Effects of progressive exercise and hypoxia on human muscle sarcoplasmic reticulum function


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Duhamel, T. A., H. J. Green, S. D. Sandiford, J. G. Perco, and J. Ouyang. Effects of progressive exercise and hypoxia on human muscle sarcoplasmic reticulum function. J Appl Physiol 97: 188–196, 2004. First published April 2, 2004; 10.1152/japplphysiol.00958.2003.—This study examined the effects of progressive exercise to fatigue in normoxia (N) on muscle sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} cycling and whether alterations in SR Ca\textsuperscript{2+} cycling are related to the blunted peak mechanical power output (PO\textsubscript{peak}) and peak oxygen consumption (VO\textsubscript{2peak}) observed during progressive exercise in hypoxia (H). Nine untrained men (20.7 ± 0.42 yr) performed progressive cycle exercise to fatigue on two occasions, namely during N (inspired oxygen fraction \( = 0.21 \)) and during H (inspired oxygen fraction \( = 0.14 \)). Tissue extracted from the vastus lateralis before exercise and at power output corresponding to 50 and 70% of VO\textsubscript{2peak} (as determined during N) and at fatigue was used to investigate changes in homogenate SR Ca\textsuperscript{2+}-cycling properties. Exercise in H compared with N resulted in a 19 and 21% lower \((P < 0.05)\) PO\textsubscript{peak} and VO\textsubscript{2peak}, respectively. During progressive exercise in N, Ca\textsuperscript{2+}-ATPase kinetics, as determined by maximal activity, the Hill coefficient, and the Ca\textsuperscript{2+} concentration at one-half maximal activity were not altered. However, reductions with exercise in N were noted in Ca\textsuperscript{2+} uptake (before exercise = 357 ± 29 \( \mu \)mol\cdot min\(^{-1}\)\cdot g protein\(^{-1}\); at fatigue = 306 ± 26 \( \mu \)mol\cdot min\(^{-1}\)\cdot g protein\(^{-1}\); \( P < 0.05 \)) when measured at free Ca\textsuperscript{2+} concentration of 2 \( \mu \)M and in phase 2 Ca\textsuperscript{2+} release (before exercise = 716 ± 33 \( \mu \)mol\cdot min\(^{-1}\)\cdot g protein\(^{-1}\); at fatigue = 500 ± 53 \( \mu \)mol\cdot min\(^{-1}\)\cdot g protein\(^{-1}\); \( P < 0.05 \)) when measured in vitro in whole muscle homogenates. No differences were noted between N and H conditions at comparable power output or at fatigue. It is concluded that, although structural changes in SR Ca\textsuperscript{2+}-cycling proteins may explain fatigue during progressive exercise in N, they cannot explain the lower PO\textsubscript{peak} and VO\textsubscript{2peak} observed during H.

AMONG THE EXCITATION-CONTRACTION (E-C) PROCESSES INVOLVED IN TRANSLATING THE NEURAL SIGNAL INTO FORCE IN THE MUSCLE CELL, THE SARCOPLASMIC RETICULUM (SR) REMAINS AS AN INVITING SITE OF REGULATION OF CYTOSOLIC FREE Ca\textsuperscript{2+} LEVELS DURING EXERCISE, especially during fatiguing submaximal exercise given its role in regulating cytosolic free Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{c}) and, consequently, activation of the myofibrillar complex and force generation (4).

