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Excitation-induced cell damage and β₂-adrenoceptor agonist stimulated force recovery in rat skeletal muscle

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Mikkelsen, Ulla Ramer, Hanne Gissel, Anne Fredsted, and Torben Clausen. Excitation-induced cell damage and β₂-adrenoceptor agonist stimulated force recovery in rat skeletal muscle. Am J Physiol Regul Integr Comp Physiol 290: R265–R272, 2006. First published October 6, 2005; doi:10.1152/ajpregu.00392.2005.—Intensive exercise leads to a loss of force, which may be long lasting and associated with muscle cell damage. To simulate this impairment and to develop means of compensating the loss of force, extensor digitorum longus muscles from 4-wk-old rats were fatigued using intermittent 40-Hz stimulation (10 s on, 30 s off). After stimulation, force recovery, cell membrane leakage, and membrane potential were followed for 240 min. The 30–60 min of stimulation reduced tetanic force to ~10% of the prefatigue level, followed by a spontaneous recovery to ~20% in 120–240 min. Loss of force was associated with a decrease in K⁺ content, gain of Na⁺ and Ca²⁺ content, leakage of the intracellular enzyme lactic acid dehydrogenase (10-fold increase), and depolarization (13 mV). Stimulation of the Na⁺-K⁺ pump with either the β₂-adrenoceptor agonist salbutamol, epinephrine, rat calcitonin gene-related peptide (tCGRP), or dibutyryl cAMP improved force recovery by 40–90%. The β-blocker propranolol abolished the effect of epinephrine on force recovery but not that of CGRP. Both spontaneous and salbutamol-induced force recovery were prevented by ouabain. The salbutamol-induced force recovery was associated with repolarization of the membrane potential (12 mV) to the level measured in unfatigued muscles. In conclusion, in muscles exposed to fatiguing stimulation leading to a considerable loss of force, cell leakage, and depolarization, stimulation of the Na⁺-K⁺ pump induces repolarization and improves force recovery, possibly due to the electrogenic action of the Na⁺-K⁺ pump. This mechanism may be important for the restoration of muscle function after intense exercise.

INTENSIVE, UNACCUSTOMED exercise leads to a loss of force, which may be long-lasting. This functional defect may be attributed to the following: 1) rundown of transmembrane Na⁺-K⁺-gradients and depolarization leading to loss of excitability (2, 17); 2) loss of energy supplies (14); 3) impaired release of Ca²⁺ from the sarcoplasmic reticulum or reduced myofibrillar Ca²⁺ sensitivity (6, 31, 34); 4) intracellular accumulation of metabolites, protons, or inorganic phosphate (14); and 5) longer-lasting leaks in the sarcolemma, leading to loss of excitability.

We have previously shown that in isolated rat skeletal muscle, intermittent electrical stimulation (40 Hz, 10 s on, 30 s off) for 30–60 min elicits cell membrane leakage, as assessed from increased [¹⁴C]sucrose space, loss of the intracellular enzyme lactic acid dehydrogenase (LDH), and loss of K⁺. This was associated with a considerable reduction in tetanic force lasting for at least 240 min (22). In another study on rat muscles, we found that sarcolemmal leaks induced by electroporation and documented by increases in [¹⁴C]sucrose space and LDH release were associated with a graded and partly reversible loss of force (11). The force recovery of these electroporated muscles was markedly improved by stimulating the Na⁺-K⁺ pumps with epinephrine, norepinephrine, the β₂-adrenoceptor agonist salbutamol, or calcitonin gene-related peptide (CGRP). Conversely, inhibition of the Na⁺-K⁺ pumps with ouabain suppressed both the spontaneous force recovery and that induced by Na⁺-K⁺ pump stimulation. It was surprising that Na⁺-K⁺ pump stimulation increased the rate of force recovery even in muscles showing evidence of cellular leakages. In this way, stimulation of the Na⁺-K⁺ pumps apparently compensates for the sarcolemmal leakages elicited by electroporation, restoring the resting membrane potential, and improving force development.

