Inactivation of human muscle Na\(^{+}\)-K\(^{+}\)-ATPase in vitro during prolonged exercise is increased with hypoxia

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Sandiford, S. D., H. J. Green, T. A. Duhamel, J. G. Perco, J. D. Schertzer, and J. Ouyang. Inactivation of human muscle Na\(^{+}\)-K\(^{+}\)-ATPase in vitro during prolonged exercise is increased with hypoxia. J Appl Physiol 96: 1767–1775, 2004. First published January 16, 2004; 10.1152/japplphysiol.01273.2003.—This study investigated the effects of prolonged exercise performed in normoxia (N) and hypoxia (H) on neuromuscular fatigue, membrane excitability, and Na\(^{+}\)-K\(^{+}\)-ATPase activity in working muscle. Ten untrained volunteers [peak oxygen consumption (\(\dot{V}_{O_2\text{peak}}\)) = 42.1 ± 2.8 (SE) ml·kg\(^{-1}\)·min\(^{-1}\)] performed 90 min of cycling during N (inspired oxygen fraction = 0.21) and during H (inspired oxygen fraction = 0.14) at ~50% of normoxic \(\dot{V}_{O_2\text{peak}}\). During N, 3-O-methylfluorescein phosphatase activity (nmol·mg protein\(^{-1}\)·h\(^{-1}\)) in vastus lateralis, used as a measure of Na\(^{+}\)-K\(^{+}\)-ATPase activity, decreased (\(P < 0.05\)) by 21% at 30 min of exercise compared with rest (101 ± 53 vs. 79.6 ± 4.3) with no further reductions observed at 90 min (72.8 ± 8.0). During H, similar reductions (\(P < 0.05\)) were observed during the first 30 min (90.8 ± 5.3 vs. 79.0 ± 6.3) followed by further reductions (\(P < 0.05\)) at 90 min (50.5 ± 3.9). Exercise in N resulted in reductions (\(P < 0.05\)) in both quadriceps maximal voluntary contractile force (MVC; 633 ± 50 vs. 473 ± 67 N) and force at low frequencies of stimulation, namely 10 Hz (142 ± 16 vs. 86.7 ± 10 N) and 20 Hz (283 ± 32 vs. 236 ± 31 N). No changes were observed in the amplitude, duration, and area of the muscle compound action potential (M wave). Exercise in H was without additional effect in altering MVC, low-frequency force, and M-wave properties. It is concluded that, although exercise in H resulted in a greater inactivation of Na\(^{+}\)-K\(^{+}\)-ATPase activity compared with N, neuromuscular fatigue and membrane excitability are not differentially altered.

membrane excitability; sodium-potassium-adenosinetriphosphatase activity; fatigue

INCREASING ATTENTION IS BEING focused on failure in one or more of the processes involved in excitation-contraction coupling as a cause of fatigue in skeletal muscle during prolonged submaximal exercise (1, 46). The processes involved in excitation-contraction coupling include transmission of the action potentials initiated by neural stimulation in the sarcolemma and T tubules; mechanical-chemical linking between the voltage sensors in the T tubules, the dihydropyridine receptors, and the Ca\(^{2+}\) release channels or ryanodine receptors of the sarcoplasmic reticulum (SR); and release of stored Ca\(^{2+}\) into the cytosol (20). Control of cytosolic free Ca\(^{2+}\) concentration, and consequently the mechanical response, is also, in part, dependent on Ca\(^{2+}\) uptake by the SR, which is, in turn, dependent on the catalytic activity of the Ca\(^{2+}\)-ATPase.

For one or more of these processes to be implicated in fatigue, induced by repetitive activity, it must be demonstrated that disturbances occur. Disturbances in SR Ca\(^{2+}\)-cycling properties assessed “in vitro,” including both reductions in Ca\(^{2+}\) uptake and Ca\(^{2+}\) release (16, 25), have been demonstrated to occur during prolonged exercise. The reductions in Ca\(^{2+}\) uptake and Ca\(^{2+}\) release have been attributed to structural alterations in the Ca\(^{2+}\)-ATPase enzyme (42) and the Ca\(^{2+}\) release channels (16), respectively. Evidence is accumulating that the structural alterations occur via oxidation and/or nitrosylation as a result of accumulation of reactive oxygen species (ROS) (36). Oxidant stress has been reported to increase in skeletal muscle during repetitive activity (54, 55).

