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Prolonged exercise to fatigue in humans impairs skeletal muscle Na^+-K^+-ATPase activity, sarcoplasmic reticulum Ca^{2+} release, and Ca^{2+} uptake

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Leppik, James A., Robert J. Aughey, Ivan Medved, Ian Fairweather, Michael F. Carey, and Michael J. McKenna. Prolonged exercise to fatigue in humans impairs skeletal muscle Na^+-K^+-ATPase activity, sarcoplasmic reticulum Ca^{2+} release, and Ca^{2+} uptake. *J Appl Physiol* 97: 1414–1423, 2004. First published May 21, 2004; 10.1152/japplphysiol.00964.2003.—Prolonged exhaustive submaximal exercise in humans induces marked metabolic changes, but little is known about effects on muscle Na^+-K^+-ATPase activity and sarcoplasmic reticulum Ca^{2+} regulation. We therefore investigated whether these processes were impaired during cycling exercise at 74.3 ± 1.2% maximal O_2 uptake (mean ± SE) continued until fatigue in eight healthy subjects (maximal O_2 uptake of 3.93 ± 0.69 l/min). A vastus lateralis muscle biopsy was taken at rest, at 10 and 45 min of exercise, and at fatigue. Muscle was analyzed for in vitro Na^+-K^+-ATPase activity [maximal K^+-stimulated 3-*O*-methylfluorescein phosphatase (3-*O*-MFPase) activity], Na^+-K^+-ATPase content ([^{3}H]ouabain binding sites), sarcoplasmic reticulum Ca^{2+} release rate induced by 4 chloro-*m*-cresol, and Ca^{2+} uptake rate. Cycling time to fatigue was 72.18 ± 6.46 min. Muscle 3-*O*-MFPase activity ($nmol \cdot min^{-1} \cdot g\ protein^{-1}$) fell from rest by 6.6 ± 2.1% at 10 min ($P < 0.05$), by 10.7 ± 2.3% at 45 min ($P < 0.01$), and by 12.6 ± 1.6% at fatigue ($P < 0.01$), whereas ^{3}[H]ouabain binding site content was unchanged. Ca^{2+} release ($mmol \cdot min^{-1} \cdot g\ protein^{-1}$) declined from rest by 10.0 ± 3.8% at 45 min ($P < 0.05$) and by 17.9 ± 4.1% at fatigue ($P < 0.01$), whereas Ca^{2+} uptake rate fell from rest by 23.8 ± 12.2% at fatigue ($P = 0.05$). However, the decline in muscle 3-*O*-MFPase activity, Ca^{2+} uptake, and Ca^{2+} release were variable and not significantly correlated with time to fatigue. Thus prolonged exhaustive exercise impaired each of the maximal in vitro Na^+-K^+-ATPase activity, Ca^{2+} release, and Ca^{2+} uptake rates. This suggests that acutely downregulated muscle Na^+, K^+, and Ca^{2+} transport processes may be important factors in fatigue during prolonged exercise in humans.

calcium ion ATPase; sodium-potassium pump; potassium

MUSCLE FATIGUE IS A HIGHLY COMPLEX phenomenon, acting at numerous sites and via multiple mechanisms, which include within the central nervous system, but fatigue predominantly acts through mechanisms residing within skeletal muscle (7, 9, 30, 59). Extensive research over many decades has identified the importance of metabolic disturbances in muscle fatigue, including the depletion of glycogen, phosphocreatine, and ATP, as well as an accumulation of P_i and ADP (9, 26). However, the mechanisms of muscle fatigue remain both highly controversial and incompletely defined, particularly within contracting muscles of exercising humans.

There is considerable recent interest in the possible causality of impaired muscle cation [e.g., potassium (K^+), sodium (Na^+), and calcium (Ca^{2+})] regulation in fatigue during exercise in humans (15, 35). Impairment of membrane excitability and in the maximal rates of sarcoplasmic reticulum (SR) Ca^{2+} release and Ca^{2+}-ATPase activity with fatigue would be an energetically conservative and thus efficient muscular strategy and provide mechanisms additional to myofibrillar regulation for reducing muscle power with fatigue. These mechanisms have recently been studied during short-term intense contractions in humans. Brief, high-intensity, fatiguing muscle contractions depressed the maximal Na^+-K^+-ATPase activity in skeletal muscle (11, 12), which may exacerbate the marked muscular K^+ efflux and Na^+ gain that occur with membrane excitation during contractions (19, 24, 25, 35, 50, 52, 57). Impaired maximal Na^+-K^+-ATPase activity was associated with membrane inexcitability in human muscle measured via altered M-wave characteristics (11). This is consistent with a recent proposal that reduced membrane excitability may be an important cellular protective mechanism under metabolic stress (43). There is also substantial evidence favoring a vital role for impaired SR Ca^{2+} regulation in muscle fatigue (1, 7, 30, 60). In humans, brief, repeated maximal muscle contractions impaired the maximal rate of SR Ca^{2+} release, which was associated with reduced muscle force with fatigue (22, 33). Furthermore, depressed maximal rates of SR Ca^{2+} uptake and/or SR Ca^{2+}-ATPase activity have also been found with intense fatiguing exercise in human muscle (14, 20, 22, 33).

Studies investigating muscle metabolism during prolonged, continuous exercise in humans span nearly four decades. However, surprisingly little is known about the possible impairment of muscle cation transport regulatory proteins in human muscle with prolonged exercise and their possible role in fatigue. In rats, prolonged running (~2 h) followed by 45 min of low-intensity running depressed muscle maximal Na^+-K^+-ATPase activity (10). This does not appear to reflect an acute loss of Na^+-K^+-ATPase enzymes (11, 37, 44). Submaximal exercise also depressed Na^+-K^+-ATPase activity (48). Importantly, no studies have investigated whether muscle maximal Na^+-K^+-ATPase activity is impaired with fatigue induced by prolonged exercise in humans. Furthermore, the time course of any such depression including fatigue is unknown and has important

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implications for understanding the significance of these changes in muscle fatigue. Several prolonged exercise studies in humans point to SR dysfunction with fatigue. Depressed muscle SR Ca^{2+} uptake and Ca^{2+}-ATPase activity occurred with prolonged exercise in human muscle (2) and developed after only 30 min of exercise (16). In rats, muscle SR Ca^{2+} release was depressed after prolonged exercise in three studies (8, 23, 53) but unchanged in another (49). No studies have investigated whether prolonged exercise reduces SR Ca^{2+} release in human muscle. This is important due to the critical regulatory role of Ca^{2+} in muscle contractile force development. It is also unclear whether any depression in muscle SR Ca^{2+} release (8, 23) would develop progressively during exercise or exhibit a sudden decline of late onset, as occurs in single muscle fibers (59). Finally, although depressed SR Ca^{2+} uptake and muscle force after prolonged exercise were not related (2), the relationships of muscle Na^+-K^+-ATPase activity and SR Ca^{2+} release with exercise performance during prolonged exercise in humans remain unknown.

