Reduced activity of muscle Na\textsuperscript{+}-K\textsuperscript{+}-ATPase after prolonged running in rats

J. R. Fowles, H. J. Green, J. D. Schertzer, and A. R. Tupling

Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Received 9 July 2001; accepted in final form 20 July 2002

Fowles, J. R., H. J. Green, J. D. Schertzer, and A. R. Tupling. Reduced activity of muscle Na\textsuperscript{+}-K\textsuperscript{+}-ATPase after prolonged running in rats. J Appl Physiol 93: 1703–1708, 2002.—The purpose of this study was to investigate the hypothesis that Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity is reduced in muscle of different fiber composition after a single session of aerobic exercise in rats. In one experiment, untrained female Sprague-Dawley rats (weight 275 ± 21 g; means ± SE; n = 30) were run (Run) on a treadmill at 21 m/min and 8% grade until fatigue, or to a maximum of 2 h, which served as control (Con), or performed an additional 45 min of low-intensity exercise at 10 m/min (Run+). In a second experiment, utilizing rats of similar characteristics (weight 258 ± 18 g; n = 32), Run was followed by passive recovery (Rec). Directly after exercise, rats were anesthetized, and tissue was extracted from Soleus (Sol), red vastus lateralis (RV), and extensor digitorum longus (EDL) and frozen for later analysis. 3-O-methylflourescein phosphatase activity (3-O-MF-Pase) was determined as an indicator of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity, and glycogen depletion identified recruitment of each muscle during exercise. 3-O-MF-Pase was decreased (P < 0.05) at Run+ by an average of 12% from Con in all muscles (P < 0.05). No difference was found between Con and Run. Glycogen was lower (P < 0.05) by 65, 57, 44, and 33% (Sol, EDL, RV, and WV, respectively) at Run, and there was no further depletion during the continued low-intensity exercise period. No differences in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity was observed between Con and Rec. The results of this study indicate that inactivation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase can be induced by aerobic exercise in a volume-dependent manner and that the inactivation that occurs is not specific to muscles of different fiber-type composition. Inactivation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase suggests intrinsic structural modifications by mechanisms that are unclear.

Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, or Na\textsuperscript{+}-K\textsuperscript{+} pump, catalyzes coupling of the chemical hydrolysis of ATP to the vectorial transport of Na\textsuperscript{+} out of and K\textsuperscript{+} into the cell. In skeletal muscle, basal activity of the Na\textsuperscript{+}-K\textsuperscript{+} pump depends primarily on the distribution of Na\textsuperscript{+} and K\textsuperscript{+} on either side of the plasma membrane and utilizes only 2–8% of maximum pumping capacity in vivo (23). During contractile work, transport of the Na\textsuperscript{+} and K\textsuperscript{+} by the Na\textsuperscript{+}-K\textsuperscript{+} pump rapidly restores ionic gradients after an excitatory electrical potential or “action potential” (35). The capacity of the Na\textsuperscript{+}-K\textsuperscript{+} pump can be challenged by heavy contractile demands and by changes in the local environment (i.e., ionic and metabolite buildup, substrate depletion), and, therefore, appropriate regulation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity is essential for maintaining transport capacity and muscle excitability. Despite a number of articles and reviews published recently on the role of the Na\textsuperscript{+}-K\textsuperscript{+} pump in muscle excitability and fatigue (1, 12, 13, 20, 21, 35, 37), the factors controlling both the acute and long-term regulation of the pump with exercise are still largely underdetermined.

Acute regulation of the Na\textsuperscript{+}-K\textsuperscript{+} pump can occur by influencing the activity of the pumps and by modulating the number of pumps at the cell surface (7). With contraction, the passive Na\textsuperscript{+} influx and K\textsuperscript{+} efflux can stimulate up to a 20-fold increase in Na\textsuperscript{+}-K\textsuperscript{+} pump activity (12, 33). Substrates, cytoskeletal components, catecholamines, and hormones can also provide additional short-term activation (6, 16, 40). In addition, an increase in the number of pumps at the sarcolemma is believed to occur acutely by translocating a specific pool of Na\textsuperscript{+}-K\textsuperscript{+} pump subunits from intracellular sites to the muscle membrane (27), potentially increasing the number of functional Na\textsuperscript{+}-K\textsuperscript{+} pumps. Collectively, these cellular processes increase the transport capacity of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase during exercise and, therefore, help to maintain ion gradients, excitability, and contractility.

