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Electrical and ionic regulation of Na^+,K^+-ATPase isoform mRNA expression in isolated fast-twitch muscle in the rat

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Acute exercise has been shown to elevate Na^+,K^+-ATPase isoform mRNA expression in both human (1) and in rat muscle (2). Whether electrical stimulation exerts similar effects is unknown and was therefore investigated here in rat skeletal muscle. This study also explored factors regulating Na^+,K^+-ATPase transcription in isolated EDL muscle by utilising interventions designed to induce increased intracellular $[Na^+]$ ($[Na^+]_i$), cytosolic free $[Ca^{2+}]$ ($[Ca^{2+}]_{cyto}$), and membrane depolarisation.
Rats were humanely killed and dissected muscles were equilibrated for 30 min at 30°C in standard Krebs-Ringer bicarbonate buffer (KR) (3). Soleus and EDL muscles were mounted on force transducers and stimulated electrically (3 stimulation bouts, comprising 10 s of stimulation given every 10 min, at 60 Hz, 12 V) to evoke short tetani at regular intervals. Ouabain (1 mM, 120 min), veratridine (0.1 mM, 30 min) and monensin (0.1 mM, 30 min) were used to induce increased $[Na^+]_i$ or Na^+ influx, with muscle Na^+ content measured by flame photometry. Caffeine (5 mM, 30 min) and high extracellular $[K^+]$ ($[K^+]_e$) (13 mM, 60 min) were used to induce elevated $[Ca^{2+}]_{cyto}$ and membrane depolarisation, respectively. Muscles exposed to electrical stimulation were either immediately removed or allowed to rest for 3 h in KR buffer, while muscles incubated in ouabain, monensin, veratridine, caffeine or high $[K^+]_e$ were allowed to rest for 3 h in normal KR buffer. Muscles were then analysed for Na^+,K^+-ATPase $\alpha1$, $\alpha2$, $\alpha3$, $\beta1$, $\beta2$ and $\beta3$ mRNA expression (real-time RT-PCR).
Electrical stimulation had no immediate effect on Na^+,K^+-ATPase mRNA in either the EDL or the soleus, but at 3 h post-stimulation increased $\alpha1$, $\alpha2$ and $\alpha3$ mRNA by 3.2-, 7.2- and 9.9-fold, respectively, only in the EDL ($P<0.02$). In the EDL, ouabain increased muscle Na^+content by 90% ($P<0.01$), but Na^+,K^+-ATPase isoform mRNA was unchanged; neither muscle Na^+ content nor isoform mRNA were increased with veratridine or monensin. Caffeine elevated $\alpha1$, $\alpha2$ and $\beta1$ mRNA by 2.6-, 2.8- and 2.7-fold, respectively ($P<0.03$), while high $[K^+]_e$ did not affect Na^+,K^+-ATPase isoform mRNA in the EDL.
In conclusion, the effect of high-frequency electrical stimulation on Na^+,K^+-ATPase isoform mRNA expression was both isoform- and muscle-specific. Furthermore, Na^+,K^+-ATPase transcription appears to be regulated by different stimuli in rat EDL muscle, including the cellular changes associated with caffeine (such as elevated $[Ca^{2+}]_{cyto}$).

Murphy et al. (2004). J Physiol 556, 507-519.

Tsakiridis et al. (1996). J Appl Physiol 80, 699-705.

Macdonald et al. (2005). J Physiol 563, 459-469.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C48

Carbacholine-induced loss of sarcolemmal excitability and muscle contractility in isolated muscles: recovery with Na^+-K^+ pump stimulation