Thus the aims of this study were to investigate the magnitude and time course of prolonged exercise effects on muscle Na^+-K^+-ATPase activity and on SR Ca^{2+} regulation in humans, and whether these changes would be correlated with muscle endurance exercise, measured as exercise time to fatigue. We tested the hypotheses that *1*) prolonged exercise in humans would depress each of the maximum Na^+-K^+-ATPase activity, SR Ca^{2+} release, and SR Ca^{2+} uptake rates in skeletal muscle; *2*) these changes would develop progressively with exercise time; and *3*) the magnitude of these changes at fatigue would be correlated with endurance exercise performance.

METHODS

Subjects

A total of eleven healthy volunteers participated in the study. Eight subjects, seven male and one female (age 27 ± 9 yr, height 177.9 ± 9.0 cm, body mass 74.1 ± 9.8 kg, mean ± SD), participated in the prolonged exercise trials. A further three healthy men (age 37.3 ± 7.4 yr, height 179 ± 7.5 cm, body mass 75.7 ± 9.5 kg) participated in separate trials to determine intrasubject variability and the effects of a protease inhibitor on muscle SR variables. All subjects gave written, informed consent, and all procedures were approved by the Victoria University of Technology Human Research Ethics Committee.

Exercise Tests

Subjects underwent three exercise test sessions 3–7 days apart, at least 2 h after a light meal. They abstained from strenuous physical activity in the previous 24 h and avoided caffeine, nicotine, or any medications in the previous 12 h. All tests were conducted on an electronically braked cycle ergometer at ~80 rpm (Lode NV, Groningen, Netherlands). During all tests, expired gases were collected to determine oxygen consumption ($\dot{V}O_2$).

Incremental exercise trial. The initial trial comprised a maximal incremental test (33). Subjects breathed through a Hans-Rudolph three-way nonrebreathing valve, with expired air passed through flexible tubing into a mixing chamber; expired volume was measured using a ventilometer (KL Engineering, Sunnyvale, CA); mixed expired oxygen and CO_2 contents were analyzed by rapidly responding gas analyzers (Applied Electrochemistry S-3A O_2 and CD-3A CO_2, Ametek, PA). The gas analyzers were calibrated immediately before and rechecked after each test using commercially prepared gas mixtures. The ventilometer was calibrated before each test using a standard 3-liter syringe. Subjects cycled for 3 min at each of 60, 90, and 120 W, followed by a 25-W increment each minute until fatigue, defined as an inability to maintain pedal cadence above 55 rpm. The highest $\dot{V}O_2$ over a 30-s interval was termed peak $\dot{V}O_2$ ($\dot{V}O_{2\ peak}$). The workrate corresponding to 75% $\dot{V}O_{2\ peak}$ was then calculated from the linear regression of submaximal $\dot{V}O_2$ vs. power output for use in all subsequent exercise sessions.

Prolonged exercise fatigue trial. In the second visit, subjects performed a familiarization trial, comprising submaximal cycling exercise at a workrate corresponding to 75% $\dot{V}O_{2\ peak}$ continued to the point of fatigue, defined as the inability to maintain cadence above 55 rpm. In the final laboratory visit, subjects performed an identical prolonged cycling exercise test, with inclusion of muscle biopsy and blood sampling procedures. Pulmonary $\dot{V}O_2$, CO_2 output, and respiratory exchange ratio were measured during the periods at 4–9, 25–30, and 39–44 min and then continuously from 50 min until fatigue. A muscle biopsy was taken at rest, at 10 and 45 min of exercise, and at the point of fatigue. Arterialized venous blood samples were taken at rest, at 9, 30, and 44 min of exercise, and at fatigue.

Muscle torque measurements. To verify earlier observations of a decline in quadriceps maximal muscle torque with fatigue (2), two subjects returned for an additional prolonged exercise trial, with measurement of the quadriceps muscle maximal isometric torque before, during, and after exercise. Subjects were familiarized with isometric knee extensor exercise, performed on a Cybex dynamometer (Cybex Norm 770, Henley Health Care, Sugar Land, TX) using standard procedures (33). Subjects warmed up for 10 min at 50% $\dot{V}O_{2\ peak}$ and then performed three maximal voluntary isometric contractions (MVC). Subjects then commenced the prolonged exercise trial with three further MVCs conducted at the same time points that muscle biopsies were taken in the previous invasive trial.

Repeat biopsy and intense exercise trial. To determine the intrasubject and interassay variability of SR Ca^{2+} uptake and Ca^{2+} release, three healthy men underwent two vastus lateralis muscle biopsies at rest. A third muscle biopsy was taken immediately after 50 maximal isokinetic knee extensor contractions performed at a cadence of 180°/s at 0.5 Hz to test the effects of addition of the Ca^{2+}-activated protease inhibitor leupeptin to the SR homogenizing buffers (detailed below). This exercise protocol was used because this depressed muscle SR Ca^{2+} uptake, Ag^+-induced SR Ca^{2+} release, and Na^+-K^+-ATPase activity (12, 33). Each biopsy sample was immediately separated into two portions, with one piece homogenized in the standard homogenizing buffer and a separate portion in the standard buffer plus 1 mM leupeptin (Sigma Aldrich).

Blood Sampling and Analyses

Before the prolonged exercise trial was commenced, an indwelling catheter (Jelco 20–22 gauge) was inserted into a dorsal hand vein and kept patent by periodic infusions of isotonic heparinized saline. To obtain arterialized blood samples, the hand was sheathed by a waterproof glove and heated in a 45°C water bath for 10 min before all sampling. Arterialized venous blood samples (2.5 ml) were drawn into heparinized syringes (Rapidlyte, Ciba Corning Diagnostic), well mixed, and then divided into two separate tubes for measurement of plasma K^+ concentration ($[K^+]$), whole blood hematocrit, and hemoglobin concentration. For plasma $[K^+]$ determinations ($n = 6$), an aliquot of whole blood was centrifuged at 4,000 rpm for 4 min; plasma was then separated, stored in liquid N_2, and later analyzed in triplicate using a K^+-selective electrode housed in an automated blood-gas electrolyte analyzer (Ciba Corning 865, Bayer). Blood hematocrit and hemoglobin concentration ($n = 4$) were measured in triplicate using an automated hematology analyzer (Sysmex K800, Roche Diagnostics). The decline in plasma volume from rest ($n = 4$) was calculated from changes in hematocrit and hemoglobin concentration, as previously described (12, 38).

Muscle Biopsy Sampling, Processing, and Analyses

On arrival at the laboratory, subjects rested on a laboratory bed, and four small incisions (2 per leg) were made under local anesthesia (1% xylocaine) in the skin overlying the middle third of the vastus lateralis. A muscle biopsy was taken by the percutaneous biopsy technique modified for suction at rest, at 10 and 45 min of exercise, and at fatigue. Approximately 100–120 mg of muscle tissue were removed, rapidly divided into portions with one immediately frozen, and stored in liquid N_2 for later analysis of Na^+-K^+-ATPase content. The remaining two portions were blotted on filter paper, rapidly weighed, immediately homogenized in the respective buffer, and then stored in liquid N_2 for later analysis of Na^+-K^+-ATPase activity and for SR Ca^{2+} release and Ca^{2+} uptake rates.