A decrease in the ability of the sarcolemma and T tubules to maintain action potential generation and propagation represents another possible failure processes (11). Electromyographic (EMG) evidence, and particularly the properties of the muscle compound action potential (M wave), have been used as evidence for decreases in membrane excitability with sustained activity. As an example, reductions in M-wave amplitude and/or area have been frequently reported (4, 19, 23, 44, 46). Decreases in membrane excitability with exercise could be elicited by alterations in the catalytic activity of the Na\(^{+}\)-K\(^{+}\)-ATPase enzyme, which is primarily embedded in the sarcolemma and T tubule and which serves to reestablish membrane potential by active Na\(^{+}\) and K\(^{+}\) transport (46). Recent evidence from our laboratory (18, 19) and others (21) has documented that repetitive, fatiguing isometric and dynamic leg extension exercise elicits a decrease in maximal Na\(^{+}\)-K\(^{+}\)-ATPase activity as assessed in vitro. Given the susceptibility of the Na\(^{+}\)-K\(^{+}\)-pump to ROS attack (38), it is strongly suspected that the reduction in Na\(^{+}\)-K\(^{+}\)-ATPase activity is mediated by oxidation, secondary to ROS accumulation (19, 21). Because ROS accumulation also appears to occur in prolonged submaximal exercise (50), inhibition of Na\(^{+}\)-K\(^{+}\)-ATPase activity might also be expected with this type of exercise.

It is well known that repetitive contractile activity performed in hypoxia compared with normoxia results in a more rapid onset of fatigue (24, 51). It is possible that, under hypoxic conditions, given the greater metabolic stress that occurs (12) and the probable greater ROS production as a result of higher inosine monophosphate accumulation (26, 52) and the xanthine oxidase reaction (54), greater disturbances in membrane excitability and fatigue may occur. These functional impairments could be secondary to reductions in Na\(^{+}\)-K\(^{+}\)-ATPase activity.

The purpose of this study was to investigate the effects of prolonged submaximal exercise, performed in normoxia and hypoxia, on mechanical function, membrane excitability, and Na\(^{+}\)-K\(^{+}\)-ATPase activity. We have hypothesized that pro-

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longed exercise in normoxia would result in reductions in mechanical function, membrane excitability, and Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity and that the changes would be more pronounced when the exercise was performed in hypoxia.

METHODS

Participants

Ten untrained, healthy men (n = 7) and women (n = 3), with a mean age of 20.5 ± 0.1 (SE) yr, volunteered to participate in the study. Peak aerobic power [peak oxygen consumption (\(\dot{V}O_2\) peak)], as assessed during progressive exercise to fatigue, was 42.1 ± 2.8 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}. This study was approved by the Office of Human Research at the University of Waterloo, and all participants were fully informed of all experimental procedures and associated risks before written consent was obtained.

Experimental Design

This study involved one \(\dot{V}O_2\) peak test followed by two randomized, blind 90-min sessions of cycle exercise, performed at ~50% \(\dot{V}O_2\) peak as determined in normoxia. During the prolonged exercise trials, the volunteers breathed either room air (inspired oxygen fraction = 0.21), which represented the normoxic condition (N), or a hypoxic gas mixture (inspired oxygen fraction = 0.14), which represented the hypoxic (H) condition. During both N and H, the volunteers inspired from a 350-liter Tissot gasometer. The gas level was maintained constant by controlling the rates of flow into the tank from the appropriate gas mixture. This protocol ensured that the conditions were blind. At least 2 wk were provided between the initial \(\dot{V}O_2\) peak test and the first submaximal exercise session and at least 4 wk between the N and H conditions. Respiratory gases, heart rates, and arterial oxygen saturation (Sa\textsubscript{O}2) were collected at rest and at selected times during the exercise. In addition, muscle samples were obtained from the vastus lateralis muscles at rest and at 30 and 90 min of exercise, regardless of the condition. All resting biopsies were done under N. Muscle samples were rapidly frozen and stored at ~80°C until further analysis. Before and after each prolonged exercise session, measurements of mechanical function and membrane excitability were performed by using isometric knee extension.