Despite the extensive research into mechanisms that may increase pump transport capacity during activity, it is unclear whether intrinsic Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity can be altered by exercise, as has been observed for the other ATPases in the cell. As an example, Williams et al. (41) observed that maximal activity of the actomyosin ATPase in frog muscle was reduced by 20% with a 5-min stimulation protocol involving repeated maximal contractions. Several investigators have also observed decreased Ca\textsuperscript{2+}-ATPase activity after fatiguing exercise in rats (4, 9, 41). Although the exact mechanisms involved in the inactivation of these ATPases remain unclear, structural damage induced by the generation of free radicals is strongly suspected (33). The evidence...
from cardiac muscle Na\(^{+}\)-K\(^{+}\)-ATPase indicates that damage by circulating free radicals can occur (32). Collectively, the results suggest that catabolic processes associated with exercise may alter the structure of the skeletal Na\(^{+}\)-K\(^{+}\)-ATPase, reduce membrane excitability, impair conduction of action potentials, and contribute to fatigue. No published study presently exists, however, that examines the intrinsic activity of skeletal muscle Na\(^{+}\)-K\(^{+}\)-ATPase in response to exercise.

The goal of this study was to determine whether the Na\(^{+}\)-K\(^{+}\)-ATPase activity is altered in muscles of different fiber composition after prolonged endurance running in rats. Our hypotheses were that the Na\(^{+}\)-K\(^{+}\) pump is intrinsically modified during exercise, which results in a reduced Na\(^{+}\)-K\(^{+}\)-ATPase activity, and that the reduction in Na\(^{+}\)-K\(^{+}\)-ATPase activity is not specific to the fiber-type composition of the muscle. These hypotheses were tested in muscle homogenates using a 3-O-methylfluorescein K\(^{+}\)-stimulated phosphatase assay (3-O-MFPase) as an indicator of Na\(^{+}\)-K\(^{+}\)-ATPase activity.

METHODS

Animals. Untrained, female Sprague-Dawley rats (age 12.1 ± 0.7 wk; weight 275 ± 21 g; means ± SE) were utilized for the study. Rats were housed in a room where the light cycle was controlled (12:12-h light-dark cycle), and rat chow and water were provided ad libitum. Care and treatment of the animals was in accordance with procedures outline by the Canadian Council on Animal Care. All procedures were approved by the University of Waterloo Office for Ethics in Research.

Experimental design. To investigate the effect of a single session of aerobic exercise on Na\(^{+}\)-K\(^{+}\) pump function, rats were randomly assigned to one of three groups (n = 10 per group). In one group (Run), rats were run on a treadmill at 21 m/min and 8% grade (~65% peak aerobic power) until fatigue or to a maximum of 2 h. A second group of rats (Run+) were run on the treadmill as for the Run protocol, and then they were kept on the treadmill for an additional 45 min of low-intensity exercise (i.e., continued fast walking at 10 m/min). By reducing the speed of the treadmill, we were able to increase exercise duration. A third group of rats (Con) served as control for the anesthetic and surgical procedures. This design, including the exercise protocol, is comparable to that previously used to investigate Ca\(^{2+}\)-ATPase activity after running and recovery (18).

Directly after exercise (or at rest in Con), rats were anesthetized with pentobarbital sodium (6 mg/100 g body wt), and a muscle sample was obtained from soleus (Sol), extensor digitorum longus (EDL), red vastus lateralis (RV), and white vastus lateralis (WV) and plunged into liquid nitrogen for later analysis of muscle metabolites, glycogen content, and Na\(^{+}\)-K\(^{+}\) pump characteristics. Time for anesthetization and surgery after exercise averaged ~5 min. Surgery was not initiated until animals showed no reflex response to pinching the foot. In an additional experiment, performed after the first experiment, we have investigated the effect of resting or passive recovery after Run on the changes in Na\(^{+}\)-K\(^{+}\) pump function. For this purpose, rats with similar characteristics (weight 258 ± 18 g; n = 32) were randomized into four groups (n = 8 per group), namely a control (Con) and groups that received 10 (Rec 10), 25 (Rec 25), and 45 (Rec 45) min of passive recovery after Run.

