Differential effects of repetitive activity on sarcoplasmic reticulum responses in rat muscles of different oxidative potential

G. P. Holloway, H. J. Green, and A. R. Tupling
Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada

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Holloway, G. P., H. J. Green, and A. R. Tupling. Differential effects of repetitive activity on sarcoplasmic reticulum responses in rat muscles of different oxidative potential. Am J Physiol Regul Integr Comp Physiol 290: R393–R404, 2006. First published September 22, 2005; doi:10.1152/ajpregu.00006.2005.—We investigated the hypothesis that muscles of different oxidative potential would display differences in sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} handling responses to repetitive contractile activity and recovery. Repetitive activity was induced in two muscles of high oxidative potential, namely, soleus (SOL) and red gastrocnemius (RG), and in white gastrocnemius (WG), a muscle of low oxidative potential, by stimulation in adult male rats. Measurements of SR properties, performed in crude homogenates, were made on control and stimulated muscles at the start of recovery (R0) and at 25 min of recovery (R25). Maximal Ca\textsuperscript{2+}-ATPase activity (V\textsubscript{max}, \(\mu\text{mol}\cdot\text{g protein}^{-1}\cdot\text{min}^{-1}\)) at R0 was lower in stimulated SOL (105 ± 9 vs. 135 ± 7) and RG (269 ± 22 vs. 317 ± 26) and higher (P < 0.05) in WG (795 ± 32 vs. 708 ± 34). At R25, V\textsubscript{max} remained lower (P < 0.05) in SOL and RG but recovered in WG. Ca\textsuperscript{2+} uptake, measured at 2,000 nM, was depressed (P < 0.05) in SOL and RG by 34 and 13%, respectively, in stimulated muscles at R0 and remained depressed (P < 0.05) at R25. In contrast, Ca\textsuperscript{2+} uptake was elevated (P < 0.05) in stimulated WG at R0 by 9% and remained elevated (P < 0.05) at R25. Ca\textsuperscript{2+} release, unaltered in SOL and RG at both R0 and R25, was increased (P < 0.05) in stimulated WG at both R0 and R25. We conclude that SR Ca\textsuperscript{2+}-handling responses to repetitive contractile activity and recovery are related to the oxidative potential of muscle.

skeletal muscles; contractile activity; calcium handling

The generation of specific force levels by the muscle cell is intimately dependent on effectively translating the excitation signal into a precisely regulated increase in the cytosolic free Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}). In skeletal muscle, the regulation of [Ca\textsuperscript{2+}]\textsubscript{i} is primarily dependent on the kinetics of Ca\textsuperscript{2+} release and Ca\textsuperscript{2+} uptake by the sarcoplasmic reticulum (SR) (3). The rate at which Ca\textsuperscript{2+} release occurs is dependent on the open state of the calcium release channel (CRC), also known as the ryanodine receptor (RyR), whereas Ca\textsuperscript{2+} uptake is dependent on recruitment of Ca\textsuperscript{2+}-ATPase, the enzyme responsible for sequestering [Ca\textsuperscript{2+}]	extsubscript{i} from the cytosol into the lumen of the SR (3). The factors involved in the acute regulation of both the CRC and the Ca\textsuperscript{2+}-ATPase to achieve a given [Ca\textsuperscript{2+}]	extsubscript{i} transient, and consequently an expected mechanical event, remain largely undefined (31).

It has become clear that the SR Ca\textsuperscript{2+} handling can display a variety of behaviors depending on the characteristics of the task. The particular Ca\textsuperscript{2+} handling response also appears to depend on the fiber type characteristics of the muscles involved. Repetitive contractile activity, as an example, results in reductions in Ca\textsuperscript{2+} uptake (5, 18, 54) and Ca\textsuperscript{2+} release (16, 26) in soleus and in red gastrocnemius/red vastus lateralis muscles of the rat. The decreases that are observed in these properties appear to be due to structural alterations in the Ca\textsuperscript{2+}-ATPase (30) and CRC (16), respectively. In contrast, no reductions in SR Ca\textsuperscript{2+} cycling properties have been observed in white gastrocnemius/white vastus lateralis muscles following repetitive stimulation (5, 16, 18, 40). Because the red vastus lateralis and gastrocnemius muscles are composed primarily of fast-twitch (type II) fibers and the soleus of slow-twitch (type I) fibers (11), it would appear that the “red” character of the fiber, which associates with properties supporting oxidative phosphorylation (39), may be important in the SR Ca\textsuperscript{2+} cycling responses observed in response to repetitive stimulation. In addition, it is also possible, given that prolonged treadmill running was used to induce repetitive muscle activity, that the SR Ca\textsuperscript{2+} handling responses observed between muscles also may reflect different patterns of recruitment during the activity (2).

Reports also have been published describing an overshoot in Ca\textsuperscript{2+}-ATPase activity (17, 40) and Ca\textsuperscript{2+} uptake (40) during recovery following exercise. To date, these observations have been restricted to muscles of predominantly fast, white composition. In muscles that display a reduction in Ca\textsuperscript{2+} cycling behavior with exercise, which, as emphasized, are highly oxidative in character, it is unclear what occurs during the recovery period following exercise. However, given the observation that normalization of Ca\textsuperscript{2+}-ATPase activity following exercise-induced structural damage to the enzyme appears to take several days (33), it might be expected that Ca\textsuperscript{2+}-ATPase activity would remain depressed in the late recovery period following exercise.

Differential responses in SR Ca\textsuperscript{2+} cycling behavior between muscles in response to repetitive activity might also be expected to associate with changes in mechanical behavior, because disturbances in Ca\textsuperscript{2+} handling have been mechanistically linked to fatigue (1). If such is the case, low-frequency fatigue, observed at low frequencies of stimulation and known to persist for hours after exercise, should be restricted to muscles showing disturbances in Ca\textsuperscript{2+} cycling behavior and, in
particular, depressions in Ca\(^{2+}\) release (52). It also would be expected that prolongations in relaxation rate would accompany reductions in Ca\(^{2+}\) uptake (14). Reductions in both Ca\(^{2+}\) release and Ca\(^{2+}\) uptake have been shown to accompany the decrease in the [Ca\(^{2+}\)]\(_i\) transient observed in isolated single fibers at fatigue induced by repetitive stimulation (1).