The SR, an intracellular membranous network that envelopes the myofibrils, regulates [Ca\textsuperscript{2+}]\textsubscript{c} levels by selective control of the rate of Ca\textsuperscript{2+} release from Ca\textsuperscript{2+} stored inside the SR and by the rate of Ca\textsuperscript{2+} uptake (4). Ca\textsuperscript{2+} release depends on the open state of the Ca\textsuperscript{2+}-release channels (CRC) or ryano time receptors, an ~450-kDa protein embedded in the SR membrane, most of which is localized to the terminal cisternae, which is in close apposition to the T tubules (12). It is now generally acknowledged that, in skeletal muscle, the action potentials that are conducted along the sarcolemma and into the T tubule result in a physical-chemical coupling between the T tubules and the CRC and opening of the CRC, enabling the rapid release of Ca\textsuperscript{2+} (4). The sequestration of [Ca\textsuperscript{2+}]\textsubscript{i} back into the SR is controlled by the Ca\textsuperscript{2+}-ATPase, an ~110-kDa protein located primarily in the longitudinal SR, which uses the energy from the hydrolysis of ATP to pump Ca\textsuperscript{2+} against its concentration gradient for storage in the SR (27). Increases in [Ca\textsuperscript{2+}]\textsubscript{i} are a potent stimulus for increasing the activity of Ca\textsuperscript{2+}-ATPase and, consequently, the rate of Ca\textsuperscript{2+} uptake into the SR (32).

The possibility that disturbances in SR Ca\textsuperscript{2+} cycling may be involved in fatigue during submaximal exercise is also supported by several studies that have demonstrated reductions in both Ca\textsuperscript{2+}-ATPase activity (6, 7, 14), Ca\textsuperscript{2+} uptake (6, 7), and Ca\textsuperscript{2+} release (10) in both rat and human locomotor muscles. Information is emerging to indicate that the disturbances in Ca\textsuperscript{2+} uptake and Ca\textsuperscript{2+} release are mediated by structural adaptations in the Ca\textsuperscript{2+}-ATPase enzyme (26) and the CRC (9) secondary to oxidation and/or nitrosylation mediated by reactive O\textsubscript{2} species (ROS) (23, 24).

During intense dynamic exercise, increases in the rate of Ca\textsuperscript{2+} cycling in the muscle cell and consequently in the rate of Ca\textsuperscript{2+} release and Ca\textsuperscript{2+} uptake are necessary to realize increases in PO. A disturbance in either Ca\textsuperscript{2+} release and Ca\textsuperscript{2+} uptake would be expected to alter the [Ca\textsuperscript{2+}]\textsubscript{i} integral and disrupt force profiles (1).

Intuitively, one might expect that supramaximal fatiguing exercise would produce even more dramatic reductions in SR Ca\textsuperscript{2+} cycling. There is evidence to support this idea since pronounced reductions in Ca\textsuperscript{2+}-ATPase activity and Ca\textsuperscript{2+} uptake have been reported with exhaustive running in horses (8) and rats (52) and with intense knee extensions in humans (13, 25). Pronounced depressions in SR Ca\textsuperscript{2+} release have also been reported (25, 43). However, not all studies are consistent, because reports have been published indicating no change in Ca\textsuperscript{2+}-ATPase activity (19, 31) and in Ca\textsuperscript{2+} uptake (18, 31) with intense, repetitive contractions.

Progressive dynamic exercise to fatigue is commonly employed to assess peak aerobic power [peak O\textsubscript{2} consumption (VO\textsubscript{2peak})], one of the most important properties in applied physiology. However, it is unclear whether the peak mechanical power output (PO\textsubscript{peak}) attained is limited by a failure in SR Ca\textsuperscript{2+} cycling.

Hypoxia is known to result in a reduction in PO\textsubscript{peak} and VO\textsubscript{2peak} during progressive exercise to fatigue (15, 33). It is
The interpretation could be simplistic because it assumes that the viability of the E-C processes in muscle are protected, allowing power output (PO) to increase and available O2 to be fully utilized for ATP regeneration to contribute the increasing demands of the cellular ATPases involved in E-C. It is possible that the reverse may be true, namely that E-C failure may occur prematurely, resulting in fatigue and an inability to utilize available O2 for oxidative phosphorylation (OxPhos).

