Effects of lactic acid and catecholamines on contractility in fast-twitch muscles exposed to hyperkalemia

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Hansen, Anders Krogh, Torben Clausen, and Ole Bægaard Nielsen. Effects of lactic acid and catecholamines on contractility in fast-twitch muscles exposed to hyperkalemia. Am J Physiol Cell Physiol 289: C104-C112, 2005. First published March 2, 2005; doi:10.1152/ajpcell.00600.2004.—Intensive exercise is associated with a pronounced increase in extracellular K⁺ ([K⁺]o). Because of the ensuing depolarization and loss of excitability, this contributes to muscle fatigue. Intensive exercise also increases the level of circulating catecholamines and lactic acid, which both have been shown to alleviate the depressive effect of hyperkalemia in slow-twitch muscles. Because of their larger exercise-induced loss of K⁺, fast-twitch muscles are more prone to fatigue caused by increased [K⁺]o, than slow-twitch muscles. Fast-twitch muscles also produce more lactic acid. We therefore compared the effects of catecholamines and lactic acid on the maintenance of contractility in rat fast-twitch [extensor digitorum longus (EDL)] and slow-twitch (soleus) muscles. Intact muscles were mounted on force transducers and stimulated electrically to evoke short isometric tetani. Elevated [K⁺]o (11 and 13 mM) was used to reduce force to ~20% of control force at 4 mM K⁺. In EDL, the β₂-agonist salbutamol (10⁻⁵ M) restored tetanic force to 83 ± 2% of control force, whereas in soleus salbutamol restored tetanic force to 93 ± 1%. In both muscles, salbutamol induced hyperpolarization (5–8 mV), reduced intracellular Na⁺ content and increased Na⁺⁻K⁺ pump activity, leading to an increased K⁺ tolerance. Lactic acid (24 mM) restored force from 22 ± 4% to 58 ± 2% of control force in EDL, an effect that was significantly lower than in soleus muscle. These results amplify and generalize the concept that the exercise-induced acidification and increase in plasma catecholamines counterbalance fatigue arising from rundown of Na⁺ and K⁺ gradients.

During exercise, contracting muscles lose K⁺, leading to increased extracellular K⁺ ([K⁺]o) (19, 25). In humans, a plasma concentration of 8–9 mM K⁺ has been reported (32) and in the interstitium of active muscles, [K⁺]o might reach values of 10–12 mM (23, 29, 33). Elevated [K⁺]o leads to depolarization, which has been shown to cause slow inactivation of voltage-dependent Na⁺ channels (42) and to some degree, inactivation of the dihydropyridine receptors (8). Hence, increased [K⁺]o induces a loss of excitability and contractility and thereby potentially contributes to muscle fatigue (2, 9, 28, 41, 43). Recent in vitro studies show that the loss of K⁺ during stimulation is considerably larger in fast-twitch muscles than in slow-twitch muscles (14), indicating that elevated [K⁺]o may contribute more to fatigue in fast-twitch muscles than in slow-twitch muscles.

Intensive exercise also causes an increase in the level of circulating catecholamines (22). Studies (10) on isolated slow-twitch muscles have shown that the depressing effects of high [K⁺]o on muscle contractility can be counteracted by the action of these compounds on muscular β₂-adrenoceptors. β₂-Agonists such as salbutamol stimulate the Na⁺⁻K⁺ pumps (13, 40), which has two implications. First, it improves and accelerates the restoration of the transmembrane chemical gradients for Na⁺ and K⁺ (35). Second, it increases the electrogenic contribution of the Na⁺⁻K⁺ pumps to the membrane potential (Vₘ) and thereby further protects the excitability of the muscle fibers (24). Early studies (18, 40) indicate that the stimulating effect of β₂-agonists on the activity of the Na⁺⁻K⁺ pumps is smaller in fast-twitch muscles than in slow-twitch muscles. On the basis of this, we hypothesize that at high [K⁺]o, the protective effect of β₂-agonists on excitability is less in fast-twitch muscles than in slow-twitch muscles. We therefore examined the effects of β₂-agonists on contractility in extensor digitorum longus (EDL) muscles at high [K⁺]o and compared this with their effects in soleus muscles.

Concomitant with the increases in [K⁺]o, and the level of circulating catecholamines, intensive exercise also causes an increase in the level of lactic acid (20, 21, 45). Recent in vitro studies (30, 31, 36) on slow-twitch muscles have shown that in these muscles, the depressing effect of high [K⁺]o on excitability and contractility can also be counteracted by lactic acid. The effect on excitability is secondary to the ensuing reduction in muscle pH and might be related to an inhibitory effect of acidosis on the chloride conductance (38, 39). It is well established that fast-twitch muscles have a higher rate of lactic acid production than slow-twitch muscles. During intermittent electrical stimulation at 100 Hz (200 ms/s for 5 min), the in vivo lactic acid production was 22.4 μmol/g wet wt in the rat EDL and 1.3 μmol/g wet wt in soleus muscle (3). Moreover, it has been proposed that there is a “lactate shuttle,” where especially fast-twitch muscles produce lactic acid, which is then transported to other areas of the body (e.g., slow-twitch muscles), where it is used as an energy source (4, 5). Measurements of biopsies showed that in human fast-twitch muscles, the content of lactic acid reached 17–29 mmol/kg wet wt during intensive work, whereas in slow-twitch muscles, values of 13–19 mmol/kg wet wt were reached (16). On the basis of this, we hypothesize that fast-twitch muscles are better protected by lactic acid, than slow-twitch muscles, against loss of force induced by high [K⁺]o. Moreover, we selected the concentration range 20–30 mmol/l buffer (20–30 mM) of lactic acid as appropriate for in vitro experiments with EDL and soleus muscles.