During each test day, volunteers were required to ingest an Ensure meal replacement consisting of 9.4 g protein, 6.7 g fat, and 38 g carbohydrate (Ross Products Division, Saint-Laurent, PQ, Canada) ~4 h before exercise. This was done to control for differences in timing, type and amount of the nutrients ingested, which could alter some of the properties being investigated. Participants were also asked to refrain from caffeine for 24 h and strenuous exercise for at least 48 h before the submaximal exercise protocols. All women included in the study were tested only during the follicular phase of the menstrual cycle, confirmed through blood samples analyzed for estrogen levels. All tests were conducted in temperatures that ranged between 23 and 24°C and relative humidity that ranged between 50 and 60%. There were no differences in the environmental conditions between N and H.

Description of Specific Protocols

\(\dot{V}O_2\) peak. For all exercise protocols, upright exercise was performed on an electrically braked cycle ergometer (model 870, Quinton) that was calibrated on a daily basis. The protocol used to measure \(\dot{V}O_2\) peak and related measures consisted of a 4-min baseline period of cycling at 25 W, followed by 15-W step increases in power output (PO) each minute until the subject could no longer maintain a pedalling cadence of ~60 cycles/min. Ventilation, gas exchange, and heart rate were monitored both before exercise and throughout exercise by previously published methods (32). Venti-
to deliver the electrical impulse to the quadriceps muscle. The ground electrode was placed centrally on the anterior aspect of the thigh just above the patella, whereas the active electrode was toward the hip on the proximal portion of the belly of the vastus lateralis. Twitches and tetani were elicited by using a Grass model S48 stimulator with an isolation unit. Twitches were evoked by using a single supramaximal (~150 V) impulse of 50-μs duration. Tetani were produced by using a pulse duration of 50 μs and train duration of 1 s. During a typical trial, subjects were first assessed for supramaximal twitch properties followed by the tetanic stimulations of different frequencies and concluding with the MVC. Two trials were provided for the twitch and MVC measurements.

The twitch the properties assessed included the peak force (P0), contraction time (CT), one-half relaxation time (RT1/2) and the maximal rate of force development (+dF/dtmax) and the maximal rate of force decline (−dF/dtmax). For the tetanic stimulations, peak force was measured as well as the +dF/dtmax and −dF/dtmax for each frequency. We have also assessed motor unit activation by using the interpolated twitch technique (5). In this technique, supramaximal twitch is applied at the point of maximal force during MVC. The calculation of motor unit activation is calculated as the potentiated twitch is applied at the point of maximal force during MVC. The interpolated twitch technique (5). In this technique, supramaximal twitch is applied at the point of maximal force during MVC. The calculation of motor unit activation is calculated as the potentiated twitch is applied at the point of maximal force during MVC.

To perform the 3-O-MFPase assay, homogenates were prepared by using a buffer containing (in mM) 10 Tris, 2 EDTA, and 250 sucrose (pH 7.4) and ~30 mg of tissue (5% wt/vol) that was stored at −80°C after sampling. Homogenization was performed at 0° for 2 × 20 s at 25,000 rpm (Polytron). Homogenates were freeze-thawed four times to break up vesicles and fully expose binding sites. Homogenates were diluted 1:4 in cold homogenerate buffer and incubated at 37°C in an assay medium containing (in mM) 5 MgCl2, 1.25 EDTA, and 100 Tris (pH 7.4). The K+-stimulated activity of the Na+/K+-ATPase was determined by the increase in activity after the addition of 10 mM KCl and 160 μM 3-O-MFP. The K+-stimulated 3-O-MFPase activity was quantified using fluorescence spectrophotometry (excitation wavelength light = 475 nm; emission wavelength = 515 nm; slit width = 5 nm) and calculating the difference in slopes obtained with and without KCl. We have previously demonstrated that the K+-stimulated 3-O-MFPase activity can be completely eliminated by the specific inhibitor, ouabain (H. Green, unpublished observations). The protein content of the homogenates was determined spectrophotometrically by using the method of Lowry as modified by Schacterle and Pollock (53).

All measurements for 3-O-MFPase activity and protein were performed in triplicate.

Data Analysis

Two-way ANOVA procedures for repeated measurements were used to examine for differences between conditions (N and H) and exercise time (preexercise, 30 min, 90 min) for VO2, SaO2, and 3-O-MFPase activity. A two-way ANOVA for repeated measures was also employed to determine the effects of condition (N and H) and exercise (preexercise and postexercise) on muscle mechanical properties and membrane excitability. Where significance was found, the Newman-Keuls technique was applied to determine which means were significantly different. The probability for statistical significance was accepted at P < 0.05. Descriptive statistics include only means and SE.