Salbutamol has been shown to enhance isotonic contractile properties of rat diaphragm muscle (30). In fatigued diaphragm muscles, force recovery was improved by isoproterenol (18) and selective β₂-agonists (1, 12, 29). In the present study, effects of the β₂-agonists epinephrine and salbutamol and CGRP on force recovery in fatigued and damaged muscles are investigated. The β₂-agonists act via β₂-adrenoceptors and G protein to activate adenylyl cyclase in the cell membrane. Adenylyl cyclase then converts ATP to cAMP, thus increasing the intracellular concentration of cAMP, which in turn stimulates the Na⁺-K⁺ pumps via protein kinase A (8). CGRP increases the intracellular concentration of cAMP (8) via binding to specific CGRP receptors. In this way, CGRP stimulates the Na⁺-K⁺ pump via the same second messenger as the β₂-agonists. Therefore, the effects of CGRP on fatigued muscles are also investigated.

To examine the specificity of the receptors mediating the effects, the β-antagonist propranolol is used to block the β₂-adrenoceptors in experiments with epinephrine and CGRP. The effect of direct addition of cAMP is tested as well. The dibutyryl-derivative of cAMP (db-cAMP) more readily gains access to the cytoplasm and is used for these experiments.

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Furthermore, the influence of salbutamol on sarcolemmal integrity evidenced by the release of LDH is investigated. The focus of this paper is the force loss after fatiguing electrical stimulation, the subsequent recovery of force, and the extent to which simultaneous stimulation of four muscles in incubation chambers can compensate the commonly occurring muscle fatigue seen after heavy workload or exercise, and restore force. Cell membrane leakages frequently arise in skeletal muscles during strenuous work, but also after bruises or electrical shocks. It was of general interest, therefore, to test the working hypothesis that the Na\(^+\)-K\(^+\) pumps contribute to the restoration of contractility/force generation in muscles that have lost cell membrane integrity or have a functional defect after fatiguing stimulation.

Some of the results of this study have been presented in a preliminary version (21).

**MATERIALS AND METHODS**

**Animals**

All experiments were carried out using fed 4-wk-old female or male Wistar rats (own breed) weighing 60–70 g. Animals of this size were chosen to obtain muscles of a relatively small size to improve diffusion and oxygenation during incubation. The rats had free access to food and water and were maintained at a constant temperature (21°C) with constant day length (12 h). The animals were handled and maintained in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. The animal facilities were checked by the Danish Inspectorate for Experimental Animals and the Animal Welfare Officer of the Medical Faculty of the University of Aarhus. All handling and use of animals complied with APS Guiding Principles in the Care and Use of Animals.

**Muscle Preparation and Incubation**

Animals were killed by cervical dislocation followed by decapitation and intact extensor digitorum longus (EDL) muscles, weighing 20–30 mg, were excised, as previously described (7). The standard incubation medium was a Krebs-Ringer bicarbonate buffer (pH 7.2–7.4) containing (in mM) 122.1 NaCl, 25.1 NaHCO\(_3\), 2.8 KCl, 1.2 KH\(_2\)PO\(_4\), 1.2 MgSO\(_4\), 1.3 CaCl\(_2\), and 5.0 D-glucose. After preparation, the muscles were mounted for isometric contractions on muscle holders with electrodes on either side of the central part of the muscle. The buffer was continuously gassed with a mixture of 95% O\(_2\)-5% CO\(_2\). All muscles were equilibrated for a minimum of 30 min in the standard medium before further treatment. This procedure has been shown to allow the maintenance of constant membrane potential and intact extensor digitorum longus (EDL) muscles, weighing 30–50 mg, were exposed to 30 min of fatiguing stimulation. Immediately after the end of stimulation, the muscles were moved to the membrane potential measurement setup and mounted at resting length. The chamber was perfused with Krebs-Ringer bicarbonate buffer, continuously gassed with a mixture of 95% O\(_2\)-5% CO\(_2\). The first membrane potential recording was performed ~15 min after the end of stimulation. Contralateral control muscles were mounted in the transducers, and force was tested, but they were not fatigued before the recording of membrane potential. The effect of Na\(^+\)-K\(^+\) pump stimulation was examined in both resting and fatigued muscles by the addition of salbutamol (10\(^{-5}\) M) 130 min after the muscles had been removed from the force transducer.

