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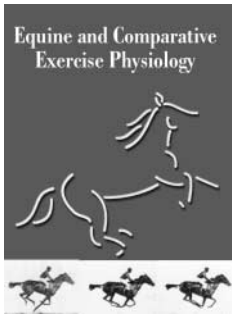

Determinants of surface membrane and transverse-tubular excitability in skeletal muscle: implications for high-intensity exercise

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Abstract

The fatigue of high-intensity exercise is now believed to reside primarily within the excitation–contraction coupling processes associated with the plasma membrane of skeletal muscle (sarcolemm) and calcium-mediated events leading to myofilament sliding. This paper summarizes recent developments and advances in the identification of factors that contribute to changes in sarcolemmal excitability of mammalian skeletal muscle as a consequence of high-intensity exercise. There is an increasing recognition of the probable role that is played by the transverse tubular system (T-system), a system that comprises *c.* 80% of the total sarcolemmal surface capable of ion exchange. Furthermore, the fluid within the T-system has limited access to interstitial fluid bathing myofibres; hence, T-system fluid is probably markedly different from interstitial fluid during high-intensity exercise. Mechanically skinned fibre preparation is providing many new insights into functions of the surface membrane and T-system in fatigue. A scenario is developed whereby accumulation of potassium within the T-system ($[K^+]_o$) contributes to reduced membrane excitability, as well as lowering of T-system sodium and chloride, concomitant with loss of intracellular potassium ($[K^+]_i$) and accumulation of intracellular sodium ($[Na^+]$) and chloride ($[Cl^-]$). Lowering the $[Na^+]_o/[Na^+]_i$ ratio and raising myoplasmic $[Na^+]_i$ have been shown to decrease membrane excitability and impair action potential propagation. Maintained high $[Cl^-]_o$ may also have a protective effect in maintaining membrane excitability, and this effect appears to be very pronounced in the presence of raised $[K^+]_o$. In contrast to dogma associating high $[H^+]$ to fatigue, recent studies have also shown that induced acidosis that results in increased $[H^+]_o$ and $[H^+]_i$ restores force production in muscles and skinned fibres fatigued by intermittent tetanic stimulation. This effect may be due to a decrease in surface membrane Cl^- permeability that serves to restore membrane excitability. During high-intensity exercise, simultaneous changes in transmembrane ion concentrations and membrane ion conductances may serve to reduce impairment of membrane excitability that provides for a maintained, though reduced, contractile function.

Keywords: membrane potential; potassium; sodium; T-tubule; contraction; chloride

Introduction

High-intensity exercise in mammalian athletes is accompanied by a rapid onset of skeletal muscle fatigue that is associated with a decline of plasma membrane excitability. This reduction of excitability is gradual and progressive during the period of exercise, allowing continued muscle contractions, albeit at slowed shortening and relaxation velocities. Therefore, the racing Thoroughbred, greyhound or human sprinter that crosses the finish line first is an individual that has decreased its muscle-shortening velocity, and hence stride rate or sprint velocity, to a lesser extent than its competitors during the final seconds of the race. The mechanisms for this delayed slowing of muscle function may be the result of factors that affect sarcolemmal excitability. In the context of this paper, the sarcolemma is separated into the surface membranes that are proximal to the interstitial fluids and to the transverse tubule system (T-system); these represent *c.* 20% and *c.* 80% of the total sarcolemmal membrane area, respectively[1].

The purpose of this paper is to summarize recent developments and advances in the identification of

factors that contribute to mammalian skeletal muscle sarcolemmal excitability, or inexcitability, as a consequence of the many changes occurring in the vicinity of the surface membrane and T-system during high-intensity exercise. High-intensity exercise is defined as contractile activity generating power outputs equal to or greater than those achieved at VO_2 peak, such as that which occurs during the performance of maximal or supramaximal sprinting activities in humans, horses and dogs. The emphasis is on discoveries made over the past 5 years, of which there are several. These discoveries contribute to an increased understanding of the roles of the Na,K ATPase, chloride concentrations and sarcolemmal chloride conductance, effects of lactic acid and intracellular acidosis on membrane excitability and possible roles for the ATP-sensitive potassium channels (K_{ATP}) and sodium–potassium–two–chloride–cotransporter (NKCC) in cell ion and volume regulation. For summaries prior to these developments, please refer to reviews by Nielsen *et al.*[2], Sejersted and Sjogaard[3], Renaud[4], Gosmanov *et al.*[5], Clausen[6] and Green[7].

What is membrane excitability?

