Pflügers Arch – Eur J Physiol (1995) 430 : 909–915

ORIGINAL ARTICLE

Simeon P. Cairns · John A. Flatman · Torben. Clausen

Relation between extracellular [K^+], membrane potential and contraction in rat soleus muscle: modulation by the Na^+-K^+ pump

Received: 31 January 1995/Received after revision: 25 April 1995/Accepted: 26 April 1995

Abstract An increased extracellular K^+ concentration ($[K^+]_0$) is thought to cause muscle fatigue. We studied the effects of increasing $[K^+]_0$ from 4 mM to 8–14 mM on tetanic contractions in isolated bundles of fibres and whole soleus muscles from the rat. Whereas there was little depression of force at a $[K^+]_0$ of 8–9 mM, a further small increase in $[K^+]_0$ to 11–14 mM resulted in a large reduction of force. Tetanus depression at 11 mM $[K^+]_0$ was increased when using weaker stimulation pulses and decreased with stronger pulses. Whereas the tetanic force/resting membrane potential (E_M) relation showed only moderate force depression with depolarization from −74 to −62 mV, a large reduction of force occurred when E_M fell to −53 mV. The implications of these relations to fatigue are discussed. Partial inhibition of the Na^+-K^+ pump with ouabain (10^{-6} M) caused additional force loss at 11 mM $[K^+]_0$. Salbutamol, insulin, or calcitonin gene-related peptide all stimulated the Na^+-K^+ pump in muscles exposed to 11 mM $[K^+]_0$ and induced an average 26–33% recovery of tetanic force. When using stimulation pulses of 0.1 ms, instead of the standard 1.0-ms pulses, force recovery with these agents was 41–44% which was significantly greater ($P < 0.025$). Only salbutamol caused any recovery of E_M (1.3 mV). The observations suggest that the increased Na^+ concentration difference across the sarcolemma, following Na^+-K^+ pump stimulation, has an important role in restoring excitability and force.

Key words Na^+-K^+ pump · Potassium · Salbutamol · Insulin · Skeletal muscle · Fatigue

S. P. Cairns[1] · J. A. Flatman · T. Clausen (✉)
Institute of Physiology, University of Aarhus,
DK-8000 Århus-C, Denmark

Present address:
[1] Department of Physiology, School of Medicine,
University of Auckland, Private Bag 92019, Auckland,
New Zealand

Introduction

During intense exercise or fatiguing electrical stimulation an efflux of K^+ from working skeletal muscle gives rise to peak K^+ concentrations in the effluent venous plasma or interfibre space of 7–10 mM [12, 15, 21, 27]. The increased extracellular K^+ concentration ($[K^+]_0$), and associated decline in the K^+ concentration difference across the sarcolemma, is thought to be a major cause of muscle fatigue [14, 27, 28]. In addition, non-working muscle fibres may be subjected to the elevated $[K^+]_0$ and suffer due to a reduced excitability. Several groups have tested these proposals by examining the effects of increased $[K^+]_0$ on contraction in non-fatigued muscle. The usual result is a depression of tetanic contractions [6, 8, 13, 17, 25] but sometimes a potentiation of submaximal contractions is observed [5, 6, 18, 25]. In the first part of the present study we quantified the relation between peak tetanic force and $[K^+]_0$, over the physiological range of $[K^+]_0$, in isolated rat soleus muscle fibres. Although the mechanisms for the K^+-induced depression of force are not fully understood they are linked to a depolarization and reduced excitability of the sarcolemma [14, 16, 25]. Therefore, we determined the relation between tetanic force and the resting membrane potential (E_M).

We were also interested in the ability of the Na^+-K^+ pump to counteract the depressive effects of elevated $[K^+]_0$ on contraction. Indeed, agents which stimulate the Na^+-K^+ pump, such as β-adrenergic agonists and insulin, cause recovery of force in K^+-depressed whole muscles [3, 8, 13, 29]. We examined whether the Na^+-K^+ pump normally acts to resist K^+-induced force depression by partially reducing pump activity with ouabain. Acute stimulation of the Na^+-K^+ pump with salbutamol (a β_2-adrenergic agonist), insulin or calcitonin gene-related peptide (CGRP), all caused a recovery of force in K^+-depressed whole muscles and salbutamol also induced a force recovery in fibre bundles. Possible mechanisms for these effects were

investigated. The results indicate that the Na^+-K^+ pump has an important role in reducing the force depression induced by elevated $[K^+]_0$, possibly by lowering the intracellular Na^+ concentration ($[Na^+]_i$).

Materials and methods

Muscle preparations

Four-week-old Wistar rats (60–70 g) were killed by decapitation and intact soleus muscles were dissected out. These muscles weighed 24.7 ± 0.8 mg (mean ± SEM, n = 17). Contraction studies were performed on either whole muscles or small bundles of fibres. Muscles were pinned into a Petri dish lined with Sylgard and bathed in a modified Krebs-Ringer buffer in which all $NaHCO_3$ had been replaced by Tris (pH 7.35). Fibre bundles were then dissected from tendon-to-tendon [4]. The bundles weighed 1.8 ± 0.3 mg (mean ± SEM, n = 11). We estimated that these bundles had an average of around 150 fibres. The ratio of peak tetanic force to preparation wet weight was 1.3 ± 0.1 g/mg (mean ± SEM) in fibre bundles and 1.7 ± 0.1 g/mg in whole muscles which indicates that they had a similar contractile status.