Na^+-K^+-ATPase Activity

Muscle homogenates for Na^+-K^+-ATPase activity measurements were prepared as previously described (12, 13). Muscle samples (30 mg) were immediately blotted on filter paper, weighed, then homogenized (5% wt/vol) at 0°C for 2 × 20 s, 15,000 rpm (Omni 1000, Omni International), in an homogenate buffer containing (in mM) 250 sucrose, 2 EDTA, and 10 Tris (pH 7.40). Muscle homogenates were rapidly frozen and stored in liquid N_2 for later determination of activity. Before analysis, homogenates were freeze-thawed four times and then diluted 1/5 in cold homogenate buffer. Muscle Na^+-K^+-ATPase activity was determined in quadruplicate using the K^+-stimulated 3-*O*-methyl fluorescein phosphatase (3-*O*-MFPase) activity assay (12, 13). The assay medium in which 3-*O*-MFPase activity was measured contained 5 mM $MgCl_2$, 1.25 mM EDTA, 100 mM Tris, and an 80 nM 3-*O*-methyl fluorescein standard (pH 7.40). The freeze-thawed, diluted homogenate (30 μl) was incubated in 2.5 ml of assay medium at 37°C for 5 min before addition of 40 μl of 10 mM 3-*O*-MFP to initiate the reaction. After 60 s, 10 μl of 2.58 M KCl (final concentration, 10 mM) was added to stimulate K^+-dependent phosphatase activity, and the reaction was measured for a further 60 s. All assays were performed at 37°C, using continuous stirring, with data sampled at 1 Hz, on a spectrofluorimeter (Aminco Bowman AB2 SLM, Thermospectronic, Madison, WI). Excitation wavelength was 475 nm, and emission wavelength was 515 nm, with 4-nm slit widths. The 3-*O*-MFPase activity was calculated from the slope after addition of 10 μM KCl minus the slope before KCl addition (the latter comprises unspecific ATPase activity and any spontaneous hydrolysis of 3-*O*-MFP). All slopes were measured over 20–50 s. A least squares linear regression was also calculated for each slope, and any results with $r < 0.97$ were excluded from the analyses.

[^{3}H]Ouabain Binding

Muscle Na^+-K^+-ATPase content was determined by the vanadate-facilitated [^{3}H]ouabain binding content (37). Vanadate facilitates the binding of [^{3}H]ouabain and allows quantification of the Na^+-K^+-ATPase content in biopsies of skeletal muscle (40). Samples were cut into 2- to 4-mg pieces. In all experiments, freshly made vanadate solution was used. The binding of [^{3}H]ouabain was determined by incubating cut muscle specimens for 120 min at 37°C in a buffer containing 10^{-6} M [^{3}H]ouabain (0.6 μCi/ml), 10 mM Tris chloride, 3 mM $MgSO_4$, 1 mM Tris vanadate, and 250 mM sucrose, pH 7.3. After washout for 4 × 30 min at 0°C, the tissue samples were soaked in 0.3 M TCA and taken for counting of ^{3}H activity using a beta counter. The content of [^{3}H]ouabain binding sites was determined and expressed as picomoles per gram wet weight (5).

SR Homogenization, Ca^{2+} Release, and Ca^{2+} Uptake Measurements

Assay procedures and modifications. Muscle homogenates for SR Ca^{2+} release and Ca^{2+} uptake measurements were prepared as described earlier (33). Approximately 30 mg of muscle were weighed, diluted 1:11 (wt/vol) in a cold buffer containing 40 mM Tris·HCl (pH 7.9), 0.3 M sucrose, 10 μM L-histidine, 10 mM EDTA, 10 mM sodium azide, and then homogenized on ice at 15,000 rpm for 3 × 15 s (Omni 1000, Omni International). The homogenate was then rapidly frozen in liquid N_2 for later analyses of SR Ca^{2+} release and Ca^{2+} uptake. All assays were conducted in triplicate, using magnetic stirring and at 37°C. The Ca^{2+} release and Ca^{2+} uptake rates were measured in triplicate in a standard buffer containing 20 mM HEPES (pH 7.0), 150 mM KCl, 4.5 mM Mg-ATP, 1 μM Indo-1 (Calbiochem), 7.5 mM oxalate, 10 mM sodium azide, and 5 μM TPEN. The assay medium utilized several important procedural and analytical improvements compared with our laboratory's previous methods (2, 33, 36, 45). The reaction was initiated by addition of 40 μl homogenate (Fig. 1). After the SR Ca^{2+}-ATPase-mediated reduction in the F_{410}-to-F_{485} ratio had reached a plateau, the Ca^{2+}-ATPase-specific inhibitor cyclopiazonic acid (CPA) was added to the cuvette at a final concentration of 20 μM to inhibit further vesicular Ca^{2+} uptake (45). The addition of CPA produced a small rise in Ca^{2+} concentration ([Ca^{2+}]; Fig. 1) consistent with the initial Ca^{2+} leak via the inhibited Ca^{2+}-ATPase (55). Initiation of SR Ca^{2+} release was then induced by the addition of the specific pharmacological activator of the SR Ca^{2+} release channels 4-chloro-*m*-cresol (4-CmC; Ref. 21) at a final concentration of 5 mM (Fig. 1) (41, 55). This was followed by determination of Rmin (minimum 410/485 ratio value at zero [Ca^{2+}]) and Rmax (maximum 410/485 ratio value at saturating [Ca^{2+}]) with sequential addition of 3.5 mM EGTA and 5 mM $CaCl_2$, respectively (Fig. 1). The sample was excited by a Xenon lamp at 349 nm with a band pass of 1 nm; emission was measured at 410 nm for Ca^{2+}-bound and at 485 nm for Ca^{2+}-free forms of Indo, with 8-nm band passes, using a spectrofluorimeter with an additional external photomultiplier tube (Aminco Bowman AB2, Thermospectronic, Madison, WI). All 410/485 nm fluorescent ratiometric data were sampled at 10 Hz, which was the limit of the Aminco Bowman data acquisition software. The maximal rates of Ca^{2+} uptake and Ca^{2+} release were determined from individual curves applied to smoothed data portions using custom-made software (LabView, Austin, TX). Curve fits were used to reduce variability resulting from noisy data points and thus enhance precision of the method.

Determination of SR Ca^{2+} uptake rate. The Ca^{2+} uptake rate was calculated from the entire Ca^{2+} uptake curve data, which typically comprised 200–300 s. This excluded data artifacts with homogenate addition (Fig. 1), which most likely reflects Ca^{2+} binding by hemoglobin (45), EGTA, and myoglobin in the homogenate and by oxalate in the assay medium. The raw F_{410} and F_{485} data were each initially smoothed using a 15-point running average. A higher order polynomial curve was then fitted to each of the smoothed F_{410} and F_{485} data sets, with the best curve fit determined on the basis of the lowest mean

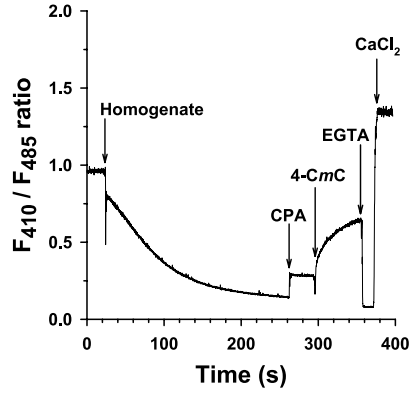

Fig. 1. A typical sarcoplasmic reticulum (SR) Ca^{2+} uptake and Ca^{2+} release curve in human muscle homogenate. CPA, cyclopiazonic acid (20 μM); 4-CmC, 4 chloro-*m*-cresol (5 mM); $CaCl_2$, calcium chloride (5 mM). EGTA concentration was 3.5 mM.