Muscle metabolites and glycogen depletion. Muscle glycogen and metabolites, including ATP, phosphocreatine, creatine, and lactate, were analyzed fluorometrically after extraction from freeze-dried tissue, according to procedures previously published (22). In addition, we have also measured the contents of the adenine nucleotides (ATP, ADP, AMP) and inosine monophosphate by using ion-pair reversed-phase high-performance liquid chromatography (28) as modified by our group (22). All samples were analyzed in duplicate. On a given analytical day, an equal number of tissue samples from each muscle and group were measured.

Na\(^{+}\)-K\(^{+}\)-ATPase activity. Activity of Na\(^{+}\)-K\(^{+}\)-ATPase was assessed by using the K\(^{+}\)-stimulated 3-O-MFPase modified from the procedures of Huang and Askari (26) and Horgan and Kuypers (25) but using higher substrate concentration (19, 26). We have confirmed in a separate set of experiments (results not shown) that maximal activity was achieved at ~160 μM substrate concentration in rat tissue. Additionally, the use of 1.25 mM EGTA and 5 mM Na\(_{3}\) was also employed to optimize enzyme activity in rat muscle samples (2). Similarly, the use from frozen muscle samples was homogenized (5% wt/vol) at 0°C for 2 × 20 s at 25,000 rpm (Polytron) in a buffer containing (in mM) 250 sucrose, 2 EDTA, 1.25 EGTA, 5 Na\(_{3}\), and 10 Tris (pH 7.40). Homogenates were freeze thawed four times and diluted 1:5 in cold homogenate buffer. Approximately 30 μg of protein (~30 μl homogenate) were incubated for 4 min in medium containing (in mM) 5 MgCl\(_{2}\), 1.25 EDTA, 1.25 EGTA, 5 Na\(_{3}\), and 100 Tris (pH 7.40). The K\(^{+}\)-stimulated activity of the Na\(^{+}\)-K\(^{+}\)-ATPase was determined by the increase in activity after the addition of 10 mM KCl at a substrate concentration of 160 μM 3-O-MFPase. The activity of 3-O-MFPase was determined by the difference in slope before and after the addition of KCl. We have shown that the change in slope with the addition of KCl is completely eliminated with ouabain (H. Green, unpublished observation). Na\(^{-}\)-K\(^{-}\)-ATPase activity, which was based on the average of three trials, is expressed in nanomoles per milligram of protein per hour. Protein content of the homogenate was determined by the method of Lowry as modified by Schoenfeld and Pollock (36). The intra-assay coefficient of variation, defined as the standard deviation divided by the mean, was 10.9% when averaged over all muscles.

Data analysis. Statistical analysis was performed on Statistica for Windows ver. 4.5 software (Statsoft, Tulsa, OK). Descriptive statistics included means ± SE. Two-way ANOVA with repeated measures was used to analyze difference in Na\(^{+}\)-K\(^{+}\) pump activity between the three conditions (Con, Run, Run+) and within muscle groups. Similarly, a two-way ANOVA was used to examine the effects of passive recovery (Con, Rec 10, Rec 25, and Rec 45) and muscle (WV, RV). Identical procedures were employed for analyses of the metabolite data. Paired analysis was used to assess the activity response to exercise between muscles. Post hoc analysis of mean values was performed by using Tukey’s test. The probability level of statistical significance was accepted at P < 0.05.

RESULTS

Exercise protocol, metabolites, and glycogen. The average running duration at 21 m/min for the exercise was 102.4 ± 5.2 min for both Run and Run+ groups. The Run+ group exercised for an additional 45 min at 10 m/min. The glycogen depletion pattern indicated that each muscle was used during the prolonged, low-intensity protocol (Fig. 1). Muscle glycogen was reduced (P < 0.05) between 33 and 66% in the Run group.
in the muscles examined. No differences were observed between muscles in the Run group for glycogen content. No recovery in glycogen content was observed in the Run+ group.