The overall aim of this study was to identify and quantify the protective effects of catecholamines and lactic acidosis on the
maintenance of contractility in EDL muscles exposed to physiological elevations in [K⁺]o, and to compare this with soleus muscles. The importance of catecholamines and lactic acid is of particular interest in fast-twitch muscle fibers because they are known to be recruited during intense exercise, a situation where the plasma [K⁺] is known to reach their maximum.

**METHODS**

**Animal handling and muscle preparation.** All experiments were carried out using 4-wk-old male or female Wistar rats of own breed and weighing 60–70 g. Animals were fed ad libitum and maintained under 12:12-h light-dark conditions at a thermostatically controlled temperature of 21°C. The rats were euthanized by cervical dislocation, followed by decapitation, and intact soleus or EDL muscles (17–26 mg) were dissected out. 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.

Muscles were incubated in standard Krebs-Ringer bicarbonate buffer (pH 7.4 at 30°C) containing (in mM) 122 NaCl, 25 NaHCO₃, 2.8 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄, 1.3 CaCl₂, and 5.0 d-glucose. All buffers were equilibrated with a 95% O₂–5% CO₂ mixture. In buffers with high [K⁺], an equivalent amount of Na⁺ was omitted to maintain isosmolarity. To avoid exposing muscles to damaging fluctuations in pH, the buffer to which t-lactic acid had been added was equilibrated for at least 20 min with a 95% O₂–5% CO₂ mixture before use. The pH of standard buffer and buffers containing lactic acid or elevated CO₂ were, in all experiments, determined with the use of a pH meter (model PHM 92 Lab, Radiometer, Copenhagen, Denmark).

**Isometric force.** Muscles were mounted on isometric force transducers at optimal length and exposed to field stimulation across the central part of the muscle through platinum electrodes. The stimulation paradigm was 2×60-Hz trains of 0.2-ms pulses of 10 V, given every 10 min for soleus muscle and 0.5×90-Hz trains of 0.2-ms pulses of 10 V given every 20 min for EDL. The choice of stimulation frequency (60 or 90 Hz) was based on a force-frequency analysis to ensure that full tetanic force was obtained. Force was recorded on a chart recorder and/or digitally on a computer.

Na⁺/K⁺ pump activity and muscle Na⁺ content. The activity of the Na⁺/K⁺ pump was determined from the ouabain-suppressible ⁸⁶Rb⁺ uptake in resting muscles as previously described (6). Briefly, the muscles were mounted at resting length. After equilibration for 30 min in standard Krebs-Ringer bicarbonate buffer containing 11 mM K⁺ (soleus) or 13 mM K⁺ (EDL), the muscles were preincubated for 15 min in the absence or presence of ouabain (10⁻⁵ M). This was followed by incubation for 10 min in buffer containing ⁸⁶Rb⁺ (0.1 μCi ml⁻¹) without or with salbutamol (10⁻⁶ M). Finally, the muscles were washed for 4×15 min at 0°C in Na⁺-free Tris-sucrose buffer containing (in mM) 263 sucrose, 10 Tris-HCl, 4.7 KCl, 1.3 CaCl₂, 1.2 MgSO₄, and 1.2 KH₂PO₄ (pH 7.4) to remove extracellular ⁸⁶Rb⁺ and Na⁺. After washout, the muscles were blotted, weighed for determination of wet weight, soaked in 0.3 M trichloroacetic acid, and taken for counting of ⁸⁶Rb⁺ activity by Cerenkov radiation in a beta counter (Packard). The amount of ⁸⁶Rb⁺ activity retained after the cold wash and the uptake of K⁺ was then calculated by converting the relative uptake of ⁸⁶Rb⁺ to K⁺ using the concentration of K⁺ in the incubation medium. The uptake of K⁺ could thus be expressed as nanomoles per gram wet weight per minute. Earlier studies (12) with isolated resting muscles showed that the use of ⁸⁶Rb⁺ and ⁴²K⁺ gave closely similar results for the Na⁺/K⁺ pump-mediated K⁺ uptake. Thus ⁸⁶Rb⁺ is a suitable and comparable tracer for the transport of K⁺ via that system. Ouabain was used to block the activity of the Na⁺/K⁺ pumps, with subtraction of the ouabain-insensitive ⁸⁶Rb⁺ uptake from the total ⁸⁶Rb⁺ uptake, giving the ouabain-sensitive ⁸⁶Rb⁺ uptake. The concentration of Na⁺ in the trichloroacetic acid extract was determined by flame photometry (model FLM3, Radiometer). Part of the intracellular Na⁺ was, however, lost during the washout in the Na⁺-free Tris-sucrose buffer. As shown in an earlier study (17), this could be corrected for by using semilogarithmic plots of the time course of Na⁺. After the early rapid loss of Na⁺ representing washout of Na⁺ from the extracellular phase there was a rectilinear reduction in Na⁺ content, decreasing by a factor of 1.46 per hour. Because four 15-min washouts in the Na⁺-free buffer removed extracellular Na⁺, the concomitant loss of intracellular Na⁺ was corrected for by multiplying the Na⁺ content of the muscles at the end of washout by a factor of 1.46.

