Fatigue Conditions Alter Sarcoplasmic Reticulum Function of Striated Muscle

MARK ANTHONY W. ANDREWS and THOMAS M. NOSEK

*Department of Medical Physiology, New York College of Osteopathic Medicine of NYIT, Old Westbury, New York 11568-8000, USA

*Department of Physiology and Endocrinology, Medical College of Georgia, Augusta, Georgia 30912-3000, USA

The decline in muscle function that occurs with repeated stimulation and under hypoxic and/or ischemic conditions (fatigue) results from decreases in maximal Ca\(^{2+}\)-activated force generation (\(F_{\text{max}}\)) and Ca\(^{2+}\) sensitivity of the contractile apparatus, along with alterations in Ca\(^{2+}\) uptake and release by the sarcoplasmic reticulum (SR).\(^1\)

These effects are thought to be due to a number of factors, including the accumulation of metabolic products generated within the cells. While a number of putative metabolic factors, for example, inorganic orthophosphate, and H\(^+\), have been under investigation for some time,\(^2\) it is becoming increasingly evident that the accumulation of lactate has significant deleterious effects on striated muscle function, independent of pH.\(^3\)

\(L(+)-\)Lactate (LL) accumulates in skeletal muscle and in the myocardium, as the aerobic pathways of ATP regeneration cannot keep pace with the rate of ATP utilization, primarily due to a limited oxygen availability. Under these conditions, ATP production becomes increasingly dependent upon anaerobic glycolysis, and concentrations of LL rapidly increase from 1–2 mM (at rest) to 20 mM or more.\(^1,3\) We have demonstrated that such concentrations of LL significantly decrease \(F_{\text{max}}\) in striated muscle independent of pH changes.\(^3\) In experiments presented here, we have furthered our investigations, attempting to determine whether or not LL alters Ca\(^{2+}\) uptake by Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) from the SR of striated muscle cells at a constant pH.

METHODS

All experiments were carried out at room temperature (22°C) on single muscle fibers of fast-twitch extensor digitorum longus (EDL) muscles taken from mature male rats. All solution compositions were formulated by solving the set of simultaneous equations describing the multiple equilibria of ions in solution.\(^4\) The control bathing solution (REL), upon which all experimental solutions were also based, contained (in mM): 1.0 Mg\(^{2+}\), 5.0 MgATP, 15 phosphocreatine, 140 mM potassium methanesulphonate, 5 imidazole, and 10 EGTA (differences from these levels are noted in parentheses), with a final ionic strength of 170 mM, pH of 7.0, and a pCa (-\(\log[Ca^{2+}]\)) > 8.5 (no added Ca\(^{2+}\))

\(^*\) Corresponding author: Phone: 516-686-3776; fax: 516-686-3832; e-mail: mawandrews@compuserve.com

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unless otherwise noted. Appropriate amounts of potassium L(+)-lactate were added as required.

Fiber preparations involved excision of the EDL from a euthanized rat, rinsing the muscle with REL, and immediate dissection of the muscle into small fiber bundles. These bundles were then stored overnight at 4°C (to stabilize the function of the muscle fibers) in REL containing (in mM) 0.1 mM phenylmethylsulfonyl fluoride, 0.1 leupeptin, 1.0 benzamidine, 0.01 μM aprotinin, and 1 dithiothreitol (to inhibit proteolysis). The following day, fiber bundles were taken and the sarcolemma and t-tubules were permeabilized by immersion of the fibers in REL plus 25 μg/mL saponin for 30 min, leaving the SR intact. Single fibers were then dissected in REL and mounted on an optoelectric force transducer apparatus (Scientific Instruments GmbH, Heidelberg, Germany).

Ca\(^{2+}\) loading and release from the SR involved five steps: (1) initial depletion of SR Ca\(^{2+}\) by immersion in REL plus 25 mM caffeine for one minute; (2) washing in REL to remove any caffeine; (3) loading with Ca\(^{2+}\) for 15 s in REL (pCa = 6.6) containing 0, 10, 20, or 30 mM LL (15 SR loading at 0 mM LL yielded 90% of the maximal Ca\(^{2+}\) loading achieved in 60 s); (4) washing in REL (0.05 EGTA, 0.1 Mg\(^{2+}\)) for 45 s; and (5) emptying the SR of Ca\(^{2+}\) by exposure to REL (0.05 EGTA, 0.02 Mg\(^{2+}\)) containing 25 mM caffeine. The magnitude of the contractile response in step 5 functioned as the assay for Ca\(^{2+}\) content of the SR.

CICR was determined by following steps 1–3, then blocking further Ca\(^{2+}\) uptake by immersion of the fiber in a rigor solution (pCa > 8.5, 5 EGTA, 0 ATP for 2 min). CICR was then induced by exposure of the fiber to REL, or to REL containing 10 μM Ca\(^{2+}\) (pCa = 5), for 30 s. The release process was then terminated by placing the fiber in REL with 10 mM procaine for 30 s. The amount of Ca\(^{2+}\) remaining in the SR was determined by processing the fibers through steps 4 and 5.

