kinetics are difficult to measure directly, the drug 2,3-butanedione monoxime (BDM) can be used to investigate the relationship between cross-bridge kinetics, force enhancement and relaxation rates. BDM slows phosphate release in the cross-bridge cycle (Herrmann *et al.* 1992), thereby inhibiting ATPase activity (Higuchi & Takemori 1989), and placing cross-bridges in a pre-power-stroke state (Regnier *et al.* 1995; Zhao *et al.* 1995). As a result, BDM reversibly inhibits force generation (Higuchi & Takemori 1989; Herrmann *et al.* 1992; Zhao & Kawai 1994; Regnier *et al.* 1995; Zhao *et al.* 1995). Additionally, BDM reduces the rate of force development and the relaxation time (Sun *et al.* 1995, 2001).

Recently, we have performed experiments in which muscle fibres were treated with BDM and observed that force enhancement was significantly increased compared to control experiments without BDM, suggesting that force enhancement is associated with the cross-bridge kinetics (Rassier & Herzog 2004). Motivated by these observations, the purpose of the current study was to systematically investigate the relation between force enhancement and relaxation time, for conditions in which cross-bridge kinetics were changed by BDM. Force enhancement was found to be systematically

* Author for correspondence (rassier@kin.ucalgary.ca).

accompanied by an increase in relaxation time. Furthermore, force enhancement and relaxation time increased with BDM in a dose-dependent manner, suggesting that force enhancement is associated in part with an increased proportion of attached cross-bridges, caused by a decrease in the rate of cross-bridge detachment.

2. MATERIAL AND METHODS

(a) *Muscle fibre preparation*

Experiments were performed with 10 single muscle fibres (~2 mm length) dissected from lumbrical muscles of the frog *Rana pipiens*. The treatment of these animals and all experimental procedures were approved by the University of Calgary committee for the ethical use of animals in research.

The tendons of the isolated fibres were gripped with small pieces of T-shaped aluminium foil close to the end of the fibres. The fibres were transferred to an experimental chamber containing Ringer's solution (NaCl 115 mM, KCl 3 mM, CaCl₂ 3 mM, NaH₂PO₄ 2 mM, NaHCO₃ 20 mM, pH = 7.5), and suspended between a servomotor length controller (Aurora Scientific) and a force transducer (Sensomotor). The temperature of the solution was regulated at ~9 °C during all experiments.

Stimulation (Grass S88, Grass Instruments) was given through two platinum wire electrodes placed in the chamber parallel to the muscle fibres, with square wave pulses (0.4 ms duration) delivered at an amplitude of 25% above the voltage that gave maximal force production (mean voltage: 50 V). The frequency of stimulation was set individually for each fibre to produce a fused tetanic contraction in regular Ringer's solution with the smallest possible frequency (range in these experiments: 20–35 Hz) to avoid fatigue effects.

(b) *Fibre length and force measurements*

At the beginning of the experiments, fibres were paced for ~60 min with repeated twitch contractions (90 s intervals). At the end of this period, fibres were inspected for any apparent damage, and were evaluated for a possible decrease in force. If damage was found or force had decreased, the fibres were discarded from analysis. Reference contractions (1 s) were repeated throughout the experiments, and testing was stopped any time the reference force was decreased.

A force-length relationship was obtained from isometric tetanic contractions (2 s duration, 5 min intervals). The plateau of the force-length relationship, referred to as L_0 hereafter, was identified. Following, fibres were placed at an initial length (L_i) of approximately 20% beyond L_0 , and activated to produce maximal isometric force. At 1000 ms after the onset of activation, fibres were stretched (5 and 10% of fibre length, at a speed of 40% fibre length s⁻¹), and held isometric at the final length (L_f ; total contraction time = 4 s). Before and after the stretch contractions, isometric contractions were performed at L_0 , L_i and L_f . The whole stretch protocol was repeated following administration of 2, 5 and 10 mM of BDM. Two fibres showed a significant decrease in force during contractions elicited after treatment with 5 mM. Therefore, data presented in this paper for 5 and 10 mM BDM are from eight fibres.

After fibres were treated with 10 mM BDM, they were exposed again to the regular Ringer's solution, to test if the effects of BDM were reversible. In a few cases, force and relaxation rates were not reversed back to the values observed

at the beginning of the experiment; these fibres were not incorporated into this study.

(c) *Data analysis and statistics*

Forces were measured at the end of the activation period (3.8 s), and force enhancement was defined as the increase in steady-state force after active stretch compared to the purely isometric force at the corresponding length. Half-time of force development during isometric contractions (50% F_t) was measured from the onset of stimulation to the point where 50% of maximal force was obtained. Half-relaxation time in all contractions (50% R_t) was calculated from the onset of deactivation to the point where force had decreased to 50% of the active force.

A two-way ANOVA for repeated measures was used for the evaluation of the effects of BDM and stretch amplitude on isometric force, 50% F_t and 50% R_t . A one-way ANOVA for repeated measures was used for comparisons between forces following stretch and forces produced during isometric contractions. Regression analysis was used to determine the relationship between force enhancement and changes in relaxation times. When significant differences were observed, contrasts chosen *a priori* were used for comparison. A significance level of $p < 0.05$ was used for all analyses.

