Recovery of force during postcontractile depression in single Xenopus muscle fibers

RICHARD A. HOWLETT, CREED M. STARY, AND MICHAEL C. HOGAN

Department of Medicine, University of California, San Diego, La Jolla, California 92093-0623

Received 26 April 2000; accepted in final form 10 January 2001

Howlett, Richard A., Creed M. Stary, and Michael C. Hogan. Recovery of force during postcontractile depression in single Xenopus muscle fibers. Am J Physiol Regulatory Integrative Comp Physiol 280: R1469–R1475, 2001.—This study examined the relationship between force and cytosolic free calcium concentration ([Ca$^{2+}$]$_{c}$) in different fiber types from Xenopus before, during, and after cells underwent postcontractile depression (PCD). During a standardized fatigue run, force in the two fast fatiguing (FF) fiber types (types 1 and 2, n = 10) fell more quickly (5.8 vs. 8.1 min) and to a greater degree (0.36 vs. 0.51 of initial (P$_{o}$) than in the slow fatiguing (SF) fiber type (type 3, n = 11). After the initial fatigue run, both FF and SF experienced a drop in force to <15% P$_{o}$ (PCD) at a similar time (20.6 vs. 21.4 min). A second stimulation period, undertaken during PCD, produced significant recovery of force in both groups, but significantly more so in SF than FF (64 ± 7 vs. 29 ± 2% P$_{o}$). This force recovery during PCD was accompanied by a significant increase in peak [Ca$^{2+}$]$_{c}$, particularly in SF. However, despite the significant recovery of force during stimulation while in PCD, the amount of force produced for a given peak [Ca$^{2+}$]$_{c}$ was significantly lower in both groups during PCD than at any other point in the experiment. A final stimulation period, initiated when all fibers had recovered from PCD, demonstrated a recovery of both force and peak [Ca$^{2+}$]$_{c}$ in both groups, but this recovery was significantly greater in SF vs. FF. These data demonstrate that with continuous electrical stimulation, it is possible to produce a significant recovery of force production during the normally quiescent period of PCD, but that it occurs with a decreased muscle force production for a given peak [Ca$^{2+}$]$_{c}$. This suggests that factors other than structural alterations of the sarcoplasmic reticulum are likely the cause of PCD in these fibers.

fatigue; calcium; electrical stimulation; action potential; transverse tubules; adenosine 5-triphosphatase; fiber types

IT HAS BEEN WELL DOCUMENTED that after a period of fatiguing contractions, skeletal muscle can undergo a period marked by a decreased ability to produce force (7). In low-frequency fatigue (LFF), this decrease in force-producing ability occurs immediately after contractions and can last several hours or even days. It is termed LFF because it usually occurs only in response to stimulation at lower frequencies (<30 Hz), whereas force production in response to high-frequency stimulation is maintained (13, 23). However, a similar phe-

The costs of publication of this article were defrayed in part by the payment of page charges. The author must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
METHODS

Animal care. The female adult *Xenopus laevis* was used for this study. These animals were doubly pithed and decapitated, the hind feet were removed, and the lumbral muscles (II-IV) were dissected free. All procedures were performed in accordance with the University of California, San Diego institutional animal care and use committee and conform to National Institutes of Health guidelines.

Measurement systems. Single living muscle fibers (n = 21) with tendons intact were microdissected, and the cells were microinjected (World Precision Instruments PV830 pneumatic picopump, Sarasota FL) with the Ca²⁺ indicator dye fura 2 (Molecular Probes, Eugene, OR).