Solutions

The standard Krebs-Ringer bicarbonate buffer, used as a control solution, contained 4 mM K^+ which is close to the resting plasma $[K^+]$ in rats [24]. Its composition was (in mM): 122.2 NaCl, 25.1 $NaHCO_3$, 2.8 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.3 $CaCl_2$, and 5 D-glucose. Solutions with an increased $[K^+]$ were made by exchanging equal amounts of KCl for NaCl to maintain osmolarity. The $[K^+]$ in these solutions was checked by flame photometry and it did not vary from the expected value by more than 0.05 mM. The small reduction in $[Na^+]$ when $[K^+]$ was increased from 4 to 14 mM reduced the Na^+ equilibrium potential (E_{Na}) by only 1.5 mV. All solutions were oxygenated with carbogen (95% O_2, 5% CO_2) and pH was maintained at 7.4. Force recordings and incubations took place at 30 °C.

Force recording

Preparations were mounted vertically by their tendons in a temperature-controlled chamber [6]. Isometric contractions were evoked by direct electrical stimulation from two platinum electrodes placed close to surface fibres. Supramaximal rectangular pulses (1 ms, 8–12 V) were obtained from a pulse generator and force was recorded on a chart recorder via a force transducer (Grass-FTO_3). The length of the preparation was adjusted to give maximum twitch force. Maximum tetanic force was obtained by stimulating at 100 Hz until a force plateau was achieved (1–2 s in the control solution). Muscles with a low maximum force, a rapid run-down of force, or incomplete recovery of force at 4 mM $[K^+]_0$, were rejected. Following a 30-min equilibration period, when a steady tetanic force level was achieved, the standard buffer containing 4 mM K^+ was replaced by one containing an elevated $[K^+]$. Tetani were evoked every 5 min. Elevated $[K^+]$ solutions were usually applied in a random order, although in some experiments $[K^+]_0$ was increased cumulatively to reduce the duration of the experiment. Drugs and hormones were added when a steady-state force had been achieved at 11 mM $[K^+]_0$. The maximum change in force obtained 5–15 min later was noted.

Membrane potentials

In parallel experiments we measured the E_M in surface fibres of whole soleus muscles [7] in conditions which matched those in the contraction studies. Briefly, glass microelectrodes (tip resistance 20–30 MΩ) filled with 3 M KCl were used and the potential, recorded via an Axoclamp-2A amplifier, was displayed simultaneously on an oscilloscope and X-t chart recorder. The maximum drop in potential upon penetration was taken as the E_M. Recordings were rejected if the electrode resistance, which was monitored continuously, changed by more than 10% upon fibre penetration and during continuous recording. Muscles were equilibrated for about 30 min in the standard buffer containing 4 mM K^+ and then the E_M was measured. The bulk solution was then rapidly changed to one containing an elevated $[K^+]$ and, when the E_M had changed to a steady level (≈20 min), a further series of penetrations were made. The effect of salbutamol, insulin, CGRP or ouabain on the E_M was determined as the steady E_M obtained 10–15 min after their addition to muscles equilibrated at 11 mM $[K^+]_0$ (for comparison see [7]).

Intracellular Na^+ and K^+ contents

Whole muscles were equilibrated in the standard buffer containing 4 mM K^+ for about 30 min, then further incubated at an elevated $[K^+]$ for 75 min prior to the determination of Na^+ and K^+ contents. In some experiments muscles were incubated for 75 min in buffer containing 11 mM K^+ with either salbutamol (10^{-5} M), insulin (100 mU/ml), CGRP (10^{-7} M) or ouabain (10^{-6} M). After the incubation, the muscles were washed 4 times each for 15 min in ice-cold Na^+-free Tris-sucrose buffer to remove extracellular Na^+, blotted, weighed and homogenized in 0.3 M trichloroacetic acid (TCA). Following centrifugation, Na^+ and K^+ contents were determined by flame photometry of the TCA extracts of the muscle, using a FLM flame photometer with Li^+ as an internal standard (Radiometer, Copenhagen, Denmark). This procedure for the determination of intracellular Na^+ content is described in detail elsewhere [11].

Analysis and statistics

Results are expressed as the mean value ± SEM of n preparations, unless stated otherwise. Statistical significance was assessed using the Student's t-test or analysis of variance. To compare effects in preparations of different sizes the steady-state force at elevated $[K^+]_0$ was expressed as a percentage of the control peak tetanic force at 4 mM $[K^+]_0$. The control tetanic force was determined by averaging the peak force from tetani obtained immediately before, and after maximum recovery from, exposure to solutions with an elevated $[K^+]$.