The primary purpose of this study was to investigate the effects of repetitive contractile activity and contractile activity with subsequent recovery on the regulation of the SR Ca\(^{2+}\) cycling properties in rat muscles of different fiber type composition and oxidative potential. We hypothesized that in response to repetitive stimulation, the changes in SR Ca\(^{2+}\) handling properties would be directly related to the oxidative potential of the muscle and not to the major fiber type composition as dictated by the myosin heavy chain composition and contractile speed. Moreover, fatigue in the soleus, both with exercise and recovery, would be directly related to the reduction in Ca\(^{2+}\) cycling properties. As a subsidiary problem, we also examined fatigue manifestations during exercise and recovery in the whole gastrocnemius muscles. Because this muscle contains, on average, the same oxidative potential as the soleus but has a different fiber type composition (11), we were only able to investigate the hypothesis that fatigue, both with exercise and recovery, could be more pronounced in a muscle of predominantly type II fiber composition compared with a muscle of predominantly type I composition.

METHODS

Animals

Adult male Sprague-Dawley rats were randomly divided into different groups to determine the effects of contractile activity plus recovery on homogenate SR Ca\(^{2+}\) cycling properties and “in situ” mechanical properties. The animals were fed water and laboratory chow ad libitum and were housed in an environmentally controlled room on a reverse light-dark cycle until the time of the study. All experiments were conducted at approximately the same time, between 8:00 AM and 3:00 PM, to limit diurnal variations in muscle glycogen (7). The experimental protocols were approved by the University of Waterloo Animal Ethics Committee.

Experimental Design

To investigate the effects of contractile activity and contractile activity plus recovery on soleus (SOL), red gastrocnemius (RG), and white gastrocnemius (WG) muscles, we applied electrical stimulation directly to the sciatic nerve of one limb, whereas the opposing limb acted as the control. Adult male Sprague-Dawley rats were randomly divided into two different groups: one group, weighing 416 ± 3.0 g (mean ± SE), was used for studying crude homogenate (HOM) Ca\(^{2+}\) cycling properties (n = 20); a second group, weighing 421 ± 2.0 g, was used to determine the mechanical properties of isolated muscles stimulated in situ (n = 16). The first group of rats, used for the SR measurements, was randomly assigned to one of two subgroups (n = 10 per subgroup): one group received stimulation, whereas the other group received the same stimulation plus 25 min of recovery. The second group of rats also was divided into two subgroups: one subgroup was used for assessment of SOL contractile characteristics, and the second subgroup was used for assessment of the whole gastrocnemius (GAS). In general the conditions investigated were patterned on earlier work for which investigators in our group reported both increases in Ca\(^{2+}\) -ATPase activity with short-term exercise (22) and an overshoot in Ca\(^{2+}\) -ATPase activity in recovery (41). The actual specifics of the stimulation protocol were refined by pilot work. Using electrical stimulation, we were able to sample the tissue much more rapidly after the repetitive contractions.

The stimulated muscles received 150 ms/s trains at supramaximal voltage (determined at optimal length, L\(_{opt}\)) at 60 Hz for 5 s, followed by 5 s of relaxation, applied for a duration of 5 min. Immediately after the stimulation and at 25 min of recovery, the muscles were removed and separated into the SOL, RG, and WG tissues, and a portion of each muscle was rapidly frozen in liquid N\(_2\). We labeled the recovery times, namely, immediately after the exercise and after recovery, as R0 and R25, respectively. The RG and WG tissue were separated visually by color, and samples were always collected from similar areas; RG was taken at the proximal end of the muscle belly just superficial to the plantararis (which was previously removed and discarded), and WG was taken from the superficial muscle belly just lateral to the saphenous artery. The RG and WG tissue would appear to represent the proximal and distal compartments, respectively, that have been characterized by de Ruiter et al. (8). For the muscles and regions selected, oxidative potential, estimated by maximal citrate synthase (CS) activity was 5.03 ± 0.33, 7.50 ± 0.34, and 2.72 ± 0.26 μmole g\(^{-1}\) protein\(^{-1}\) min\(^{-1}\) for the SOL, RG, and WG, respectively. Maximal CS activity, measured as previously described (20), was different (P < 0.05) among each group of muscles.

The tissue was diluted 1:10 (wt/vol) in ice-cold homogenizing buffer (pH 7.5) containing (in mM) 250 sucrose, 5 HEPES, 0.2 phenylmethylsulfonyl fluoride (PMSF), and 0.2% sodium azide (NaN\(_3\)) and was mechanically homogenized on ice using a Polytron homogenizer (PT 3100) at 16,500 rpm for two 30-s bursts separated by a 30-s break. Approximately 1,000 μl of HOM sample were frozen in liquid N\(_2\) and stored at −80°C for later analysis of Ca\(^{2+}\) cycling properties.

The additional subgroups of animals also underwent the same stimulation protocol; however, in this group mechanical data were collected both before and during recovery following the 5 min of stimulation. Mechanical characteristics were collected in separate experiments for isolated SOL and whole GAS. Surgery was performed similar to that for tissue sampling, except that this time, instead of leaving the whole plantar flexors intact, either the SOL or the GAS was isolated. These isolations were accomplished by separating the SOL at the proximal end of the Achilles tendon, just distal to the muscle belly. The entire SOL was isolated from the GAS and the plantararis. If the SOL was the muscle of interest, the other plantar flexors were cut from the Achilles tendon; if, on the other hand, the GAS was the muscle of interest, the SOL tendon was cut. In addition, the plantaris was isolated from the rest of the GAS and severed close to the Achilles tendon.

Stimulation was applied directly to the sciatic nerve with a Grass S48 stimulator. Before the 5-min stimulation (rest), after the 5-min stimulation (R0), and during recovery at 5 (R5), 10 (R10), 15 (R15), 20 (R20), and 25 min (R25), mechanical properties were measured for both control and stimulated limbs. Tetanies were produced at L\(_{opt}\) and at various frequencies of stimulation (10, 20, 30, 50, and 100 Hz), using a 0.2-ms pulse at a supramaximal voltage, and were utilized to determine the maximal rates of relaxation (−dP/dt\(_{max}\)) and force development (+dP/dt\(_{max}\)) and the peak force (F\(_0\)). Invoked twitches were also used to characterize these properties, as well. During the determination of L\(_{opt}\), muscle twitches were separated by 30 s to prevent fatigue and potentiation. All mechanical parameters were expressed relative to the initial preexercise resting value, which was set at 100% (rest). Because of the length of this article, only the twitch properties have been included. Force data were collected online by using a 640 A signal interface (Aurora Scientific) connected to a National Instruments 16-bit analog-to-digital card and were analyzed using the Dynamic Muscle Control and Data Acquisition and Dynamic Muscle Analysis software (Aurora Scientific). The general procedures used and properties assessed can be found in earlier reports from our laboratory (6). An independent shunt calibration was performed daily for each force transducer.
Animal Surgical Preparation