Membrane excitability may be defined as that characteristic of membranes that allows for a rapid change in electrical potential in response to a stimulus; with respect to skeletal muscle, excitability specifically refers to the amount of inward current required to depolarize the sarcolemma sufficiently to trigger the opening of enough Na^+ channels to elicit an action potential[3]. The main factors contributing to sarcolemmal excitability include the presence and quantity of fixed, negatively charged particles on the intracellular side of the sarcolemma, the distribution of ions across the sarcolemma, the unidirectional and net flux of ions through transporters and channels and the regulators of ion transport/channel proteins. The electrical membrane potential (E_m) of a non-fatigued skeletal muscle cell ranges from -65 to -90 mV depending on fibre type and species. In rodent muscle, slow-twitch oxidative fibres have a more depolarized E_m (-75 to -65 mV), while fast glycolytic fibres have an E_m that is more polarized, from -90 to -80 mV[8]. The negative electrical potential of cells results from the presence of fixed negative charges on intracellular proteins[9], and these proteins are in greater abundance in fast glycolytic fibres than in slow oxidative fibres[10].

In excitable cells such as neurons, cardiac and skeletal muscle, there is a requirement for rapidly changing the electrical potential of the plasma membrane, and this requirement is met by the presence of ion-selective channels that allow for the flux of ions across surface and T-system membranes. In order to generate rapid changes in membrane electrical potential, cells take advantage of the electrochemical driving forces for each ion. For example, in striated muscle, the excitation–contraction coupling sequence initiates with the release of acetylcholine (ACh) from motor nerve endplates at the neuromuscular junction, which results in binding of ACh to its receptor (ACh-R), specialized cation channels on the surface membrane at the location of neuromuscular junctions[11]. The binding of ACh to the ACh-R results in a conformational change of the protein that allows rapid entry of sodium into the cell, causing localized sarcolemmal depolarization. Voltage-gated sodium channels in proximity to the ACh-R are in high density[11] and not sensitive to ACh. When sarcolemmal voltage adjacent to the ACh-R increases (depolarization), these voltage-gated sodium channels open, allowing additional influx of sodium (inward sodium current) and localized spreading of surface membrane depolarization. Surface membrane depolarization (action potential) spreads as a wave over the myofibre surface and into the T-system. Depolarization of the T-system in turn signals the sarcoplasmic reticulum (SR) to release calcium[12–14]. The increase in free myoplasmic calcium concentration resulting from the large, rapid release of calcium from the SR also serves as a feedback mechanism to open the high-conductance, calcium–activated potassium channels (BKCa), which are twofold more abundant in T-system membranes than in the surface membrane[15]. This in turn results in a large outward K^+ current that helps repolarize the E_m.

In skeletal muscle, the force of contraction is determined, in part, by the relationship between resting E_m, the action potential and SR calcium release. The resting E_m is that which occurs while the muscle is quiescent and not undergoing depolarization or repolarization (Fig. 1). The threshold potential is that E_m at which the voltage-gated Na^+ channels open, resulting in rapid depolarization of the E_m and the upstroke of the action potential. In general, the more depolarized the E_m, the

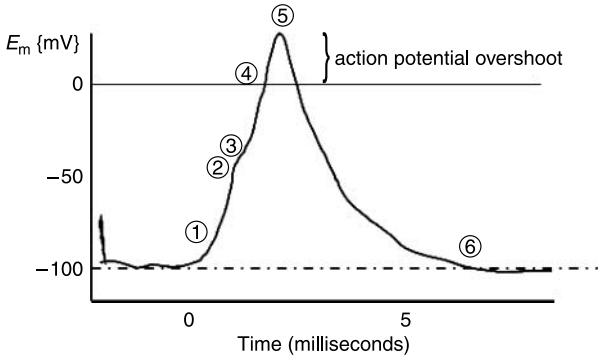

Fig. 1 Resting membrane potential and the action potential. A nerve impulse arrives at the neuromuscular junction at the arrow. The depolarization phase comprises: 1. Opening of AChR channels letting Na^+ rapidly enter the cell; 2. Opening of voltage-dependent Na^+ channels; 3. T-system depolarization results in calcium release from SR and initiates contraction. Repolarization comprises: 4. Opening of K^+ channels letting K^+ rush out; 5. Closing of Na^+ channels; 6. Membrane potential restored

greater will be the action potential. The frequency of action potential generation is directly proportional to the magnitude of SR calcium release and force of contraction[16].