**Fatiguing Protocol**

EDL muscles were exposed to fatiguing stimulation using a protocol previously shown to elicit muscle cell damage and long-lasting loss of force (22). This standard fatiguing stimulation protocol was applied in all experiments. The muscles were stimulated intermittently (10 s on, 30 s off) at 40 Hz (1-ms pulses of 10 V). Stimulation was applied through platinum electrodes on either side of the central part of the muscle. Different groups of muscles were stimulated for 0, 5, 10, 30, or 60 min. Because the distance between the stimulation electrodes was 0.4 cm, 10-V pulses correspond to an electrical field of 25 V cm\(^{-1}\).

**Force Measurement**

The experimental setup used for force measurements allowed for the simultaneous stimulation of four muscles in incubation chambers containing 8 or 23 ml buffer. EDL muscles were mounted on a force displacement transducer (Grass FTO3; W. Warwick, RI) and during repeated stimulation with single pulses adjusted to optimal length for twitch force development. Force was recorded with chart recorders (Servogor; Kolding, Denmark) and a PowerLab data acquisition system (ADI Instruments). Before stimulation, all four muscles were tested at 90 Hz (1-ms pulses of 10 V) for 0.5 s to achieve maximal tetanic activation of all muscle fibers. Testing was done three times at 15-min intervals before the fatiguing stimulation, and the mean of these force determinations was defined as the initial force level. The mean initial force was 452 mN [59 (SD), n = 72]. Force recovery in each group of muscles is given as % initial force of the same muscles. The muscles were then allowed to rest or were fatigued for 5, 10, 30, or 60 min. Force development was recorded during the fatiguing stimulation and, as previously described (22), declined by 88% within 10 min (data not shown). Subsequently, force recovery was tested at 90 Hz (0.5-s pulse trains) at intervals up to 240 min after cessation of stimulation. Force was on average 12% immediately after 30-min fatiguing stimulation and 8% immediately after 60-min fatiguing stimulation. At different time points, epinephrine (10\(^{-8}\) M–10\(^{-5}\) M), salbutamol (10\(^{-5}\) M), db-cAMP (1 mM), or CGRP (10\(^{-7}\) M) were added to evaluate the effect of Na\(^+\)-K\(^+\) pump stimulation on force recovery. In some experiments, propranolol (10\(^{-5}\) M) or ouabain (10\(^{-3}\) M) were added before these agents to test their specificity. In experiments with epinephrine at concentrations <10\(^{-5}\) M, ascorbic acid (1 mM) and EDTA (0.05 mM) were added to the buffer to prevent metabolic degradation of epinephrine. These agents were also added to the controls.

**Membrane Potential Recordings**

Resting membrane potentials were recorded using standard electrophysiological techniques as previously described in detail (25). In brief, each muscle was impaled by a glass microelectrode filled with 3 M KCl (tip resistance, 15–35 MΩ) and the potential, recorded via an Axcopamp-2A amplifier, was displayed on an oscilloscope and a chart recorder. The muscle bath was grounded with an Ag/AgCl wire. In each muscle, ~10 fibers were impaled. To avoid measuring from the same fiber twice, the electrode was moved a small distance across the muscle between each impalement. EDL muscles were exposed to 30 min of fatiguing stimulation. Immediately after the end of stimulation, the muscles were moved to the membrane potential measurement setup and mounted at resting length. The chamber was perfused with Krebs-Ringer bicarbonate buffer, continuously gassed with a mixture of 95% O\(_2\)-5% CO\(_2\). The first membrane potential recording was performed ~15 min after the end of stimulation. Contralateral control muscles were mounted in the transducers, and force was tested, but they were not fatigued before the recording of membrane potential. The effect of Na\(^+\)-K\(^+\) pump stimulation was examined in both resting and fatigued muscles by the addition of salbutamol (10\(^{-5}\) M) 130 min after the muscles had been removed from the force transducer.

**LDH Release**

As an indicator of cellular integrity, the release of LDH from the muscles into the incubation medium was determined, as previously described (15). In short, after being mounted on muscle holders in 5-ml chambers but before measurement, the muscles were prewashed 4 × 30 min to remove LDH released from cells damaged during excision of the muscles. Buffer samples for determination of the level of LDH release before the fatiguing stimulation were taken from the last of the prewash tubes. During recovery after the fatiguing stimulation, the muscles were moved to new tubes every 30 min. Buffer samples were taken immediately after removal of the muscle, and BSA was added to a final concentration of 0.1%. A 250-μl sample was mixed with 2.65 ml phosphate buffer (0.1 M K\(_2\)HPO\(_4\), titrated with KH\(_2\)PO\(_4\) to pH 7.0) containing NADH (0.3 mM) and pyruvate (0.8 mM), and the decline in the absorbance of the substrate NADH...
was monitored at 340 nm (Lambda 20, Perkin Elmer) at 30°C. Activity of LDH was expressed as units per gram wet weight per 30 min, 1 unit being the amount of enzyme that catalyzes the use of 1 µmol substrate/min.

