Activity-induced recovery of excitability in K⁺-depressed rat soleus muscle

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Activity-induced recovery of excitability in K⁺-depressed rat soleus muscle. Am J Physiol Regulatory Integrative Comp Physiol 280: R48–R55, 2001.—Increased extracellular K⁺ concentration ([K⁺]o) can reduce excitability and force in skeletal muscle. Here we examine the effects of muscle activation on compound muscle action potentials (M waves), resting membrane potential, and contractility in isolated rat soleus muscles. In muscles incubated for 60 min at 10 mM K⁺, tetanic force and M wave area decreased to 23 and 24%, respectively, of the control levels. Subsequently, short (1.5 s) tetanic stimulations given at 1-min intervals induced recovery of force and M wave area to 81 and 90% of control levels, respectively, within 15 min (P < 0.001). The recovery of force and M wave was associated with a partial repolarization of the muscle fibers. Experiments with tubocurarine suggest that the force recovery was related to activation of muscle Na⁺-K⁺ pumps caused by the release of some compound from sensory nerves in response to muscle activity. In conclusion, activity produces marked recovery of excitability in K⁺-depressed muscle, and this may protect muscles against fatigue caused by increased [K⁺]o during exercise.

fatigue; skeletal muscle; contractility; M waves

DURING ACTIVITY, skeletal muscles are exposed to elevated extracellular K⁺ concentration ([K⁺]o) due to the loss of K⁺ from the muscle fibers. This may lead to muscle fatigue due to the force-depressing effects of high [K⁺]o (6). Recently, it was shown that in rat soleus, frequent electrical stimulation elicits a protective effect against the K⁺-induced decrease in force. Thus, in muscles where force was depressed by high [K⁺]o, a marked recovery of force could be produced simply by reducing the intervals between short tetanic stimulations (18). This bootstrapping phenomenon has been termed excitation-induced force recovery. Because it was associated with a decrease in intracellular Na⁺ concentration ([Na⁺]i) and was blocked by ouabain, it was most likely related to an excitation-induced increase in the activity of the Na⁺-K⁺ pump (18). We therefore hypothesized that the force recovery was caused by an improvement of the excitability brought about by an increase in the electrogenic contribution of the Na⁺-K⁺ pump to the membrane potential (17). This idea is supported by the recent observation that stimulation of the Na⁺-K⁺ pump with the β₂-agonist salbutamol or insulin induces recovery of both tetanic force and M waves (compound action potentials) in muscles exposed to high [K⁺]o and low extracellular Na⁺ concentration (21).

The excitability or “action potential activity” of a muscle fiber is determined by several factors such as the magnitude of the chemical gradients for Na⁺ and K⁺, the resting membrane potential, and the density and activation state of the voltage-sensitive Na⁺ channels. Here we have used M wave recordings to assess muscle excitability. The aims of this study were to examine the effects of muscle activity on excitability of muscles and to test whether the reported activity-induced recovery of force in muscles depressed by high [K⁺]o (18) relates to an improved excitability of the muscle fibers. Some of the results were presented previously in a preliminary form (19, 20).

MATERIALS AND METHODS

Animals. All handling and use of animals complied with Danish animal welfare regulations. All experiments were carried out using isolated soleus muscles from 4-wk-old male or female Wistar rats weighing 60–70 g (own breed). The rats were fed ad libitum and were maintained at a constant temperature (21°C) and day length (12 h).