In addition to the ACh-R and voltage-gated sodium channels, there are different types of potassium channels[3], chloride channels[17], an NKCC[5], a ouabain-sensitive Na,K ATPase[6] and possibly an ouabain-insensitive Na,K ATPase[18] that contribute to surface membrane and T-tubule excitability.

Changes in E_m due to passive distribution of ions

The E_m is determined by the concentrations of extracellular ($[\,]_o$) and intracellular ($[\,]_i$) ions, as described by the Goldman equation:

$$E_m = \frac{RT}{F}\ln\left(\frac{p_k[K^+]_o + p_{Na}[Na^+]_o + p_{Cl}[Cl^-]_i}{p_k[K^+]_i + p_{Na}[Na^+]_i + p_{Cl}[Cl^-]_0}\right)$$

where R is the gas constant, T is absolute temperature, F is Faraday's constant and P is the relative permeability of the membrane for the ion; $RT/F = 26.7$ mV at 37°C[21].

The resting E_m is negative due to the presence of fixed negative charges in the cell interior in excess of diffusible cation charge[19]. The surface and T-tubule membranes are highly permeable for Cl^-, and this high Cl^- conductance is a primary determinant of membrane excitability and membrane stability upon depolarization[20]. Surface membranes of excitable cells have a much higher permeability to Cl^- than to cations, and Cl^- flux effectively maintains charge balance for net cation fluxes. Therefore, changes in membrane excitability are typically effected through changes in intracellular [Cl^-] and extracellular [K^+], while changes in intracellular and extracellular [Na^+] have minimal effect on E_m because of its relatively low permeability compared with K^+ and Cl^-[21].

Upon excitation (transmission of the nerve impulse to the muscle fibre at the neuromuscular junction), surface membrane depolarization and the generation of the action potential arises directly from the inward sodium current. The resultant accumulation of Na^+ in the subsarcolemmal region exceeds the sum of fixed negative charges in this region, resulting in the more positive membrane potential. Repeated stimulation of skeletal muscle, such as that which occurs during very high-intensity exercise, results in changes in intracellular and extracellular (interstitial fluid) ion concentrations. An example with very large concentration changes, with E_m estimated using the Goldman equation, is provided in Table 1.

Ion transporters and channels

Disturbances in the trans-membrane concentrations of ions are tolerated for only very brief periods, and a number of ion transport and channel systems are activated that serve to restore ion concentrations back towards those seen in resting, un-contracted muscle. Transport will be defined loosely to refer to flux of ion across membranes against the electrochemical gradient for at least one of the ions being transported, i.e. the NKCC or Na,K ATPase. In addition to transport, ions may cross membranes through channels, most of which are highly specific for a given ion (Fig. 2).

From the preceding descriptions, it is apparent that changes in intracellular, interstitial or T-system ion concentrations resulting from net flux of ions across membranes will affect membrane excitability and E_m. Such changes result in the activation of regulatory mechanisms. For example, increased Na,K ATPase activity, in the absence of altered ion flux through other pathways such as that which occurs in response to increased intracellular [Na^+] and beta-adrenergic stimulation[6], results in an increased net flux of K^+ into the cell and Na^+ out of the cell. The increase in Na,K ATPase

Table 1 Changes in intracellular and interstitial fluid ion concentrations and calculated effects on E_m

Ion	Change in $[ion]_i$ during high-intensity exercise (mmol l^{-1})	Effect on resting E_m	Change in $[ion]_o$ during high-intensity exercise	Effect on resting E_m
K^+	−60	−74.2	+10 mmol l^{-1}	−60.2
Na^+	+20	−84.7	No change	
Cl^-	+30	−66.9	No change	

E_m was estimated using the Goldman equation, assuming no changes in relative membrane permeability for each ion. However, it is likely that surface or T-system membrane permeabilities change during high-intensity exercise and early recovery. The estimated E_m prior to the manipulations is −84.7. The combined effects of all changes noted in the table yield an E_m of −41.5 mV. Initial values are: $[K^+]_o$ 4 mM, $[K^+]_i$ 155 mM, $[Na^+]_o$ 140 mM, $[Na^+]_i$ 10 mM, $[Cl^-]_o$ 100 mM, $[Cl^-]_i$ 4 mM, the permeability ratio for sodium:potassium 0.009 and the permeability ratio for chloride:potassium 0.208[21] (see Lindinger and Heigenhauser, 1991). The changes in $[ion]_i$ and $[ion]_o$ during high-intensity exercise were approximated from measurements in rodent[24] (Lindinger and Heigenhauser, 1988) and human (Lindinger *et al.*, 1995) skeletal muscle.