**Membrane potential.** In experiments where resting Vm was measured, the muscles were placed in an experimental setup as previously described in detail (13). Shortly thereafter, the Vm was recorded in surface fibers using standard techniques with glass microelectrodes filled with 3 M KCl and tip resistances of 10–30 MΩ. The potential was recorded via an Axoclamp-2 amplifier and displayed simultaneously on an oscilloscope and a chart recorder.

**Chemicals and isotope.** All chemicals were of analytical grade. Salbutamol, t-lactic acid, and ouabain were from Sigma-Aldrich and ⁸⁶RbCl (0.4 Ci/mmol) was from Amersham International (Aylesbury, Buckinghamshire, UK). Rat calcitonin gene-related peptide (rCGRP) was from Bachem (Bubendorf, Switzerland).

**Statistics.** All data were expressed as means ± SE. The statistical significance of any difference between groups was ascertained with the use of Student’s two-tailed t-test for nonpaired observations.

**RESULTS**

**Effect of salbutamol on Na⁺/K⁺ pump activity in EDL and soleus muscles incubated at high [K⁺]o.** Everts et al. (18) showed that when muscles are incubated at normal [K⁺]o, the stimulating effect of β₂-agonists on the Na⁺/K⁺ pumps is larger in soleus than in EDL muscle. To examine whether a similar difference occurs in muscles incubated at high [K⁺]o, the effect of the β₂-agonist salbutamol on the ouabain-suppressible ⁸⁶Rb⁺ uptake was determined in EDL and soleus muscles incubated at [K⁺]o of 13 and 11 mM, respectively. These levels of [K⁺]o corresponded to the [K⁺]i, needed to depress tetanic force of the respective muscles to 20% of control force. In these experiments, as well as in those with other agents used to stimulate the Na⁺/K⁺ pumps, we used supramaximal concentrations. Table 1 shows that the basal activity of the Na⁺/K⁺ pumps in EDL was similar to that of soleus, but the increase in the activity of the Na⁺/K⁺ pumps on addition of salbutamol was almost 50% less. Thus salbutamol augmented the ouabain-suppressible ⁸⁶Rb⁺ uptake by 88% in soleus and by 47% in EDL. The control level of intracellular Na⁺ was almost 50% lower in EDL compared with soleus, and the increase in Na⁺/K⁺ pump activity after the addition of salbutamol led to a decrease in intracellular Na⁺ content by 37% and 53% in the EDL and soleus, respectively. In the presence of ouabain, salbutamol caused no change in ⁸⁶Rb⁺ uptake (Table 1) and intracellular Na⁺ content (data not shown).

Effects of salbutamol, catecholamines, and rCGRP on tetanic force in EDL and soleus muscles incubated at high [K⁺]o. The smaller effect of salbutamol on the Na⁺/K⁺ pump activity in EDL (Table 1) would suggest that the protecting effect of salbutamol against the inhibitory effect of high [K⁺]o on contractility was less in EDL than in soleus. To examine
The muscles were weighed and soaked overnight in 4 ml of 0.3 M TCA. 86Rb EDL force was reduced to 23% of the force obtained at 4 mM K⁺. When the force was depressed to 10 min in similar buffer containing 86Rb, the effect of salbutamol on tetanic force in EDL and soleus muscles was reduced to 25% of the control force. When [K⁺]o was changed to 11 or 13 mM K⁺, the muscles were incubated at varying [K⁺]o caused a graded depression of tetanic force. It appears that over the range of concentrations from 11 to 15 mM K⁺, salbutamol induced a control force obtained at 4 mM K⁺, addition of 10⁻⁵ M salbutamol recovered force to 93 ± 1% of the force obtained at 4 mM K⁺. This indicates that despite the smaller stimulation of the Na⁺-K⁺ pump activity in EDL than in soleus muscle, salbutamol induced almost the same force recovery in EDL as in soleus, when exposed to 13 or 11 mM [K⁺]o, respectively. To further quantify the salbutamol-induced force recovery in EDL muscle, the experiment shown in Fig. 1 was repeated using a range of [K⁺]o (11–16 mM), with the data shown in Fig. 3. The data were fitted to a Boltzmann sigmoid equation. In control muscles, elevation of [K⁺]o caused a graded depression of tetanic force. It appears that over the range of concentrations from 11 to 15 mM K⁺, salbutamol induced a control force obtained at 4 mM K⁺, addition of 10⁻⁵ M salbutamol recovered force to 93 ± 1% of the force obtained at 4 mM K⁺. This indicates that despite the smaller stimulation of the Na⁺-K⁺ pump activity in EDL than in soleus muscle, salbutamol induced almost the same force recovery in EDL as in soleus, when exposed to 13 or 11 mM [K⁺]o, respectively. To further quantify the salbutamol-induced force recovery in EDL muscle, the experiment shown in Fig. 1 was repeated using a range of [K⁺]o (11–16 mM), with the data shown in Fig. 3. The data were fitted to a Boltzmann sigmoid equation. In control muscles, elevation of [K⁺]o caused a graded depression of tetanic force. It appears that over the range of concentrations from 11 to 15 mM K⁺, salbutamol induced a control force obtained at 4 mM K⁺, addition of 10⁻⁵ M salbutamol recovered force to 93 ± 1% of the force obtained at 4 mM K⁺. This indicates that despite the smaller stimulation of the Na⁺-K⁺ pump activity in EDL than in soleus muscle, salbutamol induced almost the same force recovery in EDL as in soleus, when exposed to 13 or 11 mM [K⁺]o, respectively.

