Na\(^+\)-K\(^+\)-ATPase in rat skeletal muscle: muscle fiber-specific differences in exercise-induced changes in ion affinity and maximal activity

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Juel C. Na\(^+\)-K\(^+\)-ATPase in rat skeletal muscle: muscle fiber-specific differences in exercise-induced changes in ion affinity and maximal activity. *Am J Physiol Regul Integr Comp Physiol* 296: R125–R132, 2009. First published November 5, 2008; doi:10.1152/ajpregu.90760.2008.—It is unclear whether muscle activity reduces or increases Na\(^+\)-K\(^+\)-ATPase maximal in vitro activity in rat skeletal muscle, and it is not known whether muscle activity changes the Na\(^+\)-K\(^+\)-ATPase ion affinity. The present study uses quantification of ATP hydrolysis to characterize muscle fiber type-specific changes in Na\(^+\)-K\(^+\)-ATPase activity in sarcomemmal membranes and in total membranes obtained from control rats and after 30 min of treadmill running. ATPase activity was measured at Na\(^+\) concentrations of 0–80 mM and K\(^+\) concentrations of 0–10 mM. \(K_m\) and \(V_{\text{max}}\) values were obtained from a Hill plot. \(K_m\) for Na\(^+\) was higher (lower affinity) in total membranes of glycocolytic muscle (extensor digitorum longus and white vastus lateralis), when compared with oxidative muscle (red gastrocnemius and soleus). Treadmill running induced a significant decrease in \(K_m\) for Na\(^+\) in total membranes of glycocolytic muscle, which abolished the fiber-type difference in Na\(^+\) affinity. \(K_m\) for K\(^+\) (in the presence of Na\(^+\)) was not influenced by running. Running only increased the maximal in vitro activity (\(V_{\text{max}}\)) in total membranes from soleus, whereas \(V_{\text{max}}\) remained constant in the three other muscles tested. In conclusion, muscle activity induces fiber type-specific changes both in Na\(^+\) affinity and maximal in vitro activity of the Na\(^+\)-K\(^+\)-ATPase. The underlying mechanisms may involve translocation of subunits and increased association between PLM units and the \(\alpha\beta\) complex. The changes in Na\(^+\)-K\(^+\)-ATPase ion affinity are expected to influence muscle ion balance during muscle contraction.

Adaptational modifications; Na\(^+\)-K\(^+\)-pump; ion sensitivity

Ion gradients across the outer membrane vary during muscle activity. These activity-induced changes in ion distribution affect muscle excitability and may lead to impairment of force development.

The Na\(^+\)-K\(^+\)-ATPase (Na\(^+\)-K\(^+\)-pump) maintains normal transmembrane gradients of Na\(^+\) and K\(^+\) and counteracts changes in ion gradients due to muscle activity; therefore, regulation of this pump is important for muscle function. The minimal functional pump consists of a heterodimer of one \(\alpha\) and one \(\beta\) subunit. Each subunit exists in a number of isoforms, among which the \(\alpha_1\), \(\alpha_2\), \(\beta_1\), and \(\beta_2\) forms are important in skeletal muscle. The \(\alpha_1\) and \(\alpha_2\) isoforms are found in all muscle fiber types; however, they exhibit different cellular localization patterns. Both the \(\alpha_1\) and \(\alpha_2\) isoforms are found in the sarcolemmal fraction, but only the \(\alpha_2\) isoform is present in the T-tubules (29). The \(\beta_1\) isoform is mainly found in oxidative fibers, whereas the \(\beta_2\) isoform is found in glycolic fibers (9, 28). The different dimers have different ion affinities (unpublished data), and the different localization of the isoforms suggests that the heterodimers play different roles (11) and perhaps are regulated differently.

In recent years, it has become evident that the Na\(^+\)-K\(^+\)-ATPase pump complex of different tissues can include members of the FXYD protein family. In skeletal muscle, the PLM (FXYD1) protein is associated with the pump (5, 25). The association of PLM with the \(\alpha\beta\) dimer has been investigated in expression systems. In *Xenopus* oocytes, association of PLM with the \(\alpha\beta\) complex significantly increases the \(K_m\) for Na\(^+\) (5). In HeLa cells, association of PLM with the complex also increases the \(K_m\) for Na\(^+\), whereas in *Pichia pastoris*, this association reduces the \(K_m\) (17). It was hypothesized that the contradictory effects of PLM on Na\(^+\) affinity observed in these systems are caused by differences in its degree of phosphorylation (17). The association between PLM units and the \(\alpha\) units increases during muscle activity in rats (24), which may give rise to changes in pump activity, but this has not yet been demonstrated.