Exercise had little effect in altering adenine nucleotides (Table 1). The only difference observed was an increased ADP content in Run relative to Con ($P < 0.05$) in Sol. Exercise also had minimal effects on high-energy phosphates and metabolites, namely ATP, phosphocreatine, creatine, and lactate (Table 1). Increases in creatine in Run relative to Con ($P < 0.05$) were observed but only for EDL ($P < 0.05$). When averaged over all conditions, differences were observed for adenine nucleotides and for the high-energy phosphates and metabolites between muscles, typical to what our laboratory has previously published (14).

**Na⁺-K⁺-ATPase activity.** Na⁺-K⁺-ATPase activity, measured by 3-O-MFPase (Fig. 2), decreased ($P < 0.05$) by $\sim 12\%$ from Con to Run+ when averaged over all muscles ($168 \pm 7$ vs. $149 \pm 7$ nmol mg protein$^{-1}$ h$^{-1}$, respectively). The decrease was not specific to muscle. No differences were observed between Con and Run and between Run and Run+ for any muscle. No differences were found in nonspecific or background activity between Con, Run, and Run+ for any muscle.

In an additional experiment designed to investigate the effect of the type of recovery on 3-O-MFPase activity, rats were allowed to recover for varying periods of time without exercise after Run. Using RV and WV as representative tissue, we could find no main effects between Con and Rec 10, Rec 25, or Rec 45 on 3-O-MFPase activity (Table 2). As with the previous experiment, no differences were found in nonspecific activity between Con and Rec groups. In this experiment, the average run time for each group was similar to the initial experiment.

**DISCUSSION**

As hypothesized, we have found that the exercise protocol that we employed induced a reduction in Na⁺-K⁺-ATPase activity when measured in muscle homogenates in vitro. Although there is a strong indication for Na⁺-K⁺-ATPase activity to decrease in all muscles except the EDL at the Run ($P = 0.12$), an additional 45 min at reduced exercise intensity were needed to obtain a significant reduction. In addition, we have also found that with up to 45 min of resting recovery, Na⁺-K⁺-ATPase activity is not different from nonexercised control.