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marked and highly significant increase of tetanic force. The 
$[K^+]_o$ required to reduce tetanic force to 50% of the force 
measured at 4 mM K$^+$ (IC$_{50}$) increased from 12.2 mM in 
control muscles to 14.2 mM with salbutamol. Taken together, 
the data in Figs. 1 and 3 show that salbutamol induces consid-
erable force recovery in EDL muscles exposed to high $[K^+]_o$, 
$\text{despite a relatively small stimulation of Na}^+-K^+$ pump activity 
(Table 1).

In soleus muscles, the Na$^+-K^+$ pumps can also be stimu-
lated by rCGRP, epinephrine, and norepinephrine (9). We 
therefore examined the effect of these compounds on force 
development in EDL muscles that had been exposed to 13 mM K$^+$. Table 2 shows that in muscles where tetanic force had 
been reduced to 25–35% of control force by increasing $[K^+]_o$ to 
13 mM, all three compounds induced a significant two- to 
threefold increase of tetanic force.

Effect of lactic acid and salbutamol on tetanic force in EDL 
muscles at high $[K^+]_o$. Recent studies (36) showed that in rat 
soleus, where force had been reduced to 25% of the control 
level by exposure to 11 mM K$^+$, 20 mM lactic acid could 
almost fully restore tetanic force. The following experiments 
were undertaken to evaluate the effect of lactic acid on force in 
EDL muscles exposed to high $[K^+]_o$. Figure 4 shows that when 
$[K^+]_o$ was increased to 13 mM, tetanic force was reduced to 
22 ± 4% of that obtained at 4 mM K$^+$. The addition of 24 mM 
lactic acid recovered force to 57 ± 2% of that obtained at 4 
mM K$^+$, and the subsequent addition of $10^{-5}$ M salbutamol 
rapidly restored force to 88 ± 2%.

To further clarify the extent to which lactic acid increases 
the tolerance of EDL muscles to elevated $[K^+]_o$, the experi-
ment shown in Fig. 4 was repeated with varying $[K^+]_o$ (11–18 
mM). Figure 5 shows the relationship between the tetanic force 
and $[K^+]_o$ in EDL before (control) and after exposure to 24 
mM lactic acid, $10^{-5}$ M salbutamol, alone or combined. The 
data were fitted to a Boltzmann sigmoid equation. The addition 
of 24 mM lactic acid increased the $[K^+]_o$ required to reduce the 
force to 50% of the control force at 4 mM K$^+$ (IC$_{50}$) from 12.2 
mM in control muscles to 12.8 mM. Thus, whereas salbutamol 
increased IC$_{50}$ by 2.0 mM (Fig. 3), lactic acid alone increased 
IC$_{50}$ by 0.6 mM, and in combination lactic acid and salbutamol 
increased IC$_{50}$ by 2.3 mM K$^+$. Figure 5 also shows that the 
effect of salbutamol on the tolerance to increased $[K^+]_o$ was 
almost fully maintained in acidified muscles, producing a 1.7 
mM increase in IC$_{50}$.

Table 2. Effect of rCGRP, epinephrine, and norepinephrine 
on tetanic force in rat EDL muscles at 13 mM K$^+$

<table>
<thead>
<tr>
<th></th>
<th>Force Before Addition</th>
<th>Force After Addition</th>
<th>$P$ Value</th>
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<tbody>
<tr>
<td></td>
<td>(% of tetanic force at</td>
<td>(% of tetanic force at</td>
<td></td>
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<tr>
<td></td>
<td>4 mM K$^+$)</td>
<td>4 mM K$^+$)</td>
<td></td>
</tr>
<tr>
<td>rCGRP ($10^{-7}$ M)</td>
<td>25 ± 6</td>
<td>63 ± 9</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Epinephrine ($10^{-5}$ M)</td>
<td>35 ± 4</td>
<td>83 ± 3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Norepinephrine ($10^{-5}$ M)</td>
<td>27 ± 10</td>
<td>84 ± 2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are presented as the percentage of tetanic force at 4 mM K$^+$ and are 
means ± SE of observations on 3 muscles. rCGRP, rat calcitonin gene-related 
peptide. EDL muscles were mounted on force transducers at optimal length for 
isometric contractions in Krebs-Ringer buffer containing 4 mM K$^+$ and 
exposed to field stimulation using 0.5- to 0.90-Hz trains of 0.2-ms pulses of 10 V 
every 20 min. Data show tetanic force after 180-min incubation at 13 mM K$^+$ 
(before addition) and 10 min after addition of the indicated compounds.

Fig. 4. Effects of 24 mM lactic acid and $10^{-5}$ M salbutamol on tetanic force in 
EDL muscles incubated at 13 mM K$^+$. EDL muscles were mounted for 
isometric contractions in Krebs-Ringer bicarbonate buffer containing 4 mM K$^+$. 
Experimental conditions are as described in Fig. 1. When the force was 
depressed to ~20% of control force, 24 mM lactic acid was added, followed by 
$10^{-5}$ M salbutamol as indicated. Values are the percentage of control force 
at 4 mM K$^+$. Each point indicates the mean ± SE of observations on 4 muscles.