A number of experiments using human muscle samples have revealed depressed maximal in vitro Na\(^+\)-K\(^+\)-ATPase pump capacity after both maximal and submaximal exercise; however, the data from rat muscle are conflicting. Two studies have reported a decrease in maximal pump capacity after muscle activity (8, 20), two studies reported an unchanged maximal capacity (10, 16), whereas one study reported a substantial increase in activity (26). These results were all obtained using the 3-O-methylfluorescein phosphate (3-O-MFP) assay, which employs 3-O-MFP as an artificial substrate, without involving ATP hydrolysis (23). This assay cannot be used to measure Na\(^+\)-dependent activation, and the K\(^+\) sensitivity obtained may not reflect the sensitivity of the intact ATPase. In addition, in these studies, the in vitro maximal pump capacity was measured at only one combination of Na\(^+\) and K\(^+\) concentrations, which were higher than the physiological concentrations; thus, these ion-induced activity values may not reflect in vivo values. It is important to note that the in vitro maximal pump activity does not reflect the activity in the intact organism, where the pump is also stimulated by hormones. The possibility that muscle contractions can also induce changes in \(K_m\) (ion affinity) has not been addressed. In the present study, we used ATP hydrolysis to quantify pump activity, and the affinity for Na\(^+\) and K\(^+\) was measured in rats both before and after treadmill running. Treadmill running (rather than isolated rat muscle) was used because this model is comparable to voluntary exercise in humans.

Adaptational modifications; Na\(^+\)-K\(^+\)-pump; ion sensitivity

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It has previously been reported, for both rat and human muscle, that α, β, and PLM pump subunits can be translocated from internal stores and/or from caveolae during muscle activity (13, 14, 16, 24); however, the occurrence of subunit translocation has been disputed (19, 22).

A previous study with mixed rat muscle failed to detect any effect of treadmill running on Na\(^+\)-K\(^+\)-ATPase activity in total membranes (24). In the present study, we used both total membranes and sarcolemmal membranes comprising different fiber types obtained before and after treadmill running to investigate contraction-induced changes in Na\(^+\)-K\(^+\)-ATPase pump activity. We hypothesize that contraction-induced changes in pump activity involve both translocation of pump subunits and changes in Na\(^+\)-K\(^+\)-ATPase ion affinity and that these changes are fiber type specific.

**MATERIALS AND METHODS**

**Animals and muscle sampling.** Male Wistar rats (body weight 130–150 g, age 6 wk) were provided with unlimited food and water and kept under a 12:12-h dark-light cycle. The handling of animals was conducted in accordance with Danish Animal Welfare Regulations. Animals were randomly allocated to the control and exercise treatments. The rats were exercised (without conditioning) using a treadmill equipped with an airpuff system. The exercise session lasted ~30 min and consisted of 7–8 bouts of 3-min running (20 m/min, 10° slope) separated by 1 min of rest. Immediately after the final bout of exercise, the rats were killed with a blow to the neck, and muscle tissue was immediately removed. All sample preparations were carried out at a maximum temperature of 4°C unless otherwise stated. The protein content of samples was determined using a BSA standard (DC protein assay, Bio-Rad).

**Muscle preparations and membrane fractionating—total membranes.** Two identical muscles (red gastrocnemius, soleus, extensor digitorum longus, or white vastus lateralis) from the same rat were minced, and the muscle fragments were homogenized for 30 s (Polytron PT 2100) in 250 mM mannitol, 30 mM L-histidine, 5 mM EGTA, and 0.1% deoxycholate, pH 6.8. The sample (crude homogenate) was subjected to 3,000 g spinning for 30 min, and the supernatant was subjected to 190,000 g spinning for 90 min (4°C). The final pellet, called “total membranes,” was resuspended in assay buffer (see **Measurement of K\(^+\)**- and Na\(^+\)**-stimulated Na\(^+\)/K\(^+\)**-ATPase activity in sarcolemmal membranes and total membranes) and used for the activity measurements. Sample protein recovery was about 5% of the total protein content (including soluble proteins) in the crude homogenate, and the Na-K-ATPase α2 isoform protein content in the final total membrane fraction (quantified with Western blot analysis) was more than half of the content in the crude homogenates. The production of total membranes removed some of the background ATPase activity, thereby increasing the maximal Na\(^+\)-stimulated Na-K-ATPase activity to about 11% of the total ATPase activity (including Ca\(^2+\)-ATPase).