Ca²⁺, Na⁺, and K⁺ Contents

At the end of the experiment, the tendons were removed and the muscles were blotted, weighed, and soaked overnight in 3 ml 0.3 M TCA to extract all ions. Previous studies showed that this procedure was as efficient in extraction of ions as homogenization and subsequent centrifugation of the TCA extract (10, 15). Ca²⁺ content was determined by atomic absorption spectrophotometry (Solaar AAS; Thermo Elemental, Franklin, MA) using 1.5 ml of the TCA extract mixed with 150 µl of 0.27 M KCl. The muscle extracts were measured against a blank and standards containing 12.5 or 25 µM Ca²⁺ and the same amount of TCA and KCl. Na⁺ and K⁺ contents of the TCA extracts were determined with the use of a FLM3 flame photometer (Radiometer, Copenhagen, Denmark) with lithium as the internal standard. For each 0.5-ml sample of the TCA extract, 1.5 ml of 5 mM LiCl and 0.5 ml of 0.3 M TCA were added.

Chemicals

All chemicals were of analytical grade. Salbutamol (racemic), db-cAMP, and ouabain were purchased from Sigma (St. Louis, MO); propranolol (racemic) was from Ferrostan (Soeborg, Denmark); NADH and pyruvate were from Boehringer-Mannheim (Mannheim, Germany). (R-isomer) Epinephrine was from Pharmacies of Danish Hospitals; tCGRP was from Bachem AG (Bubendorf, Switzerland). Ascorbic acid was from Merck (Albertslund, Denmark).

Statistics

Results are given as mean values with SD. The statistical significance of any difference between groups was ascertained using Student’s two-tailed t-test for unpaired observations. All comparisons made were between separate groups of muscles. When required, a one-way ANOVA and a post hoc Student’s t-test were performed to ascertain the significance of the differences between the groups.

RESULTS

Force Recovery

Effect of β₂-agonists. As shown in Fig. 1, 30 min of fatiguing stimulation caused a drop in tetanic force to ~12% of the initial level followed by a slow spontaneous recovery to 20–25%. Addition of salbutamol (10⁻⁵ M) after a 140-min recovery period improved tetanic force by an average of 77% (P = 0.007, at 180- to 240-min recovery) compared with the controls (Fig. 1A). Control experiments with unfatigued muscles showed that the same dose of salbutamol (10⁻⁵ M) induced no significant change in tetanic force (90 Hz, 0.5 s, n = 4 vs. 4 muscles, data not shown). The role of the Na⁺-K⁺ pump was examined using the Na⁺-K⁺ pump inhibitor, ouabain. Ouabain (10⁻³ M), added at 115-min recovery completely abolished spontaneous force recovery in all muscles, and the stimulating effect of salbutamol (10⁻⁵ M, added at 130-min recovery) was alleviated. As shown in Fig. 1B, epinephrine (10⁻⁵ M) added to fatigued muscles after 130-min recovery improved tetanic force by an average of 90% (P = 0.0004, at 180- to 240-min recovery).

Effects of salbutamol on force recovery after less fatiguing stimulation was investigated. As shown in Fig. 2, after 5- and 10-min stimulation, spontaneous force recovery was 63 and 56%, respectively. Addition of salbutamol (10⁻⁵ M) significantly improved force recovery by 30 and 34%, respectively (n = 4–6 muscles, P < 0.01). To examine whether this force recovery was also present at physiological concentrations of epinephrine, the dose-response relation was determined and is shown in Fig. 3. After 30-min stimulation and 240-min recovery, the control muscles regained 20% [3 (SD), n = 7] of their initial force. Epinephrine significantly improved tetanic force compared with control muscles at concentrations down to 10⁻⁸ M. This concentration is close to the plasma level of epinephrine (0.6 × 10⁻⁸ M) reached in running rats (27). Tetanic force (at 240 min) after treatment with the different doses of epinephrine was improved by 47% with 10⁻⁸ M, 74% with 10⁻⁷ M, 95% with 10⁻⁶ M, and 85% with 10⁻⁵ M epinephrine, respectively. These effects were all highly significant. Salbutamol also improved force recovery at a lower concentration.
Thus 10⁻⁷ M induced a 26% increase in force recovery (P < 0.03, n = 12; data not shown).