Platinum clips were attached to the tendons of the cells, and they were mounted in a chamber filled with Ringer solution consisting of (in mM) 112 NaCl, 1.87 KCl, 0.82 CaCl₂, 2.38 NaHCO₃, 0.07 NaH₂PO₄, 0.1 EGTA, pH 7.0. One end of the fiber was fixed, and the other free end was attached to an adjustable force transducer (Aurora Scientific, model 400A, Aurora, Ontario), allowing the muscle to be set at a length that produced maximal tetanic force (Po). The analog signal from the force transducer was sampled at 200 Hz and converted to a digital signal via an MP100WSW analog-to-digital converter and analyzed with AcqKnowledge III 3.2.6 analysis software (Biopac Systems, Santa Barbara, CA). Relative force was standardized by comparing force to the maximal tetanic force (Po).

Cytosolic [Ca²⁺] was measured using an epifluorescent microscope system that consisted of a Nikon inverted microscope with a ×40 fluor objective and a DeltaScan illumination and detection system (Photon Technology International, South Brunswick, NJ) (24). Injected fibers were illuminated sequentially (20 Hz) with two excitation wavelengths of 340 and 380 nm, and the resulting fluorescence emission was measured at 510 nm. The ratio of 340/380-nm fluorescence was used to obtain the Ca²⁺-dependent signal (14). Relative peak [Ca²⁺] measurements were standardized by comparing individual 14 lines to the highest ratio within that run. Individual resting [Ca²⁺] measurements (340/380-nm baseline) were compared with the lowest resting level within that run (24).

Stimulation protocol. Tetanic contractions were elicited using direct (8–10 V) stimulation of the muscle (Grass model S48, Warwick, RI). Stimulation consisted of 200-ms trains of 70-Hz impulses with a 2-ms duration. Cells were subjected to three fatigue runs in which stimulation frequency was increased every 2 min sequentially (0.25, 0.33, 0.5, 1.0, and 2.0 contractions/s) or until <50% initial force was produced.

After the initial fatigue run, tetanic tension was monitored with one tetanic contraction every 5 min. When force output had declined to <15% Po, (PCD), a second fatigue run was started (PCDstim). After this second fatigue run, tension was again monitored with single tetanic contractions every 5 min until force output had returned to >50% Po, and was obviously recovering. At this point, a third and final fatigue run was performed (recovery). The stimulation protocol for the second and third fatigue runs was identical to the initial fatigue run for each individual fiber. Force and [Ca²⁺] were monitored continuously throughout the duration of the experiment (Fig. 1). All experiments were performed at 25°C.

Fibers were classified into types by their fatigue response in the initial fatiguing stimulation. Type 1 fibers (fast fatiguing [FF]) showed a rapid fall in force at 0.25 or 0.33 contractions/s, whereas type 2 fibers (intermediate) did not begin to fatigue until 0.5 contractions/s. Type 3 fibers (slow fatiguing [SF]) showed no fatigue until 1 contraction/s but force rapidly declined at 2 twitches/s. Because the PCD and Ca²⁺ responses were similar in types 1 and 2 fibers, they were pooled into one group of FF fibers (n = 10) for comparison with the more SF (n = 11) type 3 fibers.

Statistics. All values are presented as the means ± SE. Results were evaluated by using a two-way ANOVA (fiber type × time point) with repeated measures. When significant differences were found between groups, a Tukey least-significant difference post hoc test was used to determine which groups were different. Significance was set at P < 0.05 throughout.

RESULTS

Force. Absolute force developed at the start of the protocol (Po) averaged 1.11 ± 0.12 mN or 340 ± 37 kN/m² for the SF vs. 2.02 ± 0.28 mN or 355 ± 54 kN/m² for the FF. Figure 2 shows the relative force produced by both groups at various points in the experimental protocol. Relative force decreased significantly from initial in both groups during the first fatiguing run (fatigue). However, force fell significantly more in FF fibers than SF in a shorter time (5.8 vs. 8.1 min). Force for a single tetanic contraction reached the lowest point at ~20 min after fatigue (PCD) in both groups of fibers, and force at this time was not different between groups but was significantly lower than initial and fatigue. Stimulation during PCD (PCDstim) resulted in a significant increase in force compared with PCD in both groups. Between groups, SF had significantly greater recovery of force during PCDstim than FF. After recovery from PCD (recovery), relative force in SF had increased until it was no longer significantly different from initial, whereas force in FF had increased above PCDstim and fatigue, but was still significantly lower than initial.