### Table 1. Effects of exercise on high-energy phosphates and metabolites in the different muscles

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Sol</th>
<th>RV</th>
<th>EDL</th>
<th>WV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>Con 21.0 ± 0.7</td>
<td>26.2 ± 1.0</td>
<td>30.0 ± 0.3</td>
<td>29.5 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Run 22.7 ± 0.8</td>
<td>28.2 ± 0.6</td>
<td>29.4 ± 0.9</td>
<td>30.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Run+ 22.0 ± 0.7</td>
<td>26.8 ± 1.1</td>
<td>31.3 ± 0.5</td>
<td>30.9 ± 0.5</td>
</tr>
<tr>
<td>ADP</td>
<td>Con 4.15 ± 0.13</td>
<td>3.77 ± 0.11</td>
<td>4.57 ± 0.14</td>
<td>5.44 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Run 4.80 ± 0.20*</td>
<td>3.75 ± 0.09</td>
<td>4.69 ± 0.08</td>
<td>4.19 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>Run+ 4.15 ± 0.20</td>
<td>3.90 ± 0.20</td>
<td>4.86 ± 0.05</td>
<td>4.31 ± 0.05</td>
</tr>
<tr>
<td>AMP</td>
<td>Con 0.32 ± 0.03</td>
<td>0.21 ± 0.02</td>
<td>0.15 ± 0.02</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Run 0.36 ± 0.03</td>
<td>0.20 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Run+ nd</td>
<td>0.19 ± 0.04</td>
<td>0.17 ± 0.02</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>TAN</td>
<td>Con 25.6 ± 0.8</td>
<td>30.2 ± 1.0</td>
<td>34.8 ± 0.3</td>
<td>33.7 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Run 27.8 ± 1.0</td>
<td>32.1 ± 0.7</td>
<td>34.2 ± 0.9</td>
<td>35.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Run+ 25.1 ± 0.8</td>
<td>30.9 ± 1.1</td>
<td>36.3 ± 0.5</td>
<td>35.3 ± 0.5</td>
</tr>
<tr>
<td>IMP</td>
<td>Con nd</td>
<td>0.24 ± 0.03</td>
<td>0.27 ± 0.04</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Run nd</td>
<td>0.30 ± 0.04</td>
<td>0.32 ± 0.03</td>
<td>0.12 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Run+ nd</td>
<td>0.29 ± 0.04</td>
<td>0.27 ± 0.03</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>PCr</td>
<td>Con 45.7 ± 2.3</td>
<td>58.8 ± 4.7</td>
<td>85.4 ± 5.9</td>
<td>74.7 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>Run 41.2 ± 1.8</td>
<td>69.9 ± 3.0</td>
<td>82.5 ± 6.8</td>
<td>78.3 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>Run+ 44.0 ± 1.6</td>
<td>57.9 ± 5.9</td>
<td>75.0 ± 7.9</td>
<td>77.1 ± 4.2</td>
</tr>
<tr>
<td>Cr</td>
<td>Con 44.3 ± 2.8</td>
<td>78.9 ± 3.8</td>
<td>51.5 ± 4.4</td>
<td>72.7 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>Run 57.5 ± 2.3</td>
<td>71.2 ± 3.3</td>
<td>61.5 ± 7.2</td>
<td>75.4 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>Run+ 60.1 ± 3.0</td>
<td>75.5 ± 4.7</td>
<td>76.0 ± 8.4*</td>
<td>74.2 ± 6.1</td>
</tr>
<tr>
<td>Lactate</td>
<td>Con 8.2 ± 0.9</td>
<td>12.6 ± 1.8</td>
<td>12.9 ± 2.0</td>
<td>16.9 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>Run 9.1 ± 0.9</td>
<td>7.4 ± 2.6</td>
<td>11.5 ± 2.2</td>
<td>15.1 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>Run+ 5.7 ± 0.8</td>
<td>7.8 ± 1.6</td>
<td>14.9 ± 4.1</td>
<td>15.6 ± 4.0</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/kg dry wt for $n = 10$ rats for all muscles except extensor digitorum longus (EDL; $n = 7$). Sol, soleus; RV, red vastus; WV, white vastus. Glycogen depletion ranged between 33 and 66% from Sol to WV muscle. There was a main effect for group whereby recovery from Run was different from Con ($P < 0.05$).
Table 2. Effects of resting recovery after prolonged exercise on 3-O-methylfluorescein phosphatase activity in rat-vastus lateralis muscle

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>Rec 10</th>
<th>Rec 25</th>
<th>Rec 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>WV</td>
<td>138 ± 6.4</td>
<td>140 ± 7.4</td>
<td>131 ± 7.8</td>
<td>127 ± 6.0</td>
</tr>
<tr>
<td>RV</td>
<td>246 ± 9.6</td>
<td>266 ± 15</td>
<td>241 ± 23</td>
<td>249 ± 17</td>
</tr>
</tbody>
</table>

Values are means ± SE in nmol-mg protein⁻¹-h⁻¹ of n = 8 rats per group. Rec 10, 10 min of resting recovery; Rec 25, 25 min of resting recovery; Rec 45, 45 min of resting recovery.

control animals. Our results for Run are similar to what has been found for the Ca²⁺-ATPase activity in response to a similar bout of exercise (18). However, unlike Ca²⁺ activity, where an overshoot was observed to occur during the additional exercise (18), our results for Na⁺-K⁺-ATPase activity indicate that further exercise depresses activity. The effect of the additional exercise on Na⁺-K⁺-ATPase activity did not depend on the muscle examined.