**Sarcolemmal giant vesicles—sarcolemmal membranes.** Because the total membranes contained a large background from other ATPases, we also used sarcolemmal giant vesicles as a membrane purification method. The sarcolemmal membranes were isolated as giant vesicles in accordance with the technique by Juel (12). Vesicles contain solubile proteins from the cell; however, previous characterizations have demonstrated that the vesicles contain no T-tubule membranes and very low contamination with sarcoplasmic reticulum. Briefly, muscle tissue was incubated for 45 min at 34°C in KCl-MOPS buffer (140 mM KCl, 5 mM MOPS, pH 7.4) containing 150 U/ml collage-nase. The samples were washed and subjected to 50 g three-layer gradient centrifugation for 45 min at room temperature. After harvesting, the giant vesicles were washed in KCl-MOPS buffer and recovered by centrifugation at 830 g for 30 min at room temperature. For the K\(^+\)-affinity experiments, the vesicles (sarcolemmal membranes) were further washed in K\(^+-\)free assay media and sedimented at 20,000 g for 30 min. This procedure removed soluble proteins. The activity values in these measurements were therefore based on membrane proteins, whereas the sarcolemmal membranes (vesicles) used in the Na\(^+\)-affinity studies also contained soluble protein; the activity values can, therefore, not be directly compared.

**Fiber-type-specific samples containing glycolytic (white) and oxidative (red) muscle fibers were made from the following muscles: white gastrocnemius, white vastus lateralis (VLw) and white tibialis anterior (glycolytic), and soleus (SOL), red gastrocnemius (RG), and vastus intermedius (oxidative). The fiber-type distribution IIA:IID/X:IIB in these preparations has been calculated, in accordance with Delph and Duan (6), to be about 0:0:4:96 (glycolytic) and 69:23:7:1 (oxidative).

**Western blot analysis.** Samples were mixed with sample buffer (2 mM Tris-HCl, 0.2 mM EDTA, 20 mM DTT, 4% SDS, 10% glycerol, 0.04% bromophenol blue, pH 8.0). Equal amounts of protein were loaded into each lane and separated by 8–18% SDS-PAGE (Excel gel; Amersham) or 12.4% SDS-PAGE (ExcelGel; Amersham) for PLM measurements. The proteins were then electroblotted onto a polyvinylidene difluoride membrane (Immobilon-P; Millipore). The membrane was blocked for 60 min at room temperature in TS-buffer (10 mM Tris-Base, 0.9% NaCl, pH 7.4) containing 2% BSA, 1% skim-milk powder, and 0.1% Tween-20 before incubation with primary antibody diluted in a similar buffer overnight at 4°C. After treatment with a horseradish peroxidase-coupled secondary antibody (Dako) for 90 min at room temperature, the membrane was repeatedly washed in TS-buffer with or without 0.05% Tween-20. The membrane was incubated with enhanced chemiluminescence reagent (ECL; Amersham) and visualized on Hyperfilm (Amersham). Samples to be compared were loaded on the same gel. Relative protein concentrations were quantified by scanning the film and analyzing band intensities with UN-SCAN-IT ver. 5.1 software. For each isoform, the values are calculated relative to the mean of the highest value for that isoform. This method does not allow a comparison of isoform protein levels.

**Antibodies.** The α1 isoform was detected with the monoclonal mbF antibody (Iowa Hybridoma Bank). A monoclonal antibody (McB2) used to detect the α2 isoform and a polyclonal antibody to detect the β1 isoform were generously provided by Dr. P. A. Pedersen, University of Copenhagen, Copenhagen, Denmark. The β2 isoform was detected with a polyclonal antibody from Upstate (#06–171). For detection of PLM, we used the polyclonal C2 antibody generously provided by Dr. J. Cheung, Geisinger Medical Center.