[Ca²⁺]o. Figure 3 shows the relative baseline and peak [Ca²⁺] for both groups of fibers at various points in the experimental protocol. Baseline [Ca²⁺] increased significantly from initial to the end of the first fatigue run in both SF and FF, followed by a decrease to levels similar to initial in both groups for subsequent time points. No significant differences in baseline
[Ca$^{2+}$]$_c$ were found between SF and FF at any time point in the experimental protocol.

Peak [Ca$^{2+}$]$_c$ decreased similarly in both groups of fibers at PCD until it was significantly different from initial or fatigue. During PCD$_{stim}$, peak [Ca$^{2+}$]$_c$ increased significantly in both FF and SF compared with PCD, but SF increased significantly more than FF so that it was no longer different from initial. At recovery,

---

**Fig. 1.** Representative data for relative force (A) and cytosolic free calcium concentration ([Ca$^{2+}$]$_c$, 340/380 ratio, B) for a single fiber at the various points in the experimental protocol. PCD, postcontraction depression.

**Fig. 2.** Relative force produced in fast fatiguing (FF) and slow fatiguing (SF) fibers at the various points in the experimental protocol. All data are reported as means ± SE. *Significantly (P < 0.05) different from SF. †Significantly (P < 0.05) different from initial. ‡Significantly (P < 0.05) different from fatigue.

**Fig. 3.** Relative peak (open symbols) and baseline (closed symbols) [Ca$^{2+}$]$_c$ in SF and FF at the various points in the experimental protocol. All data are reported as means ± SE. *Significantly (P < 0.05) different from SF. †Significantly (P < 0.05) different from initial. ‡Significantly (P < 0.05) different from fatigue.
peak \([Ca^{2+}]_c\) in SF was still similar to initial and significantly higher than FF, which, despite increasing significantly from PCD, was still lower than initial.

**Force/[Ca\(^{2+}\)]_c.** Figure 4 shows the relationship between relative force and relative peak \([Ca^{2+}]_c\) during the experimental protocol. The ratio of force to \([Ca^{2+}]_c\) fell significantly in both groups at fatigue, compared with initial, with FF decreasing significantly more than SF. At PCD, FF and SF were not different, but were significantly lower than fatigue. During PCD\(_{stim}\), force/[\(Ca^{2+}\)]\(_c\) rose significantly in SF so that it was not different from initial but was significantly greater than FF, which was still not different from during PCD. At recovery, force/[\(Ca^{2+}\)]\(_c\) increased significantly compared with PCD in the FF group but was still less than initial and significantly lower than SF.

**DISCUSSION**

The results of the present study demonstrated that 1) continuous electrical stimulation of fibers during PCD caused a significant recovery of force, particularly in slow fatiguing fibers; 2) this force recovery was correlated with an increase in peak \([Ca^{2+}]_c\); and 3) the normal relationship between force and \([Ca^{2+}]_c\) is altered during PCD.

**PCD.** PCD was first reported in single skeletal muscle fibers from *Xenopus* by Westerblad and Lannergren (28). It is characterized by a variable period of severely reduced force-generating ability following fatiguing contractions, the length of which is determined primarily by fiber type, followed by a subsequent complete recovery of force-generating ability. Unlike low-frequency fatigue (7), cells that are in PCD show decreased force production across a wide range of stimulation frequencies (26). A definitive cause of PCD is still currently unclear, but alterations in Ca\(^{2+}\) metabolism are thought to have a key role (1).