RESULTS

VO2 and Related Properties

Steady-state VO2 was increased at 30 min of exercise, the point at which the first measurement was recorded (Fig. 1). No further changes were noted in VO2 during the remainder of the exercise. A similar pattern was found for VO2 in H. No differences occurred between the N and H conditions. As expected, H resulted in increased heart rate both at rest and during exercise. Heart rate was also increased during the latter part of the exercise, regardless of condition. The steady-state heart rate observed at 90 min of exercise was greater than at either 30 or 60 min of exercise. SaO2, as measured by an oximeter, averaged between 95 and 97% during both rest and exercise in N. In contrast, the SaO2 at rest during H was 91%. With exercise in H, SaO2 declined to 85% during the first 30 min of exercise. This decline persisted throughout the remainder of the exercise. A decline in SaO2 did not occur during exercise in N.

Na+/K+-ATPase Activity

Both exercise and oxygen condition altered maximal Na+/K+-ATPase activity (Fig. 2). During N, Na+/K+-ATPase activity decreased by ~21% by 30 min of exercise. During the remaining 60 min of exercise in N, no further decline in activity was found. The changes in Na+/K+-ATPase activity with exercise in H were not different from N at 30 min of exercise. However, at 90 min of exercise, Na+/K+-ATPase activity was lower in H compared with N. No effects of either exercise or condition were detected for nonspecific Na+/K+-ATPase activity.
In addition, $P_t$ was higher in N compared with H. Twitch relaxation properties assessed included $RT_{1/2}$, during exercise compared with rest and during H compared

Muscle Mechanical Performance

Both exercise and oxygen state affected mechanical function of the quadriceps but in a property-specific manner (Fig. 3). Twitch force ($P_t$) was lower after exercise for both N and H (Table 1). In addition, $P_t$ was higher in N compared with H. Of the two properties used to assess the characteristics of force development after the single supramaximal stimulus, namely CT and $+\frac{dF}{dt_{max}}$, only $+\frac{dF}{dt_{max}}$ was affected by exercise and oxygen condition. For $+\frac{dF}{dt_{max}}$, lower values resulted during exercise compared with rest and during H compared with N. Twitch relaxation properties assessed included $RT_{1/2}$, and $-\frac{dF}{dt_{max}}$. Exercise resulted in a faster $RT_{1/2}$, an effect that was independent of condition. Neither exercise nor oxygen level altered $-\frac{dF}{dt_{max}}$. The tension-time index, defined as the area under the twitch-response curve, was reduced by exercise, regardless of condition.

The effect of exercise on tetanic force was frequency dependent (Fig. 3). At low frequencies of stimulation, namely 10 and 20 Hz, exercise resulted between a 17 and 39% reduction in force during N. At the higher frequencies of stimulation (30, 50, and 100 Hz), no effect of exercise was noted. No differences were found between N and H either at rest or after exercise for any of the frequencies examined.

The peak isometric force generated during an MVC was reduced $-21\%$ with the prolonged exercise protocol (Table 2). The differences between N and H conditions were not significant. The percentage of motor unit activation in a MVC, as assessed by the interpolated twitch, was reduced by $-11\%$ with exercise in N. A similar effect occurred for H. Significant reductions were also found after exercise in the average rectified EMG (AEMG). The exercise effect was not different between N and H.

M Wave

The properties of the M wave were measured in conjunction with the muscle mechanical properties. No effects of exercise or condition were found for any of the properties examined, namely the amplitude, duration, and area of the M wave (Table 3).