The same surgical preparation was used for all experimental groups and was similar to that reported by Tupling et al. (47). Rats were initially anesthetized using intraperitoneal injections of pentobarbital sodium (6 mg/100g body wt). Throughout the experiments, the animals were given booster injections as required to maintain anesthesia. For HOM experiments, hindlimbs were shaved, connective tissue and overlying fascia were removed, and the distal section of the Achilles tendon was dissected, cut, and tied with surgical silk to an S-hook that later was attached to a Grass linear variable differential transducer. A vertical incision was made through the gluteal muscles, and the sciatic nerves were isolated from surrounding connective tissue. Rats were then placed on their stomachs in a neutral position and positioned on a heated holding platform. Both legs were prepared identically, and random assignment was used to determine the S leg. The knee joint was stabilized at 90° flexion by inserting a steel needle through the capsule of the knee (hip also under 90° flexion with no rotation), the previously secured S-hook was attached to the transducer, and electrodes (attached to a Grass model FT03 stimulation apparatus) were placed around the sciatic nerve. During surgical preparation and throughout the experimental protocol, muscle tissue was covered by gauze and repeatedly moistened with warm saline (9% NaCl) to prevent dehydration. Muscle surface temperature was monitored with a surface thermometer and maintained between 28 and 32°C.

Analytical Procedures

To investigate the changes in SR Ca2+ handling properties with our experimental protocol, we measured Ca2+-ATPase, Ca2+ uptake, and Ca2+ release in crude homogenates. SR Ca2+-ATPase activity. Measurements of Ca2+-induced SR Ca2+-ATPase activity were performed on crude homogenates prepared from SOL, RG, and WG tissue. Ca2+-ATPase enzyme kinetics were assessed by measuring the Ca2+-ATPase activity at progressively increasing [Ca2+]i. Ca2+-ATPase kinetic parameters of interest included the maximal Ca2+-ATPase activity (Vmax), the Hill coefficient (nH), an indicator of the cooperative Ca2+ binding affinity, and the [Ca2+]i needed to elicit 50% Vmax (Ca50). Ca2+-induced Ca2+-ATPase activity was measured using a Beckman DU900 spectrophotometer according to the regenerating assay developed by Simonides and van Hardeveld (42) with minor modifications (49). Samples were run with and without the Ca2+ ionophore A-23187 to provide information regarding the effects of exercise and recovery on SR membrane integrity. The reaction buffer contained (in mM) 20 HEPES, 1.0 MgCl2, 1.0 Na2ATP, 0.1 EDTA, 5.0 NaCl, 9.0 glucose, 3.0 pyruvate (PEP), pH 7.0. Immediately before the reaction with Ca2+, and the absorption was measured for 0.5 min. Once Vmax was obtained, 40 μM (1.0 μl of 40 mM stock) cyclopiazonic acid, a specific inhibitor of the Ca2+-ATPase, was added to determine basal activity. The [Ca2+]i corresponding to the additions of 100 mM CaCl2 and the absorption was measured for 2 min. At this time, and every 2 min thereafter until Vmax was reached, 0.5 μl of CaCl2 was added and, again, absorption was measured. Once Vmax was obtained, 40 μM (1.0 μl of 40 mM stock) cyclopiazonic acid, a specific inhibitor of the Ca2+-ATPase, was added to determine basal activity. The [Ca2+]i corresponding to the additions of 100 mM CaCl2 were measured separately using dual-wavelength spectrofluorometry and the Ca2+ fluorescent dye indo-1. Details of the procedure for calculating [Ca2+]i have been previously published by our group (50).

The Ca2+-dependent Ca2+-ATPase activity was used to calculate nH and Ca50. These properties were determined by nonlinear regression with computer software (GraphPad Software) using the following sigmoidal dose-response equation:

$$ Y = Y_{\text{max}} + \left( Y_{\text{top}} - Y_{\text{bot}} \right) \frac{1 + 10^{(\log V_{\text{max}} - X) \times n_H}}{1 + 10^{(\log V_{\text{max}} - X) \times n_H}} $$

(1)

where $Y_{\text{max}}$ is the value at the bottom of the curve, $Y_{\text{top}}$ is the value at the top of the plateau, and log $V_{\text{max}}$ is the logarithm of the Ca50 (the pCa value that gives a response halfway between $Y_{\text{bot}}$ and $Y_{\text{top}}$). For calculation of nH, only the linear portion corresponding to 10–90% of Vmax was used.

SR Ca2+ uptake. Oxalate-supported Ca2+ uptake was measured using the Ca2+ dye indo-1 at 37°C according to the methods outlined by O’Brien et al. (36) with minor modifications by our group (47). Fluorescence measurements were collected on a dual-emission wavelength spectrofluorometer (Ratiomaster system; Photon Technology International). The excitation wavelength was set to 355 nm, and 405 and 485 nm correspond to the emission wavelengths for bound (F) and free (G) indo-1, respectively. The measurement of [Ca2+]i with this system is based on the difference between the maximal emission wavelengths between the F and G indo-1. The reaction buffer contained (in mM) 200 KC1, 20 HEPES, 10 Na2ATP, 0.005 mM N,N',N''-tetrakis(2-pyridylmethyl)ethylenediamine, 5 oxalate, 15 MgCl2, and 10 PEP, pH 7.0. The ratio of F/G (R) was used to calculate [Ca2+]i and decreases with Ca2+ uptake. Using FeliX software (Photon Technology International), we calculated [Ca2+]i, from the following equation:

$$ [\text{Ca}^{2+}]_i = K_d \times \left( \frac{G_{\text{max}}}{G_{\text{max}} - R_{\text{max}}} \right) \left( R - R_{\text{min}} \right) $$

(2)

where $K_d$ represents the equilibrium constant for the interaction between Ca2+ and indo-1. A $K_d$ value of 250 was used (23). $R_{\text{max}}$ is the minimum value of R, $R_{\text{max}}$ is the maximum value of R, and $G_{\text{max}}$ is the maximum value of G (corresponding to the addition of 1 mM CaCl2).

Photon counts were simultaneously collected for each wavelength. Before each analytical session, the background fluorescence was determined in the absence of indo-1 and implemented before each assay was started.

Before data collection was initiated, the assay buffer was heated to 37°C and 1.5 μM indo-1, 2.0 μl of 10 mM CaCl2, and 150 μl of SOL HOM (100 μl of RG HOM and 50 μl of WG HOM) were added to a cuvette containing 2.0 ml of buffer. Once these additions were made, data collection was started. After a brief period, 40 μl of 250 mM ATP were added to initiate Ca2+ uptake. Initial [Ca2+]i, defined as the [Ca2+]i obtained just after addition of ATP and before any noticeable Ca2+ uptake by the SR, was ~3.0 μM.