3. RESULTS

During isometric tetanic contractions at L_0 , force decreased (figure 1, inset), 50% F_t increased (figure 1a), and 50% R_t decreased (figure 1b) with increasing concentrations of BDM, confirming previous findings (Sun *et al.* 1995, 2001). These results were confirmed statistically across all fibres investigated in this study (table 1).

Figure 2 shows the result of active stretches of different amplitudes on the total force produced by a typical fibre during the experiments. The total force produced after stretch of activated fibres was higher than the force produced during isometric contractions at the corresponding lengths before (figure 2a, FE) and after BDM treatment (figure 2b). Force enhancement was greater for the 10% than the 5% fibre length stretch in both situations. After 10 mM BDM, the total force during isometric contractions at $L_0 + 25\%$ and $L_0 + 30\%$ decreased by 76.6 and 74.8% of the force produced by the untreated fibres, respectively, but the relative force enhancement was increased (figure 2b). Figure 3 shows the relative force enhancement values for all conditions investigated in this study, statistically confirming the results observed with the fibre shown above, i.e. increasing magnitudes of stretch and increasing concentrations of BDM gave greater levels of force enhancement.

After stretch of the activated fibres, 50% R_t increased in untreated (control) fibres (figure 4a). Similar to the results on force enhancement, the increase in 50% R_t was dependent on the stretch amplitude, i.e. a 10% stretch increased 50% R_t more than a 5% stretch (figure 4a). Relaxation time was longer after stretch in BDM-treated fibres, and showed the same dependence on stretch amplitude found before BDM administration (figure 4b). BDM increased the relaxation time after stretch beyond that observed during regular stretches, which rendered a significant interaction between the BDM condition and stretch amplitude (figure 5), such

Figure 1. Time of force development (50%FD_t) and relaxation time (50%R_t) for one fibre during contractions performed at L_0 in Ringer's solution (control), and after adding 2 and 10 mM BDM to the solution, are shown in panels (a) and (b). Contractions with 5 mM BDM are not shown for clarity. Thick vertical bar indicates onsets of activation and deactivation (considered as the time of the first and last pulses of activation, respectively). After BDM, 50%FD_t increases and 50%R_t decreases substantially. Inset: entire force-time histories of the isometric contractions performed in Ringer's solution (control), and after adding 2, 5 and 10 mM BDM to the solution. Note that force decreases substantially with increasing concentrations of BDM.

Table 1. Total force, time of force development (50%FD_t) and relaxation time (50%R_t) during isometric contractions at optimal length.

(Results are given as mean $\pm$ s.e.m. Force decreased, 50%FD_t increased and 50%R_t decreased with increasing concentrations of BDM ($p < 0.05$).)

BDM	force (mN)	50%FD _t (s)	50%R _t (s)
control	0.36 $\pm$ 0.05	0.07 $\pm$ 0.01	0.28 $\pm$ 0.04
2 mM	0.22 $\pm$ 0.03	0.12 $\pm$ 0.02	0.21 $\pm$ 0.04
5 mM	0.15 $\pm$ 0.02	0.20 $\pm$ 0.05	0.20 $\pm$ 0.02
10 mM	0.05 $\pm$ 0.01	0.29 $\pm$ 0.07	0.19 $\pm$ 0.04

that the increase in relaxation time was more pronounced after BDM when compared with before BDM. In order to relate increases in force with increases in relaxation times, we plotted all data (before and after BDM, 5 and 10% stretches) in figure 6. There was a

Figure 2. Force-time histories of isometric contractions performed at $L_0 + 25\%$ and $L_0 + 30\%$, and stretches of 5 and 10% ending at $L_0 + 25\%$ and $L_0 + 30\%$, respectively. (a) Fibre experiments in Ringer's solution. Force enhancement (FE) was 14.1 and 24.2% following a 5 and 10% stretch, respectively. (b) Fibre experiments in Ringer's solution after adding 10 mM BDM. FE was 77.5 and 156.3% following a 5 and 10% stretch, respectively.

Figure 3. Force enhancement (relative to the isometric reference force at the corresponding length) during the different conditions investigated. Force increases with increasing stretch amplitudes and with increasing BDM concentrations.

strong linear relationship between force enhancement and the increase in 50%R_t observed after stretch (figure 6).

4. DISCUSSION

The main finding of this study was that stretch-induced force enhancement is accompanied by an increased relaxation time during regular contractions, and also in conditions in which the cross-bridge kinetics were altered by BDM. These results suggest that force enhancement

Figure 4. Relaxation time ($50\%R_t$) for one fibre in (a) Ringer's solution and (b) after adding 10 mM BDM, during isometric contractions performed at $L_0 + 25\%$ and $L_0 + 30\%$, and stretches of 5 and 10% ending at $L_0 + 25\%$ and $L_0 + 30\%$, respectively. Thick vertical bar indicates onset of deactivation, considered as the time of the last pulse of activation.