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Intense exercise results in increases in intracellular Na^+ and extracellular K^+ concentrations, leading to depolarisation and a loss of muscle excitability and contractility. Here, we use carbacholine to chronically activate the nicotinic acetylcholine (nACh) receptors, mimicking the changes in membrane permeability, chemical Na^+ and K^+ gradients and membrane potential, observed during intense exercise. Intact soleus muscles obtained from humanely killed rats were mounted on force transducers and stimulated electrically to evoke short tetani of 60 Hz, 12 V, 2 s, every 10 min, at 30°C. Carbacholine (10^{-4} M) produced a 2.6-fold increase in Na^+ influx, that was tetrodotoxin (TTX, 10^{-6} M) insensitive but abolished by tubocurarine (10^{-5} M), resulting in a significant 36% increase in intracellular Na^+ and 8% decrease in intracellular K^+ content. The mid region, near the motor end plate, had larger changes in Na^+ and K^+ contents than did more distal regions of the muscle, and was associated with a greater membrane depolarisation of 13 mV compared to 9 mV. Carbacholine significantly reduced tetanic force to ~30% of controls, which was significantly recovered by Na^+-K^+ pump stimulation with salbutamol (10^{-5} M), adrenaline (10^{-5} M) and calcitonin gene related peptide (CGRP, 10^{-7} M). The salbutamol induced force recovery was accompanied by a restoration of intracellular Na^+ and K^+ contents and a small 4-5 mV recovery of membrane potential. Similar results were obtained using succinylcholine (10^{-4} M), indicating that Na^+-K^+ pump stimulation may prevent or restore depression of muscle finction caused by succinylcholine-induced hyperkalemia. Stimulation of the Na^+-K^+ pump allows muscle to partially recover contractility, by regaining excitability through electrogenically driven repolarisation of the muscle membrane.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Extracellular potassium concentration, muscle electrical activity and efficiency during voluntary exercise with a small muscle group

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The elevation of extracellular potassium concentration depolarizes the sarcolemma (1) and therefore can result in a loss of excitability. This effect is widely discussed as a possible cause of muscle fatigue. On the other hand, muscle activity stimulates the Na^+-K^+ pump too, which contributes to recovery of RMP and therefore excitability and contractility. In our study the effects

of the elevated venous $[K^+]$ ($[K^+]_v$) has been proved during handgrip exercise.

The study was performed according to the Declaration of Helsinki. Each of nine male volunteers performed 12 exercise bouts of 1 min separated by 4 min of rest after the 15 min warm-up phase and following 4 min resting. During warming-up the subjects lifted a 7.5 kg weight for 2.5 s. During the test phase dynamic (DE) and static (SE) exercise bouts were altered. The subjects had to lift the weight and to keep it at a constant level during SE and to lift the weight with maximal speed during DE using the forearm muscles only. The weight was varied randomly between 5 and 30 kg with steps of 5 kg. Blood was drawn from the cubital vein draining the working forearm. To conserve blood composition during measurements, the blood flow in the exercising arm was occluded after 55 s of exercise for 15-20 s. M-wave recording and blood sampling took place just before and immediately after each exercise period. Acid-base state, lactate, $[K^+]_v$ and $[Na^+]_v$ in plasma were determined. EMG was recorded via surface electrodes. The motor point of the muscle was stimulated supramaximally with rectangular current pulses. M-waves were analysed for area of the negative phase and duration. A t test was used to identify different means. All data are expressed as mean ± S.D.

After warming-up and following 4 min resting the M-wave area increased by 24.9±19.6% ($p<0.01$, n=9). $[K^+]_v$ decreased thereby from 4.1±0.3 to 3.5±0.2 mM ($p<0.01$, n=9). Thereafter pre-exercise values of both $[K^+]_v$ and M-wave area remained at these levels. Muscle force during SE and power during DE decreased intensity-dependently. $[K^+]_v$ correlated with changes of total EMG both in DE ($r^2=0.75$, $P<0.001$, n=54) and SE ($r^2=0.62$, $P<0.001$, n=54). Changes of $[K^+]_v$ correlated negatively with M-wave area ($r^2=0.37$, $p<0.001$, n=54) (Fig. 1). The median power frequency of EMG decreased during exercise intensity-dependent ($r^2=0.43$ for SE, $r^2=0.21$ for DE, $p<0.001$, n=54). Nevertheless, M-waves did not show any sign of decrease of the muscle AP propagation velocity and the M-wave area never decreased below the initial value. It can be concluded, that in spite of decrease of M-wave area with elevation of $[K^+]_v$ there should be another cause, maybe of the central origin, for the decrease of muscle force.

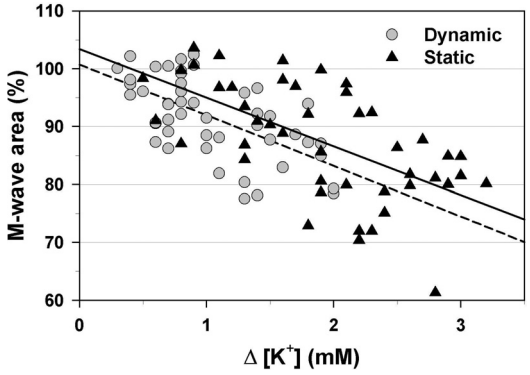

Figure 1. Relations between venous potassium and M-wave area Hodgkin AL & Horowicz P (1960). J Physiol 153, 370-385.