The basis of our hypothesis, namely that a reduction in Na⁺-K⁺-ATPase activity would occur with prolonged exercise, was based on previous reports of declines in SR Ca²⁺-ATPase that occurred in response to similar types of exercise (4, 8, 41). However, not all studies report a reduction in Ca²⁺-ATPase activity with exercise (11, 14, 18). Although the reasons for the discrepancies remain unclear, differences in exercise protocols, muscles examined, assay procedures, and species appear important (20). Our study suggests that at least some of these factors may be important with Na⁺-K⁺-ATPase activity given the additional exercise that was needed to induce changes.

The diminishing Na⁺-K⁺-ATPase activity that we have observed with exercise is likely to represent structural modifications to the enzyme since our measurements were performed under optimal conditions in vitro. There are a number of cellular mechanisms that may explain the intrinsic changes to the enzyme and the specific effect on Na⁺-K⁺-ATPase activity. The most notable possibilities for acute inactivation may result from free radical damage (33), Ca²⁺-activated proteolysis (3), and heat denaturation (17), all of which can increase with exercise (38). Free radical damage has been demonstrated to reduce Na⁺-K⁺-ATPase activity in cardiac tissue (32) in a time- and concentration-dependent manner after response to a reactive compound (31). Evidence from studies on mouse diaphragm indicates that excessive intercellular Ca²⁺ can inhibit Na⁺-pump activity (39). Prolonged exposure to heat stress has been demonstrated to alter a number of metabolic processes in skeletal muscles, including Ca²⁺-ATPase activity (17). Further study is required to determine which of these potential mechanisms may predominate, as well as identifying the site on the enzyme that is altered.

In the experiment, we have also examined whether a muscle-specific response in Na⁺-K⁺-ATPase activity occurred with exercise. As indicated by a planned comparison, the response of EDL muscle was significantly different from that of the other muscles. EDL muscle exhibits considerably faster fatigue than Sol (15), and this is attributed, at least in terms of muscle excitability, to a greater number of Na⁺-channels relative to the muscle examined.
Na⁺–K⁺ pumps (24). This increased ion “leak” relative to ion “pump” capacity can result in a run down of ion gradients and result in fatigue (35). This fact may explain why EDL Na⁺–K⁺-ATPase activity was unaffected in the Run protocol compared with other muscles, possibly because of early fatigue and a lack of involvement in the treadmill running. Glycogen content measurements showed that EDL was similarly depleted to other muscles, so EDL was used either early or late in the Run protocol and/or was continued once the active recovery phase began. It is clear that additional exercise was needed for depressions in Na⁺–K⁺-ATPase activity to occur in EDL in the Run+ protocol.

A final noteworthy issue when results of this experiment are interpreted is whether Na⁺–K⁺-ATPase activity was measured in homogenates by using the 3-O-MPFase assay. K⁺-dependent hydrolysis of the 3-O-methylfluorescein phosphate chromogenic substrate substitutes for the aspartylphosphate intermediate of ATPase (25) to represent the terminal step in ATP hydrolysis (26). These phosphatase assays relate to actual ATPase activity (25, 26) but yield results that are less than the activity assessed by direct methods, possibly because of a reduced affinity for the artificial substrates. Although a direct measurement of Na⁺–K⁺-ATPase activity using a measurement of inorganic phosphate accumulation or a regenerating assay involving NAD/NADH changes would have been more desirable, such measurements on whole muscle homogenates are problematic given the low relative Na⁺–K⁺-ATPase activity in the cell relative to other ATPases.

The observations from this experiment are consistent with previous findings (34) for cardiac muscle Na⁺–K⁺-ATPase and confirm a preliminary report that activity is reduced after repeated submaximal leg extension exercise to fatigue in skeletal muscle. These findings reintroduce the possibility that Na⁺–K⁺-ATPase may contribute to acute neuromuscular fatigue in skeletal muscle and highlight the role of the Na⁺–K⁺-ATPase activity in the cell relative to other ATPases. A study by Ferrington et al. (172) showed that the sodium-potassium pump in rat skeletal muscle is responsible for maintaining the sodium-potassium gradient, which is important for the regulation of nerve impulses and muscle contraction. The sodium-potassium pump is also involved in the maintenance of the resting membrane potential, which is critical for the proper functioning of nerve and muscle cells.

This study was supported by the National Sciences and Engineering Research Council of Canada.

REFERENCES