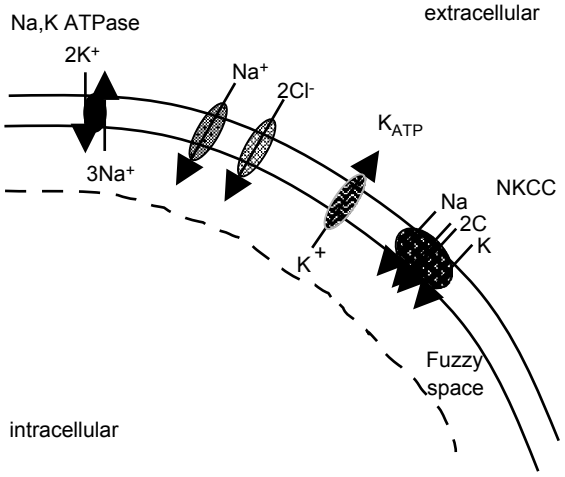

FIG. 2 Schematic representation of some of the key ion transport and channel systems in surface membranes of skeletal muscle

activity contributes to restoration of membrane potential; the decrease in intracellular positive charge is effected by the pumping of $2K^+$ into the cell for each $3Na^+$ removed (this also decreases intracellular tonicity and hence cell volume). In contrast, increased flux of Na^+, K^+ and $2Cl^-$ through the NKCC has the effect of lowering excitability, depolarizing E_m[22] and increasing intracellular tonicity and cell volume[5,23] (Fig. 3).

The Na,K ATPase plays a fundamental and important role in the maintenance and restoration of membrane excitability during periods of high-intensity exercise[2,6,7]. This role is evident from the Goldman equation: membrane excitability is maintained when

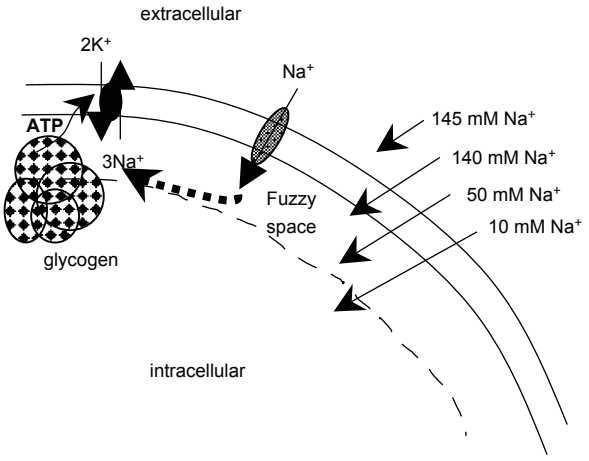

FIG. 3 Representation of functional coupling of ion channel and transport systems through intracellular compartmentation in response to an action potential. The concentrations of free/diffusible Na^+ in the fuzzy space region (0–50 nm below the inside membrane surface) are estimated from the data of Silverman *et al.*[32] in ventricular myocytes. The implication is that immediately following an action potential there is negligible $[Na^+]$ gradient at this membrane site, resulting in an inexcitable region of the membrane, but not of the entire cell

$[K^+]_i$ is high and $[K^+]_o$ is low. By simultaneously pumping Na^+ out of the cell, the Na,K ATPase essentially recharges the membrane in time for the next depolarization event. With high-intensity exercise (frequent and rapid depolarization–repolarization cycles), this recharging of the membrane potential is incomplete and is associated with extracellular K^+ accumulation, intracellular K^+ depletion and increased intracellular $[Na^+]$ and $[Cl^-]$[2,24]. While Na,K ATPase activity is rapidly and maximally activated within *c.* 10 s of the onset of high-intensity exercise, pump rate is inadequate to maintain trans-membrane Na^+ and K^+ gradients, resulting in progressive loss of membrane excitability[25,26]. In view of this, any signal that increases Na,K ATPase activity will be helpful in maintaining excitability and hence muscle contractility. In addition to the important roles played by ion substrates for the pump, a number of important extracellular signalling agents have been identified (e.g. epinephrine, norepinephrine, amylin, insulin) and these have been recently reviewed in detail[6].

Intracellular compartmentation

It is recognized that the inside of a cell is highly compartmentalized with respect to functional and structural activities. Intracellular compartmentation makes good sense with respect to localized signalling, transduction of signals amongst intracellular compartments through discrete signal transduction pathways and protection of other regions of the cell against undesirable changes in ion and metabolite concentrations[27]. For example, there exists functional coupling of K_{ATP} channels and the Na,K ATPase in skeletal muscle[28]. Furthermore, regions of the sarcolemmal well endowed with Na,K ATPase and K_{ATP} channels have high densities of glycogen granules and glucose transporters, with requisite glycolytic and glycogenolytic enzyme pathways to rapidly provide ATP at times of increased ATP demand by the Na,K ATPase[29,30].