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Effects of halothane on depolarisation-induced Ca^{2+} release from the sarcoplasmic reticulum in isolated rat skeletal muscle fibres

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The skeletal muscle disorder malignant hyperthermia (MH) is associated with increased sensitivity of RYR1 to activation by volatile anaesthetics. Previous work suggests that the inhibitory effect of Mg^{2+} on SR Ca^{2+} release is impaired in MH-susceptible muscle (Laver *et al.* 1997). In the present study, we have investigated the effects of halothane on Ca^{2+} release from the SR induced by t-tubule depolarisation, in the presence of normal (1 mM) or reduced levels of cytosolic $[Mg^{2+}]$. Rats (200-250 g) were humanely killed. Single extensor digitorium longus (EDL) muscle fibres were mechanically skinned under oil and then superperfused with solutions approximating to the intracellular milieu containing (mM): HDTA, 50; ATP, 8; Na^+, 37; K^+,126; phosphocreatine, 10; EGTA, 0.03; Hepes, 90; Ca^{2+}, 0.0001; fluo-3, 0.002; pH 7.0, 22°C. Following skinning, the t-tubules reseal and repolarise, allowing the physiological depolarisation-induced Ca^{2+} release process to be initiated by field stimulation. Ca^{2+} release from the SR was measured using line-scan confocal microscopy to detect changes in fluo-3 fluorescence. A train of brief twitch responses was initiated by applying suprathreshold square-wave stimuli (70 V, 2 ms duration) at 2 second intervals, via platinum electrodes running parallel to the fibre. At 1 mM free Mg^{2+}, 1 mM halothane induced a significant (17.4 ± 7 %, n=6, mean ± SE, $p<0.05$) increase in the time taken for the Ca^{2+} transient amplitude to decline by 75%. At 0.4 mM free $[Mg^{2+}]$, the effects of halothane were much more pronounced: Ca^{2+} transients failed to decline fully to baseline between stimuli and often exhibited a slow secondary Ca^{2+} release phase, consistent with regenerative Ca^{2+} -induced Ca^{2+} release (CICR). However, in the absence of depolarisation-induced Ca^{2+} release, perfusion with 1 mM halothane failed to induce Ca^{2+} release from the SR at either 1 mM or 0.4 mM free Mg^{2+}. These results suggest that in MH, impaired Mg^{2+} inhibition of RYR1 may facilitate the action of halothane on the SR, such that the physiological Ca^{2+} release mechanism fails to terminate. These effects may be of particular importance when susceptibility to MH is combined with (i) hypomagnesemia or (ii) depolarising muscle relaxants.

Laver DR et al. (1997). *Biophys J* **73**, 1913-1924.

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SA19

The potentiating and depressing effects of K^+ on muscle contraction and their implication in the aetiology of muscle fatigue

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Interstitial K^+ concentration increases up to ~10 mM during a muscle activity leading to fatigue. It is well known that increases in extracellular K^+ concentration depolarize cell membrane. As the membrane depolarizes more Na^+ channels become inactivated resulting in lower action potential amplitudes. It was believed that lower action potential amplitude results in lower amount of Ca^{2+} release and force development.
However, recent studies have demonstrated that at 37°C twitch and tetanic force is depressed only if the extracellular K^+ concentration exceeds 12 mM, a concentration usually not observed during exercise. More importantly, increases in extracellular K^+ up to 12 mM from control level (4.7 mM) potentiate twitch force. Force-frequency relationship shows that the K^+-induced force potentiation also occurs at stimulation frequencies up to 100 Hz in mouse EDL and up to 30 Hz in soleus muscle.
Action potential durations are longer at 9 mM compared with those at 4.7 mM K^+. In mouse EDL muscle, mimicking the change in action potential with TTX does not give rise to twitch potentiation, suggesting that the mechanisms of action for the potentiation is downstream of action potential. In EDL muscle, the potentiation is also not related to longer contraction time, albeit it is in soleus muscle. K^+-induced and post-tetanic twitch potentiation are additive in EDL muscle, suggesting that the K^+ induced twitch potentiation is not due to a phosphorylation of myosin light chain. V_{MAX} is also unaffected at 9 mM K^+. The experiments involving post-tetanic twitch potentiation and V_{MAX} suggest that the K^+-induced potentiation may not involve an effect at the level of the contractile apparatus.
The next question is whether or not K^+ is an important factor contributing to the decrease in force during fatigue. While the concentration of K^+ must exceed 12 mM before it depresses force under resting conditions, a decrease in Na^+ concentration gradient, involving a decrease in extracellular Na^+ as small as 20 mM, is large enough to switch the potentiating effect of K^+ to a depressing effect at 12 mM.
In summary, increases in extracellular K^+ concentration during exercise does not necessarily lead to force depression as it can also potentiate force. However, the capacity of K^+ to potentiate and depress force appears to depend on the physiological state of muscles. Muscles were removed from mice under terminal sodium pentobarbitone anaesthesia.

