Active force inhibition and stretch-induced force enhancement in frog muscle treated with BDM

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Rassier, Dilson E., and Walter Herzog. Active force inhibition and stretch-induced force enhancement in frog muscle treated with BDM. J Appl Physiol 97: 1395–1400, 2004. First published June 11, 2004; 10.1152/japplphysiol.00377.2004.—There is evidence that the stretch-induced residual force enhancement observed in skeletal muscles is associated with 1) cross-bridge dynamics and 2) an increase in passive force. The purpose of this study was to characterize the total and passive force enhancement and to evaluate whether these phenomena may be associated with a slow detachment of cross bridges. Single fibers from frog lumbral muscles were placed at a length 20% longer than the plateau of the force-length relationship, and active and passive stretches (amplitudes of 5 and 10% of fiber length and at a speed of 40% fiber length/s) were performed. Experiments were conducted in Ringer solution and with the addition of 2, 5, and 10 mM of 2,3-butanedione monoxime (BDM), a cross-bridge inhibitor. The steady-state active and passive isometric forces after stretch of an activated fiber were higher than the corresponding forces measured after isometric contractions or passive stretches. BDM decreased the absolute isometric force and increased the total force enhancement in all conditions investigated. These results suggest that total force enhancement is directly associated with cross-bridge kinetics. Addition of 2 mM BDM did not change the passive force enhancement after 5 and 10% stretches. Addition of 5 and 10 mM did not change (5% stretches) or increased (10% stretches) the passive force enhancement. Increasing stretch amplitudes and increasing concentrations of BDM caused relaxation after stretch to be slower, and because passive force enhancement is increased at the greatest stretch amplitudes and the highest BDM concentrations, it appears that passive force enhancement may be related to slow-detaching cross bridges.

METHODS

Muscle fiber preparation. The experiments were performed with 10 single muscle fibers (~2 mm length) dissected from lumbral muscles of the frog Rana pipiens. Treatment of these animals and all experimental procedures were approved by the University of Calgary committee for the ethical use of animals in research. After dissection, the tendons were gripped with small pieces of T-shaped aluminum foil close to the end of the isolated fibers. Fibers
were mounted in an experimental chamber between a servomotor length controller (Aurora Scientific) and a force transducer (Sensomotor). The chamber was bathed with a Ringer solution (in mmol: 115 NaCl, 3 KCl, 3 CaCl₂, 2 NaH₂PO₄, and 20 NaHCO₃, pH = 7.5), and the temperature 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 fibers, with square-wave pulses (0.4-ms duration) delivered at an amplitude of 25% above the voltage that gave maximal force production (range: 30–70 V). The frequency of stimulation was set individually for each fiber to produce a fused tetanic contraction with the smallest possible frequency (range in these experiments: 20–35 Hz).

**Fiber length and force measurements.** After the optimal voltage and frequency of stimulation were defined with 1-s contractions, the fibers were paced for 60 min with twitch contractions (90-s intervals). At the end of this period, fibers were inspected visually for any apparent damage and were evaluated for a possible decrease in force with 1-s tetanic contractions. If damage was found or force had decreased, the fibers were discarded. Reference contractions were repeated throughout the experiments, and testing was stopped when the reference force was decreased.

An active (total force – passive force) 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, and the descending limb of the force-length relationship were identified. Fibers were then placed at an initial length ($L_i$) of ~20% beyond the plateau of the force-length relationship and were activated to produce maximal isometric force. Experiments were performed on the descending limb of the force-length relationship because this is the region where total force enhancement is most prominent (7, 14, 21) and where passive force enhancement has been observed in the past (14, 21). At 1,000 ms after the onset of activation, fibers were stretched (5 and 10% of fiber length, at a speed of 40% fiber length/s), and held isometric at the final length ($L_f$) (total

Fig. 1. Force-time histories of isometric contractions of one fiber in Ringer (control) solution and after the addition of 2, 5, and 10 mM 2,3-butanedione monoxime (BDM). Notice that increasing concentrations of BDM decrease isometric force significantly.

were identified. Fibers were then placed at an initial length ($L_i$) of ~20% beyond the plateau of the force-length relationship and were activated to produce maximal isometric force. Experiments were performed on the descending limb of the force-length relationship because this is the region where total force enhancement is most prominent (7, 14, 21) and where passive force enhancement has been observed in the past (14, 21). At 1,000 ms after the onset of activation, fibers were stretched (5 and 10% of fiber length, at a speed of 40% fiber length/s), and held isometric at the final length ($L_f$) (total

Fig. 2. Effects of BDM and length on force production. There was no significant statistical interaction, but BDM decreased force significantly across all lengths investigated.