**Measurement of K\(^+\)**- and Na\(^+\)**-stimulated Na\(^+\)/K\(^+\)**-ATPase activity in sarcolemmal membranes and total membranes. Na\(^+\)- or K\(^+\)-stimulated Na\(^+\)-K\(^+\)-ATPase activity was determined by measuring the hydrolysis of \(3^{3}P\)-ATP (25). Sarcolemmal membranes or total membranes were suspended in assay buffer (10 mM KCl or 80 mM NaCl, 5 mM MgCl\(_2\), 50 mM Tris-base, 5 mM EGTA, pH 7.4). The sarcolemmal membranes (vesicles) were subjected to five freeze-thaw cycles to permeabilize the membranes (9).

In the studies with sarcolemmal membranes, each sample contained 9–95 μg of protein, including soluble protein (Na\(^+\) affinity studies), and 3–12 μg purified membrane protein only (K\(^+\) affinity studies). In total membranes, each sample contained 30–100 μg of protein. Na\(^+\) was added to the samples to a final concentration of 0–80 (0, 2, 4, 6, 10, 20, 40, 80, 0) mM (ionic strength was kept constant by substituting NaCl with choline chloride) or K\(^+\) was added to the samples to a final concentration of 0–10 (0, 0.5, 1, 2, 3, 5, 7.5, 10, 0) mM with Na\(^+\) kept constant at 80 mM. After 15 min of preincubation at 37°C, the reaction was started by adding \(3^{3}P\)-ATP (PerkinElmer) plus Mg-ATP (Sigma) to a final concentration of 5 mM. After 30 min, the samples were placed on ice and the reaction was stopped by adding 1 ml of a solution containing 1 M H\(_2\)SO\(_4\) and 0.5% (NH\(_4\))\(_2\)Mo\(_7\)O\(_2\)\(_4\). Inorganic phosphate ions were isolated by the addition of 2 mls isobutanol,
RESULTS

Na\(^{+}\)-stimulated Na\(^{+}\)-K\(^{+}\)-ATPase activity in total membranes from four different muscles—effect of running. The ATPase activity was measured at Na\(^{+}\) concentrations from 0 to 80 mM, with the K\(^{+}\) concentration kept constant at 10 mM. A comparison of control experiments demonstrated that the stimulatory effect of Na\(^{+}\) reached saturation at high concentrations in RG, SOL, and EDL, whereas the pump activity in the glycolytic VLw fibers increased throughout the [Na\(^{+}\)] interval used (Fig. 1). The mean Hill parameters are shown in Table 1. Overall, oxidative fibers had a lower K\(_{m}\) (higher affinity), when compared with glycolytic fibers. Pooled data from glycolytic muscle (EDL and VLw) yielded a significant (P < 0.05) decrease in K\(_{m}\) for Na\(^{+}\) associated with running, whereas treadmill running had no significant effect on K\(_{m}\) in oxidative muscle (SOL and RG). These changes caused that the fiber-type difference in K\(_{m}\) obtained in control samples disappeared with running.

V\(_{\text{max}}\) for Na\(^{+}\) in RG, VLw, and EDL muscle was unchanged after running, whereas treadmill running increased the V\(_{\text{max}}\) for Na\(^{+}\)-stimulated Na\(^{+}\)-K\(^{+}\)-ATPase in SOL muscle (0.12 vs. 0.10 μmol ATP·h\(^{-1}\)·mg\(^{-1}\)) (P < 0.05). It must be noted that the determination of V\(_{\text{max}}\) for VLw was uncertain, because of a lack of saturation within the [Na\(^{+}\)] interval used.

Na\(^{+}\)-stimulated Na\(^{+}\)-K\(^{+}\)-ATPase activity in sarcolemmal membranes from oxidative and glycolytic fibers—effect of running. The Na\(^{+}\)-dependent activity (K\(^{+}\) kept constant at 10 mM) of the Na\(^{+}\)-K\(^{+}\)-ATPase pump was measured in sarcolemmal membranes from oxidative and glycolytic fibers (Fig. 2) obtained before (control) and after 30 min of treadmill running. K\(_{m}\) for Na\(^{+}\) in the sarcolemmal membranes from oxidative fibers was significantly increased (P < 0.05) with exercise, when compared with control conditions (15.8 vs. 6.1 mM), whereas K\(_{m}\) for Na\(^{+}\) in sarcolemmal membranes from glycolytic fibers remained unchanged after exercise, when compared with the control (11.6 vs. 14.0 mM). The mean Hill parameters are included in Table 1.