The curve generated from Eq. 2, [Ca2+]i vs. time, was smoothed over 21 points using the Savitsky-Golay algorithm. Linear regressions were performed on values of [Ca2+]i at 500, 1,000, 1,500, and 2,000 (range ±100) nM. Differentiating the linear fit curve allowed the determination of Ca2+ uptake rates.

Apparent coupling ratios, an indication of the number of Ca2+ ions transported per ATP hydrolyzed, was determined using the ratio of Ca2+ uptake, assessed at 2,000 nM, to Ca2+-ATPase activity (Vmax). We have used the term “apparent coupling ratio” because the two properties were not determined under identical conditions.

SR Ca2+ release. Ca2+ release was measured in a similar way to Ca2+ uptake, by using indo-1 with a dual-wave emission spectrofluorometer (Ratiomaster system; Photon Technology International) described in detail previously (47). After active loading of the SR, [Ca2+]i was calculated to be <100 nM. Ca2+ release was induced with the addition of 5 mM (10 μl of 0.5 M stock) 4-chloromercuribenzoate (4-CMC). Tupling and Green (48) have shown that 4-CMC acts directly on the RyR to induce Ca2+ release, unlike silver nitrate (AgNO3), which also induces Ca2+ release from the Ca2+-ATPase. Ca2+ release was determined on the same homogenate following the Ca2+ uptake measurements. After Ca2+ release, the Ca2+ uptake calibration was applied, and the generated curve was smoothed over 21 points by using the Savitsky-Golay algorithm, yielding a curve of [Ca2+]i vs. time. The generated Ca2+ release curve yields two visually distinct phases, a fast initial phase 1 and a slower secondary
phase 2. Linear regressions were performed on Ca\(^{2+}\) release vs. time corresponding to each phase and were differentiated to determine the maximal rate of Ca\(^{2+}\) release.

**Muscle glycogen and metabolites.** Muscle metabolites and glycogen were analyzed fluorometrically after extraction from freeze-dried tissue according to previously published procedures (21, 24). The metabolites assessed included ATP, phosphocreatine (PCr), and lactate. Glycogen was determined as glucose in a separate piece of tissue after alcohol precipitation and hydrolysis (29).

**Statistics**

For Ca\(^{2+}\) uptake measurements, a three-way ANOVA was used to investigate the effects of stimulation (control vs. stimulated), time (stimulation vs. recovery), and the biphasic release response (phase 1 vs. phase 2). For all other SR measurements and the muscle metabolites, a two-way ANOVA was used to investigate the effects of stimulation (control vs. stimulated) and time (rest, R0, R5, R10, R15, R20, and R25). The significance level was set at 0.05 (P < 0.05), and when appropriate, a Newman-Keuls post hoc test was used to compare specific means. Where only main effects were observed, the conditions affected are indicated.

**RESULTS**

**Muscle Metabolites and Glycogen**

Only in the RG and WG muscles did the stimulation protocol result in a reduction in ATP and PCr concentrations (Table 1). For these two regions of the GAS, reductions ranging between 20 and 22% and between 24 and 45% occurred for ATP and PCr, respectively. By 25 min of recovery, no differences existed between control and stimulated muscles for either of the high-energy compounds, regardless of region. For the SOL, no differences were observed between control and stimulated muscles for both ATP and PCr at either R0 or R25. Lactate concentration increased in all three tissues immediately after the stimulation, with the greatest increase occurring in the SOL, no differences were observed between control and stimulated muscles for either of the high-energy compounds, regardless of region. For the SOL, no differences were observed between control and stimulated muscles for both ATP and PCr at either R0 or R25. Lactate concentration increased in all three tissues immediately after the stimulation, with the greatest increase occurring in the RG, which persisted throughout the recovery period. In contrast, in WG, V\(_{\text{max}}\) was increased by 12% at R0 and returned to control levels at R25. The other kinetic properties examined, namely, n\(_{\text{H}}\) and C\(_{50}\), were unaffected by the stimulation and recovery protocol in both SOL and RG. For WG, although no change was observed in C\(_{50}\), a main effect of stimulation was observed such that n\(_{\text{H}}\) was less in stimulated than in control muscle at both R0 and R25. The basal ATPase activity was unaltered by the experimental protocol regardless of the muscle studied.

The ionophore ratio, a measure of the ratio of V\(_{\text{max}}\) measured with and without the Ca\(^{2+}\) ionophore A-23187, was reduced with stimulation, but only in the SOL and RG (Fig. 1). The stimulation-induced reduction in this ratio was a main effect that also persisted throughout the recovery period. Repetitive activity was without effect in altering the ionophore ratio in WG at either R0 or R25.

Ca\(^{2+}\) uptake was assessed at four different [Ca\(^{2+}\)]\(_{i}\) levels. All three muscles showed a main effect of [Ca\(^{2+}\)]\(_{i}\) level (2,000 > 1,500 > 1,000 > 500 nM). Because all Ca\(^{2+}\) concentrations showed generally the same effect, we have presented the data for a [Ca\(^{2+}\)]\(_{i}\) value of 2,000 nM. As with V\(_{\text{max}}\), repetitive activity depressed Ca\(^{2+}\) uptake in SOL and RG at R0 by 34 and 13%, respectively (Fig. 3). The depression in Ca\(^{2+}\) uptake persisted at R25. For WG, Ca\(^{2+}\) uptake increased by 9% at R0 and remained significantly elevated at R25. For all muscles, only main effects of the stimulation were observed. The apparent coupling ratio, defined as the ratio between Ca\(^{2+}\) uptake and maximal Ca\(^{2+}\).ATPase activity, was not affected by the differences between control and stimulated muscles at R25, glycogen levels remain depressed in each tissue during recovery.

**SR Ca\(^{2+}\) Cycling Properties**

The kinetic properties of the Ca\(^{2+}\).ATPase activity included V\(_{\text{max}}\), n\(_{\text{H}}\), and C\(_{50}\) (Fig. 1 and Table 2). The SOL and RG muscles displayed similar responses to the contraction and recovery protocol, namely, a main effect of stimulation. In the case of the SOL, V\(_{\text{max}}\) was reduced by 22% immediately following the stimulation. After 25 min of inactivity, no recovery in V\(_{\text{max}}\) occurred. For the RG, V\(_{\text{max}}\) was reduced by 15% at R0, which persisted throughout the recovery period. For the RG, V\(_{\text{max}}\) was reduced by 15% at R0, which persisted throughout the recovery period. In contrast, in WG, V\(_{\text{max}}\) was increased by 12% at R0 and returned to control levels at R25. The other kinetic properties examined, namely, n\(_{\text{H}}\) and C\(_{50}\), were unaffected by the stimulation and recovery protocol in both SOL and RG. For WG, although no change was observed in C\(_{50}\), a main effect of stimulation was observed such that n\(_{\text{H}}\) was less in stimulated than in control muscle at both R0 and R25. The basal ATPase activity was unaltered by the experimental protocol regardless of the muscle studied.