square error. The F_{410}/F_{485} ratio (R) was then derived and $[Ca^{2+}]$ calculated using the standard equation $[Ca^{2+}] = K_d \times [(R - R_{min})/(R_{max} - R)] \times (S_{f2}/S_{b2})$, where S_{f2} and S_{b2} represent the fluorescence at 485 nm for Ca^{2+}-free Indo and for bound-Indo (saturating $[Ca^{2+}]$), respectively (18), and K_d for Indo-1 was 167 nm (33). The maximal Ca^{2+} uptake rate was then measured as the minimum $d[Ca^{2+}]/dt$, expressed in $nmol \cdot l^{-1} \cdot s^{-1}$ and then corrected to both $mmol \cdot min^{-1} \cdot g$ muscle wet wt^{-1} and, after determination of homogenate protein content, to $mmol \cdot min^{-1} \cdot g\ protein^{-1}$ (33). Free $[Ca^{2+}]$ was calculated from ratiometric data throughout the Ca^{2+} uptake and release curves and is reported before the addition of the homogenate (prehomogenate), immediately after the homogenate artifact (posthomogenate), at the $[Ca^{2+}]$ plateau before addition of CPA (end uptake), during the plateau immediately before 4-CmC addition (prerelease), and at 10 s after 4-CmC addition (postrelease) (see Table 3).

Determination of SR Ca^{2+} release rate. The SR Ca^{2+} release rate was determined using data from a duration of 10 s after addition of 4-CmC to ensure detection of the initial fast phase of SR Ca^{2+} release. Any artifact associated with 4-CmC addition was excluded from the analysis. The F_{410} and F_{485} raw data were first smoothed using a five-point running average. Smoothing was conducted to minimize the effects of any aberrant points and was less than the 15-point smoothing for Ca^{2+} uptake analyses due to the fewer number of data points in the Ca^{2+} release phase.

To ascertain the most appropriate analysis period to reflect the fast initial phase of Ca^{2+} release under our measurement conditions, Ca^{2+} release rates were calculated for 14 analyses using curve fits of data of between 3- and 20-s durations, yielding a total of between 28 and 198 data points. The best curve fitting duration for Ca^{2+} release analysis was required to meet each of three criteria: *1*) yielded a good curve fit, evidenced by visual inspection and by a low mean square error; *2*) detected the fast phase of the Ca^{2+} release rate; and *3*) had a low variability between triplicate measures on the same homogenate, thus reflecting minimal disturbances due to any small number of aberrant or noisy points.

The Ca^{2+} release rates determined from curve fitting over 3 and 5 s were higher than for 10 s and longer durations ($P < 0.01$, Table 1). This does not reflect any physiological differences, because all analyses were conducted on the same 14 data sets. Rather, this difference reflected the bias of the curve fit to the initial steeper component of the Ca^{2+} release curve when analyzed over a short time period, because this initial portion of the data represented a higher proportion of the total data incorporated into the curve fit. The curve fit over longer durations, which included more data points, were biased toward the less steep portion of the Ca^{2+} release curve and thus yielded lesser maximal Ca^{2+} release rates (Table 1). Although the mean square error for data fits declined with longer analysis durations beyond 10 s due to the increased number of data points, these analyses failed to meet the criterion of detecting the fast phase of Ca^{2+} release and were therefore excluded. The Ca^{2+} release data for the 3-s (i.e., only 28 points) and 5-s curve fit analyses were each found to be highly variable with respect to both the magnitude of Ca^{2+} release and the time point at which the maximum $d[Ca^{2+}]/dt$ was detected. During these analyses, even a single aberrant data point during the fast initial phase of Ca^{2+} release measurement induced considerable variability and in some instances delayed the highest measured release rate to a time point well after addition of 4-CmC. Thus the highest rate of Ca^{2+} release was not found in the 3-s analysis in 7 of 14 data sets, and the 3-s analysis under these conditions was therefore excluded. Analysis of Ca^{2+} release using curve fitting over a 10-s duration (i.e., 98 smoothed data points) was minimally influenced by aberrant data points and produced much more consistent results, and the greatest Ca^{2+} release always occurred within the first few data points after application of 4-CmC. Consequently, a duration of 10 s with 5-point data smoothing was chosen for curve fitting for all Ca^{2+} release analyses. Although the 10-s analysis period underestimates the maximal in vitro Ca^{2+} release rate (Table 1), these results were reproducible, allowing testing of our hypotheses. Although these in vitro rates are clearly much less than in vivo release rates, this underestimation is not disadvantageous for this study. The maximal Ca^{2+} release rate was measured as the maximum $d[Ca^{2+}]/dt$, expressed in $nmol \cdot l^{-1} \cdot s^{-1}$ and then corrected to $mmol \cdot min^{-1} \cdot g$ wet $weight^{-1}$ or $mmol \cdot min^{-1} \cdot g\ protein^{-1}$.

Statistical Analyses

All data are reported as means ± SE and were analyzed using a one-way analysis of variance, with repeated measures for time. Post hoc analyses were determined using the Newman-Keuls test. Correlations between muscle variables and exercise performance were determined by least square linear regression. Significance was accepted at $P < 0.05$.

RESULTS

Exercise Responses

The incremental exercise $\dot{V}O_{2\ peak}$ was 3.93 ± 0.69 l/min and the mean $\dot{V}O_2$ during prolonged exercise was 2.92 ± 0.49 l/min (74.3 ± 1.2% $\dot{V}O_{2\ peak}$; Table 2). Time to fatigue was 72.18 ± 6.46 min. The respiratory exchange ratio declined from 9 min to fatigue ($P < 0.01$; Table 2), plasma $[K^+]$ increased throughout exercise and at fatigue ($P < 0.001$), whereas plasma volume declined with exercise (Table 2).

Muscle Na^+-K^+-ATPase

Maximal in vitro 3-O-MFPase activity. Muscle 3-*O*-MFPase activity (per g wet wt) was not significantly lower than rest at 10 min ($P < 0.10$) but was decreased at 45 min (−9.0 ± 3.9%; $P < 0.05$) and at fatigue (−14.0 ± 3.1%; $P < 0.01$; Fig. 2*A*). These changes did not reflect fluid shifts into muscle, with similar reductions in 3-*O*-MFPase activity expressed per gram of protein, at 10 min (−6.6 ± 2.1%; $P < 0.05$), at 45 min (−10.7 ± 2.3%; $P < 0.01$), and at fatigue (−12.6 ± 1.6%; $P < 0.001$; Fig. 2*B*). Furthermore, 3-*O*-MFPase activity per gram of protein at 45 min and fatigue were less than at 10 min ($P < 0.05$). The interassay [coefficient of variation (CV) 2.7%, $n = 8$] and intra-assay (CV 3.7%, $n = 31$) variability for 3-*O*-MFPase were low.