Chemicals and hormones

All chemicals used were of analytical grade. Salbutamol, ouabain and rat CGRP were obtained from Sigma, St. Louis, Mo., USA. Insulin was a gift from NOVO-Nordisk, Copenhagen, Denmark.

Results

Effects of elevated $[K^+]_0$ on tetanic contractions

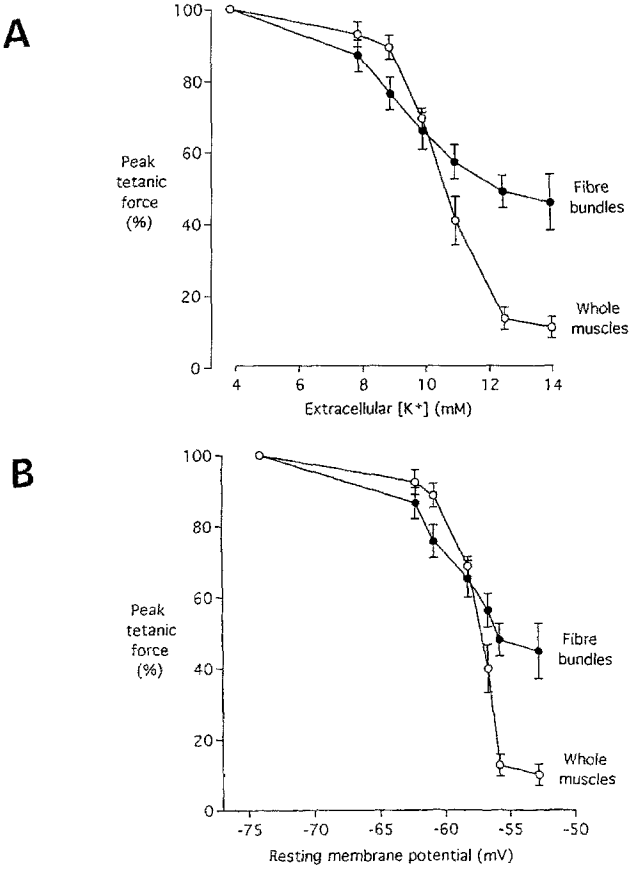
The influence of increasing $[K^+]_0$ from 4 to 8–14 mM on peak tetanic force in soleus preparations is

shown in Fig. 1A. Whole muscles displayed a moderate depression of force when $[K^+]_0$ was increased to 8–9 mM. At 9 mM $[K^+]_0$ the force was 89 ± 3% (n = 8) of the control level. Increasing $[K^+]_0$ to 12.5 mM resulted in a large, and almost complete, depression of force to 13 ± 3% (n = 12) of the control. The fibre bundle preparation was used to reduce diffusion distances and presumably better control the $[K^+]_0$ around fibres in the middle of the preparation [4, 5, 25]. The tetanic force/$[K^+]_0$ relation in fibre bundles (Fig. 1A) shows a greater resistance to force depression at 11–14 mM $[K^+]_0$ than for whole muscles. For example, at 14 mM $[K^+]_0$ the force was reduced to 10 ± 3% (n = 6) in whole muscles but to only 45 ± 8% (n = 5) in fibre bundles. Surprisingly, further increasing $[K^+]_0$ to 16–20 mM did not cause much additional force loss in fibre bundles (n = 3) with the largest depression being to 25% of the control.

Following exposure to an elevated $[K^+]_0$ the reduction of tetanic force occurred more rapidly in fibre bundles than in whole muscles. The time to reach half of the maximum force depression was less than 5 ± 1 min (n = 12) in bundles and 20 ± 2 min (n = 14) in whole muscles. The maximum steady-state force depression occurred in 20 ± 2 min (n = 14) in bundles, which is comparable to the time for maximum depolarization in surface fibres (present study), and in 57 ± 5 min (n = 8) in whole muscles.

The rise of tetanic force was slowed at elevated $[K^+]_0$. This meant that the stimulation duration had to be increased from 1–2 s to several seconds to ensure that a force plateau was achieved. Furthermore, at a $[K^+]_0$ of 11 mM or greater, the rising phase of tetanic force development often displayed small downward deflections which may indicate that some fibres became inexcitable and failed to contribute to the total force production.

Fig. 1. A Relation between peak tetanic force and extracellular K^+ concentration ($[K^+]_0$) in whole soleus muscles, and in bundles of fibres from soleus muscle. Tetani were evoked at 100 Hz using supramaximal stimulation pulses (1.0 ms, 8–12 V) at 30 °C. The steady-state tetanic force at elevated $[K^+]_0$ is expressed relative to the control level at 4 mM $[K^+]_0$. Each point is the mean ± SEM of data from 5–12 whole muscles or 4–9 fibre bundles. The tetanic force at 11–14 mM $[K^+]_0$ was significantly greater in fibre bundles than in whole muscles ($P < 0.001$). **B** Relation between peak tetanic force and resting membrane potential (E_M) in whole soleus muscles, and in fibre bundles from soleus muscle. Shown are the mean values ± SEM. The SEM for membrane potentials are less than the size of the *symbols*. E_M values are from surface fibres of whole muscles during exposure to elevated $[K^+]_0$. The corresponding tetanic force levels were those used in **A**