Table 1. Concentrations of selected metabolites and substrates after stimulation and recovery in different muscles

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>SOL</th>
<th>RG</th>
<th>WG</th>
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</thead>
<tbody>
<tr>
<td>ATP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>16.4 ± 0.6</td>
<td>15.8 ± 0.9</td>
<td>24.2 ± 0.7</td>
</tr>
<tr>
<td>S</td>
<td>15.7 ± 0.05</td>
<td>16.0 ± 0.4</td>
<td>19.1 ± 1.0</td>
</tr>
<tr>
<td>PCr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>46.0 ± 2.3</td>
<td>45.8 ± 2.7</td>
<td>70.5 ± 3.5</td>
</tr>
<tr>
<td>S</td>
<td>47.1 ± 5.7</td>
<td>44.8 ± 1.7</td>
<td>53.6 ± 3.5#</td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6.7 ± 0.4</td>
<td>4.2 ± 0.4</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>S</td>
<td>24.6 ± 4.1#</td>
<td>4.6 ± 0.2*</td>
<td>19.4 ± 1.7#</td>
</tr>
<tr>
<td>Glycogen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>318 ± 28</td>
<td>359 ± 41</td>
<td>408 ± 30</td>
</tr>
<tr>
<td>S</td>
<td>232 ± 13#</td>
<td>314 ± 43#</td>
<td>269 ± 19#</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/kg dry wt for ATP, phosphocreatine (PCr) and lactate. For glycogen, values are in glucosyl units/kg dry wt; n = 10. SOL, soleus; RG, red gastrocnemius; WG, white gastrocnemius; R0, 0 min of recovery; R25, 25 min of recovery; C, control; S, stimulated. *P < 0.05, significantly different from R0. #P < 0.05, significantly different from C.
by either stimulation or recovery in any of the muscles examined.

The rate of Ca\(^2+\) release was assessed for two distinct phases following active Ca\(^2+\) loading (Fig. 4). In contrast to the decrease in Ca\(^2+\) sequestering properties observed in SOL and RG, no differences at either R0 or R25 were noted for either phase 1 or phase 2 Ca\(^2+\) release. For WG, the repetitive contractile activity resulted in increases in phase 1 and phase 2 at R0 by 21 and 30%, respectively. The increase in Ca\(^2+\) release for these phases was not affected by the time of recovery.

**Contractile Properties**

The 5 min of intermittent contractile activity resulted in pronounced alterations in the properties of the twitch in GAS both immediately after the stimulation and during the 25-min period of recovery (Fig. 5). Compared with rest, peak tension (P\(_1\)) was reduced by ~36% immediately after the contractile activity. No recovery in P\(_1\) was observed during the recovery period. The property used to assess the kinetics of force development during the supramaximal twitch, namely, +dP/ dt\(_{\text{max}}\), was also affected by the stimulation protocol. For +dP/ dt\(_{\text{max}}\), the depression that was observed at R0 persisted throughout the 25 min of recovery. Relaxation characteristics following the single twitch were measured as −dP/dt\(_{\text{max}}\). At R0, −dP/dt\(_{\text{max}}\) was reduced by ~19% compared with rest. At R5, −dP/dt\(_{\text{max}}\) was reduced by an additional 17%. No further change in −dP/dt\(_{\text{max}}\) was observed throughout the subsequent recovery periods. No changes were observed in the control limb between rest and each of the recovery periods for any of the twitch properties examined.

Mechanical function in the SOL muscle was also disturbed by the repetitive contractions. For the twitch properties, P\(_1\) was reduced by ~46% at R0 (Fig. 6). No recovery in P\(_1\) occurred during the recovery period. A similar effect was observed for +dP/dt\(_{\text{max}}\), namely, a pronounced reduction in the rate of force development at R0 that persisted throughout R25. For −dP/dt\(_{\text{max}}\), persistently lower values were observed for stimulated compared with control muscle throughout recovery. However, these differences were not significant.

**DISCUSSION**

As hypothesized, we found that the effects of the repetitive contraction and recovery protocol on SR Ca\(^2+\) handling properties depended on the oxidative potential of the muscle. The SOL and RG muscles, which are primarily slow twitch and fast twitch, respectively, but of high oxidative potential (8, 11), displayed similar responses. These responses consisted of a depression in V\(_{\text{max}}\) observed both after the contractile activity and after the 25 min of recovery. In contrast, the WG, a muscle of predominant fast-twitch composition but low in oxidative potential (11), responded with an increase in V\(_{\text{max}}\) that returned to normal during recovery. In general, the changes in Ca\(^2+\) uptake mirrored the changes in V\(_{\text{max}}\), namely, decreases in SOL and RG and increases in WG during the exercise and recovery phases of the protocol. We also found that differences observed in Ca\(^2+\) release appeared to relate to oxidative potential of the muscle. Repetitive mechanical activity resulted in increases in both phase 1 and phase 2 Ca\(^2+\) release in WG that persisted during the recovery period. In contrast, no differences between the stimulated and control muscles in Ca\(^2+\) release, in either phase 1 or phase 2, were observed for SOL and RG either after exercise or after recovery. From the perspective of the SR and [Ca\(^{2+}\)]\(_{\text{f}}\) regulation, it appears that...
Comparative effects of contractile activity and recovery on kinetic properties of the sarcoplasmic reticulum 

**Table 2. Comparative effects of contractile activity and recovery on kinetic properties of the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase in rat muscles of different fiber type composition**