The purpose of this study was to investigate the effects of progressive cycle exercise in normoxia and hypoxia on muscle SR function. We have hypothesized that progressive reductions in Ca\(^{2+}\)-ATPase activity, Ca\(^{2+}\) uptake, and Ca\(^{2+}\) release would occur with increases in PO. We have also postulated that, at similar PO, the depression in the Ca\(^{2+}\)-cycling properties would be more pronounced in hypoxia compared with normoxia. However, at fatigue in normoxia vs. hypoxia, the disturbances would be comparable, consistent with the lower PO\(_{peak}\) and VO2\(_{peak}\) typically observed with hypoxia.

**METHODS**

**Participants.** Participants were nine healthy male university students who were not engaged in vigorous physical activity on a regular basis. Age, height, and body mass were 20.0 ± 0.37 yr, 175 ± 2.3 cm, and 80.0 ± 3.5 kg (means ± SE), respectively. All procedures were approved by the Office of Research Ethics, and all experimental protocols and risks were carefully explained to each volunteer before written consent was obtained.

**Experimental design.** To investigate the effects of progressive cycle exercise during normoxia and hypoxia, a randomized cross-over design was employed. In this design, all participants completed each condition within a minimum of 3 wk between test sessions for each individual. At least 1 wk before the beginning of the experiment, volunteers reported to the laboratory and completed a preliminary incremental exercise test to fatigue during normoxia. This test was used to ensure a normal response to exercise and to verify that a stepwise increase in PO could be achieved. It is possible that the reverse may be true, namely that E-C failure may occur prematurely, resulting in fatigue and an inability to utilize available O2 for oxidative phosphorylation (OxPhos).

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standardized for each volunteer between tests. Average environmental conditions in the testing room were not different between conditions and ranged between 50 and 60% relative humidity and 21 and 22°C in temperature.

**Measurement of SR function.** Immediately after extraction of the tissue, homogenates were prepared and stored at −80°C until analyses of Ca2+-ATPase activity, Ca2+ uptake, and Ca2+ release. Homogenates, prepared under ice-cold conditions, consisted of 11:1 (vol/wt) dilution of buffer containing (in mM): 250 sucrose, 5 HEPES, 10 Na2SO4, and 0.2 PMSF (pH 7.5). Tissue (30–40 mg) was homogenized by using a handheld glass homogenizer (Kontes, Duall 20) (29). Once prepared, the homogenate was separated into a number of aliquots and quick frozen in liquid N2 before storage. On a given analytical day and just before analysis, aliquots were thawed and processed for specific measurements. For a given property, duplicate measurements were performed. Care was taken to ensure that a complete set of measurements were made on all tissues for a given participant during a specific analytical session.

**Ca2+-ATPase activity.** Ca2+-ATPase activity was assessed spectrophotometrically using the basic procedures developed by Simonides and van Hardeveld (40), as modified in our laboratory (45). The reaction buffer contained (in mM): 200 KC1, 20 HEPES, 15 MgCl2, 1 EGTA, 10 Na2SO4, 5 ATP, and 10 phosphoenolpyruvate. The pH of the reaction buffer was adjusted to 7.0 at 37°C. Just before the reaction was started, 18 U/ml lactate dehydrogenase, 18 U/ml pyruvate kinase, 0.3 mM NADH, and 25 μl of homogenate were added to a cuvette containing 1 ml of reaction buffer. One micromolar Ca2+ ionophore A-23187 (Sigma C-7522) was also added to prevent intraluminal Ca2+ accumulation in SR and inhibition of Ca2+-ATPase activity (40). Assays were performed in duplicate at 37°C and 340 nm (Shimadzu UV 160) using −1 mg wet wt of tissue per assay. After the baseline absorbance of NADH was recorded for ~1 min, the reaction was initiated by adding 1 μl of 100 mM CaCl2 and monitored for ~2 min.