Intracellular compartmentation and functional coupling with respect to ion channel and ion transport systems may also make good sense in terms of surface membrane function (Fig. 4). For example, rapid influx of Na^+ into the cell during depolarization has the potential to increase markedly the bulk cytosolic $[Na^+]$ when active Na^+ extrusion is inadequate. It has been hypothesized that the majority of the Na^+ entering the cells through the AChR and voltage-gated Na^+ channels accumulates in the region immediately adjacent to the sarcolemma, in the so-called 'fuzzy space'[31] because of its electron microscopic appearance[32]. It may be beneficial that the Na^+ that rapidly enters the cell be readily accessible to the intracellular sodium-binding site on the Na,K ATPase. Keeping an increase in ICF $[Na^+]$ localized to the subsarcolemmal region would provide for an enhanced

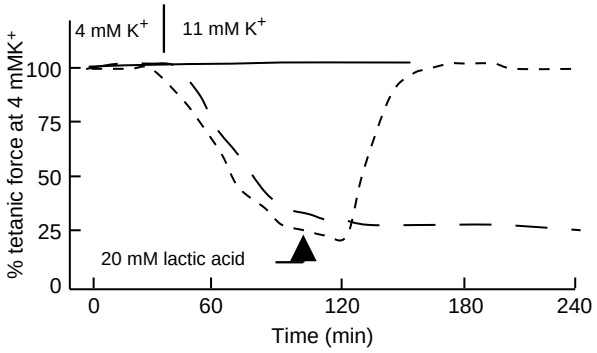

FIG. 4 Extracellular and intracellular acidosis counteracts the force-depressing effects of elevated $[K^+]_o$. Lactic acid prevents tetanic force decline (tetanic stimulation at 10 min intervals) in rat *soleus* during incubation in Krebs–Ringer bicarbonate solution containing 11 mM K^+ (solid line to 150 min) and restores force when added to muscles incubated with 11 mM K^+ (dotted line). The dashed line shows a 70% force decline after 100 min of incubation with 11 mM K^+. Adapted from Nielsen *et al.*[51]

Na^+ signal for activating the Na,K ATPase in times of altered ion balance[31]. This type of functionally coupled system need not rely on 'global' cytosolic increases in $[Na^+]_i$ and activation of all Na,K ATPases on the cell surface. Rather, selective activation of Na,K ATPases immediately adjacent to fuzzy space regions of increased $[Na^+]$ would provide for fine, localized control that uses less ATP than if all surface and T-system membrane Na,K ATPases are activated.

Recent studies in cardiac myocytes provide compelling evidence for elevated Na^+ in the subsarcolemmal fuzzy space during both systole and diastole. However, there did not appear to be an accumulation of Na^+ in this region (measured using X-ray microprobe analysis of cryo-sectioned tissue) in response to repeated stimulation, nor an increase in Na,K ATPase current[32]. Therefore, a role for increased fuzzy space $[Na^+]$ in activating the Na,K pump has yet to be supported by experimental data. An alternate proposal is that a minority (20%) subpopulation of Na,K ATPases may be responsive to increased fuzzy space $[Na^+]$, but an increase in their activities is not detectable using present techniques[32].

Gosmanov *et al.*[5] provide evidence that an increase in NKCC activity during muscle stimulation may be an alternative means by which muscle gains K^+, and thus contributes to maintaining membrane excitability during exercise. Potassium gained by this transport system would augment K^+ gained by increased Na,K ATPase activity, which, although increased maximally within *c.* 10 s of onset of high-intensity exercise, has insufficient capacity to prevent muscle K^+ losses[6]. None the less, an increase in contracting muscle NKCC activity during exercise is, on the surface, counterintuitive because its role in most cell types studied is to increase cellular volume in the face of induced cell shrinkage[23], including those in skeletal muscle[18,33]. However, contracting muscle already has increased volume due to the accumulation of intracellular osmotically active metabolites. While the mechanism by which the NKCC is increased in response to muscle contraction is not known, an increase in its activity may yet be beneficial.