<table>
<thead>
<tr>
<th></th>
<th>SOL R0</th>
<th>SOL R25</th>
<th>RG R0</th>
<th>RG R25</th>
<th>WG R0</th>
<th>WG R25</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n_H)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.63±0.04</td>
<td>1.68±0.06</td>
<td>1.70±0.03</td>
<td>1.73±0.04</td>
<td>2.05±0.03</td>
<td>1.97±0.04</td>
</tr>
<tr>
<td>S</td>
<td>1.55±0.04</td>
<td>1.62±0.06</td>
<td>1.73±0.04</td>
<td>1.67±0.05</td>
<td>1.79±0.03</td>
<td>1.82±0.05</td>
</tr>
<tr>
<td>(Ca_{50}), nM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1596±136</td>
<td>1718±184</td>
<td>1008±92</td>
<td>1076±118</td>
<td>1006±102</td>
<td>1212±114</td>
</tr>
<tr>
<td>S</td>
<td>1670±193</td>
<td>1628±209</td>
<td>1059±96</td>
<td>992±91</td>
<td>1286±113</td>
<td>1210±108</td>
</tr>
<tr>
<td>Basal, μmol·g protein(^{-1})·min(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>53±4</td>
<td>47±3</td>
<td>34±2</td>
<td>36±3</td>
<td>68±5</td>
<td>57±4</td>
</tr>
<tr>
<td>S</td>
<td>51±4</td>
<td>47±3</td>
<td>34±2</td>
<td>34±2</td>
<td>61±3</td>
<td>60±2</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 10). \(V_{max}\), maximal Ca\(^{2+}\)-ATPase activity; \(n_H\), Hill coefficient defined as the slope of the relationship between Ca\(^{2+}\)-ATPase activity and the cytosolic free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_c\)) between 10 and 90% of \(V_{max}\); \(Ca_{50}\), amount of [Ca\(^{2+}\)] required for 50% activation of \(V_{max}\). Basal ATPase activity, residual activity following inhibition of Ca\(^{2+}\)-ATPase activity. A main effect of stimulation (\(P < 0.05\)) was found for \(n_H\) in WG. In WG, values for control were greater than for stimulated muscle.

- **Ca\(^{2+}\)**, indicated that the Ca\(^{2+}\) release during a short period of tetanic contraction, whereas muscles of low oxidative potential respond with increases in both Ca\(^{2+}\) release and Ca\(^{2+}\) uptake.

- Interestingly, at least with the stimulation protocol employed, these effects generally persist for at least 25 min after the activity. Because these measurements were performed in vitro under supposedly optimal activating conditions (42), the changes probably reflect second messenger regulatory influences and/or structural modifications to the Ca\(^{2+}\)-ATPase enzyme (3, 32, 46) and/or the RyR (15, 19).

- A second objective of the study was to investigate the hypothesis that the depression in mechanical function observed both during the contractile activity and during recovery in SOL would be related to the changes in the SR Ca\(^{2+}\) cycling properties. We employed the twitch to investigate the time-dependent changes of repetitive activity on the mechanical properties of SOL. On the basis of the responses we observed, our hypothesis must be partially rejected, namely, that the mechanical changes observed after exercise and during recovery are associated with changes in SR Ca\(^{2+}\) cycling behavior. In the SOL, a muscle of high oxidative potential, \(P_i\), was depressed in stimulated muscle at R0 and remained depressed at R25. In SOL, although depressions were noted in Ca\(^{2+}\) uptake, no changes occurred in Ca\(^{2+}\) release, regardless of the time of measurement.

- Of particular importance are the mechanisms underlying the different responses observed in the SR Ca\(^{2+}\) handling properties with our stimulation protocol and how the oxidative potential of the fiber is implicated. The depression in \(V_{max}\) with contractile activity was expected on the basis of earlier studies that found similar reductions in both the SOL (54) and RG (5), using a variety of exercise protocols. The reductions in \(V_{max}\) have been attributed to structural alterations in the enzymes, specifically in the region near the adenine nucleotide binding site (30, 33). This effect appears to be mediated by oxidation and nitrosylation as a result of accumulation of reactive oxygen species (ROS) (28). As in previous studies (5, 54), the reduction in \(V_{max}\) occurred in isolation, because two other kinetic properties of the enzyme that were examined, namely, \(n_H\) and \(Ca_{50}\), indicated that the Ca\(^{2+}\) sensitivity had not changed with the tetanic contractions. Because Ca\(^{2+}\) uptake responded in a similar fashion to \(V_{max}\) in SOL and WG, the apparent coupling ratio was unaltered. This would suggest that the energy cost of the transport of a given amount of Ca\(^{2+}\) ions into the SR was unaltered. This result is similar to that previously observed with both SOL (54) and RG (5) following contractile activity.

- Somewhat surprising, however, was the reduction in the ionophore ratio observed in the SOL and RG with the repetitive contractions, an effect that persisted throughout recovery. Because the ionophore ratio is based on the ratio of \(V_{max}\) measured with and without the Ca\(^{2+}\) ionophore A-23187, reductions in this ratio suggest that increases in SR membrane permeability to Ca\(^{2+}\) have occurred as a result of the tetanic contractions. The increased Ca\(^{2+}\) permeability could reduce the accumulation of Ca\(^{2+}\) in the SR and prevents back inhibition of the enzyme (31). Exercise-induced decreases in the ionophore ratio should result in increased leak of Ca\(^{2+}\) back into the cytosol during Ca\(^{2+}\) sequestration, providing an erroneously reduced Ca\(^{2+}\) uptake to what actually occurred (27). It is possible, given the apparent increased SR membrane permeability, that the decrease in Ca\(^{2+}\) uptake that we observed in SOL and RG with the contractile activity in this study could have been even more pronounced if a Ca\(^{2+}\) leak through the membrane was present. Previous studies using different exercise protocols have not reported any changes in the ionophore ratio in SOL (54), red vastus/RG (5), or diaphragm (34).

- The limitations of our assay notwithstanding (see Appendix), our findings do suggest a causal association between changes in \(V_{max}\) and Ca\(^{2+}\) uptake that was observed with our stimulation and recovery protocol. It is clear from our results that the dominant mechanism acting to reduce Ca\(^{2+}\) uptake with repetitive activity is a reduced \(V_{max}\).

- Mechanisms underlying the increase in \(V_{max}\) that were observed in WG with exercise present a unique challenge given the novelty of these results. Others (17), including investigators in our laboratory (41), have reported an overshoot in \(V_{max}\) in muscles of predominant fast-twitch fiber composition and low oxidative potential, but only during the recovery period following prolonged exercise in rats. No changes in \(V_{max}\) were observed immediately after running (41). We have suggested, on the basis of our earlier observations using a NCD-4 probe, that the overshoot could be mediated by increased activation
secondary to increased availability of the Ca\textsuperscript{2+} binding site, possibly induced by changes in the oligomeric status of the Ca\textsuperscript{2+}-ATPase (40). At present, there are no known acute regulatory mechanisms that could result in increasing $V_{\text{max}}$ of the enzyme, short of decreasing sarcolipin content or increasingly actual Ca\textsuperscript{2+}-ATPase content (46). Because we did not find increases in SERCA1a isoform content in previous studies using a more sustained exercise protocol (49), it is unlikely that the 5 min of contractile activity used in this study elevated SERCA1a content. Similarly, increased degradation of sarcolipin appears unlikely as well, given the brevity of our exercise intervention. It is possible that dissociation of SERCA1a with sarcolipin could occur, but there are no known regulatory mechanisms to cause this.