Maximal Ca2+-ATPase activity and Ca2+ dependency of Ca2+-ATPase activity were measured using a 0.5-μl addition of 100 mM CaCl2. The additions were continued until a plateau and subsequent decline in Ca2+-ATPase activity were observed. Basal or Mg2+-ATPase activity was determined in the presence of 40 μM of the specific inhibitor of the Ca2+-ATPase cyclopiazonic acid (38). Ca2+-ATPase activity is based on the difference between the total and basal ATPase activities. The validity of SR Ca2+-ATPase activity assay has been previously established in whole muscle homogenates (34, 40). It should be emphasized that a potential limitation of the regenerating assay employed is the possible loss of ADP to AMP via the myokinase reaction. However, we have shown using a myokinase inhibitor (AP5A) that Ca2+-ATPase activity is unaffected (unpublished observations).

**Measurement of [Ca2+]i**. which is used to assess Ca2+ dependency of the Ca2+-ATPase reaction, was assessed with dual-wave spectrophotometry and the Ca2+ fluorescent dye indo 1 according to procedures previously detailed (46, 48). In summary, the measurement of [Ca2+]i is based on the difference in maximal emission wavelengths between the Ca2+-bound indo 1 complex and the Ca2+-free indo 1 complex. An excitation wavelength of 355 nm and the emission maxima were recorded at 405 and 485 nm for Ca2+-bound and Ca2+-free indo 1. Felix software (Photon Technology International) was used to calculate the ionized Ca2+ concentration according of Eq 2.5 of Grynkiewicz et al. (17). The dissociation constant that was used for the interaction between Ca2+ and indo 1 was 250 nM (17). It should be emphasized that in homogenates the actual dissociation constant can be significantly affected by the protein composition. As such, given the uncertainty as to the true value of the dissociation constant, the pCa values used throughout the text are only apparent.

The kinetic properties of the Ca2+-ATPase activity that were measured included the maximal activity (Vmax), the [Ca2+]i, needed to obtain half-Vmax (Ca50), and the Hill coefficient (nH). To obtain these properties, Ca2+-ATPase activity was plotted against the negative logarithm of [Ca2+]i (pCa). Vmax represented the peak value, Ca50 represented the [Ca2+]i obtained from a sigmoid fit of the data that yields 50% of Vmax, and nH was obtained through nonlinear regression with computer software (GraphPad Software) by using a portion of the curve that corresponded to between 20 and 80% of Vmax. These procedures have been described in recent papers from our laboratory (37, 49).

**Ca2+ uptake and Ca2+ release.** Both Ca2+ uptake and Ca2+ release were measured during the same assay. In general, the procedure employed involved using the Ca2+ fluorescent dye indo 1 according to the methods of O’Brien et al. (30) as modified by our group (46). The reaction buffer for these assays contained (in mM) 200 KC1, 20 HEPES, 15 MgCl2, 10 Na2SO4, 0.005 NaN3, N4-tetraakis(2-pyridylmethyl)-ethylenediamine, 5 oxalate, and 10 phosphoenolpyruvate. Before each assay, 1.5 μM indo 1, 1, 8 U/ml lactate dehydrogenase, and 18 U/ml pyruvate kinase were added to 2 ml of reaction buffer. In addition, 2.5 μl of CaCl2 (10 mM) were added to the cuvette to produce a consistent starting [Ca2+]i of ~3.5 μM. After a constant [Ca2+]i was achieved, 5 mM ATP was added to the cuvette to initiate Ca2+ uptake. [Ca2+]i uptake rates were assessed over a range of [Ca2+]i by using a single assay. [Ca2+]i was measured as described earlier. The maximal rate of [Ca2+]i uptake for each [Ca2+]i was determined by differentiating the linear fit curve using four concentrations of cytosolic free Ca2+, namely 500, 1,000, 1,500, and 2,000 μM. We have found that with this procedure there is a rapid initial drop in [Ca2+]i. A large component of the initial drop in [Ca2+]i has been credited to the binding of Ca2+ with proteins in the homogenates (34). As a consequence, the initial changes in [Ca2+]i were not used in the calculation of [Ca2+]i uptake (34).