Fig. 3. Force-time histories of an isometric contraction, and active and passive stretches of a single fiber in Ringer solution (A) and after administration of 10 mM BDM (B). The isometric reference force decreased by 72% from normal force after administration of 10 mM BDM. Total and passive forces were enhanced after stretch in both conditions but more prominently after BDM administration. Iso, isometric contraction; Str, active stretch contraction (speed: 40% fiber length/s); Pas, passive stretch (speed: 40% fiber length/s); FE, force enhancement; PFE, passive force enhancement.

Fig. 4. Force-time histories of stretch contractions at different BDM concentrations. Isometric force before stretch decreased with increasing concentrations of BDM, and stretch increased force substantially in all conditions.
contraction time = 4 s). Before and after the stretch contractions, isometric contractions were performed at \( L_0 \), \( L_s \), and \( L_f \). Stretches with equivalent stretch amplitude and speed were also performed for the passive fiber.

To test whether force enhancement is associated with cross-bridge kinetics, the whole stretch protocol described above was repeated after adding different concentrations (2, 5, and 10 mM) of BDM to the Ringer solution.

Data analysis and statistics. Force enhancement was defined as the increase in steady-state force after active stretch compared with the corresponding purely isometric force at the corresponding length. Force was measured at 3.8 s after the onset of activation. Passive force enhancement was defined as the increase in passive force after active stretch compared with the corresponding passive force obtained after purely isometric contractions at the same length and after stretches of identical magnitude and speed with the passive fiber. Passive force enhancement was evaluated at 5 and 10 s after fiber deactivation. The time of force decay (50% FDt) after stretch at different BDM concentrations was evaluated from the end of the stretch, where maximal force was obtained, to the point where force had decreased by 50% of the total force decay observed during that phase.

A two-way ANOVA for repeated measures was used for evaluation of the effects of BDM and length on force production. A one-way ANOVA for repeated measures was used for comparisons between total and passive forces after active stretch and forces produced during the isometric reference contractions at the corresponding lengths or after stretches of the passive fiber. A one-way ANOVA for repeated measures was used for comparisons of 50% FDt between the different BDM conditions. When significant differences were observed, contrasts that were chosen a priori were used for comparisons. A significance level of \( P < 0.05 \) was used for all analyses.

RESULTS

Figure 1 shows tetanic contractions recorded at \( L_0 \) at Ringer solution and after the addition of 2, 5, and 10 mM of BDM. Increasing concentrations of BDM decreased tetanic force in a dose-dependent fashion (Fig. 1), and this effect was independent of fiber length (Fig. 2), as evidenced by a lack of statistical interaction.

Total force produced after stretch of activated fibers was higher than the force produced during the isometric reference contractions at the corresponding lengths; i.e., force enhancement was observed in normal Ringer solution (Fig. 3A, FE) and after BDM administration (Fig. 3B, FE). Passive force after deactivation of the actively stretched fibers was higher than the passive force after the isometric reference contraction, and it was also higher than the force produced when the fiber was stretched passively. Therefore, passive force enhancement was observed in Ringer (Fig. 3A, PFE) and after BDM administration (Fig. 3B, PFE). Figure 4 shows the time-histories of stretch tests (10% amplitude) for the same fiber as shown in Fig. 1 for the control and the three BDM conditions. Note that the absolute force is higher during and after the stretch in Ringer solution than after BDM administration. Furthermore, there is a slower rate of force decay after stretches after adding 5 and 10 mM BDM.

Table 1 shows the mean values for the total forces recorded in all conditions investigated in this study. Absolute and relative force enhancement was observed in all conditions. Relative force enhancement was higher for the large-amplitude (10%) compared with the small-amplitude (5%) stretches for all experimental conditions. Furthermore, force enhancement for both stretch magnitudes increased with increasing concentrations of BDM. However, caution should be exercised when interpreting these results, because force did not reach a complete steady state for the tests involving BDM, because force relaxation after stretch was much slower than for the reference contractions (Fig. 5).

The mean passive force across all fibers increased after an active stretch compared with passive stretches or isometric contractions at the corresponding lengths (Tables 2 and 3). When 5% stretches were applied, passive force enhancement was unchanged by BDM. When 10% stretches were performed, passive force was not changed by 2 mM BDM, but it was higher after administration of 5 and 10 mM BDM, compared with the passive force recorded at control conditions or after administration of 2 mM BDM (Tables 2 and 3).