As with the SOL and RG, we found that the changes in Ca\textsuperscript{2+} uptake generally track the changes in $V_{\text{max}}$, because no changes in the apparent coupling ratio were found. The ionophore ratio in WG also was unchanged with our experimental protocol.

**Fig. 2.** Comparative effects of contractile activity and recovery on the ionophore ratio (ratio of $V_{\text{max}}$ measured with and without the Ca\textsuperscript{2+} ionophore A-23187) in rat SOL, RG, and WG muscles. Values are means ± SE ($n = 10$). A main effect ($P < 0.05$) of stimulation was found for both SOL and RG: values for control were greater than for stimulated muscle.

**Fig. 3.** Changes in sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} uptake with repetitive activity and repetitive activity plus recovery in rat SOL, RG, and WG muscles. Values are means ± SE ($n = 10$); pro, protein. Ca\textsuperscript{2+} uptake was measured at 2,000 nM. Main effects ($P < 0.05$) of stimulation were found for all muscles: for SOL and RG, values for control were greater than for stimulated muscle; for WG, values for stimulated muscle were greater than for control.
suggesting that no changes in the membrane permeability for Ca\(^{2+}\) occurred. However, for the WG, we did find evidence that the Ca\(^{2+}\) cooperativity of the enzyme had changed, because \(n_H\) was reduced after exercise and after recovery. The reduction in \(n_H\) was not accompanied by any changes in Ca\(_{50}\), the \([Ca^{2+}]_f\) value required for 50% activation of \(V_{max}\). Because \(n_H\) was defined as the slope of the relationship between Ca\(^{2+}\)-ATPase activity and \([Ca^{2+}]_f\) between 10 and 90% of \(V_{max}\), it appears that exercise reduced Ca\(^{2+}\) cooperativity during the steep activation phase. As a consequence, in this range, a higher \([Ca^{2+}]_f\) value would be needed to elicit a given Ca\(^{2+}\)-ATPase activity and, consequently, Ca\(^{2+}\) uptake. At present, the mechanism for decreases in \(n_H\) appears unclear, particularly given that \(V_{max}\) was increased. In previous studies reporting increases in \(V_{max}\) during recovery from prolonged exercise, Ca\(^{2+}\) sensitivity was not modified (40).

Reductions in Ca\(^{2+}\) release assessed in vitro with exercise have commonly been reported in human (13), rat (16, 37), and frog (51) skeletal muscle. When assessed in vitro, reductions in Ca\(^{2+}\) release have been associated with reductions in \(^3\)H-labeled RyR binding, implying structural alterations to the RyR (15). These findings would appear to not apply to our study, because we found either no change (SOL and RG) or an increase (WG) in Ca\(^{2+}\) release with exercise. Because reductions in Ca\(^{2+}\) release with exercise have been reported in both rat SOL (54) and extensor digitorum longus (37), muscles of high oxidative potential but differing fast- and slow-twitch fiber composition (11), the most logical explanation for the different results would appear to be the differences in exercise protocols. Our exercise protocol was intense but intermittent in nature, interspersed with rest periods, whereas other exercise protocols were either submaximal and performed for sustained periods (16) or intense and continuous (37).

The increase in Ca\(^{2+}\) release in WG with our exercise protocol has not been previously reported, regardless of the fiber type composition of the skeletal muscle. The increases that we observed were reflected in both phase 1 and phase 2, the fast and slow kinetic phases, respectively. Previous studies measured what is essentially the slower phase of Ca\(^{2+}\) release (16, 37). We elected to report on two phases of release on the basis of our previous work (48), which demonstrated that two distinct phases occur as demonstrated by a sharp break in the

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**Fig. 4.** Comparative effects of contractile activity and contractile activity plus recovery on Ca\(^{2+}\) release in rat SOL, RG, and WG muscles. Values are means ± SE (\(n = 10\)). **Phase 1**, early fast phase of Ca\(^{2+}\) release; **phase 2**, more delayed phase of Ca\(^{2+}\) release. A main effect (\(P < 0.05\)) of stimulation was found for WG: values for stimulated muscle were greater than for control for both **phase 1** and **phase 2**.
relationship of Ca\(^{2+}\) release vs. time. At present, the functional significance of these phases remains unclear. What is clear from our current study is that the overshoot was reflected in both phase 1 and phase 2 of Ca\(^{2+}\) release.

The overshoot in Ca\(^{2+}\) release could be explained by increases in RyR receptor protein or increases in the open state of the RyR channels. It is highly improbable that increases in RyR protein resulted, given the observation that RyR protein has not been found to change in studies utilizing much more extended exercise protocols. Increases in the open status of the RyR leading to increases in Ca\(^{2+}\) release have been reported to occur in muscle secondary to phosphorylation of calmodulin kinase II, a regulatory RyR protein (45). Whether this occurred in our study and whether detection of this effect is possible in an in vitro assay needs to be determined.

The repetitive stimulation protocol that we employed resulted in changes in force and the maximal rates of force development and relaxation in both SOL and GAS muscles. To characterize these effects, we used the properties of twitch. In both the SOL and GAS, twitch force was reduced at R0, and no recovery in force was detected in either muscle during the 25-min period of recovery.

An inviting possibility to explain the fatigue observed with our repetitive stimulation protocol is loss of SR Ca\(^{2+}\) regulation. Reductions in [Ca\(^{2+}\)]\(_r\) with repeated tetani have been commonly associated with loss of force in single fibers of mouse muscle (1). The reduction in [Ca\(^{2+}\)]\(_r\) has been attributed primarily to a reduction in Ca\(^{2+}\) release (1). In this study, we found no changes in Ca\(^{2+}\) release at R0 in SOL and RG and an increase in WG. These findings would suggest that if fatigue in our study occurred as a consequence of reduced Ca\(^{2+}\) release, it did not occur as a direct modification of the Ca\(^{2+}\) release channel. This possibility has been suggested previously on the relationship of Ca\(^{2+}\) release vs. time. At present, the functional significance of these phases remains unclear. What is clear from our current study is that the overshoot was reflected in both phase 1 and phase 2 of Ca\(^{2+}\) release.

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basis of the ability of caffeine, a known Ca$^{2+}$ release agent, to reverse the depression in Ca$^{2+}$ release and [Ca$^{2+}$]; (1). The impaired Ca$^{2+}$ release has been attributed to other mechanisms such as impaired Ca$^{2+}$ loading into the SR or defective t-tubule (44) SR coupling. The sustained depression in force that we observed particularly at the lower frequencies of stimulation is a characteristic of low-frequency fatigue that occurs secondarily to disturbances in Ca$^{2+}$ release and reduced [Ca$^{2+}$]; (4). Even though Ca$^{2+}$ uptake was depressed in SOL at R25, it is difficult to implicate Ca$^{2+}$ loading as a problem given the 25-min period of low-frequency fatigue.