Muscle preparation and buffers. Animals were killed by decapitation. Muscles were dissected out with tendons intact. The bony attachment of the proximal end of the muscle and ~10 mm of the nerve were left attached. The cross-sectional area over the midportion of the muscles was 1.6 mm², and the average weight of the preparation was 23 mg. The muscles were measured 2.3 cm from tendon to tendon when mounted at optimal length. The standard incubation medium was Krebs-Ringer (KR) bicarbonate buffer containing (in mM) 122 NaCl, 25 NaHCO₃, 2.8 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄, 1.3 CaCl₂, and 5.0 D-glucose (standard KR). The buffer was maintained at 30°C and equilibrated with a mixture of 95% O₂ and 5% CO₂ (pH = 7.4) throughout the experiments. After preparation, muscles were equilibrated in this buffer for at least 30 min before starting the experiment. To produce buffers with 10 mM K⁺, KCl was added, and an equal amount of NaCl was withheld to keep the osmolarity constant. All chemicals used were of analytic grade. D-Tubocurarine and propranolol were purchased from Sigma Chemical (St. Louis, MO).
Mounting and incubation of muscles. As shown in Fig. 1 the combined recording of contractile force, M waves, and membrane potential was performed using a thermostatically controlled chamber (set to 30°C) where the muscles were mounted horizontally with their tendons intact. At the proximal end of the muscle, the tibial bone was fixed between two metal rods, and at the distal end a metal hook was inserted in the Achilles tendon. The metal hook had a nylon wire attached, which was connected to the force transducer via a pulley. The volume of the muscle chamber was 30 ml, and buffer was flowing through at a rate of 15 ml/min from and to a reservoir. The buffer was equilibrated with a 5% CO₂-95% O₂ gas mixture. Changing buffers was done by emptying the flow chamber and replacing with the new buffer, and at the same time the buffer in the reservoir was replaced. This was done to avoid mixing of the buffers and to ensure immediate change. In some experiments, where membrane potentials were not recorded, the muscles were mounted vertically in another chamber as previously described (21).

Force development and electrical stimulation. Isometric force development was measured using a force displacement transducer (Grass FTO3) calibrated with standard weights and was recorded both with a chart recorder and digitally on a computer. Muscles were adjusted to optimal length, and initial tests of contractility were performed. Tetanic contractions were evoked by trains of 1.5-s duration at 30 Hz. The duration of the train used assured complete development of tetanic force also in the modified buffers. A stimulation frequency of 30 Hz was chosen because it is close to the maximal motor unit discharge rates observed for soleus muscles in vivo (4, 12). Control measurements showed that at 30 Hz, the maximum fluctuations in force were 0.4 ± 0.3% (n = 4) of the force produced, indicating that the contractions were close to smooth tetani. At the start of each experiment, the muscles were equilibrated in standard KR buffer for 30 min without stimulation. Next, three to four tetanic control contractions (1.5 s, 30 Hz) were elicited at 5-min intervals, and the average force of these contractions was used as the control value.

Muscles were stimulated with constant current pulses by an eight-channel programmable stimulator (Master 8; AMPI) through a stimulus isolator (ISU 165; Cibertec). Nerve stimulation was elicited through a glass suction electrode with a tip diameter of 350 μm closely fitting the nerve twig. The anode was inside the glass tube, and the cathode was coiled around the outside of the glass tube. If not otherwise noted, fixed current pulses of 5 mA and 0.2-ms duration were used, which was found to be supramaximal for stimulation of the motor nerve fibers without producing any direct stimulation of muscle fibers. In some experiments, field stimulation was applied through two platinum wire electrodes passing current over the central part of the muscle.

Fig. 1. Diagram of the setup for measurements of force, M wave, and membrane potential (E_m). The muscle-nerve preparation was mounted in a thermostatically controlled chamber containing oxygenated Krebs-Ringer (KR) buffer. The nerve twig was stimulated through a suction electrode. Surface M wave electrodes were placed in contact with the muscle. Recordings of membrane potential were performed with a microelectrode inserted in a muscle fiber. M wave, membrane potential, and force signals were filtered, amplified, and stored digitally. A/D, analog to digital; PC, personal computer.
**RESULTS**

**Effects of muscle activity on M wave configuration at high or normal [K+]o.** Soleus muscles were preincubated for 30 min in standard KR buffer containing 4 mM K+. After control tetanic force and M waves were recorded (1.5-s trains at 30 Hz), the muscles were exposed to 10 mM K+ for 60 min during which they received no electrical stimulation. Figure 2 shows that after this treatment the tetanic force and M wave area had decreased to 23 ± 4 and 24 ± 4%, respectively, of the control value in standard KR buffer. When trains of electrical stimulation (1.5 s, 30 Hz) were subsequently given at 1-min intervals, tetanic force and M wave area recovered to 81 ± 2 and 90 ± 6% of the control levels, respectively, within 15 min. The time courses of the recovery of M wave area and tetanic force were closely similar. When corresponding data for tetanic force and M wave area from all six muscles were plotted against each other, a significant linear correlation was obtained (r = 0.94). As shown in Table 1, the 60-min incubation at 10 mM K+ also reduced twitch force and propagation velocity considerably. After the muscles were stimulated at 1-min intervals for 15 min, the twitch force had recovered completely to control levels. The recovery of propagation velocity was also significant although incomplete (Table 1).