[^{3}H]ouabain binding site content. Despite reduced 3-*O*-MFPase activity with exercise, no significant differences in the [^{3}H]ouabain binding site content were found between rest, 10 min, 45 min, and fatigue (332.9 ± 19.2, 350.0 ± 12.8, 336.7 ± 22.8, and 316.6 ± 18.9 pmol/g wet wt, respectively; $P = 0.21$).

Table 1. *Effect of curve fit durations on SR Ca^{2+} release rates ($d[Ca^{2+}]/dt$, $nmol \cdot l^{-1} \cdot s^{-1}$)*

	3 s	5 s	7.5 s	10 s	12.5 s	15 s	20 s
Ca^{2+} release	65.4±5.4	60.1±5.3*	44.8±4.9†	32.1±2.9†	28.1±2.8†	25.6±2.3†	17.9±1.7†

Values are means ± SE; $n = 14$. SR, sarcoplasmic reticulum; $[Ca^{2+}]$, Ca^{2+} concentration. Significantly different from 3-s analysis: *$P < 0.05$; †$P < 0.01$.

Table 2. *Respiratory variables, plasma [K^+], and fluid shifts during prolonged cycling exercise at 75% peak $\dot{V}O_2$ to fatigue*

		Exercise Time, min			
	Rest	9	30	44	Fatigue
$\dot{V}O_2$, l/min		2.77±0.46	2.92±0.51	2.96±0.53	3.06±0.52
RER		1.01±0.01	0.98±0.01†	0.97±0.01†	0.94±0.01‡
Plasma [K^+], nM	4.17±0.05	4.95±0.18*	5.27±0.14*	5.42±0.05*	5.43±0.16*
ΔPV, %		−9.6±2.1*	−12.4±1.6*	−12.5±1.7*	−13.1±1.6*

Values are means ± SE; $n = 8$ for O_2 uptake ($\dot{V}O_2$) and respiratory exchange ratio (RER); $n = 6$ for plasma K^+ concentration ([K^+]); $n = 4$ for decline in plasma volume (ΔPV). *Significantly different from rest ($P < 0.05$). Significantly different from 9 min: †$P < 0.05$; ‡$P < 0.01$.

Muscle SR Ca^{2+} Regulation

Assay [Ca^{2+}]. Starting [Ca^{2+}] before homogenate addition was 1,734 ± 202 nM (overall mean ± SE) and did not differ significantly between time points (Table 3). The posthomogenate [Ca^{2+}] fell to 568 ± 21 nM and was higher in the 10 min than in the rest and fatigue assays ($P < 0.05$; Table 3). The end-uptake [Ca^{2+}] was low and similar between assay times, as was the prerelease [Ca^{2+}] (Table 3). The postrelease [Ca^{2+}] did not differ between the first three assay times but was less in the 45-min than in the fatigue assays ($P < 0.05$; Table 3).

Maximal in vitro SR Ca^{2+} uptake rate. SR Ca^{2+} uptake expressed per gram wet weight was not significantly reduced from rest to fatigue (−25.3 ± 11.6%, $P = 0.09$; Fig. 3*A*). However, Ca^{2+} uptake expressed per gram of protein fell by 23.8 ± 12.2% from rest to fatigue ($P = 0.05$) and declined between 10 min and fatigue ($P < 0.05$; Fig. 3*B*). The intra-assay CV for SR Ca^{2+} uptake was 15.1% ($n = 31$), whereas the interassay CV was not determined due to insufficient biopsy sample obtained. The SR Ca^{2+} uptake for the two resting biopsies ($n = 3$) were 0.90 ± 0.21 and 1.16 ± 0.32 μmol·min^{-1}·g wet wt^{-1}.

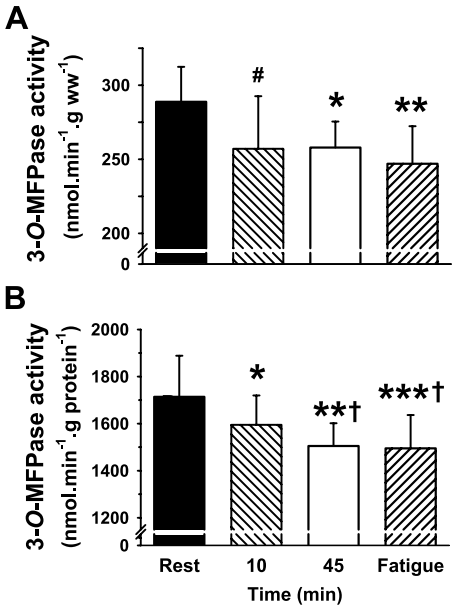

Fig. 2. Depressed maximal 3-*O*-methylfluorescein phosphatase (3-*O*-MFPase; Na^+-K^+-ATPase) activity in skeletal muscle during prolonged cycling exercise to fatigue at 75% peak O_2 uptake ($\dot{V}O_{2\ peak}$). Activity is expressed as nmol·min^{-1}·g wet wt (ww)$^{-1}$ (*A*) and nmol·min^{-1}·g protein^{-1} (*B*). Data are means ± SE; $n = 8$, except at 45 min where $n = 7$. Significantly less than at rest: #$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. †Significantly less than at 10 min ($P < 0.05$).

Maximal in vitro SR Ca^{2+} release rate. SR Ca^{2+} release expressed per gram of wet weight (Fig. 4*A*) was unchanged from rest by 10 min but then fell below 10-min levels at 45 min ($P < 0.05$), although this was not significantly less than at rest ($P < 0.10$). A clear reduction in SR Ca^{2+} release was evident at fatigue, when Ca^{2+} release had decreased by 19.4 ± 5.1% from rest ($P < 0.01$), and was also less than at 10 min ($P < 0.05$). When expressed per gram of protein (Fig. 4*B*), SR Ca^{2+} release was less than rest at 45 min (−10.0 ± 3.8%; $P < 0.05$) and at fatigue (−17.9 ± 4.1%; $P < 0.01$). The SR Ca^{2+} release both at 45 min ($P < 0.01$) and at fatigue ($P < 0.05$) was less than at 10 min (Fig. 4*B*). The intra-assay CV for SR Ca^{2+} release was 15.8% ($n = 28$), whereas the interassay CV was not determined due to insufficient tissue sample size. The SR Ca^{2+} release for the two resting biopsies ($n = 3$, measured at 1 Hz) were 0.61 ± 0.10 and 0.56 ± 0.02 μmol·min^{-1}·g wet wt^{-1}.

Leupeptin effects on SR variables. SR Ca^{2+} uptake and release were compared in two resting biopsies and in a biopsy taken after 50 fatiguing maximal contractions ($n = 3$) between muscle homogenized either in normal buffer or in homogenizing buffer plus 1 mM leupeptin.

Ca^{2+} uptake. Addition of leupeptin to the homogenizing buffer lowered Ca^{2+} uptake (control homogenizing buffer 1.07 ± 0.12 vs. buffer + leupeptin 0.60 ± 0.07 μmol·min^{-1}·g wet wt^{-1}; $P < 0.05$; $n = 9$ rest and exercise pooled observations). No difference was found between rest and exercise (each $n = 3$) for either the control (mean rest, 1.03 ± 0.25 vs. fatigue 1.16 ± 0.11 μmol·min^{-1}·g wet wt^{-1}) or buffer + leupeptin (mean rest, 0.64 ± 0.08 vs. fatigue 0.53 ± 0.13 μmol·min^{-1}·g wet wt^{-1}).