Transverse tubules: the potassium and sodium hypotheses for fatigue

The fatigue of high-intensity exercise does not have a primarily metabolic origin and probably occurs at multiple sites within the excitation–contraction coupling process[1,7] and notably at surface membranes. Historically, the focus had been on the sarcolemmal surface membrane. In recent years, however, the focus for impairment of excitability has shifted to the T-system. This recognizes that, morphologically, the T-system membrane surface area for ion exchange (and hence altered trans-membrane ion concentrations) is fourfold greater than that of the surface membrane[34,35]. In addition, fluid within the T-system has only limited exchange with the interstitial fluid surrounding the myofibres because the T-tubule openings are very small with respect to the volume contained within the tubule and the density of ion transport and channel proteins[6,36]. Therefore, while T-tubular fluid is extracellular, ion concentrations in this compartment are different than those in the interstitial compartment. Furthermore, while T-tubular volume is only ~1% that of the intracellular fluid volume[37], this volume appears to play a very important role in muscle excitability because moving even small quantities of ions will produce a relatively large change in ion concentrations.

The potassium hypothesis for fatigue states that, during high-intensity exercise, excitation-induced increases in $[K^+]_o$ contribute to membrane depolarization[38], inactivation of voltage-gated Na^+ channels responsible for action potential propagation[39–41] and hence reduced excitability[3,38]. Arguments in support of the potassium hypothesis are strengthened when one fully considers the potential (potential because it cannot be directly studied) role of the T-system[42]. Microdialysis experiments of skeletal muscle in humans[43] report that interstitial $[K^+]_o$ increases from ~4 to 12 mmol l^{-1} in response to moderately high-intensity exercise. These values are in agreement with those obtained previously using microelectrode measurements in skeletal muscle of cats[44] and humans[45]. Using the Goldman equation, it can be calculated that an increase in $[K^+]_o$ to 15 mmol l^{-1} would decrease E_m from −85 to −59 mV. Given that this magnitude of increase in $[K^+]_o$ would be accompanied by a ~40 mmol l^{-1} decrease in $[K^+]_i$[18,24], the combined effect of increased $[K^+]_o$ with decreased $[K^+]_i$ would decrease E_m to −52 mV. Experimentally, Cairns *et al.*[46]

have reported an E_m of about -50 mV and complete inexcitability.

The primary channels for K^+ release during muscle contraction are the large conductance calcium-activated potassium channels (BKCa)[3], which have twice the density in the T-system than surface membrane[15]. This, coupled with the restricted diffusion between T-system and interstitial fluids, raises the likelihood that T-tubular $[K^+]$ is significantly higher than that measured in interstitial fluid by microdialysis or microelectrode. It may be possible that a refined application of electron microprobe analysis for studying the ion composition of intracellular organelles[47,48] may provide insight into the ion concentrations within the T-system. It is therefore probable that the K^+ contribution to decreased membrane excitability is greater than that estimated from interstitial $[K^+]_o$.

The sodium hypothesis for muscle fatigue provides that a decrease of T-tubular Na^+ during high-intensity exercise, with localized increase in ICF $[Na^+]$, substantially decreases T-tubular excitability, which in turn reduces SR calcium release and force production[2,16]. This hypothesis is not exclusive of the potassium hypothesis for fatigue and, in fact, loss of membrane excitability during high-intensity exercise may be better explained by evoking both hypotheses than either one alone[49].

In a recent study employing mouse *soleus* and EDL, Cairns *et al.*[16] took advantage of the fact that membrane excitability is a function of $[Na^+]_o/[Na^+]_i$ (see Goldman equation); thus effects of increased $[Na^+]_i$ can be simulated by raising $[K^+]_o$. With high-intensity exercise, $[Na^+]_i$ can double, thus reducing $[Na^+]_o/[Na^+]_i$ by 50%; this same reduction in $[Na^+]_o/[Na^+]_i$ was achieved by decreasing $[Na^+]_o$ from 147 to 75 mmol l^{-1}. With this change in $[Na^+]_o/[Na^+]_i$, Cairns *et al.*[16] demonstrated a 15% reduction in tetanic force at the first tetanus and a 30% reduction in force at the tenth tetanus. The decrease in tetanic force production was associated with: (1) increased rate of force decline during the tetanus in EDL but not in *soleus* (indicating impaired ability to produce closely spaced action potentials in EDL); (2) a reduction in action potential amplitude, and (3) an increased number of inexcitable fibres[16].

An intriguing possibility is that voltage-gated Na^+ influx from the T-tubule fluid into the cytosol down its chemical gradient may markedly reduce tubular $[Na^+]$ because of the restricted entry of Na^+ from interstitial fluid. This may reduce the rate and amount of Na^+ influx, thus decreasing SR calcium release and the force of contraction. These combined localized effects of lowered $[Na^+]_o$ and raised $[Na^+]_i$ on membrane excitability and force production have yet to be studied.