During the excitation-induced recovery of M waves, there was an appearance of double-peaked M waves measured in the extrajunctional region as shown in Fig. 3. This phenomenon appeared in all muscles tested between 1 and 4 min after starting the 1-min interval stimulation. The double-peaked M waves fused into a single-peaked M wave within 7 min after the start of the stimulation at 1-min intervals.

In a series of experiments with a time course identical to the one shown in Fig. 2, muscles were incubated for 60 min without stimulation in standard KR (as opposed to high [K+]o) and subsequently were stimulated tetanically (30 Hz for 1.5 s) at 1-min intervals. In these experiments, no significant changes in twitch force, tetanic force, M wave amplitude, or area were observed (n = 5, data not shown).

**Effects of M wave electrode location.** The M wave recordings described above were obtained from the
electrode placed at the extrajunctional region of the muscle (see Fig. 1). However, the changes in M wave shape depended on the location of the recording electrode relative to the innervation zone of the muscle. As shown in Table 1, recordings obtained from the recording electrode placed in the junctional region showed no significant decrease in M wave area after a 60-min incubation in 10 mM K\(^+\). The amplitude of the M waves was, however, considerably reduced, but this was compensated for by an increase in M wave width; therefore, no significant decrease in M wave area was observed (Fig. 4). Compared with this, the M waves recorded in the extrajunctional region showed a much more pronounced decrease in amplitude and, combined with only a small increase in M wave width, the final result of the 60-min incubation in 10 mM K\(^+\) was a 76% decrease in M wave area. In both cases, tetanic stimulation of the muscles at 1-min intervals led to a partial recovery of both M wave amplitude and width (Fig. 4).

Resting membrane potential. Because the Na\(^+\)-K\(^+\) pump is electrogenic, it could be anticipated that activating the pump would result in a hyperpolarization. As shown in Fig. 5, a 60-min incubation at 10 mM K\(^+\) led to a substantial depolarization of the muscle fibers that was partially recovered when the muscles were stimulated tetanically (30 Hz for 1.5 s) at 1-min intervals for 10–15 min. When the muscles were returned to standard KR buffer, the resting membrane potential recovered completely to control values within 20 min (data not shown). In another series of experiments where muscles were incubated in standard KR buffer for 60 min without stimulation, there was also a significant depolarization, albeit considerably smaller than the depolarization seen at 10 mM K\(^+\). When these muscles were subsequently stimulated every minute for 15 min, a significant repolarization was observed (Fig. 5) indicating that, also at normal [K\(^+\)], muscle activity can increase the electrogenic effect of the Na\(^+\)-K\(^+\) pump.

Effect of tubocurarine on force recovery induced by nerve stimulation. Although substantial evidence argues for a central role for the Na\(^+\)-K\(^+\) pump in the excitation-induced force recovery, less is known about the exact mechanism through which excitation leads to increased activity of the Na\(^+\)-K\(^+\) pump. Kuiack and McComas (16) have shown that in rat soleus a local spread of some compound released from nerve endings in the muscle may contribute to the excitation-induced hyperpolarization by activating the Na\(^+\)-K\(^+\) pump.

### Table 1. Effects of high [K\(^+\)]\(_o\) and repeated electrical stimulation on junctional and extrajunctional M wave area and twitch force