V\(_{\text{max}}\) for the Na\(^{+}\)-dependent activation of the Na\(^{+}\)-K\(^{+}\)-ATPase pump was significantly increased in the sarcolemmal membranes from oxidative fibers obtained after 30 min running when compared with the control (0.071 vs. 0.051 μmol ATP·h\(^{-1}\)·mg\(^{-1}\) protein). Exercise had no significant effect on V\(_{\text{max}}\) when sarcolemmal membranes from glycolytic fibers obtained after running were compared with the control (0.052 vs. 0.041 μmol ATP·h\(^{-1}\)·mg\(^{-1}\) protein).

K\(^{+}\)-stimulated Na\(^{+}\)-K\(^{+}\)-ATPase activity in total membranes from oxidative and glycolytic muscle—effect of running. The ATPase activity was measured at K\(^{+}\) concentrations from 0 to 10 mM, with the Na\(^{+}\) concentration kept constant at 10 mM. Oxidative muscle was represented by RG muscle, and glycolytic muscle was represented by VLw muscle. Exercise had no significant effect on either K\(_{m}\) or V\(_{\text{max}}\) for K\(^{+}\)-dependent

Table 1. Changes in Hill parameters with running

<table>
<thead>
<tr>
<th></th>
<th>K(_{m}) mM</th>
<th>V(_{\text{max}}) μmol ATP·h(^{-1})·mg(^{-1})</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>After Running</td>
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<tr>
<td>Na(^{+})</td>
<td></td>
<td></td>
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<tr>
<td>Total membranes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red gastrocnemius</td>
<td>5.1±1.1</td>
<td>7.5±1.0</td>
</tr>
<tr>
<td>Soleus</td>
<td>5.4±2.2</td>
<td>7.3±1.7</td>
</tr>
<tr>
<td>Extensor digitorum longus</td>
<td>12.7±4.2#</td>
<td>8.3±1.6</td>
</tr>
<tr>
<td>White vastus lateralis</td>
<td>16.2±5.3#</td>
<td>6.3±0.9†</td>
</tr>
<tr>
<td>Sarcolemmal membranes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidative fibers</td>
<td>6.1±1.3</td>
<td>15.8±2.4*</td>
</tr>
<tr>
<td>Glycolytic fibers</td>
<td>14.0±1.7#</td>
<td>11.6±1.2</td>
</tr>
<tr>
<td>K(^{+})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total membranes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red gastrocnemius</td>
<td>1.6±0.6</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>White vastus lateralis</td>
<td>1.4±0.3</td>
<td>1.9±0.4</td>
</tr>
<tr>
<td>Sarcolemmal membranes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidative fibers</td>
<td>0.70±0.09</td>
<td>1.5±0.7</td>
</tr>
<tr>
<td>Glycolytic fibers</td>
<td>3.1±1.1‡</td>
<td>4.6±1.6</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE, obtained from each individual experiment. Na\(^{+}\) and K\(^{+}\) affinities and V\(_{\text{max}}\) of the Na\(^{+}\)-K\(^{+}\)-ATPase in total membranes and sarcolemmal membranes from oxidative and glycolytic fiber; the effect of treadmill running. After running: samples obtained immediately after 30 min of treadmill running. Total membranes: n = 8 (8 rats). Sarcolemmal membranes: n = 7, each sample consisted of muscle material from three rats; 21 rats included.

*Different from control (P < 0.05). †Based on pooled data (red gastrocnemius + soleus versus extensor digitorum longus + white vastus lateralis). ‡Different from oxidative fibers.
Na^+−K^+-ATPase activity in total membranes of oxidative and glycolytic muscle (Fig. 3).

K^+-stimulated Na^+−K^+-ATPase activity in sarcolemmal membranes from oxidative and glycolytic fibers—effect of running. Treadmill running had no effect on $K_m$ for K^+-dependent ATPase activity in sarcolemmal membranes from oxidative and glycolytic fibers (Fig. 4). In addition, $V_{\text{max}}$ remained unchanged after running.

Relative distribution of pump subunits. The relative distribution of pump subunits in the sarcolemmal membranes used in Fig. 2 is depicted in Fig. 5. The $\alpha_1$ and $\alpha_2$ isoforms were present in both oxidative and glycolytic fibers. The $\beta_1$ isoform was mainly present in oxidative fibers, whereas the $\beta_2$ isoform was mainly present in glycolytic fibers. PLM was also present in both fiber types but was detected at a higher density in oxidative fibers. Treadmill running significantly increased the expression of PLM in both fiber types and significantly increased the presence of the $\alpha_2$ isoform in glycolytic fibers.