Ca2+ release was measured immediately after Ca2+ uptake according to the general procedures of Ruell et al. (34). However, unlike Ruell et al., who employed AgNO3, we have employed 4-chloro-m-cresol (4-CMC). We have recently shown that effects of 4-CMC are specific to the CRC and do not cause Ca2+-ATPase pump reversal, as was noted for AgNO3 (47). To assess Ca2+-release rates, 20 μM of 4-CMC was added to the cuvette after [Ca2+]i declined to a plateau after the measurement of Ca2+ uptake. We have found that a biphasic Ca2+ release can be distinguished, an early release, which we have labeled phase 1, and a slower, more delayed release, which we have labeled phase 2 (47). Maximal release rates for each phase were calculated using the same method as for Ca2+ uptake and differentiating using a linear-fit curve.

It should be noted that, because Ca2+ uptake and Ca2+ release were measured in the same assay, the reaction buffers were the same. It is now possible that optimal assay conditions may vary with the property measured. As an example, 15 mM MgCl2 was used for both Ca2+ uptake and Ca2+ release. In the case of Ca2+ release, optimal MgCl2 levels appear to be between 20 and 30 mM (unpublished observations).

For all SR properties, protein was determined by the method of Lowry as modified by Schacterle and Poll洛克 (36). This involved separate protein assessments in homogenate used for Ca2+-ATPase activity and in homogenates used for Ca2+-uptake and Ca2+-release assays. There were no significant differences in protein levels between normoxic and hypoxic conditions. The coefficients of variation for duplicate measurements for Ca2+ uptake and Ca2+-ATPase activity were 7.6 ± 0.9 and 7.9 ± 2.2%, respectively. Only single measurements were used for Ca2+-release determinations.

**Statistics.** Group data are expressed throughout the text as means ± SE. To examine the effects of progressive exercise or O2 condition on SR function, linear regression techniques were employed, and the significance of the regression line for each condition was established. Student’s t-tests were used to examine differences between slopes and, hence, conditions. Conventional two-way ANOVA procedures were not possible because of the unbalanced nature of the tissue-
sampling schedule (see Fig. 1). Where only one variable was of interest (such as during exercise in normoxia or hypoxia), a one-way ANOVA with repeated measures was employed. Two-way ANOVA procedures were used to examine the effects of condition and exercise on the gas-exchange properties. We have also performed a secondary analysis, using two-way ANOVA procedures, to determine the effects of exercise and O2 condition on SR properties using matched samples. Where significance was found, Newman-Keuls techniques were applied to locate differences between specific means. The significance level was set at P < 0.05 for all comparisons.

RESULTS

Peak mechanical and aerobic power. POpeak generated during progressive cycle exercise to fatigue in normoxia was 300 ± 8.7 W. During hypoxia, POpeak was 250 ± 7.9 W, ~19% lower than the value obtained during normoxia. As expected, VO2peak was also reduced with hypoxia compared with normoxia (4.07 ± 0.15 vs. 3.21 ± 0.11 l/min, respectively).

Measurements of exchange during normoxia and hypoxia were obtained at matching POs of 137 ± 51, 214 ± 6.9, and 250 ± 7 W (Table 1). These data indicated that no differences existed in VO2 (l/min) between normoxia and hypoxia at corresponding POs. As expected, CO2 production, respiratory minute volume (BTPS), and respiratory exchange ratio were all higher during exercise in hypoxia compared with normoxia. In the case of CO2 production and respiratory minute volume, the differences were observed both at rest and during each PO.