<table>
<thead>
<tr>
<th></th>
<th>Junctional</th>
<th>Extrajunctional</th>
<th>Twitch Force, g</th>
<th>Conduction Velocity, m/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control period (4 mM K(^+) KR)</td>
<td>4.2±0.6</td>
<td>5.6±0.5</td>
<td>7.6±0.5</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>60 min Rest (10 mM K(^+) KR)</td>
<td>3.6±0.6</td>
<td>1.3±0.3*</td>
<td>3.3±0.6*</td>
<td>0.8±0.05*</td>
</tr>
<tr>
<td>15 min Tetanic stimulation</td>
<td>3.7±0.7</td>
<td>4.9±0.5†</td>
<td>8.4±0.6†</td>
<td>1.3±0.05†</td>
</tr>
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Values are means ± SE; n = 6 muscles in all groups. KR, Krebs-Ringer. Data are from the same experiment as depicted in Fig. 2. The M waves were recorded at the time points corresponding to points a (control), b (60 min rest), and c (tetanic stimulation) in Fig. 2. *Significantly different from control period, paired t-test, P < 0.05. †Significantly different from 60 min rest, paired t-test, P < 0.05.
test whether a similar mechanism contributes to the excitation-induced recovery of force in muscles exposed to elevated $[K^+]_o$, the ability of nerve stimulation per se to produce force recovery was examined in muscles exposed to 10 mM $K^+$ for 90 min. In these experiments, the recovery of force elicited by nerve stimulation was tested by comparing the tetanic force elicited by direct field stimulation of the muscle fibers before and after a 15-min period with tetanic stimulation of the nerve at 1-min intervals (see Fig. 6 for details). The nerve was stimulated with pulses of either 0.02 or 2 ms duration. Sakaguchi et al. (23) have demonstrated that electrical stimulation of the mixed nerve of rat soleus muscles using pulse intensities three times the threshold for the motor nerve fibers fails to excite most sensory fibers. Thus stimulation with 0.02-ms pulses presumably only excited the motor fibers, whereas 2-ms pulses most likely excited both motor and sensory fibers in the nerve. Figure 6A shows that when 0.02-ms pulses were used, 15 min of nerve stimulation at 1-min intervals led to a significant recovery of tetanic force (tested with direct stimulation) from $84 \pm 2$ to $91 \pm 3\%$ of control force at 4 mM $K^+$. However, the recovery of force was prevented completely by the addition of $10^{-5}$ M tubocurarine, which fully blocked force production during nerve stimulation (Fig. 6A). In contrast, in experiments where the nerve was stimulated with 2-ms pulses (Fig. 6B), a significant recovery of force (again tested with direct stimulation) was produced both in the absence and in the presence of tubocurarine.

The recovery of force elicited by nerve stimulation was not prevented by preincubation of the muscles with $10^{-6}$ M propranolol (data not shown).

**DISCUSSION**

Repeated electrical stimulation has previously been shown to induce considerable force recovery in rat muscles depressed by high $[K^+]_o$ (18). The present data demonstrate that this effect of excitation is caused by a marked recovery of excitability. Thus the results confirm the hypothesis that muscle activity in itself is a potent regulator of muscle excitability.

Consistent with previous observations on skeletal muscle (14, 15, 21), the exposure of resting rat soleus muscles to high $[K^+]_o$ led to depolarization, broadening of the M wave, slowing of the action potential conduction velocity, and to a reduction in contractile force. Moreover, the area of the extrajunctional M wave was reduced. Because the area of M waves recorded in the junctional region was affected much less, the change in area of the extrajunctional M wave could not be related to a failure of the neuromuscular transmission but was most likely caused by a reduction in the ability of the muscle fibers to propagate the action potentials from the neuromuscular end plate to the more distal parts of the fibers. This idea is supported by the observed decrease in the conduction velocity of the action potential and by the finding that extrajunctional regions have a lower content of voltage-activated Na$^+$ channels (2); therefore, more current is required to generate an action potential here than at the innervation zone (22,
wave area was observed. Interestingly, there was an occurrence of double-peaked M waves in the initial phase of the excitation-induced recovery. It is likely that the two separated peaks represent two subpopulations of fibers with different sensitivity to high K⁺. In the population with the highest sensitivity (seen as the peak with the longest latency), the exposure to 10 mM K⁺ led to the largest decrease in propagation velocity, as estimated from the change in time to peak. However, because the area of the M wave from this population of fibers was reduced effectively to zero, the M wave recordings made after 60 min at high K⁺ only showed a single peak. During repeated excitation, however, the most sensitive population also displayed the largest recovery of propagation velocity, and after 15 min, the two M waves had fused to a single peak. It is known that fast-twitch muscles are more resistant to high [K⁺]₀ (6) and that soleus muscles from 4-wk-old rats contain ~30% fast-twitch fibers (10, 25). Therefore, the less K⁺-sensitive population seen in the M wave signal (short latency peak) may be identical to the fast fibers present in the muscle, and the more K⁺-sensitive population may be identical to the slow-twitch fibers.