The tetanic force/resting E_M relation

The effects of $[K^+]_0$ on contraction are thought to be due to depolarization of the sarcolemma [5, 14, 18, 25]. Increasing the $[K^+]_0$ from 4 to 8 mM resulted in a depolarization from −74.1 ± 3.1 mV (± SD, n = 160/16 fibres/muscles) to −62.2 ± 2.6 mV (± SD, n = 60/6). Subsequent 1-mM increments in $[K^+]_0$ caused small further depolarizations of 1–2 mV. At a $[K^+]_0$ of 14 mM, the resting E_M was reduced to −52.8 ± 1.9 mV (± SD, n = 60/6).

The tetanic force/resting E_M relation for fibre bundles and whole muscles is shown in Fig. 1B. Whereas there was only a moderate force depression (≈10%) when the resting E_M was reduced to −62 mV, a further 7–10 mV depolarization resulted in a large reduction of force (≈55% in bundles; ≈90% in whole muscles). Possible reasons for this difference between these preparations are presented in the Discussion.

The tetanic force/$[K^+]_0$ relation depends on stimulation pulse parameters

An increased $[K^+]_0$ could reduce force by making some fibres in the preparation inexcitable [16]. Hence, we tested whether using weaker stimulation pulses (shorter duration or smaller amplitude) would cause more fibres to become inexcitable at elevated $[K^+]_0$ and whether stronger pulses would increase the safety margin for action potentials so that fewer fibres would become inexcitable. At a $[K^+]_0$ of 4 mM the peak tetanic force of whole muscles was virtually unchanged

Table 1 Effect of Na^+-K^+ pump inhibition or stimulation on intracellular Na^+, K^+ contents and resting membrane potentials at 11 mM $[K^+]_0$ in rat soleus muscles. Shown are the mean values ± SEM. Experiments were done at 30°C. (*CGRP* Calcitonin gene-related peptide). The *numbers* in *parentheses* are the number of whole muscles used to determine the Na^+, K^+ contents or the number of surface fibres/muscles used to determine the resting E_M. ΔE_M is the difference between the E_M with each agent at 11 mM $[K^+]_0$ and the 11 mM $[K^+]_0$ control. A minus sign indicates repolarization

Experimental conditions	Parameter		
	Na^+ content ($\mu mol \cdot g^{-1}$ wet wt)	K^+ content ($\mu mol \cdot g^{-1}$ wet wt)	ΔE_M (mV)
Controls (11 mM K^+)	12.2 ± 0.3 (15)	81.7 ± 1.1 (15)	–
+ Ouabain (10^{-6} M)	16.2 ± 0.5*** (12)	74.8 ± 1.4** (12)	−1.2 ± 0.4** (80/8)
+ Salbutamol (10^{-5} M)	5.4 ± 0.2*** (8)	85.3 ± 1.7$^+$ (8)	−1.3 ± 0.4*** (109/10)
+ Insulin (100 mU/ml)	9.8 ± 0.7*** (8)	84.7 ± 1.2 n.s. (8)	+0.6 ± 0.4 n.s. (60/6)
+ CGRP (10^{-7} M)	6.8 ± 0.1*** (8)	86.0 ± 0.5* (8)	−0.6 ± 0.3 n.s. (40/4)

Statistical significance for effects with each agent compared to the 11 mM K^+ controls (unpaired *t*-test) were: $^+P < 0.1$; $^*P < 0.02$; $^{**}P < 0.005$; *** $P < 0.001$; n.s. not significant ($P > 0.1$)

(4% reduction) when the pulse duration was reduced from 1.0 to 0.1 ms (constant amplitude). This indicates that nearly all fibres were maximally activated. However, at elevated $[K^+]_0$ there was a greater loss of force when stimulating with the weaker pulses (see Fig. 2). In 20 whole muscles exposed to 11 mM $[K^+]_0$, the force was reduced to 50 ± 3% when using 1.0-ms pulses compared with 28 ± 3% when using 0.1-ms pulses ($P < 0.00001$). Similar effects occurred in fibre bundles where increasing the pulse duration from 0.2 to 2.0 ms, or doubling the voltage strength, increased force by up to 35%. These data are consistent with the hypothesis that some of the K^+-induced force depression is due to inexcitable fibres.