Effect of salbutamol and lactic acid on $V_m$ in soleus and 
EDL muscles at high $[K^+]_o$. The recovery of force induced by the 
Na$^+-K^+$ pump stimulation in muscles at elevated $[K^+]_o$ has 
been associated with a partial repolarization of the muscles (13, 24). To evaluate the possible role of a hyperpolarization in the 
force recovery induced by salbutamol in the present study, 
muscle $V_m$ was measured during experiments similar to that 
shown in Fig. 1. Table 3 shows recordings of $V_m$ for both 
soleus and EDL and illustrates that at 4 mM K$^+$, EDL had a 
significantly lower resting $V_m$ than soleus ($P < 0.001$). After
Whereas lactic acid caused no significant change in salbutamol (10^{-5} M) on the EDL muscles at 13 mM K^+ and again there was no statistically significant difference between the EDL and soleus.

The membrane was depolarized to the same level (no significant difference) could be augmented by increasing the fraction of CO2 in the gas mixture used for equilibration of the Krebs-Ringer buffer.

Table 3. Effect of lactic acid and salbutamol on V_m in rat soleus muscles at 11 mM K^+ and rat EDL muscles at 13 mM K^+

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>High K^+</th>
<th>High K^+ + Salbutamol</th>
<th>High K^+ + Lactic Acid</th>
<th>High K^+ + Lactic Acid + Salbutamol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Series A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soleus</td>
<td>−75 ± 1</td>
<td>−55 ± 1</td>
<td>−63 ± 1</td>
<td>−61 ± 1</td>
<td>−61 ± 1</td>
</tr>
<tr>
<td>EDL</td>
<td>−82 ± 1</td>
<td>−57 ± 1</td>
<td>−62 ± 1</td>
<td>−61 ± 1</td>
<td>−61 ± 1</td>
</tr>
<tr>
<td><strong>Series B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soleus</td>
<td>−75 ± 1</td>
<td>−56 ± 1</td>
<td>−56 ± 1</td>
<td>−61 ± 1</td>
<td>−61 ± 1</td>
</tr>
<tr>
<td>EDL</td>
<td>−82 ± 1</td>
<td>−56 ± 1</td>
<td>−56 ± 1</td>
<td>−61 ± 1</td>
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</table>

Values are presented as means ± SE of observations on 3–4 muscles, each value representing the mean of recordings from 10–13 measurements. Soleus and EDL muscles were mounted at resting length in Krebs-Ringer buffer. Resting membrane potential (V_m) was recorded using standard electrophysiological techniques and is given in mV. First, the membrane potential was measured at 4 mM K^+ (controls). The muscles were then exposed to 11 mM K^+ (soleus) or 13 mM K^+ (EDL) and the recordings continued. The effects of salbutamol (10^{-5} M) or lactic acid (24 mM) on the membrane potential were measured alone or combined in two different experimental series (A and B). There were statistically significant differences (P < 0.001) between control values of EDL and soleus. In each individual muscle type, the response to high K^+ was significant (P < 0.001). Moreover, the response to addition of salbutamol when at high K^+ was also significant (P < 0.001) (series A).

Whereas lactic acid caused no significant change in V_m, salbutamol induced a significant hyperpolarization in both soleus and EDL muscles exposed to lactic acid (series B).

Exposure to 11 mM K^+ (soleus) or 13 mM K^+ (EDL), the membrane was depolarized to the same level (no significant difference). The subsequent addition of salbutamol repolarized the V_m by 5–8 mV (P < 0.001) in both EDL and soleus muscle.

Series B in Table 3 shows the effect of lactic acid on the V_m in EDL and soleus. Lactic acid alone caused no change in the V_m in either EDL or soleus muscle depressed with 13 and 11 mM K^+, respectively. The addition of salbutamol after lactic acid repolarized the membrane potential by 5 mV (P < 0.001), and again there was no statistically significant difference between the EDL and soleus.

Effect of pH on EDL. The increase in [K^+]_o tolerance induced by 24 mM lactic acid in EDL muscles, as illustrated in Fig. 5, was modest compared with that seen in soleus muscles (15). We therefore tested whether the protective effect of lactic acid in EDL at 13 mM K^+ could be augmented by increasing the lactic acid concentration, using an experimental procedure as depicted in Fig. 4.

Figure 6 shows that when the lactic acid concentration was increased >20 mM, recovery of force increased with the concentration of the acid, starting with 50 ± 7% at 20 mM, and increasing to 71 ± 4% at 28 mM. At values higher than 28 mM, the force recovery effect eased off, indicating that the maximum value had already been reached. It should be noted that 20% CO2, which reduced pH to 6.75, gave approximately the same force recovery as the addition of 24 mM lactic acid, which also reduced pH to 6.75 (Figs. 6 and 7). At higher levels of acidification, the recovery of force was less...
when pH was lowered by increased CO_2 than by the addition of lactic acid.

**DISCUSSION**

Previous studies (10, 15, 31, 36, 38) on K^+-induced inhibition of contractile force and the protective effects of Na^+-K^+ pump stimulation and acidosis, have focused on slow-twitch muscles. Because a large fraction of skeletal muscles are composed of fast-twitch fibers, it was of interest to characterize the response of these fibers to high [K^+]_o. Na^+-K^+ pump stimulation and acidosis, in particular because in these fibers, excitation induces a much more pronounced loss of K^+ than in slow-twitch fibers (14).