The excitation-induced recovery of M wave and force was most likely secondary to a repolarization of the muscle fibers, although the recovery of the membrane potential was incomplete. This interpretation is in keeping with the previous observations of Cairns et al. (5), who showed that there is a very steep relationship between membrane potential and tetanic force in muscles exposed to high [K⁺]₀ in the range between ~55 and ~65 mV but no further increase in force at membrane potentials more hyperpolarized than ~65 mV (5). This suggests that the excitation-induced increase in membrane potential from 61.9 to 68.5 mV observed in the present study is sufficient to explain the recovery of M wave area and force.

In concord with previous reports (13, 16), an excitation-induced hyperpolarization was also observed in muscles that had been incubated without stimulation for 60 min at 4 mM [K⁺]₀. In these muscles, incubation at rest led to a minor depolarization (3–4 mV) that was recovered completely after 15 min of tetanic stimulation at 1-min intervals. Thus both at normal and at elevated [K⁺]₀, the maintenance of the membrane potential depends in part on muscle activity, with the effect being more pronounced at elevated [K⁺]₀. One implication of this activity-induced hyperpolarization is that excitability can be maintained in active muscles even at quite high levels of extracellular K⁺ (13, 18). Interestingly, in the hyperkalemic periodic paralysis disorder, patients experience paralysis and high K⁺ levels during attacks. The paralysis but not the hyperkalemia can be prevented by maintaining muscle activity in general or even in localized muscle groups if these are kept active (9). It is possible that this phenomenon relates to the activity-induced hyperpolarization described here.

There is substantial evidence that the excitation-induced hyperpolarization is caused by an increase in

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Fig. 6. Effect of nerve stimulation on the tetanic force elicited by direct stimulation of the muscle fibers in muscle nerve preparations exposed to 10 mM K⁺. The muscle nerve preparation was incubated in 4 mM K⁺ (NKR) for 30 min, and tetanic control contractions (indicated by C on the x-axis) were tested with direct field stimulation of the muscle fibers using pulses of 1-ms duration and 12 V applied at 30 Hz for 1.5 s. Next, the preparation was incubated in 10 mM K⁺ for 60 min, with (●) or without (■) the addition of 10⁻⁵ M tubocurarine, whereafter tetanic contraction was tested again with direct field stimulation. After another 30 min of incubation, the nerve of the preparation was stimulated tetanically via a suction electrode (30 Hz for 1.5 s) with 1-min intervals for 15 min using 5-mA pulses of either 0.02 (A)- or 2 (B)-ms duration. As indicated, this stimulation produced no contraction in the tubocurarine-treated muscles, which demonstrates that excitation of the muscle fibers only took place via the nerve. After the 15 min of stimulation, tetanic force was tested again by direct field stimulation of the muscle fibers using 1-ms pulses at 12 V applied at 30 Hz for 1.5 s. ● and ■, force elicited by nerve stimulation. Force production is given as a percentage of the control force obtained at the start of the experiment with direct stimulation at 4 mM K⁺. All values are means ± SE for groups of 4 or 5 muscles. ∗Force is significantly higher than the force produced after 60 min in 10 mM K⁺ (P < 0.05, paired t-test).

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Thus, in the extrajunctional region, the propagation of action potentials may be more susceptible to depolarization, reductions in the Na⁺/K⁺ gradients, or other changes that affect Na⁺-channel function.

When muscles depressed by 10 mM K⁺ were excited repeatedly, a simultaneous recovery of force and M

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24). Thus, in the extrajunctional region, the propagation of action potentials may be more susceptible to depolarization, reductions in the Na⁺/K⁺ gradients, or other changes that affect Na⁺-channel function.
the activity of the electrogenic Na⁺-K⁺ pump (11, 13, 16, 17). In contrast, much less is known about the mechanism that causes the increase in the activity of the Na⁺-K⁺ pump in active muscles. We have shown previously that, with a stimulation regimen like the one used in the present study, the increase in Na⁺-K⁺ pump activity takes place without an increase in [Na⁺]ᵢ of the muscle fibers and may instead be related to a local release of an agent that can activate the Na⁺-K⁺ pump (17, 18).