The best recent evidence in support of the sodium hypothesis for fatigue uses muscle fibres that have the surface membrane mechanically peeled away. The T-tubular system seals off, repolarizes and represents part of the extracellular compartment, while the intracellular compartment is opened up to the bathing solution[1,37]. Electrical stimulation of the T-tubules elicits action potentials and contraction[50]. Using this preparation, Nielsen and co-workers[2] demonstrated that reduction of the Na^+ gradient across T-system membranes impaired the ability to produce successive, closely spaced action potentials. These results are consistent with those of Cairns *et al.*[16] obtained with intact surface membrane. Coincidently, increased $[Na^+]_i$ also produced recovery of excitation-induced force due to the effects of increased $[Na^+]_i$ on increasing the activity of the Na,K ATPase[2]. Thus, stripping away the surface membrane allowed Nielsen *et al.*[2] to demonstrate 'that the effect of low intracellular $[K^+]$ was caused by a reduced function of the T-tubular system, i.e. by loss of T-tubular excitability and inactivation of the voltage sensor molecules'. These results again demonstrate the importance of the Na,K ATPase in maintaining and restoring membrane excitability.

Lactate, chloride and intracellular acidification

In an elegant series of experiments employing rat *soleus*, Nielsen and co-workers[51] demonstrated that incubation in the presence of 20 mM lactic acid prevented the 70% decline in tetanic force normally seen when the muscles were incubated in the presence of 11 mM $[K^+]_o$ (Fig. 4). Furthermore, when muscles were first incubated in solutions with 11 mM $[K^+]_o$ for 1 h, force declined by 70%. Subsequently, *c.* 30 min after the addition of 20 mM lactic acid (reducing pH_o from 7.44 to 6.80, $T = 30$ or 37°C) still in the presence of 11 mM $[K^+]$, tetanic force started to increase and was fully normalized within 60 min. This effect of 20 mM lactic acid could be replicated with 23% CO_2 or with 20 mM propionic acid, suggesting that the effect was due to increases in either or both of extracellular and intracellular $[H^+]$. At the temperature at which the experiments were performed (30°C), incubation of *soleus* with 20 mM lactic acid in the presence of normal (4 mM) K^+, there was an 8% increase in tetanic force. Upon further examination, Nielsen *et al.*[51] showed that recovery of force was not related to effects on Na,K ATPase activity or cellular (but not SR) calcium handling. Measurement of fibre M-waves showed that combined extracellular–intracellular acidification fully restored muscle excitability but this was not due to repolarization of the sarcolemma. The authors suggested that elevated $[H^+]$ may have reduced the inactivation of Na^+ channels[41] by shifting the voltage-sensitivity to less negative potentials, which would reduce the sensitivity of the sarcolemma to accumulation of extra-

cellular $[K^+]$. There is also evidence that fatigue and lowered extracellular pH increases sarcolemmal ionic conductance[52,53].

Further insights regarding the effects of acidosis on sarcolemmal excitability come from studies that have investigated a role for Cl^- channels. Surface membrane and T-tubular conductance of Cl^- play an important role in stabilization of the membrane potential and controlling the depolarization threshold; therefore, altered chloride conductance modulates membrane excitability[20,54–56]. The influence of high chloride conductance and high $[Cl^-]_o$ on maintaining membrane excitability and contractility requires, however, a large inward Na^+ current to sustain a propagating action potential. The primary chloride channel in skeletal muscle is the CIC-1 chloride channel[17,57], which has distribution on the surface membrane and T-system[39]. For example, decreased chloride conductance due to mutations in the CIC-1 gene is responsible for the hyperexcitability expressed by those having hereditary myotonia[58,59]. Furthermore, fast-twitch muscle fibres used during high-intensity exercise are characterized by a higher chloride conductance with decreased sarcolemmal excitability, compared with slow-twitch fibres[20]. This, together with a higher sodium channel density in fast, compared with slow, fibres increases susceptibility of fast fibres to fatigue[60].