Fig. 1. Na^+-dependent activity of the Na^+−K^+-ATPase in total membranes obtained from four different muscles—with different isoform distribution ($K^+$ constant at 10 mM)—effect of running. Open circles denote control; closed circles denote samples obtained after treadmill running. Each value is expressed as the mean ± SE; $n = 8$. The curves represent a Hill plot constructed from the mean of the Hill parameters obtained in each experiment. For statistics, see Table 1. A: RG, red gastrocnemius. B: VLw, white vastus lateralis. C: SOL, soleus. D: EDL, extensor digitorum longus.

Fig. 2. Na^+-dependent activity of the Na^+−K^+-ATPase in sarcolemmal membranes from oxidative and glycolytic fibers—effect of running. A: sarcolemmal membranes purified from oxidative muscle. B: sarcolemmal membranes from glycolytic muscle. Open circles: control, closed circles: after activity. Each sample consisted of muscle material from three rats; $n = 7$ (total of 21 animals in each series), each value is expressed as the mean ± SE. The curves represent a Hill plot constructed from the mean of the Hill parameters obtained in each experiment. Statistics and Hill parameters are included in Table 1.
DISCUSSION

The findings of the present study confirm the hypothesis that muscle activity induces translocation of subunits and modifies the Na\(^+\)-K\(^+\)-ATPase ion affinity and that these changes are fiber type specific. Both mechanisms influence the maximal ATPase activity at physiological ion concentrations. The study also confirms previous observations that the pump affinity for Na\(^+\) and K\(^+\) is fiber type specific (unpublished data).

Underlying mechanisms. Treadmill running in rats decreased ATPase Na\(^+\) affinity (increased \(K_m\)) and increased \(V_{\text{max}}\) in sarcolemmal membranes purified from oxidative fibers (Fig. 2, Table 1). Running increased the Na\(^+\) affinity in total membranes of glycolytic fibers (pooled data, Fig. 1 and Table 1), and increased \(V_{\text{max}}\) in SOL muscle. These changes probably reflect different mechanisms.

The increase in \(V_{\text{max}}\) in sarcolemmal membranes from oxidative fibers probably involve an increase in the number of pump subunits (translocation), which is supported by the increased number of pump subunits quantified by Western blot analysis of the same samples (Fig. 5). Translocation may reflect recruitment of pump subunits from internal stores or exposure of subunits from caveolae (16). Translocation is only observed in sarcolemmal membranes, and not in total membranes, which represent the total amount of pumps; however, the translocation phenomenon can also be verified in intact muscle by surface biotinylation of \(\alpha\) subunits (16). Binding of PLM units may also be involved in the observed changes in \(V_{\text{max}}\), as PLM is reported to increase the turnover (\(V_{\text{max}}\)) in expression systems (17). This mechanism is expected to affect \(V_{\text{max}}\) in sarcolemmal membranes, which is supported by the running-induced increase in the amount of PLM, as quantified by Western blot analysis (Fig. 5). Relocation of PLM in the cell could also change \(V_{\text{max}}\) in total membranes.

Affinity changes were observed in both sarcolemmal membranes and total membranes. The overall observation was that the fiber type-dependent differences in \(K_m\) for Na\(^+\) seen when comparing oxidative and glycolytic fibers were blurred after...
treadmill running. The large decrease in Na\(^+\) affinity in sarcolemmal membranes from oxidative fibers can hardly be explained by changes in \(\alpha\) and \(\beta\) isoform distribution, as the relative amounts remained constant (Fig. 5). Another possible underlying mechanism would be a change in the phosphorylation status of the \(\alpha\) and \(\beta\) subunits, but this parameter was not measured in the preparations used here, because the long production time and high temperatures are expected to blur any potential differences. The most pronounced change in isoform content after running was the large increase in PLM levels in sarcolemmal membranes from both fiber types, which can be explained by translocation of this protein to the membrane fractions studied (24). Immunoprecipitation studies have shown that PLM associates with both \(\alpha_1\) and \(\alpha_2\) isoforms in skeletal muscle (25) and that muscle activity induces an increase in the association between PLM and \(\alpha\) units, in particular, with the \(\alpha_1\) isoform (24). Furthermore, studies using expression systems have demonstrated that association with PLM changes the activity of the pump (5). An increased association of PLM with the \(\alpha\beta\) complex is, therefore, a likely contribution to the running-induced modification in ion affinity observed in the present study.