Effect of ouabain on K^+-depressed contractions

When the resting E_M was between −62 and −53 mV tetanic force was very sensitive to small changes in E_M (Fig. 1B), and possibly also to modulation by the Na^+-K^+ pump. We first examined whether the Na^+-K^+ pump normally provides some resistance to force depression at elevated $[K^+]_0$ by partially suppressing pump activity with ouabain (10^{-6} M). Incubation with 10^{-6} M ouabain for 60 min results in an occupancy of at least 35% of the total number of Na^+-K^+ pump sites and produces a similar inhibition of active Na^+-K^+ transport [7]. Ouabain had a negligible effect (3% reduction) on peak tetanic force at 4 mM $[K^+]_0$ (1.0-ms pulses) but caused greater force depression at elevated $[K^+]_0$. At 11 mM $[K^+]_0$, ouabain reduced force from 42 ± 8% to 25 ± 8% of the control level at 4 mM $[K^+]_0$ ($n = 4$, $P < 0.025$). Ouabain treatment also increased the intracellular Na^+ content and decreased the K^+ content at 11 mM $[K^+]_0$ (Table 1) which is in keeping with a suppressed Na^+-K^+ pump activity.

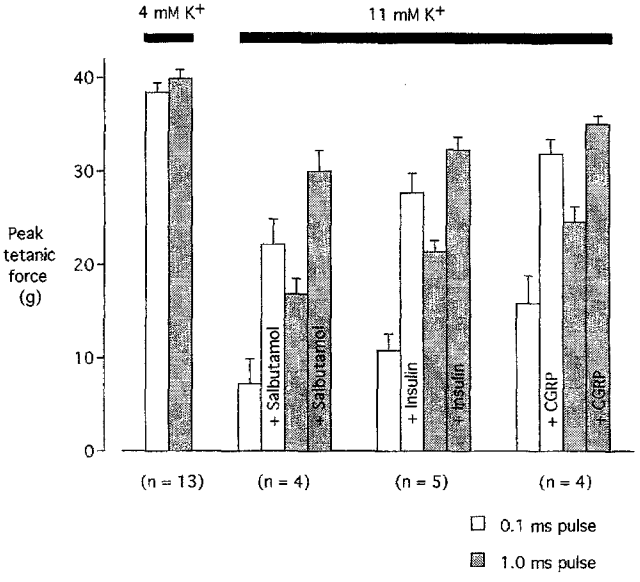


Fig. 2 Effect of salbutamol (10^{-5} M), insulin (100 mU/ml) or calcitonin gene-related peptide (CGRP, 10^{-7} M) on peak tetanic force in whole soleus muscles during exposure to solutions containing 11 mM $[K^+]$. Tetani were evoked at 100 Hz at 30°C using stimulation pulses of 0.1 ms, 8–12 V (*clear bars*) or 1.0 ms, 8–12 V (*filled bars*). The steady-state tetanic force at 11 mM $[K^+]_0$, with or without each agent, is expressed as the absolute force (g). Shown are the mean values ± SEM. The number of muscles used (*n*) are shown in *parentheses*. Each agent was added when force was maximally depressed at 11 mM $[K^+]_0$. Force restoration was significant with each agent ($P < 0.025$) and was greater when using 0.1-ms than with 1.0-ms pulses ($P < 0.025$)

Effect of Na^+-K^+ pump stimulation on K^+-depressed contractions

Three agents which have been shown to stimulate the Na^+-K^+ pump at normal as well as at elevated $[K^+]_0$: salbutamol (a β_2-agonist), insulin and CGRP [1, 2, 7, 8, 17], all induced a partial and transient restoration of tetanic force at elevated $[K^+]_0$ (Fig. 2). Salbutamol (10^{-5} M) restored tetanic force in whole muscles exposed to 11 mM $[K^+]_0$ from 44% to 77% of the control peak tetanic force at 4 mM $[K^+]_0$, i.e. by 33 ± 3% ($n = 4$, $P < 0.0025$). This was when we used our standard stimulation pulses (1.0 ms, 8–12 V). Similarly, when fibre bundles were exposed to 11–14 mM $[K^+]_0$ salbutamol restored tetanic force from 39% to 55% of the control level ($n = 7$, $P < 0.0001$). This increase (16 ± 1%) in fibre bundles was a smaller effect compared to that in whole muscles ($P < 0.00005$). Insulin (100 mU/ml) or CGRP (10^{-7} M) also restored tetanic force in whole muscles exposed to 11 mM $[K^+]_0$ (1.0-ms pulses) by 27 ± 4% ($n = 5$, $P < 0.0025$) and 26 ± 6% ($n = 4$, $P < 0.025$), respectively.

To elucidate how these agents restored contractility we used weaker stimulation pulses (0.1 ms in duration) than our standard 1.0-ms pulses. We had already shown a greater tetanus depression at 11 mM $[K^+]_0$ when using 0.1-ms pulses which we attributed to more fibres being inexcitable. We predicted that salbutamol, insulin or CGRP could re-establish excitability in some inexcitable fibres so that force recovery would be greater when using 0.1-ms pulses than with 1.0-ms pulses. These experiments with 0.1-ms pulses were done on the same muscles used earlier to describe the effects of each agent with 1.0-ms pulses. The restoration of tetanic force at 11 mM $[K^+]_0$ when using 0.1-ms pulses, as a percentage of the control force at 4 mM $[K^+]_0$, was 41 ± 3% with salbutamol ($n = 4$, $P < 0.0005$), 44 ± 2% with insulin ($n = 5$, $P < 0.00025$) and 41 ± 7% with CGRP ($n = 4$, $P < 0.01$). These increases were all significantly greater than when using 1.0-ms pulses (Fig. 2). The further increase amounted to 8% with salbutamol ($P < 0.025$), 17% with insulin ($P < 0.005$), and 15% with CGRP ($P < 0.025$).