DISCUSSION

As hypothesized, we have found that prolonged cycle exercise of moderate intensity when performed in N results in an $-28\%$ reduction in maximal in vitro $Na^+-K^+-ATPase$ activity in tissue extracted from the vastus lateralis muscle. When the exercise was performed in H (14% oxygen), the reduction in maximal $Na^+-K^+-ATPase$ activity was more pronounced, resulting in an $-44\%$ decline at 90 min of exercise. Interestingly, the effect of H was only observed later in exercise, and specifically between 30 and 90 min. As expected, prolonged exercise also resulted in reductions in MVC, $P_t$, and the force produced at stimulation frequencies of 10 and 20 Hz. Prolonged exercise did not affect force levels during the higher stimulation frequencies. In addition, we could detect no changes in membrane excitability in the vastus lateralis after prolonged exercise as assessed by the properties of the M wave. With the exception of $P_t$ and $+\frac{dF}{dt_{max}}$, which was higher and lower, respectively, during N than H, all other measures were not differentially altered by exercise during H. For both $P_t$ and $+\frac{dF}{dt_{max}}$, the differences between N and H were main effects and not specific to the exercise per se. In general, these results indicate that the greater inhibition of $Na^+-K^+-ATPase$ activity observed during exercise in H is not reflected in a greater loss of neuromuscular function when assessed after the exercise protocol.

This study appears to be the first to report a reduction in $Na^+-K^+-ATPase$ activity during prolonged moderate-intensity exercise in humans. In previous research from our laboratory, using prolonged treadmill exercise in rats, we were able to detect reductions in $Na^+-K^+-ATPase$ activity immediately...
after exercise when averaged over a range of locomotor muscles of the hindlimb (19). Reductions in Na⁺-K⁺-ATPase activity using both single-leg isometric exercise (19) and isokinetic exercise (21) have been previously reported in the vastus lateralis in humans. Collectively, these studies suggest that, at least in humans, repetitive exercise in general, if sustained for a sufficient period of time, can induce inactivation of the Na⁺-K⁺-ATPase enzyme. Moreover, on the basis of the results of the present experiment, performance of the same absolute amount of exercise in H can exaggerate the inactivation of the Na⁺-K⁺-ATPase that is observed.

The mechanisms underlying the inactivation of the Na⁺-K⁺-ATPase during exercise remains much in dispute. The reductions in $V_{\text{max}}$ that we have observed were assessed in vitro under supposedly optimal assay conditions. As a result, the decreases that occurred would be expected to be mechanistically linked to structural alterations in the enzyme, secondary to contractile-induced changes in one or more factors in the intracellular environment. Potential factors include the increase in metabolic by-products such as hydrogen ions, inorganic phosphate, ROS, and temperature (6, 8, 56). Alterations in substrate background such as free fatty acid levels and glycogen may also be involved (8, 48). Increases in ROS remain the most probable factor given the documented increase in ROS that occurs with prolonged exercise (50, 54, 55) and the suppressive effect of ROS accumulation on Na⁺-K⁺-ATPase activity (35, 38, 39). The greater inhibition of Na⁺-K⁺-ATPase activity observed with H compared with N in this study also supports the hypothesis that ROS is involved because H is thought to increase muscle ROS production (52). Because the primary effect of H occurred during the final 60 min of exercise, when reductions in Na⁺-K⁺-ATPase activity were most pronounced, progressive increases in ROS might be expected during this period. It is also possible that the recruitment of more fast-twitch-based fibers during the latter period of exercise might result in either more ROS production or greater susceptibility to damage in these fibers. These considerations provide a rationale for future studies examining both the site-specific structural alterations that occur in the Na⁺-K⁺-ATPase with exercise and hypoxia and the specific role of ROS in the changes that occur.

Because prolonged exercise in H is known to reduce the cycle time to fatigue compared with N (24), we reasoned that a loss of membrane excitability in the sarcolemma and T tubule, resulting in a decreased ability to conduct a repetitive action potential, may be involved. The loss of membrane excitability could occur as a result of a decrease in Na⁺-K⁺-ATPase activity and an impairment in the ability to reestablish transmembrane gradients for Na⁺ and K⁺ (11). To examine this possibility, we have measured the mechanical characteristics of the quadriceps muscle by using both voluntary and electrically induced contractions; both before and after the prolonged exercise session. In addition, we measured the properties of the M wave in the vastus lateralis previously shown to correlate with membrane excitability (9, 28, 29). Our results indicate that after prolonged exercise in N, a reduction
of ~25% in MVC force occurred. Using the interpolated twitch technique, we have found that at least some of the loss in the maximal isometric force-generating capability after exercise was due to reductions in central drive and motor unit activation. This observation has been previously documented by using a prolonged exercise protocol (19). The reduction in MVC noted after exercise was also accompanied by a reduction in AEMG, which has been used to indicate the active state of the muscle (14). The reduction of AEMG could reflect a reduction in neural drive or a loss of membrane excitability (10).