SR. The POpeak values at which the final tissue samples were obtained for normoxia and hypoxia were 214 ± 6.9 and 250 ± 7.9 W, respectively. Because no differences were observed between normoxia and hypoxia in VO2 at comparable POs, the SR changes have been plotted against VO2. Our results indicated that, during progressive exercise in normoxia, no alterations were observed in Vmax, nh, or Cas0 (Table 2). Similarly, we found no effect of exercise and hypoxia on these properties. No differences were found between normoxia and hypoxia. Basal ATPase was not altered by either exercise or O2 condition. For basal ATPase (μmol·min⁻¹·g protein⁻¹), the values for normoxia were 36 ± 6, 39 ± 7, 38 ± 4, and 36 ± 4 for rest and 50% VO2peak (N), 70% VO2peak (N), and fatigue, respectively. For hypoxia, the values were 44 ± 5, 44 ± 3, and 41 ± 4 for rest, 50% VO2peak (N), and fatigue, respectively. The Ca²⁺-dependent Ca²⁺-ATPase activities for a typical volunteer are plotted in Fig. 2.

Table 2. Effects of progressive exercise in normoxia and hypoxia on kinetic characteristics of sarcoplasmic reticulum Ca²⁺-ATPase activity in vastus lateralis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pre</th>
<th>1.86</th>
<th>2.98</th>
<th>3.21</th>
<th>4.07</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmax, μmol·min⁻¹·g protein⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>161±11</td>
<td>147±8.5</td>
<td>158±9.4</td>
<td>157±11</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>167±11</td>
<td>164±13</td>
<td>152±11</td>
<td></td>
<td></td>
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<tr>
<td>nh</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>1.7±0.1</td>
<td>2.0±0.2</td>
<td>2.0±0.2</td>
<td>2.0±0.2</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>2.0±0.2</td>
<td>1.8±0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cas0, nM</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>N</td>
<td>744±133</td>
<td>889±209</td>
<td>832±185</td>
<td>791±108</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>869±133</td>
<td>886±95</td>
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</table>

Values are means ± SE (n = 9). Pre, preexercise. VO2 corresponds to average VO2 when biopsies were performed. Vmax, maximal Ca²⁺-ATPase activity; nh, Hill coefficient, determined from Hill plots by using the relationship between Ca²⁺ concentration and Ca²⁺-ATPase activity and the section of the curve that corresponded to 20–80% of the maximal Ca²⁺-ATPase activity; Cas0, Ca²⁺ concentration at half-maximal Ca²⁺-ATPase activity.
Progressive exercise resulted in a reduction in Ca\textsuperscript{2+} uptake in normoxia that was independent of [Ca\textsuperscript{2+}]\textsubscript{f} but only at fatigue (Fig. 3). Reductions in Ca\textsuperscript{2+} uptake also occurred during hypoxia. No differences were observed in Ca\textsuperscript{2+} uptake between normoxia and hypoxia as determined by the slopes of the regression lines. Coupling ratios, defined as the ratio between Ca\textsuperscript{2+} uptake and Ca\textsuperscript{2+}-ATPase activity, were unaffected either during exercise or between conditions (Fig. 4).

The effects of exercise and condition on Ca\textsuperscript{2+} release were determined by analyzing two phases of Ca\textsuperscript{2+} release (phase 1 and phase 2; Fig. 5). Ca\textsuperscript{2+} release was depressed during exercise for each condition in an intensity-dependent manner but only for phase 2 (Fig. 6). For normoxia, before exercise was >70% \(\dot{V}O_2\) peak (normoxia), which was greater than fatigue. For hypoxia, before exercise was >50% \(\dot{V}O_2\) peak (normoxia), which was greater than fatigue. No differences in slopes were observed between normoxia and hypoxia for either phase.

DISCUSSION

In this study, we have investigated the hypothesis that, in normoxia, progressive disturbances in SR function would oc-
have found that exercise in hypoxia failed to exacerbate the changes in Ca\textsuperscript{2+} in hypoxia compared with normoxia, the existence of similar properties. Given that the exercise time and PO were reduced, the amount of Ca\textsuperscript{2+} release was significantly lower at the peak \(\dot{V}O_2\) in hypoxia than in normoxia. *Significantly different from before exercise (\(P < 0.05\)).