It has been argued that only the nonphosphorylated form of PLM increases the \(K_m\) for Na\(^+\), whereas \(K_m\) is decreased or unchanged for phosphorylated PLM (17). In the present study, we used the C2 anti-PLM antibody, which is produced against 16 amino acids located in the C terminus of PLM, not including the serine 68 phosphorylation site. This antibody is, therefore, expected to recognize unphosphorylated PLM. The changes in the amount of PLM after running (Fig. 5), therefore, represent unphosphorylated PLM. We have previously demonstrated, using a phosphorylation-specific anti-PLM antibody, that running had no influence on PLM phosphorylation state in rats (24). In contrast to these data, exercise increases PLM phosphorylation in human skeletal muscle (1, 27). The present data fit with the suggestion that binding of unphosphorylated PLM is increased after exercise; however, there is a lack of information regarding the differences in the way PLM interacts with different \(\alpha\beta\) dimers. An explanation for the fiber type-dependent differences in running-induced ion affinity cannot be provided at this time.

Although muscle activity resulted in a reduced difference between Na\(^+\) affinity in oxidative and glycolytic muscle in total membranes and sarcolemmal membranes, the effect was achieved in different ways. In total membranes, running reduced \(K_m\) in glycolytic muscle, whereas running increased \(K_m\) in membranes from oxidative fibers. One difference between the two preparations is the lack of T-tubuli in the sarcolemmal membranes, which results in a higher \(\alpha_1/\alpha_2\) ratio. In addition, the PLM content is higher in the sarcolemmal membranes when compared with total membranes, but this seems to be an insufficient explanation for this difference, which suggests that other mechanisms are involved. One possibility is that different pools of pumps are regulated differently.

\textit{Maximal ATPase activity—effect of running.} The majority of studies performed in humans have reported a reduced

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig5.png}
\caption{Relative distribution of Na\(^+\)-K\(^+\)-ATPase subunits in sarcolemmal membranes from oxidative (shaded columns) and glycolytic fibers (open columns) in control samples and samples prepared after 30 min of treadmill running. For each subunit, the values were calculated relative to the control value from oxidative fibers (left column 100%). The samples were identical to the samples used in Fig. 2. Each value is expressed as the mean \pm SE; \(n = 7\). *Significantly different from oxidative fibers (left column 100%). \#Significantly different from control. Western blots of pump subunits can be seen in recent papers (16, 24).}
\end{figure}

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in vitro maximal 3-O-MFPase activity after exercise. Maximal Na\(^+\)-K\(^+\)-ATPase in vitro activity in rat muscle measured using the 3-O-MFPase assay has been reported to be reduced, unchanged, or increased after muscle activity. In situ stimulation and treadmill running both reduce maximal 3-O-MFPase activity in rats (8, 20). In addition, treadmill running and in vitro electrical stimulation reportedly have no impact on maximal 3-O-MFPase activity (10, 16), and in situ electrical stimulation has been reported to substantially increase maximal 3-O-MFPase activity (26). These studies are conflicting, which can be attributed to the different experimental methods used and different muscles studied. Only one study used an ATPase assay based on ATP hydrolysis and a range of Na\(^+\) concentrations to quantify Na\(^+\)-K\(^+\)-ATPase activity; this study found no differences in pump activity between muscle homogenates of mixed muscle extracts obtained before and after treadmill running (24). It is important to note that the maximal in vitro pump activity does not reflect the pump activity in the intact organism, where the pump is also activated by hormones.

The present study compared in vitro \(V_{\text{max}}\) of the Na\(^+\)-K\(^+\)-ATPase pump before and after treadmill running in four separate series of experiments with total membranes of four different rat muscles with fiber-type composition ranging from glycolytic to mainly oxidative fibers. \(V_{\text{max}}\) remained unchanged in total membranes of RG, VLw, and EDL muscle after treadmill running. Only total membranes of SOL muscle exhibited an increased \(V_{\text{max}}\) after running. In line with the present study, the increased K\(^+\)-dependent activity (3-O-MFPase assay) after in situ stimulation reported by Sandiford et al. (26) was only obtained in soleus muscle, whereas \(V_{\text{max}}\) was unchanged in white gastrocnemius.