Increasing the duration of the fatiguing stimulation from 30 to 60 min led to a larger loss of force and a delay in the spontaneous recovery of force. However, as shown in Fig. 4, the addition of salbutamol still markedly improved tetanic force (by 61% at 180- to 240-min recovery; P = 0.01). To examine the receptors mediating the observed effects, the β-antagonist propranolol was added 15 min before epinephrine to block the β-adrenoceptors.

Fig. 5 shows that in muscles fatigued for 30 min, the addition of propranolol (10⁻⁵ M) caused no change in force development but almost completely abolished the effect of the subsequent addition of epinephrine (10⁻⁶ M, added at 130-min
recovery). This confirms that the improvement of force recovery induced by epinephrine is mediated via β-adrenoceptors.

**Effect of rCGRP.** Fig. 6 shows the average level of force recovery reached 160, 180, and 200 min after stimulation in muscles fatigued for 30 min. rCGRP (10⁻⁷ M, added at 130-min recovery) significantly improved tetanic force by 42% (P = 0.01, n = 6). After pretreatment with propranolol (10⁻⁵ M, added at 115-min recovery), rCGRP improved tetanic force by 43% (P = 0.02, n = 6), confirming that rCGRP acts via receptors different from β-adrenoceptors.

**Effect of cAMP.** Catecholamines and CGRP act via an increase in cytosolic cAMP. Therefore, the direct effect of this second messenger was tested. The db-cAMP more readily gains access to the cytoplasm and was used in these experiments. Fig. 7 shows force recovery after 30-min fatiguing stimulation and the effect of db-cAMP. Db-cAMP (1 mM) added at 130-min recovery improved tetanic force by 60% (at 220- to 240-min recovery; P = 0.008).

**Membrane Potential**

As shown in Fig. 8, the membrane potential of the resting controls remained stable around −73 mV for 240 min. In the salbutamol-treated group, the resting membrane potential was a little lower (−75 mV) before salbutamol treatment. Salbutamol caused no significant hyperpolarization. After 30 min of fatiguing stimulation, the first membrane potential recordings (at 15 min) showed values around −62 (control group) and −60 mV (salbutamol group), representing stimulation-induced depolarizations of 11 and 15 mV, respectively. Without treatment, the muscles showed no spontaneous repolarization. After the addition of salbutamol (10⁻⁵ M at 130-min recovery), a significant repolarization of the membrane potential of the stimulated muscles to −72 mV (at 240 min; P = 0.01; n = 4–5) was observed. This is an improvement of 12 mV returning the membrane potential of the stimulated muscles to values not significantly different from the resting controls.

![Fig. 6. Effect of rat calcitonin gene-related peptide (rCGRP) on force recovery after 30-min fatiguing stimulation. Muscles were fatigued as described in MATERIALS AND METHODS. The mean level of force recovery reached at 160-, 180-, and 200-min recovery is given as %initial force. Propranolol (Propr. 10⁻⁵ M) was added at 115-min recovery. CGRP (10⁻⁷ M) was added at 130-min recovery. Significance of the difference from contralateral control muscles without CGRP is given by P value. Mean values with bars denoting SD are given; n = 6 muscles.](http://ajpregu.physiology.org/)

![Fig. 7. Effect of dibutyryl-derivative of cAMP (db-cAMP) on force recovery after 30-min fatiguing stimulation. Muscles were fatigued as described in MATERIALS AND METHODS. After stimulation, force recovery was followed for 240 min and given as %initial force. db-cAMP (1 mM) was added at 130-min recovery. Open symbols represent controls (n = 5); closed symbols represent db-cAMP-treated muscles (n = 6). Significance of difference between treated and control muscles is given by P values. Mean values and SD are given.](http://ajpregu.physiology.org/)

**Membrane Integrity**

After 30–60 min of fatiguing stimulation, the cell membranes are leaky, as evidenced by an increased resting uptake of ⁴⁵Ca, resting ¹⁴C]sucrose space, and release of LDH (22). The present results indicate that despite the damaged membranes, stimulation of the Na⁺-K⁺ pump by hormones, a β₂-agonist and their second messenger cAMP, improves recovery of tetanic force. It was of interest whether stimulation of the Na⁺-K⁺ pump by salbutamol would also have effects on membrane integrity.