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Regulation of excitability in working muscle: role of pH and chloride channel function

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During exercise, loss of K+ from active muscles causes an increase in the extracellular K+ concentration ([K+]o). When exercise intensity is high, the concentration of K+ may increase to above 10 mM in the interstitium of the working muscles (see e.g. Sejersted & Sjogaard, 2000). Since exposure of isolated muscles to such concentrations of K+ leads to loss of muscle excitability and depression of excitation-induced force production, the exercise-induced increase in [K+]o has been proposed to contribute to muscle fatigue (Sejersted & Sjogaard, 2000). The loss of muscle excitability at high [K+]o is most likely related to slow inactivation of voltage gated Na+ channels (Ruff, 1996). When Na+ channels inactivate, the inward depolarising Na+ current they carry becomes to weak to overcome the re-polarising or inhibitory K+ and Cl- currents, resulting in failure of the initiation and of the propagation of action potentials. Since skeletal muscle has a large Cl- conductance, this vulnerability of membrane function to increased [K+]o may be of particular importance for the excitability of this tissue.
Lately we have found that in acidified rat muscles, excitability and force production can be maintained at a higher [K+]o than in muscles at normal pH (Nielsen et al. 2001). Thus, when isolated soleus muscles from humanely killed rats were incubated at a [K+]o of 10 mM, which reduced tetanic force and excitability (as judged from the area of compound action potentials, M-waves) by around 75%, subsequent addition of 20 mM lactic acid led to an almost complete recovery of force production and excitability. The increased tolerance of acidified muscles to elevated [K+]o could also be induced by increasing the CO2 tension and seems to be related to a reduction in intracellular pH.
In search for a mechanism for the improved excitability of acidified muscles we found that a similar increase in the tolerance to elevated [K+]o could be induced in muscles at normal pH by adding 9-AC (an inhibitor of the major muscle Cl- channel, ClC1) or by replacing buffer Cl- by methanesulfonate, which do not penetrate through the Cl- channels (Pedersen et al. 2005). Since both treatments reduces the Cl- conductance of the muscle fibres, this indicated that the improved excitability in acidified muscles could be related to an inhibition of Cl- channels leading to a lowered Cl- conductance. To examine this, the effect of acidosis on the membrane conductance of single muscle fibres was measured in muscles incubated at 11 mM K+ in normal and in Cl- free buffer at 5 or 24% CO2. Membrane conductance was measured by injecting hyperpolarizing constant current pulses into muscle fibres through one intracellular microelectrode while recording the membrane voltage response by another electrode. From these measurements, membrane conductance was calculated according to Boyd & Martin (1959). These experiments showed that in muscles at 11 mM K+, the recovery of compound action potentials and force with muscle acidification was associated with a reduction in the chloride conductance from 1731

to 938 S/cm2 ($P < 0.01$). No change was observed in the potassium conductance (405 to 455 S/cm2 ($P < 0.16$). From this study it is concluded that the recovery of excitability induced by acidification in K+-depressed muscles is related to a reduction in the inhibitory Cl- currents, possibly through an inhibition of ClC-1 channels, and that acidosis thereby reduces the Na+ current needed to generate and propagate an AP.

Together, these findings indicate that the development of muscle acidosis during intense exercise causes an up-regulation of muscle excitability, which may help muscles to maintain their excitability when [K+]o is increased. Thereby, the development of muscle acidosis may delay possible fatigue caused by loss of muscle excitability.

Boyd IA & Martin AR (1959). J Physiol 147, 450-457.

Nielsen OB et al. (2001). J Physiol 536, 161-166.

Pedersen TH et al. (2005). J Gen Physiol 125, 237-246.

Ruff RL (1996). Acta Physiol Scand 156, 159-168.

Sejersted OM & Sjogaard G (2000). Physiol Rev 80, 1411-1481.

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