Figure 5. The effects of BDM and stretch amplitude on relaxation time ($50\%R_t$). A significant statistical interaction was observed. Increasing concentrations of BDM increased relaxation times after stretches.

may be associated with the cross-bridge detachment rate and the corresponding increase in the proportion of attached cross-bridges (Huxley & Simmons 1970, 1973).

The fact that force enhancement during regular (without BDM) contractions is accompanied by an

Figure 6. Relationship between force enhancement and increase in relaxation time. Filled circles represent 5% stretches at the different BDM concentrations (written above the regression line), and empty circles represent 10% stretches at the different BDM concentrations (written below the regression line). Each circle in the graph represents mean $\pm$ s.e.m. Regression analysis yielded: $50\%R_t = 2.86(\text{FE}) - 35.74$; $r^2 = 0.98$.

increased relaxation time has been observed previously in our laboratory (Rassier *et al.* 2003). However, this relationship was discovered accidentally during *post hoc* analyses with grouped data (varying amplitudes of stretches, and varying starting and final lengths during stretch contractions), and no systematic insight could be gained from those results. In the present study, the relationship between force enhancement and relaxation times was strengthened by the dependence of both phenomena on stretch amplitude, i.e. greater amplitudes of stretch produced increased force enhancement (Julian & Morgan 1979; Edman *et al.* 1982; Sugi & Tsuchiya 1988; Rassier *et al.* 2003), and increased relaxation times. Although relaxation time has not been the focus of previous studies dealing with force enhancement, some published results seem to agree with the results observed here (e.g. Edman *et al.* 1982; figs. 2, 4, 6, 9 and 10), at least in a qualitative manner.

Relaxation in skeletal muscle is typically biphasic, starting with a slow phase, followed by a fast, exponential phase (Huxley & Simmons 1970, 1973; Cleworth & Edman 1972; Edman & Flitney 1977; Tesi *et al.* 2002; this paper, figures 1 and 4). The fast phase of relaxation is thought to be dominated by intersarcomere dynamics. Sarcomeres at the ends of the fibre lengthen, while sarcomeres in the centre of the fibre remain nearly isometric at the beginning of relaxation (Huxley & Simmons 1970, 1973). We avoided most of the non-uniform behaviour of sarcomeres that could complicate our analysis by measuring the beginning of the relaxation phase.

In the initial (slow) phase of relaxation, sarcomeres remain virtually isometric (Huxley & Simmons 1970, 1973), and relaxation is dictated by cross-bridge kinetics (Tesi *et al.* 2002). Therefore, the increased relaxation time observed in our experiments is likely associated with the cross-bridge kinetics, specifically a decrease in cross-bridge detachment rates after stretch (Huxley & Simmons 1970, 1973). Assuming everything

else to remain constant, a decrease in detachment rate would result in an increase in the proportion of attached cross-bridges for the steady-state conditions considered here.

To further elucidate the relationship between force enhancement and cross-bridge kinetics, we used BDM, a cross-bridge inhibitor (Horiuti *et al.* 1988; Higuchi & Takemori 1989) that has been shown to decrease relaxation times during isometric contractions (Sun *et al.* 1995, 2001). BDM does not significantly affect Ca^{2+} transients in frog muscles (Horiuti *et al.* 1988; Maylie & Hui 1991; Sun *et al.* 1995, 2001), and therefore its effects on relaxation time are likely caused by influencing the cross-bridge kinetics. BDM decreased isometric force production but increased the relative force enhancement. Although the reason for this increase is not apparent, it may be associated with the characteristics of the cross-bridges after treatment with BDM. Such cross-bridges, biased into the weakly bound state (Herrmann *et al.* 1992; Regnier *et al.* 1995), do not contribute significantly to active force during isometric contractions but contribute considerably to stiffness (Seow *et al.* 1997). Assuming that the weakly bound cross-bridges are preferably converted to strong-bound cross-bridges by stretch (for example by a stretch-induced enhancement of P_i release), then force enhancement should be increased with increasing amounts of BDM, because the proportion of weakly bound cross-bridges that do not contribute to the isometric reference force, but are contributing to the residual force enhancement, would be expected to increase with increasing BDM concentrations. Consistent with such a mechanism, we observed a greater increase in force during stretch (relative to the isometric contractions) after, compared with before, BDM administration (figure 2b).

If force enhancement is caused by decreased detachment rates, and the associated increase in the proportion of attached cross-bridges, then relaxation times should increase with increasing levels of relative force enhancement. Relative force enhancement increased with stretch magnitude and BDM concentration, and so did relaxation times (figure 6).

In summary, the stretch-induced force enhancement commonly seen in skeletal muscles is accompanied by an increased relaxation time in reference contractions and test contractions when cross-bridge kinetics are changed. These results support the idea that force enhancement is caused, at least in part, by a decrease in cross-bridge detachment rates, as manifested by an increased relaxation time following fibre stretch. Naturally, the results of this study raise a fundamental question: How can stretch of an activated fibre decrease the detachment rate of cross-bridges and allow for a greater proportion of attached cross-bridges? Future experiments should be aimed at thoroughly investigating the interactions between force enhancement, cross-bridge attachment and cross-bridge detachment rates.

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