Ca^{2+} release. Addition of leupeptin to the homogenizing buffer did not affect Ca^{2+} release (control buffer 0.54 ± 0.04 vs. buffer + leupeptin 0.51 ± 0.06 μmol·min^{-1}·g wet wt^{-1}; $n = 9$ pooled rest and exercise observations) For this experiment only, Ca^{2+} release was measured on data sampled at 1 Hz, thereby explaining the lesser release values than in the main study. Furthermore, Ca^{2+} release was depressed at fatigue ($P < 0.05$; $n = 3$) in both the control homogenizing buffer (mean rest, 0.59 ± 0.04 vs. fatigue 0.45 ± 0.03 μmol·min^{-1}·g wet wt^{-1}) and the buffer + leupeptin (mean rest, 0.60 ± 0.04 vs. fatigue 0.32 ± 0.05 μmol·min^{-1}·g wet wt^{-1}).

Maximal Isometric Voluntary Contraction Muscle Torque

The quadriceps maximal isometric torque was depressed at fatigue by 26% in these two subjects, similar to previous findings (2). The peak isometric quadriceps torques at rest, 10

Table 3. *Assay medium [Ca^{2+}] at selected assay time points*

		Biopsy Sample Time, min		
	Rest	10	45	Fatigue
Prehomogenate [Ca^{2+}], nM	1,478±1,231	2,395±1,554	1,512±747	1,546±806
Posthomogenate [Ca^{2+}], nM	537±27	664±54*	556±36	516±33†
End-uptake [Ca^{2+}], nM	29±3	44±8	31±4	42±9
Prerelease [Ca^{2+}], nM	70±5	107±18	73±5	92±12
Postrelease [Ca^{2+}], nM	150±12	180±19	139±4‡	169±13

Values are means ± SE; $n = 8$. *Significantly different from rest assay ($P < 0.05$). †Significantly different from 10-min assay ($P < 0.05$). ‡Significantly different from fatigue assay ($P < 0.05$).

min, 45 min, and fatigue for the two subjects were 120, 127, 109, and 88 N·m and 169, 171, 155, 126 N·m, respectively. SR Ca^{2+} release for these two subjects was 9.13, 10.28, 7.67, and 8.07 μmol·min^{-1}·g $protein^{-1}$ and 6.51, 7.34, 5.00 and 4.59 μmol·min^{-1}·g $protein^{-1}$, respectively.

Relationships Among Muscle Na^+-K^+-ATPase and SR Variables

For resting muscle ($n = 8$), 3-*O*-MFPase activity was correlated with Ca^{2+} uptake ($r = 0.79$; $P < 0.05$) but not with Ca^{2+} release ($r = 0.49$) or [^{3}H]ouabain binding ($r = 0.22$), whereas Ca^{2+} release and Ca^{2+} uptake correlations were close to significance ($r = 0.69$; $P < 0.06$). With resting and exercise muscle data pooled ($n = 31$), Ca^{2+} release and Ca^{2+} uptake were correlated ($r = 0.52$; $P < 0.01$), and each was also correlated with 3-*O*-MFPase activity (Ca^{2+} release, $r = 0.46$, $P < 0.05$; Ca^{2+} uptake, $r = 0.70$, $P < 0.01$).

Relationships Between Na^+-K^+-ATPase and SR Variables and Exercise Performance

Resting muscle ($n = 8$) 3-*O*-MFPase activity ($r = -0.70$; $P < 0.05$), Ca^{2+} uptake ($r = -0.80$; $P < 0.05$), and Ca^{2+} release ($r = -0.68$; $P < 0.07$) were inversely related to time to fatigue during prolonged exercise. No significant correlations were found with $\dot{V}O_{2\ peak}$. Fatigued muscle ($n = 8$) 3-*O*-MFPase activity was inversely related with time to fatigue ($r = -0.71$; $P < 0.05$), with no significant relationships found with Ca^{2+} release and Ca^{2+} uptake ($r = -0.45$ and -0.50, respectively). The decline in Ca^{2+} release at fatigue tended toward a negative relationship with time to fatigue ($r = -0.61$; $P = 0.11$), but no significant relationship was found between time to fatigue and decline in either 3-*O*-MFPase activity ($r = -0.24$; $P = 0.56$) or Ca^{2+} uptake ($r = -0.39$; $P = 0.35$).

DISCUSSION

We show for the first time in exercising humans that skeletal muscle in vitro maximal Na^+-K^+-ATPase activity and SR Ca^{2+} release rates were significantly depressed during prolonged exhaustive exercise, thus confirming our first hypothesis. Furthermore, these changes were progressive when vari-

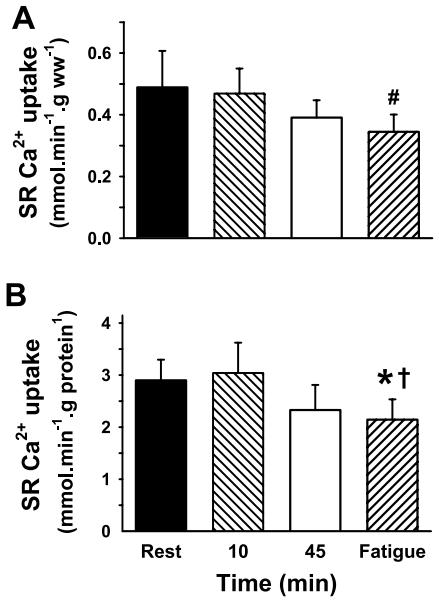

Fig. 3. Depressed maximal SR Ca^{2+} uptake rate in skeletal muscle during prolonged cycling exercise to fatigue at 75% $\dot{V}O_{2\ peak}$. Values are means ± SE; $n = 8$ except at 45 min where $n = 7$. Ca^{2+} uptake was expressed as mmol·min^{-1}·g ww^{-1} (*A*) and mmol·min^{-1}·g $protein^{-1}$ (*B*). Significantly less than rest: #$P < 0.10$; *$P = 0.05$. †Significantly less than at 10 min ($P < 0.05$).