The major new information gained from the present study is that when exposed to the inhibitory effect of high [K^+]_o, the fast-twitch EDL muscles responds to stimulation of the Na^+-K^+ pumps with a marked force recovery. This indicates that the protective effect of Na^+-K^+ pump stimulation against hyperkalemia is as important as previously shown for slow-twitch muscles. The data also show that acidosis induced by lactic acid or elevation of CO_2 induces a significant force recovery, although somewhat lower than that found in soleus muscles (36). Taken together, these observations indicate that Na^+-K^+ pump stimulation and acidosis both contribute to the maintenance of force in skeletal muscles exposed to elevated [K^+]_o, but that the combined effect of these two factors is larger in soleus than in EDL.

Compared with the time it takes for humans to reach exhaustion during intensive exercise, the time course for the effects of increased buffer K^+ and the addition of salbutamol and lactic acid on force in the present study was very slow. The reason for the slow time course is likely to be related to slow diffusion of the added substances into the core of the muscles rather than to a slow development of the biological effect within the muscle fibers. In the case of increased buffer K^+ the time until full effect may be further delayed by uptake of K^+ into more superficial fibers via the Na^+-K^+ pumps (11) and by limitation to diffusion of K^+ into the elaborate t-tubular system of the muscle fibers. Because the t-tubular network is larger in fast-twitch muscles than in slow-twitch muscles, such limitation to diffusion may also explain that the rate of force decline when exposed to elevated [K^+]_o is slower in EDL than in soleus muscle (Figs. 1 and 2). This explanation for the slow response of the muscles to manipulations of the buffers is supported by the observation that when the chemical gradient for K^+ across the t-tubular system is manipulated in experiments with mechanically skinned single fibers, the effect of changes in the K^+ gradient is observed within seconds (37).

Likewise the use of the same preparation has demonstrated that the recovery of force and excitability induced by stimulation of the Na^+-K^+ pumps or acidification of the muscle develops within seconds (37, 39). In the present study, the increase in [K^+]_o and the addition of salbutamol or lactic acid were separated in time to allow for a quantification of their separate effects on muscle function. This method represents another difference to the case of intensive exercise, when the increase in [K^+]_o may be closely followed by increased blood levels of catecholamines and muscle acidification. In earlier studies on isolated muscle (10, 36), however, it was shown that if catecholamines or lactic acid were added before or together with the increase in [K^+]_o, their presence could completely prevent the loss of force otherwise induced by elevated [K^+]_o. Together these findings indicate that changes in Na^+-K^+ pump activity or muscle pH are important for the regulation of muscle excitability, even during short bouts of intensive exercise.

**Effect of salbutamol on Na^+-K^+ pump activity and tetanic force.** The salbutamol-induced increase in the Na^+-K^+ pump mediated ^86_Rb^+ uptake at high [K^+]_o was 50% less in EDL than in soleus muscles. This would indicate that the protective effect of β-adrenergic agonists against the force-depressing effect of high [K^+]_o is smaller in EDL than in soleus muscle. However, Figs. 1 and 2 show that salbutamol (10^-3 M) produced almost as much force recovery in EDL as in soleus muscles, where force was depressed to ~20%. Considering the increase in IC_50 from 12.2 to 14.2 mM K^+ (Fig. 5), salbutamol increases the tolerance of EDL to elevated [K^+]_o by 2 mM. Figure 5 further shows that the effect of salbutamol on the K^+ tolerance of EDL was almost completely preserved in muscles acidified by addition of lactic acid. In similar experiments on rat soleus excited with 1-ms pulses, stimulation of the Na^+-K^+ pumps by the addition of 10^-5 M epinephrine, increased IC_50 of the muscles from 11.5 to 14.4 mM K^+, indicating an increase in the tolerance to increased [K^+]_o by 2.9 mM (F. de Paoli and O. B. Nielsen, unpublished observations). Previous studies (10) have shown that, like the effect of salbutamol, the effect of epinephrine on force in muscles at elevated [K^+]_o is caused by stimulation of the Na^+-K^+ pumps via β-adrenergic receptors. As in EDL, the effect of Na^+-K^+ pump stimulation is well maintained in soleus when acidified by addition of lactic acid, the increase in the tolerance to [K^+]_o being 2.2 mM (15). Together, these results demonstrate that β2-agonists induce almost the same protection of force in EDL and soleus muscles exposed to hyperkalemia, although salbutamol induced a relatively lower increase in the Na^+-K^+ pump activity of the former. This difference may, in part, be related to the lower intracellular Na^+ in EDL (Table 1), limiting the activity of the Na^+-K^+ pumps. Thus, given the size of the salbutamol-induced decrease in Na^+ content of soleus, there is no way the EDL could possibly have matched that decrease without the cells becoming devoid of Na^+. Despite this, the more modest salbutamol-induced increase of the ^86_Rb^+ uptake in EDL appeared sufficient to cause force recovery similar to that seen in soleus. It is interesting that for more modest numbers of Na^+ and K^+ ions pumped, EDL reaches the same force recovery as soleus.