The twitch rates of both force development and relaxation were also affected by the repetitive contractions. In the case of +dP/dt$_{max}$, a different effect was observed between SOL and GAS. For GAS, +dP/dt$_{max}$ was reduced at R0 and showed no recovery by R25. For SOL, a reduced +dP/dt$_{max}$ rate was observed that was not specific to a time point. These findings are inconsistent with our in vitro measurements of Ca$^{2+}$ release, because no changes in Ca$^{2+}$ release, both phase 1 and phase 2, were observed in SOL. The twitch relaxation rate also showed a dissociation with our Ca$^{2+}$ uptake measurements. In SOL, in which we observed a reduction in Ca$^{2+}$ uptake at R0 and R25, no significant effect on -dP/dt$_{max}$ was generally observed. Unfortunately, because the whole GAS was used for the mechanical studies, we were not able to investigate the role of SR Ca$^{2+}$ handling. The major portion of the GAS is dominated by type II fibers of moderate oxidative potential (11). Fibers of high oxidative potential (RG) and fibers of low oxidative potential (WG) represent a relatively small region of the muscle, comprising ~6 and 8% of the fiber mass, respectively. The calculated oxidative potential of the GAS is estimated to approximate that of the SOL. In this study, we did not isolate different branches of the sciatic nerve and investigate the mechanical responses of different regions of the GAS as has been done previously (8). These two different compartments investigated have been shown to display large differences in oxidative potential and to result in different mechanical and metabolic responses (9, 10, 38). The regions that we sampled and labeled as RG and WG would appear to be generally comparable to the two compartments studied by de Ruiter et al. (8).

Some qualification appears necessary in our study regarding the fiber type composition of the regions of the GAS that were sampled. Delp and Duan (11) in their exhaustive characterization of rat muscle reported an ~4.5-fold difference between the oxidative potential of a pure red and pure white portion of the GAS. In our study, the difference was ~2.8-fold, suggesting some contamination with the most dominant intermediate or mixed region of the GAS. Because the pure red portion contains some 50% type I fibers, it is probable that our RG contained a small percentage of type I fibers. Consequently, the possibility remains that the difference in the SR Ca$^{2+}$ cycling response observed between tissues that have been ascribed to differences in oxidative potential could also be influenced by fiber type composition. Collectively, our results suggest that processes in addition to modifications in SR Ca$^{2+}$ handling are involved in explaining the fatigue and weakness observed with repetitive stimulation.

With our experimental design, we examined the SR Ca$^{2+}$ cycling response in response to repetitive muscle contraction and recovery under optimal in vitro conditions. In contrast, the mechanical changes were measured in vivo. As such, the mechanical responses are subject to changes in the intracellular environment mediated by the contractile activity. Some of these changes involve the cellular energy status and metabolic by-product accumulation. We have assessed some of these responses in this study. Our stimulation protocol resulted in only small ATP reductions in energy potential, as assessed by ATP and PCr, and only in the RG and WG. Lactate was elevated, but only modestly, in the oxidative SOL and RG, with the greatest increase in WG as expected (25). All of these properties had returned to normal at R25, the end of recovery.

In summary, by employing a brief period of repetitive stimulation followed by 25 min of recovery, we have been able to demonstrate fundamental differences in SR Ca$^{2+}$-handling responses measured in vitro between muscles of different oxidative potential. Moreover, we also have demonstrated that the changes observed in the Ca$^{2+}$-handling properties do not generally associate with the mechanical changes that occurred as assessed by the force, rate of force development, and rate of relaxation observed with the twitch response. Collectively, these results imply that fatigue mechanisms are mediated by events other than structural changes to the Ca$^{2+}$-ATPase enzyme and the RyR channel. As a consequence, the physiological significance of the changes in the SR Ca$^{2+}$-handling properties that we have observed remains unknown. An intriguing question remains, how does the oxidative potential of the muscle impact on the Ca$^{2+}$-handling properties during contractile activity?

APPENDIX

**Methodological Issues in SR Measurements**

There are limitations in our assay that could also affect the changes in Ca$^{2+}$ uptake that were observed. It is possible that Ca$^{2+}$ could have leaked out of the SR into the cytosol via either the RyR or the Ca$^{2+}$-ATPase (27, 31). In our assay, we did not include agents that would inhibit Ca$^{2+}$-ATPase activity, such as thapsigargin or high concentrations of RyR, to examine these potential effects. We have investigated these possibilities in other studies, however, using other exercise models, and have found no additional effect of the exercise (unpublished observation). Others also have reported no effect (16).

An unavoidable limitation in our assays for Ca$^{2+}$ uptake was the need to use oxalate as the medium to precipitate Ca$^{2+}$ in SR. It is possible that the rate at which the Ca$^{2+}$-oxalate precipitate is formed could limit the kinetics of Ca$^{2+}$ uptake. In addition, it should be noted that Ca$^{2+}$-ATPase activity was measured with the Ca$^{2+}$ ionophore A-23187 to minimize inhibition in enzyme kinetics (27). For these reasons, as well as others, we have called the ratio of Ca$^{2+}$ uptake to Ca$^{2+}$-ATPase the apparent coupling ratio, to reflect the fact that the ratio has not been determined under identical measurement conditions for both properties. In our hands this was not possible, given the extremely slow rate of Ca$^{2+}$ loading into SR without oxalate and the low V$_{max}$ observed when oxalate is used (unpublished observation).

As with the measurement of Ca$^{2+}$ uptake, limitations in our assay used to measure Ca$^{2+}$ release may be involved in our findings. The measurement of Ca$^{2+}$ release followed the measurement of Ca$^{2+}$ uptake, using the same assay conditions but with the releasing agent 4-CMC. This assay procedure has been criticized because oxalate is used to precipitate the Ca$^{2+}$, and the dissociation of Ca$^{2+}$ from oxalate may be the limiting factor in Ca$^{2+}$ release and not the conductance properties of the RyR channels. However, we feel that this is highly unlikely because we have shown a graded Ca$^{2+}$ release with different concentrations of 4-CMC (unpublished observation).
Alternatively, differences in the Ca\(^{2+}\) loading might also influence the Ca\(^{2+}\) release. Because the repetitive contractions also resulted in increases in Ca\(^{2+}\) uptake in WG, it is possible that the amount of Ca\(^{2+}\) loading into the SR was greater and, consequently, Ca\(^{2+}\) release increased. However, this possibility also appears remote, because the Ca\(^{2+}\) uptake assay was continued until no further loading resulted, implying that the SR was fully loaded with Ca\(^{2+}\).

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**REFERENCES**