The effects of alterations in $[Cl^-]_o$ have recently been studied in mouse *soleus* and EDL[61] and rat *soleus*[62]. Van Emst and co-workers[62] convincingly summarize the literature and present additional evidence that in mammalian muscle at rest $[Cl^-]_i$ is greater than predicted by a trans-membrane Donnan equilibrium and therefore high (normal) extracellular $[Cl^-]_o$ exerts a depolarizing (2–3 mV) influence on E_m. Experimentally, membrane Cl^- conductance can be decreased by markedly decreasing $[Cl^-]_o$ towards $[Cl^-]_i$ of *c.* 5–10 mM[61,62]. The muscle force depression resulting from raised $[K^+]_o$, as occurs during high-intensity exercise, was significantly increased in the presence of lowered $[Cl^-]_o$ than that with normal $[Cl^-]_o$[61,62]. However, this effect of lowered $[Cl^-]_o$ appears to be short-lived, for when $[Cl^-]_o$ was lowered (5.2 mM) during exposure of twitch-stimulated muscle to elevated $[K^+]_o$ (12.5 mM), twitch force started to recover some 30 min after complete loss of force[62]. When muscles were first incubated in low $[Cl^-]_o$ prior to elevated $[K^+]_o$, the hyperkalaemia did not induce a decrease in twitch force. A scenario of lowered $[Cl^-]_o$ within the T-tubular system, resulting from increased net Cl^- flux into cells during high-intensity electrical stimulation[24], is a probability that warrants consideration. While it is improbable that T-system $[Cl^-]_o$ would approach the low concentrations used in these two studies, it is probable that some decrease in T-system $[Cl^-]_o$ would occur during high-intensity exercise. A decrease in T-system $[Cl^-]_o$ would be associated with an increased $[Cl^-]_i$, which allows the depolarizing effect of raised $[K^+]_o$ on E_m to be seen; the net effect is a decreased T-tubular membrane excitability, and hence decreased SR calcium release and decreased force of contraction[54].

T-system chloride conductance has recently been shown to be directly affected by increased intracellular acidity such as that which occurs *in vivo* during high-intensity exercise[55,56]. Using isolated, skinned rat EDL at 25°C, the authors demonstrated that the decrease in tetanic force resulting from reduced $[K^+]_i$ (60 mM) (and accompanying action potential failure) was greatly attenuated at lowered myoplasmic pH (6.6 compared with 7.1)[55]. This protective effect of reduced pH_i on muscle contractility did not occur in the absence of myoplasmic and T-system $[Cl^-]$, and pH_i also did not affect calculated resting E_m. The decrease in myoplasmic pH was calculated to have reduced T-system Cl^- permeability by 74%, thus maintaining excitability of this membrane system by 'reducing the size of the Na^+ current needed to generate a propagating action potential'[55]. The magnitude of this protective effect of lowered pH_i on muscle force production was substantial even with $[K^+]_i$ of 80 mM and $[Cl^-]_i$ of 20 mM, values close to those which may occur with very high-intensity exercise.

NKCC and cell volume regulation

A role for the NKCC in the maintenance of membrane excitability during high-intensity exercise in skeletal muscle has yet to be determined. Gosmanov *et al.*[63] present data supporting the hypothesis that the NKCC functions to replace K^+ lost during action potentials, thus helping to maintain a lowered $[K^+]_o$ and an elevated $[K^+]_i$. An increased activity of the NKCC during high-intensity exercise may also explain the elevated $[Na^+]_i$ and $[Cl^-]_i$[24]. However, the accompanying net flux of water into the cell is in the opposite direction to what may be deemed desirable with respect to cellular volume regulation. This is because the onset of high-intensity exercise results in a rapid and large increase in the concentrations of osmotically active molecules (creatine, inorganic phosphate, sodium, lactate) that drive an osmotic influx of water into the cell, thus increasing cellular volume[5,64]. The release of water bound to glycogen during glycogenolysis[65] will also contribute to the increase in free cellular water content. The effects of lowered $[K^+]_o$, increased $[Ca^{2+}]_i$ and other potential signals during high-intensity exercise on regulating the activity of the NKCC require further study. In resting muscle, inhibition of the NKCC results in a significant membrane depolarization[22]; if similar results can be demonstrated during high-intensity contractions, it may be that the demand for restoring $[K^+]_i$ and maintaining

membrane excitability may supersede that for cellular volume regulation.

Conclusions

During exercise, membrane excitability appears to be under the coordinate control of multiple ion channel and transport systems in both surface and T-system membranes. Some alterations in localized intracellular and extracellular ion concentrations, and in membrane ion permeability, reduce membrane excitability, while others appear to have the effect of simultaneously and partially restoring membrane excitability. This system appears designed to reduce muscle contractility during times of high-intensity exercise, while allowing for maintained contractile function. Such a system is conceivably of benefit for animals involved in predator avoidance and athletic competition. Further research and innovative techniques are required to elucidate the role of the T-system in control of membrane excitability.

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