Previous investigations on single *Xenopus* fibers have suggested that PCD was inducible only in faster fatiguing fibers (types 1 and 2) and that SF (type 3) fibers showed a monotonic recovery of force after fatiguing stimulations (28). Although it is unclear why PCD was found to be induced in all fiber types in the present study, there were potentially significant differences in the stimulation regimens between the previous studies and the present one. Both the previous and the present studies utilized 70-Hz stimulation, a frequency shown to be most effective in eliciting PCD in type 1 and 2 fibers. However, the previous study (28) used a 500-ms duration, 2.5-fold greater than the present study. Likewise, after the first few minutes of stimulation, the stimulation rate was increased much more slowly in the previous study, resulting in a much slower induction of fatigue in the SF fibers, whereas the behavior of the FF were very similar between the studies.

\([Ca^{2+}]_c\). Previous investigations have demonstrated that the decrease in force-producing ability during PCD is not due to either a failure of action potential transmission or the accumulation of intracellular metabolites because action potentials are normal (16) and fatigue-related metabolites such as free Pi and hydrogen ion (H\(^+\)) are not elevated when PCD is greatest (20, 26). However, using the Ca\(^{2+}\) indicator aequorin, Allen et al. (1) showed that peak \([Ca^{2+}]_c\) is lower during PCD. Therefore, an alteration in Ca\(^{2+}\) handling by the cell was strongly linked to the onset of PCD. Because action potentials propagate normally in these cells and caffeine-stimulated Ca\(^{2+}\) release is normal (29), it was suggested that the deficiency in Ca\(^{2+}\) release is most likely due to an inability for action potential signal to be transduced to the voltage-dependent Ca\(^{2+}\) release channels in the SR. The suggested locale for the impairment in action potential propagation was the triadic junctions. It has been demonstrated that there is a time- and fiber type-dependent increase in vacuole formation after fatiguing stimulations (17, 31). These vacuoles appear to be formed from transverse tubule degradation and swelling, but their role in PCD has been shown to be equivocal as the time course of formation of vacuoles does not necessarily correlate with a decline in force production and these vacuoles do not appear in mouse fibers during LFF when force production is similarly reduced and Ca\(^{2+}\) release is also affected (15).

Although these studies suggested that decreased Ca\(^{2+}\) release by the SR was a likely factor in PCD, a recent study by Tupling et al. (26) showed that Ca\(^{2+}\)-ATPase activity and Ca\(^{2+}\) reuptake into the SR decreased during a period of diminished force generation after fatiguing contractions in human subjects. These results were consistent with earlier studies in humans showing a decrease in Ca\(^{2+}\)-ATPase activity following fatigue (4, 12). This decrease in Ca\(^{2+}\) reuptake was suggested to cause a subsequent decrease in SR Ca\(^{2+}\) release and the resulting lower force production (26). The decrease in Ca\(^{2+}\) reuptake and lowered Ca\(^{2+}\)-ATPase activity has been suggested to be due to struc-

![Figure 4](http://ajpregu.physiology.org/)

**Fig. 4.** Ratio of relative force to relative peak \([Ca^{2+}]_c\) in SF and FF at the various points in the experimental protocol. All data are reported as means \(\pm SE\). *Significantly \((P < 0.05)\) different from SF. †Significantly \((P < 0.05)\) different from initial. ‡Significantly \((P < 0.05)\) different from PCD.
structural damage to the ATPase itself (26). Two suggested causes for damage of the Ca\(^{2+}\)-ATPase were calcium-activated neutral proteases (calpains), which can damage SR proteins (10), and reactive oxygen species, which can damage SR membranes (5). In the present study, induction of PCD was accompanied by severely decreased peak Ca\(^{2+}\) release, leading to a decreased \([\text{Ca}^{2+}]_c\) during PCD. Whether this was caused by damage to the Ca\(^{2+}\)-ATPase in the present study cannot be determined. However, as in a previous study (24), there was a significant increase in resting \([\text{Ca}^{2+}]_c\) in the fibers during the initial fatigue of the present study, possibly leading to the activation of calpains (2). Likewise, the generation of reactive oxygen species would be likely in these fatiguing contractions (22). Although both of these potential modulators of Ca\(^{2+}\)-ATPase activity were possibly acting during the present study, neither possibility has been directly studied in this model with respect to PCD. However, our data suggest that structural damage to the Ca\(^{2+}\)-ATPase during PCD was unlikely because 1) continuous stimulation during the PCD period resulted in significant increases in both Ca\(^{2+}\) release and force recovery and 2) the time course of the recovery from PCD was too rapid to allow for protein synthesis and repair of the SR Ca\(^{2+}\)-ATPase in the short time. Likewise, as caffeine administration elicits a large Ca\(^{2+}\) release during PCD, it is not likely that SR calcium stores are affected by decreased reuptake during this period.