It must be noted that changes in the maximal in vitro pump activity (\(V_{\text{max}}\)) may not reflect all functionally relevant changes. It is more relevant to study activity-induced changes in ATPase activity at physiological ion concentrations. Because the \(K_m\) value for Na\(^+\) and the intracellular Na\(^+\) concentration fall within the same concentration range, changes in affinity (\(K_m\)) may affect the pump activity at physiological ion concentrations without affecting \(V_{\text{max}}\). This was illustrated in the present study; pooled data from glycolytic muscle revealed a decreased \(K_m\) (higher activity at physiologicaal Na\(^+\) concentrations) after running, without any concomitant change in \(V_{\text{max}}\).

**Physiological consequences of activity-induced changes in ion affinity.** The study demonstrated that \(K_m\) for Na\(^+\) in resting muscle differ between oxidative and glycolytic muscle. The muscle-specific \(K_m\) values determine the degree of ATPase activation at physiological Na\(^+\) concentrations (~15 mM). A comparison of RG (representing oxidative muscle) and VLw (representing glycolytic muscle) show that the activation of oxidative fibers is ~77% of \(V_{\text{max}}\) at 15 mM Na\(^+\), whereas the activation of glycolytic muscle is only 47% of \(V_{\text{max}}\) in the same conditions (Fig. 1); thus, the ATPase ion activation seems to be higher in oxidative than glycolytic muscle. Theoretically, it is expected that the different Na\(^+\) affinity determines intracellular Na\(^+\) in resting muscle; however, there is no direct correlation, as intracellular Na\(^+\) at rest is slightly higher in SOL than in EDL muscle (16.5 mM vs. 12.9 mM) (3), (13.7 mM vs. 9.8 mM) (7), or (11.1 vs. 9.3 mM) (4). These Na\(^+\) concentrations indicate that other factors are involved in ion balance in resting muscle, such as hormonal activation of the pump and membrane ion leaks.

The present study demonstrated fiber-type specific Na\(^+\)-K\(^+\)-ATPase ion affinity changes during muscle activity. It is tempting to interpret the exercise-induced changes in \(K_m\) for Na\(^+\) as an adaptational modification, but this raises some questions about the physiological consequences of this phenomenon. Can the fiber-type difference be explained? Do these changes affect ion balance during and after muscle activity? Are these changes beneficial during muscle activity? The information available in the literature regarding activity-induced changes in internal Na\(^+\) is conflicting. One study used 120 s of electrical stimulation of isolated muscle. Intracellular Na\(^+\) remained constant in SOL muscle but increased in EDL muscle (from 12.9 to 20 mM) (3). This difference was related to the high passive ion leak in EDL muscle compared with soleus. In a similar study using isolated rat soleus muscle, internal Na\(^+\) increased immediately after a short (1–10 s) period of stimulation, but an undershoot was seen 10 min after stimulation. It was suggested that excitation induces a rapid increase in the Na\(^+\) affinity of the pump (2). Five minutes of swimming has been reported to increase intracellular Na\(^+\) in three rat muscles, but not in SOL, whereas electrical stimulation increases Na\(^+\) in all muscles (18). A fourth study reported that internal Na\(^+\) in SOL (but not EDL) is increased by 80% immediately after 60 min of treadmill running and is maintained as an increase of 38% 15 min after running is completed (21). At the same time, pump activity evaluated from \(^{86}\text{Rb}\) uptake was also increased in SOL. One reason for the difference in internal Na\(^+\) between these studies is probably the different lengths of activity, but changes in ion affinity could also be involved. The increased intracellular Na\(^+\) in the last study discussed fits with an activity-induced, slowly developing increase in \(K_m\) for Na\(^+\), which was found in sarcolemmal membranes from oxidative fibers, as reported in the present study. A similar increase in Na\(^+\) during stimulation was not seen in EDL muscle, which is in keeping with the increased Na\(^+\) affinity in glycolytic muscle after running reported here. In addition, the exercise-induced reduction in \(K_m\) for Na\(^+\) in glycolytic fibers could be seen as an adaptation to the high passive ion leaks in active glycolytic muscle (3).

**Perspectives and Significance**

The main finding of the present study is that acute muscle activity modifies the Na\(^+\) sensitivity of the Na\(^+\)-K\(^+\)-ATPase in a fiber type-specific manner. Although the underlying mechanism is only partly understood, this confirms the existence of a nearly unnoticed adaptive response. This mechanism may influence the changes in ion distribution associated with muscle activity.

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**REFERENCES**