Throughout all experiments, each fiber was exposed to all conditions and served as its own control with all conditions randomized and all results normalized to the maximum caffeine-induced contracture at the control condition noted. Hypotheses were tested at an α-value of 0.05 by one-way ANOVA with multiple comparisons.

RESULTS

As indicated in Figure 1, Ca\(^{2+}\) loading of the SR for 15 s (but not for 60 s) was significantly decreased to 81 ± 6%, 87 ± 4%, and 92 ± 4% (n = 12) of 15-s control loading by 10, 20, and 30 mM LL, respectively. Furthermore, Figure 2 illustrates that, following control loading of the SR for 15 s, a 30-s release period in REL (pCa = 5) caused the SR to release 15 ± 2% (n = 26) of its Ca\(^{2+}\) content, while CICR increased to 27 ± 3%, 33 ± 3%, and 37 ± 7%, in LL concentrations of 10, 20, and 30 mM, respectively (n = 6). A 30-s release period in REL, in the absence or presence of LL (not shown), did not cause significant Ca\(^{2+}\) release from the SR.

DISCUSSION

These results indicate that, in the presence of LL, there is a decrease in the rate at which SR Ca\(^{2+}\) uptake occurs (possibly related to a decrease in the rate of the Ca\(^{2+}\)-ATPase, unpublished results), but no alteration of maximal uptake when sufficient time is allowed (60 s). As can be noted in Figure 1, the decrease in SR loading is greatest at 10 mM, recovering slightly as LL concentration was increased to 20 mM and 30 mM.
FIGURE 1. Effect of l(+)-lactate on calcium loading: Ca\(^{2+}\) uptake is significantly inhibited when fibers are loaded for 15 s (to approximately 90\% of maximum) in the presence of 10, 20, and 30 mM l(+)-lactate \((n = 12)\), with no effect of l(+)-lactate on Ca\(^{2+}\) uptake at a loading time of 60 s \((n = 6)\). Data are presented as the mean amount of Ca\(^{2+}\) loaded into the SR ± SEM. Data for each fiber were normalized to the maximal level of Ca\(^{2+}\) uptake in the absence of l(+)-lactate with 60 s Ca\(^{2+}\) loading. *Indicates that there is a significant difference \((p < 0.05)\) between that condition and 60 s Ca\(^{2+}\) loading at 0 l(+)-lactate; †indicates that there is significant difference \((p < 0.05)\) between that condition and both 60- and 15-s Ca\(^{2+}\) loading at 0 l(+)-lactate.

The presence of a possible biphasic effect here is of interest because a biphasic alteration had been noted in \(F_{\text{max}}\) as LL was increased.\(^3\) However, the mechanism of such a biphasic response in \(F_{\text{max}}\) or in the present case, remains undetermined.

Given the rapid nature of SR loading in vivo, any decrease in Ca\(^{2+}\) loading of the SR by LL could result in a greater amount of Ca\(^{2+}\) remaining in the cytoplasm between repeated activations and explain, at least in part,\(^3\) the slowing of skeletal muscle relaxation noted during periods of repeated activations.\(^4\) With regard to cardiac muscle, all other factors remaining constant, such a decrease in the rate of Ca\(^{2+}\) loading of the SR could result in both a decreased Ca\(^{2+}\) content of the SR and elimination of the Ca\(^{2+}\) not loaded into the SR, by sarcolemmal Ca\(^{2+}\) ATPases. This would result in decreased contractile force on consequent beats (a "reverse treppe" effect). Such decreases in SR Ca\(^{2+}\) content might be ameliorated, or overcome, by other functional alterations of the myocardium, such as increased opening of sarcolemmal Ca\(^{2+}\) channels (known to occur); however, reduced Ca\(^{2+}\) loading of the SR might still decrease the total amount of Ca\(^{2+}\) available to the contractile apparatus and, in turn, decrease force generation. Present results also indicate that LL does not affect the "leakiness" of the SR, but does increase CICR (FIG. 2). The preceding effects were all shown to be reversible when LL was eliminated from the solutions.

Thus it is evident that l(+)-lactate not only affects \(F_{\text{max}}\) of striated muscle,\(^3\) but also
FIGURE 2. Effect of lactate on Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (CICR) from the SR; when the releasing solution contained a Ca\textsuperscript{2+} concentration of pCa 5, a 30-s exposure caused a significant CICR in the absence of L(+)lactate. This CICR increased as L(+)lactate concentration of the releasing solution was increased to 10, 20, and 30 mM. There was no significant effect of LL on the Ca\textsuperscript{2+} content of the SR when the releasing solution had a pCa > 8.5. Data are presented as the mean amount of Ca\textsuperscript{2+} remaining in the SR ± SEM. Data for each fiber were normalized to the SR Ca\textsuperscript{2+} content before exposure to releasing solution. *Indicates that there is a significant difference (p < 0.05) between that condition and 15-s Ca\textsuperscript{2+} loading at 0 L(+)-lactate (with no consequent CICR).

alters the capacity of the SR to handle Ca\textsuperscript{2+}, as it slows Ca\textsuperscript{2+} uptake by the SR and increases CICR from the SR at L(+)lactate concentrations generated in vivo. These effects of L(+)lactate on the SR are larger in magnitude than the previously determined effects of LL on the contractile apparatus.\textsuperscript{3}

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