After 60 min of fatiguing stimulation, release of the intracellular enzyme LDH was followed for 4 h. As shown in Fig. 9, 60 min of fatiguing stimulation caused a large increase in LDH release beginning during the first 30 min of recovery. Addition of salbutamol (10⁻⁵ M at 120-min recovery) had no effect on the LDH release. Thus the force recovery induced by salbutamol cannot be attributed to resealing of the cell membrane.

**Na⁺, K⁺, and Ca²⁺ Contents**

As shown in Table 1, Na⁺ content was significantly increased, and K⁺ content was significantly decreased after 60-min stimulation and 240-min recovery. None of these changes were affected by the addition of salbutamol (10⁻⁵ M at 120-min recovery). Likewise, the Ca²⁺ content of these muscles was significantly increased, and no change was observed when adding salbutamol.

After 30 min of fatiguing stimulation and 240 min of recovery, significant increases in Na⁺ (P = 0.03) and Ca²⁺ (P < 0.001) contents were observed. No significant changes were observed after the addition of salbutamol (10⁻⁵ M at 120-min recovery).
DISCUSSION

The primary purpose of the present study was to determine whether the loss of contractility induced by fatiguing electrical stimulation might be alleviated by stimulation of the Na\(^+\)-K\(^+\) pumps. The experiments show that acute stimulation of the Na\(^+\)-K\(^+\) pumps induced by catecholamines, CGRP, or the second messenger cAMP, mediating the action of these hormones on the Na\(^+\)-K\(^+\) pump, in all instances elicit a significant improvement of force recovery, both in muscles showing a large spontaneous recovery and in muscles showing a small spontaneous recovery. This recovery can be detected within 10 min after the addition of the examined compounds and lasts for at least 2 h. It is completely suppressed by ouabain, indicating that it depends on the function of the Na\(^+\)-K\(^+\) pumps.

Force recovery elicited by epinephrine is seen down to the concentrations measurable in plasma during running exercise (27). The effect is abolished by propranolol, indicating that it is mediated via \(\beta\)-adrenoreceptors. The control force is not affected by propranolol as seen in Figs. 5 and 6. Because the \(\beta_2\)-agonist salbutamol (10\(^{-5}\) M) induces almost the same maximum force recovery as epinephrine, it is reasonable to assume that the effect of salbutamol is mediated via \(\beta_2\)-adrenoreceptors. In contrast, the force recovery induced by rCGRP is not affected by propranolol, indicating that the effect of this peptide hormone is mediated via its specific receptors.

Fig. 8. Effect of salbutamol on membrane potential after 30-min fatiguing stimulation. Muscles were fatigued as described in MATERIALS AND METHODS. Immediately after stimulation, the muscles were moved to the membrane potential measurement setup and mounted at resting length. Impalements (~10 per muscle) were made, and the average value for each muscle was calculated. Salbutamol (10\(^{-5}\) M) was added at 130-min recovery. Open symbols represent resting muscles, closed symbols represent muscles exposed to 30-min stimulation. Open and closed triangles, salbutamol-treated muscles. Significance of difference between salbutamol treated and untreated muscles is given by \(P\) values. Mean values and SD are given; \(n = 3–4\) muscles.

Fig. 9. Effect of salbutamol on release of the intracellular enzyme lactic acid dehydrogenase (LDH) after 60-min fatiguing stimulation. Muscles were then fatigued as described in MATERIALS AND METHODS. LDH release was followed for 240 min. The muscles were moved to new tubes every 30 min. Immediately after removal of the muscles, buffer samples were taken for determination of LDH activity. Salbutamol (10\(^{-5}\) M) was added at 120-min recovery. Open symbols, resting control muscles \((n = 2)\); filled symbols, stimulated muscles \((n = 5)\); •, stimulation + salbutamol, •, stimulation. Mean values and SD are given.