\[ \text{Force/}[\text{Ca}^{2+}]_c\] An interesting finding of the current investigation is the observation that the force/\([\text{Ca}^{2+}]_c\) relationship is affected during PCD. The amount of force produced in these fibers for a given \([\text{Ca}^{2+}]_c\) is significantly lowered at fatigue and especially during PCD (see Fig. 3). This is true not only during single tetanic stimulations, when force production is very low, but also during continuous stimulation (PCDstim) when force recovers. This result suggests that some of the effect of PCD may be attributable to an altered sensitivity to Ca\(^{2+}\) and not solely by a decreased \([\text{Ca}^{2+}]_c\), in these cells. A previous study on single Xenopus fibers found no change in Ca\(^{2+}\) sensitivity during PCD or recovery but suggested that this parameter could have been somewhat underestimated by the Ca\(^{2+}\) probe aequorin, which can be interfered with by changes in cytoplasmic pH and/or [Mg\(^{2+}\)] (1). With use of the same Ca\(^{2+}\) indicator (fura 2) as in the present study it was subsequently demonstrated that fatigued Xenopus fibers did show a reduced Ca\(^{2+}\) sensitivity (19), but that investigation did not look directly at PCD. Changes in the intracellular milieu, such as decreased cell pH and the accumulation of Pi, can cause a reduction in Ca\(^{2+}\) sensitivity in skinned skeletal muscle fibers (11). However, because cell pH and muscle metabolites are largely recovered when PCD is greatest in these fibers, the observed decrease in Ca\(^{2+}\) sensitivity in the present study must be due to other factors.

\[ \text{Force recovery during PCD} \] If during PCD there is a decrease in the ability to release and/or resequester Ca\(^{2+}\) and a concurrent decrease in Ca\(^{2+}\) sensitivity, the question arises as to how increased force production during PCDstim is possible. It was previously shown that \([\text{Ca}^{2+}]_c\) is significantly lowered during PCD but that maximal SR Ca\(^{2+}\) release is still possible if induced with caffeine (29), suggesting that normal action potentials are not eliciting an appropriate Ca\(^{2+}\) response from the SR. In the present study, the intense stimulation protocol of PCDstim appeared to override this problem in transducing the action potential signal to the SR, allowing for increased Ca\(^{2+}\) release and subsequent increased force production. Previously, stimulation during PCD consisted of single tetanic contractions of 500 ms in Xenopus (28) and multiple tetanic contractions over 5 s in humans (26). It is possible that these stimulation protocols were not severe enough or long enough in duration to demonstrate significant recovery of force production because peak force production in the present study did not often occur until 5–6 min into PCDstim (Fig. 1).