To determine whether the prolonged exercise protocol also resulted in a loss of force in electrically stimulated muscle, we have employed different frequencies of stimulation and recorded the force response elicited by each frequency. We have found that prolonged exercise reduced force at low (10 and 20 Hz) but not at high frequencies (30, 50, and 100 Hz). The failure to observe reductions in force at the high frequencies would suggest that membrane excitability has not been perturbed (34). Support for this possibility can also be found for the M-wave measurements that were performed. Prolonged

Table 1. Effects of prolonged exercise in normoxia and hypoxia on twitch characteristics

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<th>Normoxia</th>
<th>Hypoxia</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td>P&lt;sub&gt;t&lt;/sub&gt;, N</td>
<td>159±10</td>
<td>130±9</td>
</tr>
<tr>
<td>CT, ms</td>
<td>84.1±4.6</td>
<td>73.9±3.0</td>
</tr>
<tr>
<td>+dF/dt&lt;sub&gt;max&lt;/sub&gt;, N/s</td>
<td>4,462±431</td>
<td>3,470±343</td>
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<tr>
<td>RT&lt;sub&gt;1/2&lt;/sub&gt;, ms</td>
<td>82.0±9.7</td>
<td>58.3±10</td>
</tr>
<tr>
<td>−dF/dt&lt;sub&gt;max&lt;/sub&gt;, N/s</td>
<td>1,896±279</td>
<td>1,975±269</td>
</tr>
<tr>
<td>TTI, N</td>
<td>24.2±3.5</td>
<td>14.8±2.1</td>
</tr>
</tbody>
</table>

Values are ± SE for 7 subjects. Pre, preexercise; Post, postexercise; P<sub>t</sub>, peak twitch force; CT, contraction time; +dF/dt<sub>max</sub>, maximal rate of force development; RT<sub>1/2</sub>, one-half relaxation time; −dF/dt<sub>max</sub>, maximal rate of force decline; TTI, tension-time index. Main effects of time (P < 0.05) were found for P<sub>t</sub>, +dF/dt<sub>max</sub>, RT<sub>1/2</sub>, and TTI. For all measures, Pre > Post. Main effects of condition (P < 0.05) were found for P<sub>t</sub> and +dF/dt<sub>max</sub>. For both measures, normoxia > hypoxia.

Table 2. Effects of prolonged exercise in normoxia and hypoxia on MVC and activation

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<th></th>
<th>Normoxia</th>
<th>Hypoxia</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>MVC, N</td>
<td>633±50</td>
<td>477±67</td>
</tr>
<tr>
<td>% ACT</td>
<td>93.2±13</td>
<td>82.7±3.1</td>
</tr>
<tr>
<td>AEMG, mV</td>
<td>0.603±0.14</td>
<td>0.388±0.11</td>
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</table>

Values are means ± SE for 7 subjects. MVC, maximal voluntary contraction; % ACT, percentage of motor unit activation in MVC (see text); AEMG, average integrated electromyography in a MVC. Main effects (P < 0.05) of time were found for MVC, % ACT, and AEMG. For all measures, Pre > Post.
high stimulation frequencies. Consequently, it is possible that our results underestimated the actual fatigue that occurred, particularly at response (13). The persistent depression in low-frequency force, often alluded to as low-frequency fatigue (15), has been observed in many previous studies (58).

A factor that must be considered in the interpretation of our results is the increase in muscle temperature that occurs with prolonged exercise (17). Because our electrical stimulation procedures were conducted at submaximal voltages, sufficient to induce only 50–60% of MVC at high frequencies, the temperature-induced increase in conductance would be expected to increase the electrical current and increase the force response (13). Consequently, it is possible that our results underestimated the actual fatigue that occurred, particularly at high stimulation frequencies.