Finally, cAMP, which is second messenger for the actions of epinephrine and CGRP, caused a similar, albeit slightly later, force recovery.

The long-lasting loss of force induced by fatiguing stimulation may have several causes. Depletion of metabolizable substrates, glycogen, creatine phosphate, and ATP has repeatedly been proposed (for a review, see Ref. 14). It is unlikely, however, that these stores can be repleted by stimulating active Na\(^+\)-K\(^+\) transport, which, in itself, is an energy-consuming process. Accumulation of lactic acid is another frequently mentioned cause of fatigue (14). In the present experimental

<table>
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<th>Na(^+)</th>
<th>K(^+)</th>
<th>Ca(^{2+})</th>
<th>No.</th>
</tr>
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<td>Resting controls</td>
<td>40 (SD 3)</td>
<td>101 (SD 5)</td>
<td>1.8 (SD 0.3)</td>
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<td>Stimulation 30 min</td>
<td>48 (SD 9)</td>
<td>97 (SD 9)</td>
<td>2.6 (SD 0.5)</td>
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<td>*P = 0.03</td>
<td>*NS</td>
<td>*P = 0.0005</td>
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<tr>
<td>Stimulation 30 min + salbutamol at 120 min</td>
<td>48 (SD 16)</td>
<td>94 (SD 15)</td>
<td>2.5 (SD 0.7)</td>
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<tr>
<td>†NS</td>
<td>†NS</td>
<td>†NS</td>
<td></td>
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<tr>
<td>Stimulation 60 min</td>
<td>61 (SD 6)</td>
<td>85 (SD 6)</td>
<td>2.7 (SD 0.5)</td>
</tr>
<tr>
<td>*P &lt; 0.0001</td>
<td>*P = 0.002</td>
<td>*P = 0.006</td>
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<tr>
<td>Stimulation 60 min + salbutamol at 120 min</td>
<td>62 (SD 14)</td>
<td>85 (SD 14)</td>
<td>2.9 (SD 0.6)</td>
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<td>†NS</td>
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Muscles were fatigued as described in MATERIALS AND METHODS for 30 or 60 min. Na\(^+\), K\(^+\), and Ca\(^{2+}\) contents at 240-min recovery were given in micro-moles per gram wet weight. Salbutamol (10\(^{-5}\) M) was added at 120-min recovery when indicated. Na\(^+\), K\(^+\), and Ca\(^{2+}\) contents are total muscle contents. Mean values and SD are given. Significance of differences is given by \(P\) values. *comparison to resting controls; †comparison to stimulated muscles without salbutamol. No., number of animals.
situation with a large volume of incubation medium, however, the lactic acid formed is not likely to be retained for several hours in vitro. Moreover, catecholamines induce an increase in lactic acid production, which cannot explain the force recovery observed. Finally, lactic acid causes no inhibition of contractile force in isolated rat skeletal muscle (23).

Long-term fatigue may also be related to impairment of the excitation-induced increase in cytoplasmic Ca\(^{2+}\) (6, 32, 33). There is no evidence, however, that stimulation of the Na\(^{+}\)-K\(^{+}\) pumps can improve the release of Ca\(^{2+}\) from SR or the subsequent rise in cytoplasmic Ca\(^{2+}\).

On the other hand, it has repeatedly been shown that fatiguing stimulation leads to depolarization of muscle cells, both in vivo (20) and in vitro (2, 17). This may be due to rundown of the transmembrane concentration gradients for K\(^{+}\) and the ensuing reduction in the equilibrium potential for K\(^{+}\). In the present study, fatiguing stimulation for 30 min caused no significant decrease in the K\(^{+}\) content of the muscles, whereas 60 min of stimulation gave a significant decrease. However, after 30 min of fatiguing stimulation, the muscles underwent a depolarization of 11–15 mV, showing no spontaneous repolarization. If salbutamol (10\(^{-5}\) M) was added 130 min after the end of stimulation, a rapid repolarization (12 mV) was observed returning the membrane potential to levels not significantly different from the controls.