**Mechanisms of salbutamol action.** In EDL exposed to 13 mM K^+, the improvement of contractility by salbutamol was associated with membrane hyperpolarization and a reduction in intracellular Na^+ content (Tables 1 and 3). Salbutamol reduced intracellular Na^+ content by 2.4 ± 0.5 μmol/g wet wt, which augments the equilibrium potential for Na^+ (E_Na+) (assuming constant [Na^+]_o). Together with the concomitant 5-mV hyperpolarization, this increases the driving force for the electrodiffusion of Na^+ (V_m - E_Na+). Hyperpolarization has also been shown to reduce the level of slow inactivation of the Na^+ channels (42), which increases the conductance for Na^+. Because enhanced driving force and increased conductance for Na^+ improves excitability (26), these changes are likely to contribute to the improved contractility. Because the activity of the Na^+-K^+ pumps is electroneutral, and because intracellular
[Na\(^+\)] ([Na\(^+\)]) depends on the active rate of extrusion, the salbutamol-induced hyperpolarization and reduction in [Na\(^+\)], could both be explained by an increase in the Na\(^+-K\(^+\) pump activity. This is in accordance with the salbutamol-induced increase in the ouabain-suppressible \(^{86}\)Rb\(^+\) uptake in both EDL and soleus muscles (Table 1). Another potential mechanism for the salbutamol-induced hyperpolarization is an increase in the K\(^+\) permeability of the muscle fibers, bringing the \(V_{m}\) closer to the Nernst equilibrium potential for K\(^+\), supposing that the membrane potential and the equilibrium potential for K\(^+\) are substantially different. This would, however, cause an increase in the ouabain-non-suppressible \(^{86}\)Rb\(^+\) uptake, which we do not find (Table 1).

The depression of force induced by high [K\(^+\)]\(_i\) occurred in a narrow range of [K\(^+\)] (Figs. 3 and 5), which indicates a high K\(^+\) sensitivity, and this is in accordance with an earlier study (7). Our data show that in the EDL muscles there is a large force depression between 11 and 13 mM K\(^+\). This implies that when force is depressed to 20% by exposure to 13 mM [K\(^+\)]\(_i\), a reduction of [K\(^+\)]\(_i\) of 2 mM would be expected to induce a force recovery to 80% of the force at 4 mM K\(^+\) (see Figs. 3 and 5). According to the Nernst equilibrium potential for K\(^+\), changing the [K\(^+\)]\(_i\) from 13 to 11 mM, should theoretically cause a 4.3-mV repolarization (assuming [K\(^+\)] = 140 mM). We can therefore conclude that the stimulating effect of salbutamol on the Na\(^+-K\(^+\) pumps, causing a hyperpolarization of 5 mV (Table 3), should be sufficient to explain the salbutamol-induced restoration of force.

**Effects of catecholamines and rCGRP on tetanic force.** Similar to the effects of salbutamol, addition of epinephrine, norepinephrine, and rCGRP all produced a recovery of force in EDL muscles depressed by elevated [K\(^+\)]\(_i\). Because the effects of both epinephrine and norepinephrine, in common with the effect of the \(\beta_2\)-agonist salbutamol (13), have been related to a stimulation of the muscle Na\(^+-K\(^+\) pumps via the \(\beta_2\)-adrenoceptors, these results indicate that the increase in circulating catecholamines during exercise will improve the tolerance of the skeletal muscles to elevated [K\(^+\)]\(_i\). In common with catecholamines, CGRP also stimulates the Na\(^+-K\(^+\) pumps via the adenylyl cyclase system (1), but at variance with the catecholamines, CGRP is released locally from motor and sensory nerve endings in the contracting muscles (44, 46), with little effect on the circulating levels of the neuropeptide. These findings indicate that in active muscles, the protection against elevated [K\(^+\)]\(_i\) induced by circulating catecholamines may be reinforced by local release of CGRP from nerve endings, which will further stimulate the Na\(^+-K\(^+\) pumps.

**Effects of \(CO_2\) and lactic acid.** In this study we showed force restoration in EDL by increasing the fraction of \(CO_2\) in the gas mixture used for gassing the Krebs-Ringer buffer. This is in accordance with earlier studies showing that in the soleus, the effect of lactic acid on force recovery could be mimicked by an intracellular acidification induced by \(CO_2\) (36). The same study also showed that lactic acid could fully restore force in soleus muscles where force had been depressed to \(\sim 20\%\) by a [K\(^+\)]\(_i\) of 11 mM. In EDL, lactic acid restored force to only 57 \(\pm\) 2% of that obtained at 4 mM K\(^+\), when force had been depressed to \(\sim 20\%\) by 13 mM K\(^+\). Moreover, in the soleus, 20 mM lactic acid increased IC\(_{50}\) from 11.5 to 13.4 mM K\(^+\) (15), whereas in EDL 24 mM lactic acid increased IC\(_{50}\) only from 12.2 to 12.7 mM, indicating that lactic acid offers less protection against the depressing effect of increased [K\(^+\)]\(_i\) in EDL than in soleus muscle. To investigate this, we examined whether the effect of further reducing pH by increasing the concentration of lactic acid could increase the force recovery effect in EDL. Increasing the lactic acid concentration to \(\geq 24\) mM induced a larger restoration of force, with a maximum at 28 mM lactic acid (Fig. 6). Even at 28 mM lactic acid, force recovery in EDL depressed by elevated [K\(^+\)]\(_i\) is not as great as that seen with 20 mM lactic acid in soleus muscles depressed to a similar extent. Thus the protecting effect of lactic acid against the depressing effect of increased [K\(^+\)]\(_i\) is smaller in EDL than in soleus. Furthermore, increasing lactic acid \(\geq 28\) mM did not provide greater protection of the muscles, suggesting that the physiological concentrations of lactic acid reached in rat and human working muscles (3, 16) are sufficient to exert the maximal protective effect of lactic acid observed in the present study. Recently, Kristensen et al. (31) explored the protective effect of lactic acid on the endurance of slow-twitch muscles and found no protective effect on endurance of preincubation with 20 mM lactic acid. A possible explanation might be that the work-induced generation of lactic acid in the muscles per se leads to a reduction in muscle pH that is sufficient to elicit the maximal protective effect against increased extracellular K\(^+\). Further reduction in muscle pH by addition of exogenous lactic acid would then be expected to either have no effect or even to reduce endurance by inhibiting force production.