Incubation with salbutamol, insulin or CGRP at 11 mM $[K^+]_0$ decreased the intracellular Na^+ content and increased the K^+ content (Table 1), indicating that these agents stimulated the Na^+-K^+ pump. Salbutamol was the only agent to have a significant effect on E_M, inducing a 1.3-mV repolarization (Table 1). We are uncertain whether this effect was transient, like the effect on force, due to its small magnitude. This suggests that a Na^+-K^+-pump-mediated increase in the Na^+ concentration difference may be important to increase excitability, or to re-establish excitability in inexcitable fibres, and thereby increase force in K^+-depressed muscle.

Discussion

We present two new relations for mammalian skeletal muscle: (1) the tetanic force/$[K^+]_0$ relation (Fig. 1A) and (2) the tetanic force/resting E_M relation (Fig. 1B). These relations provide new information on how K^+ influences contraction and how Na^+-K^+ pump activity modifies contraction at elevated $[K^+]_0$.

Possible mechanisms for the effects of elevated $[K^+]_0$ on contraction

The small reduction of tetanic force at a $[K^+]_0$ of 8–9 mM (Fig. 1A) might be explained if any depressive effects due to a reduced action potential amplitude [14, 25] were counteracted by a K^+-induced potentiation of excitation-contraction coupling [5]. A small further increase in $[K^+]_0$ resulted in a considerable reduction in force (Fig. 1A) which was a remarkably similar relation to that seen in patients suffering from hyperkalaemia [30].

The tetanus depression when $[K^+]_0$ was greater than 8–9 mM might be due to several effects of depolarization on action potentials which include: (1) a reduced amplitude [14, 18, 25]; (2) a reduced conduction velocity [16]; (3) inexcitability, i.e. a failure to generate action potentials [16, 25]. When $[K^+]_0$ was increased from 4 to 11 mM the E_M fell from −74 to −57 mV which would reduce the driving force for the Na^+ current, (E_{Na} - E_M), by about 15%. However, the main effect of this depolarization would be to cause slow inactivation of about 70–80% of the Na^+ channels available at 4 mM $[K^+]_0$ [26]. This would markedly reduce the Na^+ current and thereby decrease the amplitude of the action potential [26]. Certainly, K^+-induced depolarization to between −60 and −50 mV markedly diminishes the action potential in cardiac muscle [10], i.e. the critical E_M range where we observed the marked reduction in contractility (Fig. 1B). Inexcitability [16, 25] might result from an increased excitation threshold for action potentials [14]. In fact, much of the K^+-induced depression of tetanic force [17] can be accounted for by an increased number of inexcitable fibres ([16], present study). Force depression with up to 20 mM $[K^+]_0$ is unlikely to involve an impairment of excitation-contraction coupling in soleus fibres [5].

The greater depression of tetanic force at 11–14 mM $[K^+]_0$ in whole muscles than in fibre bundles might be due to greater stimulation-induced changes in ion concentrations in the extracellular space around central fibres in whole muscles due to diffusion restrictions. A higher $[K^+]_0$ in the interfibre space is likely [15] but other factors probably contribute because 16–20 mM $[K^+]_0$ did not completely depress contraction in fibre bundles. This could involve a greater decrease of the $[Na^+]$ in the interfibre space of whole muscles than in fibre bundles.

Role of the Na^+-K^+ pump in K^+ -depressed contractions

Inhibition of the Na^+-K^+ pump

Since partially suppressing Na^+-K^+ pump activity with ouabain exacerbated the force loss at elevated $[K^+]_0$, it is reasonable to assume that the Na^+-K^+ pump normally counteracts the depressive effects of increased $[K^+]_0$. The greater tetanus depression with ouabain was associated with a small increase in $[Na^+]_i$ at rest, but no further depolarization. Therefore, this extra force depression may be related to the decline of the Na^+ concentration difference across the sarcolemma at rest and/or to a much greater run-down of Na^+ and K^+ concentration differences during contraction.

Stimulation of the Na^+-K^+ pump

The observations that salbutamol, insulin, or CGRP restored the tetanic force at 11 mM $[K^+]_0$ by 26–33% in whole muscles, reduced the myoplasmic Na^+ content and increased the K^+ content, are all indicative of a stimulation of the Na^+-K^+ pump. Salbutamol induced a repolarization of 1.3 mV which, from Fig. 1B, we would predict to cause a force recovery of about 25% in whole muscles and about 8% in fibre bundles. This is slightly less than the measured force recovery. Insulin or CGRP did not induce any recovery of E_M which suggests that other mechanisms are involved. We cannot exclude the possibility, however, that stimulation of the Na^+-K^+ pump in *deeper* fibres in whole muscles decreases the $[K^+]_0$ in the adjacent interfibre space to cause repolarization in these fibres (see [2]). This effect would not occur in surface fibres where the $[K^+]_0$, adjacent to the sarcolemma, is buffered by the bulk extracellular solution. Such a mechanism might explain the greater force recovery that we observed with salbutamol in whole muscles compared with fibre bundles.