![Fig. 5. Force relaxation time after stretch (50% FDt). Point where maximal force was obtained to point where force had decreased by 50% of total force decay) of fibers tested in Ringer solution and after the addition of 2, 5, and 10 mM BDM. Notice that force decay is slower after stretch compared with control, is slower for 10% compared with 5% stretch magnitudes, and increases with increasing concentrations of BDM.](https://www.jap.org)

Table 1. Total force produced during isometric contractions and after stretches of 5 and 10% fiber length

<table>
<thead>
<tr>
<th>Condition</th>
<th>Isometric ( L_0 ) + 25%, mN</th>
<th>Stretch Active 5%, mN</th>
<th>FE, mN</th>
<th>FE %</th>
<th>Isometric ( L_0 ) + 30%, mN</th>
<th>Stretch Active 10%, mN</th>
<th>FE, mN</th>
<th>FE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.27 ± 0.03</td>
<td>0.30 ± 0.04</td>
<td>0.04 ± 0.006</td>
<td>15.7 ± 0.9</td>
<td>0.25 ± 0.03</td>
<td>0.33 ± 0.04</td>
<td>0.07 ± 0.001</td>
<td>29.4 ± 1.8</td>
</tr>
<tr>
<td>2 mM</td>
<td>0.17 ± 0.02</td>
<td>0.21 ± 0.03</td>
<td>0.03 ± 0.004</td>
<td>20.2 ± 1.3</td>
<td>0.17 ± 0.02</td>
<td>0.25 ± 0.03</td>
<td>0.07 ± 0.01</td>
<td>44.1 ± 2.8</td>
</tr>
<tr>
<td>5 mM</td>
<td>0.13 ± 0.02</td>
<td>0.17 ± 0.02</td>
<td>0.04 ± 0.006</td>
<td>31.3 ± 5.5</td>
<td>0.13 ± 0.02</td>
<td>0.23 ± 0.03</td>
<td>0.10 ± 0.01</td>
<td>76.6 ± 6.2</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.06 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>0.05 ± 0.006</td>
<td>77.6 ± 6.8</td>
<td>0.07 ± 0.01</td>
<td>0.18 ± 0.02</td>
<td>0.10 ± 0.01</td>
<td>132 ± 6.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. All stretches started at plateau of force-length relationship (\( L_0 \) + 20%). Force enhancement (FE) is given in absolute terms (mN) and relative to the isometric force produced at the corresponding length (%). Force after stretch was always higher (\( P < 0.05 \)) than force during isometric contractions at the corresponding length. Relative force enhancement increased with increasing concentrations of 2,3-butanedione monoxime.
CROSS-BRIDGE INHIBITION AND FORCE ENHANCEMENT

Table 2. Passive force measured 5 s after fiber deactivation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Isometric L₀ + 25%</th>
<th>Stretch passive 5%</th>
<th>Stretch active 5%</th>
<th>Isometric L₀ + 30%</th>
<th>Stretch passive 10%</th>
<th>Stretch active 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.014±0.001</td>
<td>0.014±0.001</td>
<td>0.016±0.002</td>
<td>0.022±0.003</td>
<td>0.022±0.003</td>
<td>0.031±0.004</td>
</tr>
<tr>
<td>2 mM</td>
<td>0.012±0.002</td>
<td>0.013±0.002*</td>
<td>0.015±0.002</td>
<td>0.020±0.002</td>
<td>0.022±0.003*</td>
<td>0.036±0.005*</td>
</tr>
<tr>
<td>5 mM</td>
<td>0.017±0.004</td>
<td>0.014±0.002</td>
<td>0.018±0.002</td>
<td>0.022±0.003</td>
<td>0.021±0.004</td>
<td>0.048±0.007†</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.012±0.001</td>
<td>0.012±0.002</td>
<td>0.017±0.002*</td>
<td>0.020±0.003</td>
<td>0.020±0.003</td>
<td>0.050±0.005†</td>
</tr>
</tbody>
</table>

Values are means ± SE given in absolute values (mN). All stretches started at L₀ + 20%. Passive force after an active stretch was usually higher (P < 0.05) than forces after passive stretches or after isometric contractions at the corresponding lengths. During 10% stretches, passive force was higher after 5 and 10 mM 2,3-butanedione monoxime than after the control and the 2 mM conditions. *Different from other lengths, P < 0.05. †Different from other 2,3-butanedione monoxime conditions, P < 0.05.

The rate of force decay after stretch was smaller after BDM treatment, as is apparent in Fig. 4. This result was confirmed statistically across all fibers; force relaxation after stretch (50% FD) became slower with increasing concentrations of BDM (Fig. 5).