Another potential explanation for the recovery of force production despite an altered force-Ca\(^{2+}\) relationship is posttetanic potentiation (PTP). PTP is characterized by an increase in peak tension after tetanic stimulation of the muscle, particularly at less than saturating \([\text{Ca}^{2+}]_c\) (13, 23, 25). It was shown previously that myosin light chain phosphorylation is actually elevated during PTP (25). In the present study, the tetrode nature of the stimulus during PCDstim could elicit PTP and cause the gradual increase in force production seen during this period. The development of PTP appears to be regulated by myosin light chain phosphorylation, which in turn is sensitive to Ca\(^{2+}\) (25). It was shown previously that myosin light chain phosphorylation is actually decreased during fatigue but that similar potentiation to nonfatigued muscle is seen even at a lower \([\text{Ca}^{2+}]_c\) (25). Therefore, despite the lower peak \([\text{Ca}^{2+}]_c\) seen during PCD in the present study, PTP could allow for a significant recovery of force for a given \([\text{Ca}^{2+}]_c\). The increase in force/\([\text{Ca}^{2+}]_c\) seen from PCD to PCDstim suggests that this may have occurred.

\[ \text{Fiber types} \] It is unclear why the SF fibers in the present study showed a greater recovery of force and \([\text{Ca}^{2+}]_c\) than FF during and after PCD. One likely explanation is that the deviations from intracellular homeostasis caused by the initial fatigue run, while large enough to cause PCD in all fibers, are not as large in SF compared with FF. In the initial fatigue run of the present study, FF fibers underwent a significantly greater fall in force in a shorter period of time. The SF fibers, having a greater oxidative potential, would be able to restore cellular homeostasis more quickly, reducing the accumulation of harmful cellular metabolites that could cause force depression (20) or cellular damage. For example, Pi, which accumulates with the breakdown of phosphocreatine (PCr), reportedly decreases SR Ca\(^{2+}\) release by binding Ca\(^{2+}\) within the SR (21, 27). Although it has been shown previously that PCr is resynthesized rapidly following fatiguing contractions in Xenopus fibers (20), in that study, the FF
type 1 fibers showed a decreased PCr-to-Cr ratio after recovery from fatiguing stimulations. This result suggests that P_i accumulation could have been greater in these less oxidative fibers during PCD, as is possible in the present study. Likewise, it has been shown that FF fibers have the largest accumulation of H^+, which inhibits normal excitation-contraction coupling, during fatiguing contractions and that they have a slower recovery to a normal intracellular pH during recovery than SF fibers (30). It is possible that differences in pH between fiber types following fatigue could be present during PCD in the present study and contribute to the differences in both force and Ca^{2+} recovery. Finally, it has been established that different skeletal muscle fiber types have different Ca^{2+} sensitivities (18), suggesting that some of the differences in the force/[Ca^{2+}]_c relationship seen between fibers at various points in the protocol are due to composition-structure differences between fibers.

In summary, the results of this study demonstrate that PCD is reversible if steady-state stimulation patterns are applied to the fiber during the depressed state. This suggests that structural damage to the SR is not likely to be the sole explanation for the PCD phenomenon and that factors related to the restoration of normal Ca^{2+} handling result in significant force recovery during this time period.

Perspectives

Currently, there is interest in the processes by which muscle, especially cardiac muscle, can modulate the amount of force it produces in response to some insult to the homeostasis of the cells. In cardiac muscle, ischemic injury often results in “stunning” or a prolonged period of decreased force production followed by complete recovery. This process is likely protective in nature, serving to reduce the force output of the heart during a period when intense contractions could possibly do damage. Because force production is intimately related to Ca^{2+} metabolism in striated muscle, the present study was undertaken to further elucidate some of the relationships between these factors during the process PCD, which is analogous to stunning, albeit on a shorter time scale. Our data show that during PCD, there is not only decreased Ca^{2+} release but also a decreased Ca^{2+} sensitivity in these single Xenopus fibers. Both of these are potential mechanisms for decreasing force output for a given contraction stimulus, providing a means to decrease the stresses on a fatigued fiber that is susceptible to tissue damage.

This study was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR-40155. R. Howlett is an National Sciences and Engineering Research Council (Canada) postdoctoral fellow.

REFERENCES