Another possibility is that force recovery with these agents is linked to the reduction in $[Na^+]_i$, as suggested previously [1, 8]. An increased Na^+ concentration difference is likely to increase the amplitude of action potentials [23]. Indeed, β_2-agonists increase the overshoot of the action potential in rat soleus muscle at normal $[K^+]_0$ [20]. Similarly, adrenaline restored the action potential amplitude in K^+-depressed cardiac muscle, which clearly did not involve any repolarization [10]. Engstfeld et al. [10] proposed that this effect was due to an increased Na^+ concentration difference. A doubling of the Na^+ concentration difference under normal conditions has little effect on peak force [14, 22]. However, under conditions when the Na^+ current is markedly reduced, a small increase in the Na^+ concentration difference can cause a large restoration of force [22]. Therefore, the increased Na^+ concentration difference may also be important in K^+-depressed conditions. A further insight as to the mechanism involved with these agents comes from the new observation of a greater recovery of force when using weaker stimulation pulses than normal, which can only be attributed to a recovery of action potentials. We propose that the increased Na^+ concentration difference, following Na^+-K^+ pump stimulation, allows the re-establishment of excitability in previously inexcitable fibres. Notably, adrenaline causes such a restoration of excitability in cardiac muscle at elevated $[K^+]_0$ [10].

The action of salbutamol may be due, in part, to the adenosine 3′:5′- cyclic monophosphate-(cAMP-) mediated [9] potentiation of Ca^{2+} release from the sarcoplasmic reticulum as seen at normal $[K^+]_0$ [4]. However the insulin-induced potentiation of force cannot involve cAMP since insulin does not increase the concentration of cAMP in skeletal muscle [9]. Since β-agonists and insulin share the ability to stimulate the Na^+ -K^+ pump, this is likely to be the common basis for the force recovery elicited by these two agents. Moreover, the relatively greater restoration of force with salbutamol, insulin and CGRP when using briefer pulses (Fig. 2) is consistent with a Na^+-K^+-pump-mediated recovery of action potentials and cannot readily be explained by a direct effect on the sarcoplasmic reticulum.

Implications for fatigue

The moderate tetanus depression at 8–9 mM $[K^+]_0$ suggests that a similar increase in $[K^+]_0$ during activity does not, by itself, cause much fatigue. However, it is speculated that the $[K^+]_0$ is even higher in the lumen of the transverse tubules [25, 27, 28]. If so, the increased $[K^+]_0$ could cause a large depression of force (Fig. 1A). In addition, the myoplasmic $[K^+]$ ($[K^+]_i$) can fall by 30–50 mM during fatiguing activity [15, 17, 19, 27, 28]. This decrease in $[K^+]_i$ combined with an increase in $[K^+]_0$ to 6–7 mM allows the calculated E_M to fall to -60 mV which would result in a large depression of force (Fig. 1B). Notably, several studies have shown that the E_M can be reduced to less than -60 mV during fatigue [15, 16, 18, 19]. Therefore, we support the hypothesis that K^+ has a role in fatigue but only when it causes a sufficiently large depolarization to the critical E_M range.

β-Adrenergic stimulation of the Na^+-K^+ pump has been shown to prevent or attenuate the impairment of force production during fatiguing stimulation and hyperkalaemia [17, 30]. From the present study, we suggest that changes in $[K^+]_i$ and $[Na^+]_i$ are likely to be necessary for such effects to occur.

Acknowledgements It was a pleasure to have the expert technical assistance of Marianne Stürup-Johansen, Ebba de Neergaard and Jette Sandgaard. This work was supported by the Danish Medical Research Council (Grant number 12-9262) and the Danish Biomembrane Research Centre.