When the forces shown in Fig. 4 were normalized relative to the force value at 3.8 s after the end of stretch, the isometric force before stretch was greatest for the control condition and decreased with increasing BDM concentrations (Fig. 6). In contrast, the peak forces during the stretch increased with increasing BDM concentrations.

DISCUSSION

The main findings of this paper are that 1) BDM increased the peak force attained during stretch and the total force enhancement produced after stretch when normalized relative to the corresponding isometric reference force, 2) BDM at a concentration of 2 mM did not change the passive force enhancement after 5 and 10% stretches, and 3) BDM at concentrations of 5 and 10 mM did not change (5% stretch) or increased (10% stretch) the passive force enhancement. These results suggest that total force enhancement is related to cross-bridge kinetics and that passive force enhancement is related to 1) non-cross-bridge structures and 2) the slow-detachment of cross bridges.

Effects of BDM. It has been shown that BDM reversibly suppresses isometric force in intact and skinned muscle fibers (2, 3, 15, 16, 22–25, 27) and myofibrils (26). BDM also decreases the maximal velocity of shortening, indicating that BDM slows the cross-bridge cycle (3, 24). BDM decreases stiffness less than force, i.e., the stiffness-to-tension ratio is increased by BDM treatment (3, 22, 23), suggesting that BDM reduces the average force produced per attached cross bridge. Biochemical studies show that BDM binds to the cross bridge and decreases the rate of Pᵢ release and accelerates the rate of ATP hydrolysis (10, 15). As a result, cross bridges should remain proportionally longer in the pre-power-stroke state than the post-power-stroke state for BDM-treated compared with untreated control fibers. Thus, after BDM application, one would expect the proportion of weakly vs. strongly bound cross bridges to be increased compared with control conditions.

The effects of BDM on the cross-bridge kinetics are consistent with the results of this study in that fiber stiffness (increase in force during the stretch) was increased relative to the isometric force and that isometric force was decreased with increasing concentrations of BDM (Figs. 4 and 6). Similar results have been observed in fibers treated with the phosphate analog vanadate (9) and polyethylene glycol (6), which are assumed to stabilize cross bridges in a pre-power-stroke state.

Total force enhancement. Our laboratory has previously suggested that total force enhancement has two components: an active component, associated with cross-bridge kinetics, and a passive component, associated with passive cellular elements (20, 21). Regarding the active component of force enhancement, the following observations made here are of particular interest: 1) BDM decreases the active isometric force but increases the (relative) force during stretch (Table 1, Fig. 1) and 2) BDM causes a decrease in the rate of force decay after stretch (Fig. 5). These findings, combined with the suggestion that BDM slows down the rate of phosphate release (10), and that force enhancement is associated with an increase in stiffness (11), lead to the following hypothesis: active force enhancement is caused by an increased proportion of attached cross bridges. This increased proportion may be achieved by a decrease in cross-bridge detachment rate and the associated

Table 3. Passive force measured 10 s after fiber deactivation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Isometric L₀ + 25%</th>
<th>Stretch passive 5%</th>
<th>Stretch active 5%</th>
<th>Isometric L₀ + 30%</th>
<th>Stretch passive 10%</th>
<th>Stretch active 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.013±0.001</td>
<td>0.014±0.001</td>
<td>0.014±0.001</td>
<td>0.021±0.003</td>
<td>0.022±0.003*</td>
<td>0.026±0.004*</td>
</tr>
<tr>
<td>2 mM</td>
<td>0.012±0.002</td>
<td>0.012±0.002</td>
<td>0.013±0.002*</td>
<td>0.019±0.002</td>
<td>0.021±0.003*</td>
<td>0.029±0.005*</td>
</tr>
<tr>
<td>5 mM</td>
<td>0.016±0.004</td>
<td>0.014±0.002</td>
<td>0.014±0.002</td>
<td>0.021±0.003</td>
<td>0.020±0.003</td>
<td>0.036±0.005†</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.012±0.001</td>
<td>0.011±0.001</td>
<td>0.013±0.002*</td>
<td>0.020±0.002</td>
<td>0.020±0.003</td>
<td>0.036±0.004**†</td>
</tr>
</tbody>
</table>