![Fig. 6. Changes in Ca\textsuperscript{2+} release during progressive exercise in normoxia and hypoxia during phase 1 (A) and phase 2 (B). Ca\textsuperscript{2+} release has been plotted against \(\dot{V}O_2\) before exercise and during progressive exercise. Reductions (\(P < 0.05\)) in phase 2 of Ca\textsuperscript{2+} release were observed during exercise for each condition. †Significantly different from before exercise (\(P < 0.05\)). ‡Significantly different from 70% \(\dot{V}O_2\) peak in normoxia (\(P < 0.05\)). §Significantly different from 50% \(\dot{V}O_2\) peak in normoxia (\(P < 0.05\)).](http://jap.physiology.org/) [View Article](http://jap.physiology.org/)

In this study, we have used 4-CMC as the CRC-releasing agent. The selection of 4-CMC, instead of AgNO\textsubscript{3}, which has previously been commonly employed as a releasing agent (9), was based on a recent publication by our group (47). In that study, our laboratory found that AgNO\textsubscript{3}, in contrast to 4-CMC,
induces Ca$^{2+}$ release by acting on both the CRC and Ca$^{2+}$-ATPase. We have also been able to identify two phases of Ca$^{2+}$ release, phase 1 and phase 2. We have observed that only reductions in phase 2 were observed with exercise. At present, the physiological significance of this observation is not clear because, in our in vitro system, the Ca$^{2+}$-release rates occur at a much slower rate than observed in single-fiber and isolated-channel preparations (9). Our homogenate system is based on a preparation from whole muscle and consequently contains all of the proteins regulating SR behavior. This factor would appear important in the regulation of Ca$^{2+}$ release and explain, at least in part, differences that exist between the homogenate system and other preparations, such as isolated vesicles and single channels. It must be acknowledged, however, that, regardless of the preparation, some functional alterations could be expected during the homogenization process.

The reduction in Ca$^{2+}$ release that we have observed with our progressive exercise protocol probably results from a decrease in the number of functional CRC (9). Although definitive evidence is lacking, it is strongly suspected that oxidative and/or nitrosylative processes mediated by ROS are involved in the structural modifications to the CRC that occur (9, 44). It would be expected that ROS would accumulate during progressive exercise given the increase in OxPhos and the increase in inosine 5'-monophosphate (IMP) that occurs (16). It has been estimated that 2–4% of OxPhos results in ROS generation by the electron transport system (39, 41). In addition, the conversion of IMP to hypoxanthine and xanthine by xanthine oxidase also increases ROS formation (39, 41). Conceivably, the increase in ROS generation by xanthine oxidase during exercise would occur given the larger IMP accumulation (35). It is possible that the increase in ROS was insufficient to result in a greater reduction in Ca$^{2+}$ release with hypoxia compared with normoxia. At present, it is not clear why reductions in Ca$^{2+}$-ATPase activities during progressive exercise did not occur in either normoxia or hypoxia, given the apparent sensitivity of this enzyme to ROS (23).

A potential factor that could influence our measurements of the SR properties has to do with potential oxidation during the homogenate preparation. In our study, as well as most similar type studies, the DTT, a sulphydryl-reducing agent, was not added. Consequently, it would be expected that some oxidation would occur, possibly masking the actual changes that occurred with exercise. DTT was not added because of the possibility that the effects of exercise, if due to oxidation, would be reversed.