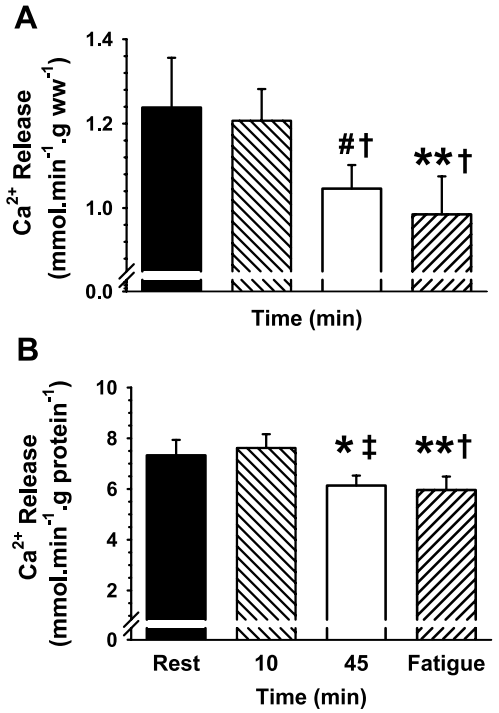

Fig. 4. Depressed maximal SR Ca^{2+} release rate in skeletal muscle during prolonged cycling exercise to fatigue at 75% $\dot{V}O_{2\ peak}$. Values are means ± SE; $n = 8$ except at 45 min where $n = 7$ units. Ca^{2+} release was expressed as mmol·min^{-1}·g ww^{-1} (*A*) and mmol·min^{-1}·g $protein^{-1}$ (*B*). Significantly less than at rest: #$P < 0.10$; *$P < 0.05$; **$P < 0.01$. Significantly less than at 10 min: †$P < 0.05$; ‡$P < 0.01$.

ables were expressed relative to muscle protein, therefore confirming our second hypothesis for these two variables. It is not known whether these impaired in vitro muscle cation transport properties also reflect impairment in vivo. If so, this would implicate membrane inexcitability and disruption to excitation-contraction coupling as important factors in muscle fatigue during prolonged exhaustive exercise in humans. We were unable to demonstrate significant correlations between these in vitro changes and time to fatigue during prolonged exercise and must therefore reject our third hypothesis. However, the absence of significant correlations may simply reflect the small sample size in this study, the variability in each of our assays, and the likely multiplicity of factors contributing to fatigue.

Depressed Maximal Na^+-K^+-ATPase Activity in Skeletal Muscle with Fatigue

The maximal 3-*O*-MFPase (Na^+-K^+-ATPase) activity was depressed by ~12% at fatigue. The decline with exercise also appeared to be progressive, being greatest at cessation of exercise due to fatigue. These findings confirm our first and second hypotheses in relation to Na^+-K^+-ATPase activity and are consistent with the importance of this decline in the progressive development of muscle fatigue (see below). The validity of these findings is demonstrated because the K^+-stimulated 3-*O*-MFPase activity is fully inhibited by ouabain, and is therefore specific to the Na^+-K^+-ATPase enzyme (13), and because the variability of the 3-*O*-MFPase assay was low and less than the observed percentage depression with exercise. Our finding of depressed maximal 3-*O*-MFPase activity during prolonged exercise is also consistent with other human studies with repeated maximal dynamic (12) and isometric contractions (11). This finding contrasts the lack of reduction in rat muscle after 2 h of running (10); in that study, a reduction was only observed after an additional 45 min of running occurred (10). Thus fatiguing exercise in humans reduces maximal 3-*O*-MFPase activity in muscle across a diverse range of exercise types, durations, and intensities, further suggesting that this is an obligatory response to exercise, as earlier suggested (12).

The decline in Na^+-K^+-ATPase activity with prolonged exercise could not be attributed to a loss of Na^+-K^+ pumps, because no reduction in [^{3}H]ouabain binding site content occurred. This is consistent with unchanged [^{3}H]ouabain binding site content in human muscle after repeated isometric contractions (11) and in rat soleus and EDL muscles after either brief, high-frequency, or prolonged low-frequency electrical stimulation-induced muscle contractions (37). A recent study did find a 10% increase in [^{3}H]ouabain binding site content with prolonged exercise (44). However, this occurred after 10 h of running, during which time Na^+-K^+-ATPase synthesis is probable (62).

Functional Implications of Impaired Maximal Na^+-K^+-ATPase Activity

The progressive decline observed in Na^+-K^+-ATPase activity during prolonged exercise is consistent with a role in fatigue. However, an important limitation in interpreting the importance of our findings is that depressed in vitro maximal activity does not directly reflect the functionally important in vivo activity. Furthermore, the magnitude and the time-dependent pattern of depression in Na^+-K^+-ATPase activity might also differ from that observed in vitro due to additional effects of any localized decline in glycogen, phosphocreatine, and ATP. Nonetheless, the marked K^+ fluxes in contracting muscle are consistent with a possible depressed maximal Na^+-K^+-ATPase activity also occurring in vivo. Muscle K^+ content is decreased during prolonged exercise (see references in Ref. 35) and muscles continually lose K^+ during submaximal contractions (19, 57), indicating a reduction in intracellular [K^+]. A widening of the arteriovenous [K^+] difference also occurs across contracting leg muscles during fatiguing isometric contractions (57) and during cycling exercise at 67% $\dot{V}O_{2\ peak}$ (46). Large increases in interstitial [K^+] also occur in contracting muscle (17, 25, 39). Although plasma [K^+] reached only 5–6 mM during prolonged exercise in this study, a far greater increase in muscle interstitial [K^+] is likely (17, 25, 39).

Although our results do not allow us to conclude that depressed maximal Na^+-K^+-ATPase activity directly contributes to fatigue, we nevertheless speculate that such a link exists. The combined effect of reduced intracellular and increased interstitial [K^+] would be a greatly reduced intracellular-to-extracellular [K^+] ratio, which together with a possible decline in the Na^+-K^+-ATPase-mediated electrogenic contribution may then reduce membrane potential and excitability in some fibers (39, 50). The functional significance of depressed Na^+-K^+-ATPase activity with prolonged exercise can be inferred from studies in isolated rat muscles in which Na^+-K^+-ATPase inhibition by ouabain markedly enhanced fatigue development and retarded subsequent recovery (6). The decline observed in Na^+-K^+-ATPase activity at fatigue was also consistent with the decline in muscle isometric MVC in two subjects, which was similar to earlier studies (2, 47). The decline in maximal in vitro Na^+-K^+-ATPase activity and time to fatigue during exercise were nonsignificantly correlated, suggesting that depressed Na^+-K^+-ATPase activity may have a limited role in muscle fatigue. However, correlational analyses are problematic with such a small sample size, especially when combined with the typical variability in these assays. Furthermore, it is highly probable that a multiplicity of factors contribute to fatigue, including impaired SR Ca^{2+} regulation (see below). Hence, the absence of a correlation between Na^+-K^+-ATPase activity and performance time is not necessarily indicative of its contribution to impaired muscle function.

The relatively small depression in Na^+-K^+-ATPase activity at fatigue with prolonged and intense exercise (~12–17%) may also question the functional significance of these findings. However, we do not know whether this depression is due to a similar, relatively small decline in Na^+-K^+-ATPase activity in all muscle fibers or reflects a more marked depression in Na^+-K^+-ATPase activity in some fibers. Interestingly, the percentage decline in Na^+-K^+-ATPase activity at fatigue is similar to the percentage gain in total Na^+-K^+ pump content ([^{3}H]ouabain binding) with intense exercise training in humans (see references in Ref. 34) and the percentage decline with inactivity (32). It is evident that the up- and downregulation of Na^+-K^+-ATPase in human muscle is much more restricted than observed in rat muscle (83%) with training and inactivity (27). Hence, a small relative decline in activity with fatigue

might also be expected to have important adverse functional implications for human skeletal muscle.

Finally, Na^+-K^+-ATPase inactivation could also be involved in the depression in muscle membrane excitability when under metabolic stress (43). Hence, depressed maximal Na^+-K^+-ATPase activity could then be an important contributory and ATP-conserving mechanism (15) in a concerted downregulation of muscle function with fatigue. Further studies combining multiple in vivo and in vitro techniques are, however, required to determine the functional importance of these findings.