Values are means ± SE given in absolute values (mN). All stretches started at L₀ + 20%. Passive force after an active stretch was usually higher (P < 0.05) than forces after passive stretches or after isometric contractions at the corresponding lengths. During 10% stretches, passive force was higher after 5 and 10 mM 2,3-butanedione monoxime than after the control and the 2 mM conditions. *Different from other lengths, P < 0.05. †Different from other 2,3-butanedione monoxime conditions, P < 0.05.
increase in the cross-bridge duty ratio. For a normal reference stretch in an untreated fiber, the relation of weakly vs. strongly bound cross bridges is low (compared with that observed in the BDM-treated fibers); therefore, the peak force during stretch is small compared with the isometric reference force. In BDM-treated fibers, the ratio of weakly vs. strongly bound cross bridges is high. During stretch, all cross bridges (weak and strong) contribute to stiffness and peak force; therefore, peak force during stretch is high compared with the isometric control force. The residual force enhancement state may be higher after great stretch amplitudes and high BDM concentrations, because both these factors decrease the rate of cross-bridge detachment, as indirectly observed by the increase in the time of force decay after stretch and BDM administration compared with isometric reference contractions. Combined, these results suggest that force enhancement is caused by an increase in the proportion of attached cross bridges, as substantiated by the increased stiffness (11), which is caused, at least in part, by a decrease in the cross-bridge detachment rate, as substantiated by the decrease in force decay after stretch.

**Passive force enhancement.** Passive force after deactivation of the actively stretched fiber was higher than that observed during isometric contractions at the corresponding length or after passive stretches. Although this result has been observed previously (12–14, 18, 21), in the present study we used different concentrations of BDM to gain further insight into the relation between passive force enhancement and cross-bridge kinetics. The addition of 2 mM BDM did not change the passive force enhancement after 5 and 10% stretches, whereas 5 and 10 mM did not change (5% stretches) or increased (10% stretches) the passive force enhancement. These results, combined with previous observations (13, 14), suggest that the passive force enhancement has two components: 1) the engagement of a passive element and 2) the slow detachment of cross bridges.

There are two observations made previously in our laboratory that support the hypothesis that passive force enhancement observed in this study is partly caused by the engagement of a passive element on activation. First, when muscles and muscle fibers are shortened while activated before stretch, total and passive force enhancement is decreased proportionally with the magnitude of shortening (11, 13). Second, when a muscle that produces passive force enhancement is deactivated for 5 s after an active stretch, and subsequently is reactivated isometrically, there is a remnant force enhancement (14). This remnant force enhancement must be associated with passive force, because it has been shown that deactivation eliminates all active force enhancement (1, 12).

The idea that a passive force is engaged on activation is consistent with findings of Bagini et al. (2, 4) that activation causes an instantaneous increase in fiber stiffness that is independent of cross-bridge attachment. This passive fiber stiffness increases with the magnitude of stretch and sarcomere length, and is independent of the speed of stretch, properties that agree with the passive force enhancement observed in the present study. Similarly, Campbell and Moss (5) observed that the initial tension and stiffness of non-cross-bridge structures are much higher in activated compared with nonactivated fibers. Studies from both groups seem to indicate the existence of a passive element that is engaged on activation.

It is reasonable to speculate that the results by Bagini et al. (2, 4) and Campbell and Moss (5) on passive stiffness are related in part to the passive force enhancement observed in this study. Our laboratory has hypothesized previously that, on activation, an increase in myoplasmic Ca2+ might increase the stiffness of titin, which could cause the passive force enhancement (14, 20, 21). A recent study by Labeit et al. (17) strengthens this hypothesis. They observed that muscle fibers from which actomyosin interactions were abolished showed an upward shift in the passive sarcomere length-tension relationship with increasing Ca2+ concentration. Furthermore, Labeit et al. demonstrated that Ca2+ binding to a defined location of titin changed titin’s persistence length and thus its stiffness.

The fact that passive force enhancement was increased after 5 and 10 mM BDM during the 10% stretch was unexpected, and we do not have a complete explanation. However, both an increase in stretch amplitude and an increase in BDM concentration decrease the rate of force decay after stretch, and thus they presumably decrease the rate of cross-bridge detachment. Half relaxation times for the 10% stretches and 10 mM BDM tests are in the 1-s time range. Therefore, it appears quite possible that, for the greatest stretches and highest BDM concentrations, the decrease in cross-bridge detachment rates gives the observed increase in passive force enhancement for these conditions.

**Summary.** From the present results, the following picture emerges for the passive force enhancement. A passive structure is engaged, or activated on muscle stimulation, thereby increasing its stiffness. If the muscle is stretched, there is an increase in the passive force compared with stretching of this structure in the deactivated state, which causes the observed passive force enhancement. The work by Labeit et al. (17) suggests that the stiffness of titin is Ca2+ dependent and, therefore, activation dependent. Thus titin might be the passive structure that contributes to the passive force enhancement in single fibers that are stretched while activated. The total force enhancement appears to be associated with a decrease in the cross-bridge detachment rate after stretch, which would increase the proportion of attached cross bridges and thus allow for increased force and stiffness.
REFERENCES