As described earlier, it has been suggested that the acidification induced by lactic acid contributes to force recovery by lowering the chloride conductance (38, 39). At elevated [K\(^+\)]\(_i\), this does not change the \(V_{m}\) (47) but causes a recovery of excitability (38, 39). In the present study we have confirmed that lactic acid has no effect on \(V_{m}\) (Table 3), although it causes considerable recovery of tetanic force in EDL depressed by [K\(^+\)]\(_i\) of 13 mM.

**EDL and soleus muscles.** The increase in the tolerance to elevated [K\(^+\)]\(_i\) (IC\(_{50}\)) induced by \(\beta_2\)-adrenoceptor stimulation of the Na\(^+-K\(^+\) pumps was almost as large in EDL as in soleus. In contrast, the effect of lactic acid on IC\(_{50}\) for extracellular K\(^+\) appeared to be somewhat smaller in EDL than in soleus. Moreover, when combined, \(\beta_2\)-adrenoceptor stimulation of the Na\(^+-K\(^+\) pumps and lactic acid was, in the present study, found to increase IC\(_{50}\) by 2.3 mM K\(^+\) in EDL, whereas the combination of Na\(^+-K\(^+\) pump stimulation and lactic acid was observed to increase IC\(_{50}\) by 4.1 mM K\(^+\) in soleus muscles (15). Thus the combined protective effect of salbutamol and lactic acid against the force-depressing effects of hyperkalemia is somewhat smaller in EDL than in soleus.

In contrast, a comparison of EDL and soleus muscles incubated in the absence of \(\beta_2\)-agonists and lactic acid indicate that EDL muscles per se can tolerate a higher [K\(^+\)]\(_i\) than soleus without any substantial force depression. Thus, in soleus muscles excited with 0.2- or 1-ms pulses, the [K\(^+\)]\(_i\) required to depress force production by 50% were 10.2 and 11.5 mM, respectively (15), whereas in EDL excited with 0.2-ms pulses, a [K\(^+\)]\(_i\) of 12.2 mM was required to depress force by 50% (Fig. 5). The tendency for a larger [K\(^+\)]\(_i\) tolerance in EDL compared with soleus is in accordance with earlier studies (7). EDL muscles may tolerate a higher [K\(^+\)]\(_i\) before starting to lose force because they maintain higher gradients for Na\(^+\) and K\(^+\) (14). The higher K\(^+\) gradient causes a more negative equilibrium potential for K\(^+\) and thereby a more negative \(V_{m}\). In the
present study this was confirmed by obtaining the resting $V_m$ of $-75$ and $-82 \text{ mV}$ in soleus and EDL, respectively. Because of their higher intracellular $[K^+]_o$, EDL muscles can tolerate a higher $[K^+]_o$ before depolarizing to a level that would depress excitability (7). Both muscle types are depolarized to the same value (Table 3) when tetanic force is depressed to 20% of that obtained at 4 mM $K^+$, by 11 mM $K^+$, and 13 mM $K^+$ in soleus and EDL, respectively. This could indicate that the slow inactivation of the $Na^+$ channels occurs at the same $V_m$ in the two muscles. However, this is not in accordance with earlier observations showing that the slow inactivation of the $Na^+$ channels occurs at a more negative $V_m$ in fast-twitch fibers than in slow-twitch fibers (42, 48).

The present study suggests an exercise scenario in fast-twitch muscle, like the one observed in slow-twitch muscle, where the decrease in excitability caused by increased $[K^+]_o$ is counteracted simultaneously by lactic acidosis and elevation of circulating catecholamines. Therefore, lactic acid, as well as $Na^+-K^+$ pump stimulation via the $\beta_2$-adrenoceptors, also provide a synergistic improvement of contractility in those fibers, which are known to be most susceptible to rapid rundown of $Na^+/K^+$ gradients and ensuing fatigue. However, our results indicate that compared with slow-twitch muscles, fast-twitch muscles show a somewhat smaller response to the protecting effect of $\beta_2$-stimulation and lactic acid. This difference may, to some extent, be compensated for by a larger tolerance of fast-twitch muscle per se to elevated $[K^+]_o$. In addition, the endogenous production of lactic acid is considerably larger in fast-twitch muscles than in slow-twitch muscle, and within a few minutes, the lactic acid level reached in working fast-twitch muscles (3) is sufficient to induce effects similar to those described in the present study. During intensive exercise, however, the reduction in intracellular pH taking place in slow-twitch fibers may be enhanced by cellular uptake of lactate and protons that are liberated from fast-twitch muscles. In that context, the lactate shuttle (4, 5) may be important not only as an improvement of the energy supplies of muscle fibers during exercise but also for the regulation of their excitability.

During exercise of increasing intensity, more fast-twitch fibers are recruited, leading to more pronounced hyperkalemia and lactate production. Concomitantly, exercise induces an increase in plasma catecholamines, which in turn stimulate the $Na^+-K^+$ pumps, leading to increased glycolysis (27). Our results indicate that optimal performance of these fibers may depend on their capacity for active $Na^+-K^+$ transport and their endogenous lactic acid production from glycogenolysis.

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