Impaired SR Ca^{2+} Release and Ca^{2+} Uptake with Fatigue

We report for the first time in human skeletal muscle that the maximal SR Ca^{2+} release rate, induced by 4-CmC, was depressed by 18% after prolonged exercise to fatigue. Greater reductions were evident at fatigue than after 10 min of exercise, suggesting a progressive decline in Ca^{2+} release. A major portion of the decline in SR Ca^{2+} release rate had already developed by 45 min of exercise with a further reduction at fatigue. These findings therefore confirm our first and second hypotheses in relation to SR Ca^{2+} release and are consistent with the proposed importance of depressed SR Ca^{2+} release as a causal factor in exercise cessation due to fatigue. The ~25% decline in SR Ca^{2+} uptake at fatigue with prolonged exercise confirms our first hypothesis and also earlier findings (2). Interestingly, there did not appear to be a progressive decline in SR Ca^{2+} uptake with exercise duration, with the fall being evident only at fatigue. Thus we reject the second hypothesis with respect to SR Ca^{2+} uptake.

The methods used are valid for measurement of SR Ca^{2+} release and Ca^{2+} uptake rates. SR Ca^{2+} release was induced by 4-CmC, a potent agonist of the Ca^{2+} release channel (21). Because assays were performed under standardized in vitro conditions, the depressed SR Ca^{2+} release with fatigue most likely reflects structural alterations to the ryanodine receptor or to associated regulatory proteins. Reduced Ca^{2+} release with fatigue occurred in both the presence and absence of leupeptin in the homogenizing buffer, suggesting that this reduction was not simply due to Ca^{2+}-activated protease degradation of the ryanodine receptor during postbiopsy sampling and processing. We have previously demonstrated that our measurement of SR Ca^{2+} uptake is mediated via SR Ca^{2+}ATPase activity, which is inhibited by CPA, and have reported a close correspondence between SR Ca^{2+} uptake and Ca^{2+} ATPase activity (2, 33, 45). Depressed SR Ca^{2+} uptake with fatigue in this study is therefore likely caused by inhibition of the SR Ca^{2+} ATPase enzyme (14, 33). The variability of our Ca^{2+} release and Ca^{2+} uptake assays (~15%) was greater than for measures of Na^+-K^+-ATPase activity. This may explain our failure to detect significant differences in Ca^{2+} uptake results between rest and 45 min of exercise. Our maximal SR Ca^{2+} uptake and release rates were also higher than our laboratory has previously reported in human muscle homogenates (2, 33, 36, 45) due to the higher frequency of ratiometric data collection (10 vs. 1 Hz), use of curve fitting techniques to restricted time points, and not normalizing the $d[Ca^{2+}]/dt/[Ca^{2+}]$ data to the corresponding assay-free $[Ca^{2+}]$ (e.g., Ref. 45).

Reduced SR Ca^{2+} release channel opening, indicated by decreased ryanodine binding, was found with fatigue after prolonged exercise in rat soleus muscle (8), consistent with the decreased SR Ca^{2+} release rate in rat red gastrocnemius and soleus muscles. Our study measured Ca^{2+} release in vastus lateralis muscle, which has a mixed fiber composition; this may explain the smaller response than in rat oxidative muscles (8, 23). Our results are also consistent with other human brief, intense exercise studies, which reported a larger 35–42% decline in SR Ca^{2+} release induced by Ag^+, after fatiguing knee extensor exercise (22, 33). Studies that used electrical stimulation of isolated rat muscles to evoke fatigue also demonstrated a similar depression in SR Ca^{2+} release rate, together with depressed maximal force (42, 58, 61). Thus depressed SR Ca^{2+} release appears to be a common factor with repeated muscle contractions, including intense and prolonged exercise, with the magnitude dependent on the intensity of contractions and on fiber composition, further suggesting an important role in fatigue.

Implications of Impaired SR Ca^{2+} Release and Uptake for Muscle Fatigue

The depressed maximal SR Ca^{2+} release and Ca^{2+} uptake with prolonged exercise to fatigue were measured in vitro and thus most likely reflect structural alterations to the ryanodine receptor, Ca^{2+} ATPase, and/or associated regulatory proteins. These effects must presumably also occur in vivo, suggesting important functional consequences. Although depressed in vivo Ca^{2+} uptake might initially act to maintain cytosolic $[Ca^{2+}]$ during contractions and thus preserve muscle force, continual depression in Ca^{2+} uptake may induce sustained exposure to elevated intracellular $[Ca^{2+}]$, possibly initiating a protease-induced protein degradation, leading to myofibrillar disruptions (31) and reduced SR Ca^{2+} release (4). Decreased Ca^{2+} uptake during exercise may also diminish SR Ca^{2+} loading, possibly leading to a decline in SR Ca^{2+} release in vivo. Such a decline would be consistent with a declining maximal muscle force, evidenced here by the 26% decline in isometric MVC at fatigue in two subjects, consistent with earlier findings (2, 47). It is possible that both the magnitude and the time-dependent pattern of depression in SR Ca^{2+} regulation with prolonged exercise may have been different in vivo due to additional effects of any localized decline in glycogen, phosphocreatine, and ATP. Reduced phosphocreatine (2), elevated P_i, and Ca^{2+}-P_i precipitation in the SR may have further depressed Ca^{2+} release (30).

Both SR Ca^{2+} release and Ca^{2+} uptake were depressed during prolonged submaximal exercise, suggesting that these may be important factors in muscle fatigue. However, no significant relationships were found between changes in either SR Ca^{2+} release or Ca^{2+} uptake with fatigue and time to fatigue during exercise. The lack of significance between these variables does not preclude their potential importance in fatigue due to the small sample size and variability within each assay and in performance time. More mechanistic studies with a more homogenous and larger sample are required to resolve the in vivo functional significance of depressed SR Ca^{2+} release and Ca^{2+} uptake during exercise in human muscles.

The mechanisms causing depressed maximal Na^+-K^+-ATPase activity, SR Ca^{2+} release and Ca^{2+} uptake with prolonged exercise are not known, but production of free radicals and cystolic Ca^{2+} accumulation might be responsible for

degradation in maximal Na^+-K^+-ATPase activity (28, 29, 51, 54) and decreased SR Ca^{2+} release (3, 4, 7, 30, 54, 56) and most likely involve structural changes to the regulatory proteins.

In conclusion, we show a reduction in the function of major cation transport regulatory proteins in human skeletal muscle with fatigue induced by prolonged exercise with depressed maximal Na^+-K^+-ATPase activity and SR Ca^{2+} release, and we confirm depressed Ca^{2+} uptake. The depression in Na^+-K^+-ATPase activity and SR Ca^{2+} release were also progressive, suggesting a role in muscle fatigue. These changes may reflect a concerted downregulation of Na^+-K^+ and Ca^{2+} transport properties with fatigue as energetically conservative mechanisms for reduction in muscle function. We were unable to demonstrate direct correlations between depressed in vitro cation transport regulatory protein function and prolonged exercise performance. If these in vitro changes are consistent with in vivo impairments, these findings then suggest important roles for membrane excitability and excitation-contraction coupling in muscle fatigue during prolonged exercise in humans. Further studies are required to determine the direct functional significance of these in vitro changes.

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