In this study, we have confirmed what has been reported on many previous occasions, namely that, with hypoxia (inspired O$_2$ fraction = 0.14), V$_{O_2\text{ peak}}$ is reduced compared with normoxia (15, 33). The ~23% reduction that was observed in V$_{O_2\text{ peak}}$ is consistent with what has been reported earlier in untrained subjects under similar conditions (20). We have also been able to demonstrate, again similar to previous studies (20), that, over a range of submaximal POs, compensatory adjustments allow OxPhos to remain similar or nearly similar to normoxia despite a lower arterial O$_2$ saturation and arterial O$_2$ content. Even at the highest PO, attained at fatigue in hypoxia, we could find no differences from normoxia when matched to the same PO. Some insight into these adjustments can be obtained from the minute ventilation and respiratory exchange ratio measurements, both of which were increased in hypoxia compared with normoxia at similar POs. Increased ventilation in conjunction with increased blood flow to working muscles appears important in minimizing the reductions in arterial O$_2$ delivery in hypoxia (50, 51). Our results indicate that a substantial blunting of PO and V$_{O_2\text{ peak}}$ can occur even though OxPhos is relatively well protected throughout submaximal exercise. This observation could suggest that fatigue may occur somewhat independently of a failure in ATP supply by OxPhos. Conceivably, fatigue could also prevent further increases in OxPhos (and V$_{O_2\text{ peak}}$) even though delivery, extraction, and utilization of O$_2$ by mitochondria have not been fully exploited. Failure in E-C coupling as a cause of fatigue remains an inviting possibility in this regard.

To examine differences between normoxic and hypoxic conditions in Ca$^{2+}$-cycling properties, it was not possible to use conventional two-way ANOVA procedures for repeated measures as a result of the unbalanced nature of our tissue-sampling schedule. Although the first exercise samples occurred at the same V$_{O_2}$ (and PO), this was not possible at the higher exercise intensities due to the randomization of conditions and ethical limitations in the number of biopsies that could be performed. To examine for differences between conditions, we have used regression analyses and slopes from the lines of best fit for the relationship between V$_O_2$ (or PO) and the property of interest. These plots clearly demonstrate a trend ($P > 0.05$) toward a greater effect of hypoxia on Ca$^{2+}$ uptake and Ca$^{2+}$ release. It should be emphasized that this approach using regression analyses depends on the assumption of linearity between the measured parameters and PO.

Although not measured in this study, reductions in the [Ca$^{2+}$]$_i$ time integral remain a viable possibility to explain the inability to continue the progressive exercise task in both normoxia and hypoxia. Decreases in [Ca$^{2+}$]$_i$ time integral could occur specifically as a consequence of decreases in Ca$^{2+}$ release. Reductions in the Ca$^{2+}$ time integral, mediated by decreases in Ca$^{2+}$ release, have been previously shown to associate with fatigue in repetitively stimulated mouse muscle fibers (2). The fact that the depression in Ca$^{2+}$ release was comparable to normoxia in hypoxia at fatigue, even though PO was considerably lower in hypoxia, is also suggestive that impaired Ca$^{2+}$ cycling may be involved in the early fatigue observed during progressive exercise in this condition.

It must be emphasized that other, more central processes may be involved in the inability to continue progressive exercise. Because our measurements were conducted on homogenates under supposedly optimal in vitro conditions, the depression in Ca$^{2+}$ release can be attributed directly to disturbances in the CRC. However, in vivo, other processes such as an inability of the sarcolemma and T tubule to conduct a repetitive action potential and/or a deficit in the mechanical signaling between the T tubule and the CRC could lead to reductions in Ca$^{2+}$ release (2). It should also be emphasized that with progressive exercise profound changes occur in the intracellular environment, which may impair Ca$^{2+}$ cycling as well as a variety of other excitation and contraction processes (53). Consequently, isolating the direct site and process involved in fatigue during voluntary activity is not possible.

In summary, our results demonstrate that, during progressive exercise to fatigue in normoxia, disturbances in SR Ca$^{2+}$ cycling occur as indicated by the in vitro measurements of Ca$^{2+}$ uptake and Ca$^{2+}$ release. With hypoxia, similar distur-
bances in Ca$^{2+}$ cycling occur but at a reduced PO$\text{peak}$ and VO$_2$peak. These findings raise the possibility that fatigue, both in normoxia and hypoxia with progressive exercise, is related to an inability to regulate the [Ca$^{2+}$]$_{i}$ integral.

GRANTS

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