References

1. Andersen SLV, Clausen T (1993) Calcitonin gene-related peptide stimulates active Na^+-K^+ transport in rat soleus muscle. Am J Physiol 264:C419–C429
2. Ballanyi K, Grafe P (1988) Changes in intracellular ion activities induced by adrenaline in human and rat skeletal muscle. Pflügers Arch 411:283–288
3. Bowman WC, Raper C (1964) The effects of adrenaline and other drugs affecting carbohydrate metabolism on contraction of the rat diaphragm. Br J Pharmacol 23:184–200
4. Cairns SP, Dulhunty AF (1993) β-Adrenergic potentiation of E-C coupling increases force in rat skeletal muscle. Muscle Nerve 16:1317–1325
5. Chua M, Dulhunty AF (1988) Inactivation of excitation-contraction coupling in rat extensor digitorum longus and soleus muscles. J Gen Physiol 91:737–757
6. Clausen T, Everts ME (1991) K^+-Induced inhibition of contractile force in rat skeletal muscle: role of active Na^+-K^+ transport. Am J Physiol 261:C799–C807
7. Clausen T, Flatman JA (1987) Effects of insulin and epinephrine on Na^+-K^+ and glucose transport in soleus muscle. Am J Physiol 252:E492–E499
8. Clausen T, Andersen SLV, Flatman JA (1993) Na^+-K^+ pump stimulation elicits recovery of contractility in K^+-paralysed rat muscle. J Physiol (Lond) 472:521–536
9. Craig JW, Rall TW, Larner J (1969) The influence of insulin and epinephrine on adenosine 3′,5′-phosphate and glycogen transferase in muscle. Biochim Biophys Acta 177:213–219
10. Engstfeld G, Antoni H, Fleckenstein A, Nast A, Hattingberg MV (1961) Die Restitution der Erregungsfortleitung und Kontraktionskraft des K^+-gelähmten Frosch- und Säugetiermyokards durch Adrenalin. Pflügers Arch 273:145–163
11. Everts ME, Clausen T (1992) Activation of the Na-K pump by intracellular Na in rat slow- and fast-twitch muscle. Acta Physiol Scand 145:353–362
12. Hnik P, Holas M, Krekule I, Kriz N, Mejsnar J, Smiesko V, Ujec E, Vyskocil F (1976) Work-induced potassium changes in skeletal muscle and effluent venous blood assessed by liquid ion-exchanger microelectrodes. Pflügers Arch 362:85–94
13. Holmberg E, Waldeck B (1980) The effect of insulin on skeletal muscle contractions and its relation to the effect produced by β-adrenoceptor stimulation. Acta Physiol Scand 109: 225–229
14. Jones DA (1981) Muscle fatigue due to changes beyond the neuromuscular junction. In: Porter R, Whelan J (eds) Human muscle fatigue: physiological mechanisms (Ciba Foundation Symposium 82). Pitman Medical, London, pp 178–192
15. Juel C (1986) Potassium and sodium shifts during in vitro isometric muscle contraction, and the time course of the ion-gradient recovery. Pflügers Arch 406:458–463
16. Juel C (1988) Muscle action potential propagation velocity changes during activity. Muscle Nerve 11:714–719
17. Juel C (1988) The effect of β_2-adrenoceptor activation on ion-shifts and fatigue in mouse soleus muscles stimulated *in vitro*. Acta Physiol Scand 134:209–216
18. Lännergren J, Westerblad H (1986) Force and membrane potential during and after fatiguing, continuous high-frequency stimulation of single *Xenopus* muscle fibres. Acta Physiol Scand 128:359–368
19. Lindinger MI, Heigenhauser GJF (1991) The roles of ion fluxes in skeletal muscle fatigue. Can J Physiol Pharmacol 69:246–253
20. McArdle JJ, D'Alonzo AJ (1981) Effects of terbutaline, a β_2-adrenergic agonist, on the membrane potentials of innervated and denervated fast- and slow-twitch muscles. Exp Neurol 71: 134–143
21. Medbø JI, Sejersted OM (1990) Plasma potassium changes with high intensity exercise. J Physiol (Lond) 421:105–122
22. Nakajima S, Nakajima Y, Bastian J (1975) Effects of sudden changes in external sodium concentration on twitch tension in isolated muscle fibers. J Gen Physiol 65:459–482
23. Nastuk WL, Hodgkin AL (1950) The electrical activity of single muscle fibers. J Cell Comp Physiol 35:39–73
24. Nørgaard A, Kjeldsen K, Clausen T (1981) Potassium depletion decreases the number of ^{3}H-ouabain binding sites and the active Na, K-transport in skeletal muscle. Nature 293:739–741
25. Renaud JM, Light P (1992) Effects of K^+ on the twitch and tetanic contraction in the sartorius muscle of the frog, *Rana pipiens*. Implication for fatigue *in vivo*. Can J Physiol Pharmacol 70:1236–1246
26. Ruff RL, Simoncini L, Stühmer W (1987) Comparison between slow sodium channel inactivation in rat slow- and fast-twitch muscle. J Physiol (Lond) 383:339–348
27. Sejersted OM (1992) Electrolyte imbalance in body fluids as a mechanism of fatigue during exercise. In: Lamb DR, Gisolfi CV (eds) Perspectives in exercise science and sports medicine vol 5., Brown and Benchmark, Dubuque, IA, pp 149–206
28. Sjøgaard G, Adams RP, Saltin B (1985) Water and ion shifts in skeletal muscle of humans with intense dynamic knee extension. Am J Physiol 248:R190–R196
29. Tomita T (1975) Action of catecholamines on skeletal muscle. In: Blaschko H, Sayers G, Smith AD (eds) Handbook of physiology, section 7. Endocrinology, part VI, adrenal gland. American Physiological Society. Williams and Williams, Baltimore, Md., pp 537–552
30. Wang P, Clausen T (1976) Treatment of attacks in hyperkalaemic familial periodic paralysis by inhalation of salbutamol. Lancet i:221–223