It was recently shown that electroporation of isolated muscles led to a large depolarization of the membrane potential. This is most likely due to the increased permeability of the membrane allowing flux of Na\(^{+}\) and K\(^{+}\) down to their electrochemical gradients. After electroporation, there was a large spontaneous repolarization of the membrane potential. However, salbutamol (10\(^{-5}\) M) not only increased the rate of recovery of the membrane potential but also the extent of the recovery. Salbutamol also improved the rate and extent of force recovery after electroporation (11).

The depolarization induced by fatiguing stimulation may also be caused by sarcolemmal damage, an interpretation supported by previous observations of increased [\(^{14}\)C]sucrose space and release of LDH (22). Also in the present study, muscles exposed to 60 min of fatiguing stimulation showed a marked increase in LDH-release. Even in these damaged muscles, salbutamol induced a significant recovery of force. This was not associated with any restoration of total Na\(^{+}\) or K\(^{+}\) contents or changes in LDH release, indicating that stimulation of the electrogenic Na\(^{+}\)-K\(^{+}\) pump in the remaining intact sarcolemma or T-tubular membranes might be sufficient to restore excitability. The restoration of excitability by the addition of salbutamol seems to be achieved by compensation of the cell membrane leakages, because no effect on membrane integrity was observed. This suggests that the stimulation of the Na\(^{+}\)-K\(^{+}\) pump is a local effect that acts to reestablish the ionic gradients and the membrane potential without changing the global content of Na\(^{+}\) and K\(^{+}\) in the muscle fibers.

All of our experiments were performed at 30°C, a temperature that ascertains optimum stability (19, 26). Because the Na\(^{+}\)-K\(^{+}\) pump has a Q10 of 2.3, elevations of the temperature cause marked increase in the pumping rate. Heating of muscles might therefore contribute to the force recovery after fatiguing stimulation.

Burniston et al. (4) have shown a myotoxic effect in vivo of the β2-agonist clenbuterol administered in doses down to 0.1 mg/kg. Assuming an extracellular volume of 20%, this dose would give a concentration in plasma of 1.6 × 10\(^{-5}\) M. In our studies, we have not observed an increase in LDH leakage with salbutamol treatment; however, we cannot rule out that high doses of salbutamol may be myotoxic as well. It should be noted that CGRP induced a significant improvement of force recovery. This is of particular interest because CGRP is stored in nerve endings in skeletal muscle from where it can be released and stimulate the Na\(^{+}\)-K\(^{+}\) pump (9).

In conclusion, fatiguing stimulation leads to severe functional impairment, depolarization, and loss of cellular integrity. Perhaps the most interesting observation of this study is that even in “leaky” muscles, stimulation of the Na\(^{+}\)-K\(^{+}\) pumps with salbutamol induces repolarization, but no recovery of integrity or Na\(^{+}\) and K\(^{+}\) contents. This is accompanied by a partial, but significant, restoration of contractile force, which is likely to reflect improved excitability.

Perspectives

Vigorous physical activity often leads to long-lasting and considerable loss of force. This may in part be attributed to loss of excitability due to depolarization or sarcolemmal leakage. The present results indicate that the ensuing impairment of contractility may be compensated by catecholamines or CGRP, agents that are both available in plasma and skeletal muscles. This information may be used for a therapeutic restoration of muscle performance after intense exercise or muscle damage due to trauma. Moreover, treatment with β2-agonists is widely used by asthmatic patients as bronchodilators to improve respiration. Because these patients often perform fatiguing or exhausting work with their respiratory muscles, the β2-agonists may in addition, compensate the ensuing fatigue in these muscles (1, 12, 30).

A possible ergogenic effect of β2-agonists in athletes has been highly disputed, and the results are inconsistent. Signorile et al. (28) found that acute inhalation of albuterol had an ergogenic effect on short-term power output on a cycle ergometer. Peak power was increased, and fatigue was reduced. With respect to endurance exercise, results are conflicting. Norris et al. (24) found no effect of salbutamol inhalation on time needed to complete a 20-km time trial. Likewise, Goubault et al. (16) found no effect on endurance time at 85% of V\(_{O2\text{max}}\) in a cycle ergometer. On the other hand, Bedi et al. (3) showed that sprint time until exhaustion after 1 h of heavy exercise was increased by pretest albuterol inhalation. In contrast, a study by Carlsen et al. (5) showed reduced running time until exhaustion after salbutamol inhalation.

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