Provided a pulse duration of 2 ms was used, the recovery of force in muscles at high [K⁺]ₒ could in the present study be elicited by electrical stimulation of the nerve even if the neuromuscular junction and thus muscle contraction were blocked by tubocurarine. This shows that excitation of the nerve per se produces force recovery in muscle depressed by high [K⁺]ₒ, possibly via a release of some compound that activates the Na⁺-K⁺ pump in the muscle fibers. This finding tallies with the observations of Kuiack and McComas (16) who showed that excitation of some of the muscle fibers in a rat soleus muscle would produce hyperpolarization both in the contracting and in the noncontracting fibers. This effect was attributed to the local spread of a compound released from nerve endings in the muscle. In contrast to the experiments with 2-ms pulses, tubocurarine completely prevented the force recovery in preparations where the nerve was stimulated with 0.02-ms pulses. Because the pulses of 0.02-ms duration presumably only stimulate motor nerve fibers (23), this could indicate that the activation of the Na⁺-K⁺ pump after stimulation of the nerve with 2-ms pulses was related to antidromic electrical stimulation of the sensory nerve fibers.

Because force recovery elicited by nerve stimulation with 0.02-ms pulses was inhibited by tubocurarine, it was most likely related to muscle contraction. Interestingly, it was previously found that, when rat soleus muscles are stimulated via the nerve with pulses of 0.02-ms duration, tubocurarine also prevents the excitation-induced stimulation of the Na⁺-K⁺ pump (17). Thus excitation of the nerve with 0.02-ms pulses per se does not lead to stimulation of muscle Na⁺-K⁺ pumps or to force recovery in muscles exposed to elevated [K⁺]ₒ. If muscles are contracting, however, both stimulation of the Na⁺-K⁺ pump and force recovery take place. The present study does not provide an explanation for this effect of muscle contraction, but one possibility is that muscle contraction per se led to excitation of sensory nerve endings, e.g., in response to mechanical or chemical stimuli. Thus both the Na⁺-K⁺ pump activation induced by muscle contraction and the Na⁺-K⁺ pump activation induced by nerve stimulation may be explained by a release of some compound from sensory nerves in the muscle.

Several hormones and neurotransmitters, including β-agonists and calcitonin gene-related peptide (CGRP), have been shown to stimulate the Na⁺-K⁺ pump (1, 7, 8). Because propranolol was unable to block the recovery of force in muscles at high [K⁺]ₒ, it is unlikely that the effect was caused by a β-agonist. At variance with this, Kuiack and McComas (16) found that the excitation-induced hyperpolarization in rat soleus was almost completely blocked by the β-antagonist propranolol. The reason for this discrepancy remains unclear. Another possible candidate is CGRP, which during excitation is released mainly from sensory nerve endings in the muscle (23). The involvement of CGRP is supported by the finding that prior CGRP depletion of muscles reduces the excitation-induced force recovery (18).

In conclusion, the present study has shown that the recovery of force induced by reducing the intervals between tetanic stimulation is due to improved excitability. This effect is secondary to Na⁺-K⁺ pump activation perhaps caused by the release of some compound from sensory nerve fibers in response to muscle contraction.

**Perspectives**

Muscle excitation is associated with a number of events that affect the membrane function and thus the membrane excitability. In particular, muscle activity leads to increased efflux of K⁺ and an ensuing rise in [K⁺]ᵢ. Because this may depolarize the membrane and thus decrease excitability, it has been proposed to be an important cause of muscle fatigue.

The present work indicates, however, that the capacity of skeletal muscles for regulating the activity of the Na⁺-K⁺ pump and thereby its electrogenic contribution to the membrane potential provide them with an intrinsic mechanism for maintaining membrane excitability in face of elevated [K⁺]ₒ. The change in Na⁺-K⁺ pump activity seems to be controlled by the degree of muscle activation partially mediated via the release of compounds from sensory nerve endings in response to muscle activity. Thus skeletal muscles may be able, via modulations in their own activity, to regulate membrane excitability. One physiological implication of this is that during intense exercise the fatiguing effect of increased [K⁺]ₒ may be postponed or even prevented by stimulation of the Na⁺-K⁺ pump in response to muscle activity.

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