We have found that prolonged exercise in H failed to increase the fatigue observed in N or to compromise membrane excitability. Our failure to find an effect of H may be explained by a number of factors. The prolonged exercise protocol employed in N was performed at an intensity equivalent to ~50% VO2 peak. When the same exercise was performed in H, the relative intensity amounted to ~60% of VO2 peak; given the reduction in VO2 peak that occurs in H (14% oxygen) (31). The increase in the relative intensity when exercising in H compared with N, has been used to explain the reduction in exercise tolerance as a result of the increased physiological strain (24). However, the specific mechanisms remain elusive. In our study, we have demonstrated, as have others (59, 60), that at the exercise intensity employed, oxidative phosphorylation is not compromised as demonstrated by the lack of difference in VO2 between N and H during exercise. With the protocol employed, VO2 remains protected during acute H primarily by increasing blood flow to the working muscles to compensate for the low-hypoxia-induced reduction in arterial oxygen content (60). The greater heart rate that we have observed in H compared with N is a classic characteristic of the compensatory response.

In the absence of changes in mechanical efficiency, no differences would be expected between N and H in the contribution of oxidative phosphorylation in satisfying the energy requirements of the contracting muscle cells. However, to sustain oxidative phosphorylation in H, adjustments in metabolic regulation appear necessary (12). These adjustments include increases in high-energy phosphate catabolism and glycolytic flux, which by altering phosphorylation potential and redox state, respectively, act to support mitochondrial respiration (12). These metabolic changes culminate in higher levels of inosine monophosphate and lactate in working muscle (26, 52). The metabolic adjustments could explain the increased inhibition of the Na+-K+-ATPase observed later in exercise as a consequence of changes in one or more of ROS generation (54, 55) or glycogen depletion (48) in the working muscle.

It should be emphasized that differences would be expected to occur in Na+-K+-ATPase activity in vitro vs. in vivo. In vivo, the activity of the enzyme is subjected to a variety of competing influences, designed to both recruit and depress the catalytic response. A number of factors, and, in particular, the catecholamines, result in increased pump recruitment during exercise (8, 11, 46) and particularly during exercise in hypoxia (43). In contrast, in vivo, the accumulation of one or more metabolic by-products can inhibit pump activity (37). The in vitro measurements were conducted under supposedly optimal conditions. As such, reductions in enzyme activity probably reflect structural alterations. These structural alterations, would be expected to exist in vivo and consequently be manifested in reduced maximal enzyme activity. Depending on the intensity of the exercise and the amount of metabolic by-product accumulation, membrane excitability could be even compromised because of the greater inhibition of enzyme activity and Na+-K+ transport.

Our ability to detect a greater depression in muscle mechanical performance and membrane excitability with prolonged exercise in N compared with H may also be due to our experimental protocol. The assessment of mechanical performance was made several minutes after completion of the cycling protocol. This was an unavoidable delay, due to the time needed to position and instrument each participant for the mechanical measurements. Previous studies have reported a substantial reversal of fatigue within the first minute of exercise, which occurs in conjunction with reductions in selected metabolic by products and normalization of phosphorylation state in the muscle (45). Numerous studies (2, 23, 33, 40, 41), including one from our laboratory (19), have reported alterations in one or more of the properties of the M wave by using a variety of different exercise protocols. In protocols employing prolonged cycle exercise, similar to what we have used to investigate the effects of H, both the amplitude and total area of the M wave decreased in the vastus medialis but not in the vastus lateralis muscle (40). This finding suggests that alterations in membrane excitability may occur but only in selected muscles of the quadriiceps. This possibility should not be a factor in this study given that M-wave measurements were obtained from the vastus medialis muscle.

In summary, our results indicate that prolonged exercise in normoxia results in reduction in Na+-K+-ATPase activity and that the reduction is more pronounced when the exercise is performed in hypoxia. Our results also indicate that mechanical function and membrane excitability are not differentially altered when the prolonged exercise is performed in hypoxia.

<table>
<thead>
<tr>
<th>Table 3. Effects of prolonged exercise in normoxia and hypoxia in characteristics of the mass action potential (M wave)</th>
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<tr>
<td><strong>Normoxia</strong></td>
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<tr>
<td><strong>Pre</strong></td>
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<tr>
<td>Amplitude, mV</td>
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<tr>
<td>Duration, ms</td>
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<td>Area, mV/s</td>
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Values are ± SE for 7 subjects. Amplitude, sum of absolute values for maximum and minimum points of the biphasic (1 positive and 1 negative deflection) M wave; duration, time from baseline to baseline from the beginning to the end of the biphasic M wave; Area, integral of the absolute value of the electromyograph waveform. See text for additional details.
REFERENCES


