The role of the Na^+,K^+-pump in delaying muscle fatigue

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Abstract Through increased electrogenic activity, the Na^+,K^+-pump can maintain, or even increase, muscle fibre membrane potentials during muscle contractions, and can thereby compensate for the rise in interstitial K^+ concentration. During submaximal contractions the enhanced pumping affects quiescent fibres as well as the contracting ones. In human subjects the pump effects can be detected by changes in the muscle compound action potential. Intramuscular release of noradrenaline is probably one of the factors stimulating the pump.

There has long been speculation that K^+, released into the interstitial spaces by contracting muscle fibres, might contribute to muscle fatigue by blocking impulse conduction (e.g. Sjøgaard 1990). Two types of experiment would appear to support this proposition. One has been the use of ion-sensitive electrodes to demonstrate substantial elevations in interstitial K^+ concentration in contracting muscles (see below). The other approach has been to bathe isolated muscles in solutions containing raised K^+ concentrations and to show reductions in muscle compound action potentials or force (Jones 1981; Clausen & Everts 1991). In this paper we shall argue a contrary view by showing that, although $[K^+]$ is indeed raised in the interstitial spaces, it does not contribute to the decline in force at physiological rates of muscle excitation, because of the powerful intervention of the electrogenic Na^+,K^+-pump. Rather it appears that the pump is able to maintain the excitability of the muscle fibre plasmalemma and to ensure that the surface action potential retains its full size, until force has started to decline. In developing this proposition, the evidence relating to K^+ efflux from contracting muscle is first reviewed; we then present the results of membrane potential measurements in mammalian muscles during maximal and submaximal contractions, and calculate the magnitude of the contribution to the resting potential by the electrogenic Na^+,K^+-pump. It will be shown that qualitative assessments of pump activity can be made in human muscles during voluntary or stimulated contractions, and also in the recovery period from fatigue. Finally, speculations are given as to the mechanisms responsible for increasing pump activity.

K^+ efflux during activity

^{42}K has been used to measure K^+ efflux during impulse activity; in two early studies the mean value for single frog muscle fibres agreed closely with that for rat diaphragm, when

expressed per impulse and per cm^2 of membrane (9.6 pmole, Hodgkin & Huxley 1959a; 10.7 pmole, Creese et al. 1958). In the preparation used by Creese et al. (1958), some K^+ would have been carried back into the fibres by the Na^+,K^+-pump; hence the K^+ efflux in this and similar studies (see Sjøgaard 1990 for review) would have been underestimated, possibly by as much as 50% (Clausen & Everts 1988; see also Everts & Clausen 1988).

Experimental determinations of interstitial K^+ concentration.
The impulse-mediated increase in $[K^+]_e$, the interstitial K^+ concentration, can be measured directly, and also calculated from morphological and biochemical data. Neither approach is free from uncertainty, but both provide some indication of the changes that are likely to ensue during contraction. In the study by Hník et al. (1976) in the cat an ion-sensitive electrode was inserted into the femoral vein and all tributaries draining sources other than the gastrocnemius were ligated. The authors found that 50 Hz stimulation of this muscle raised $[K^+]_e$ from 4.8 mM to 8.2 mM, depending on the duration of the tetanus. Such values must be regarded as minimum estimates, however. Thus, the venous effluent will be a mixture of previously stagnant blood, which had accumulated K^+ during the tetanus, and of fresh inflow to the muscle. Also, there is no certainty that the interstitial fluid is in ionic equilibrium with the capillary plasma; the long time taken for $[K^+]$ to return to normal values, at the end of a tetanus, suggests that diffusion of interstitial K^+ is impeded. The values of muscle $[K^+]_e$ in human subjects are likely to be further underestimated, because of the contamination of the muscle effluent with that from skin, subcutaneous tissue and non-contracting muscles; even so, mean values of 8.3 mM have been observed in femoral veins of subjects running on treadmills (Medbø & Sejersted 1990).

Measurements of $[K^+]_e$ in the muscle belly, made with ion-sensitive electrodes, might be expected to be more reliable. However, there are the possible effects of trauma, inflicted by the electrode, to consider; these might either increase $[K^+]_e$, by leakage of ions from damaged muscle fibres, or decrease it, by blocking impulses in those fibres round the electrode tip. Also spurious potentials, due to pressure at the electrode tip, may be comparable to those generated by changes in $[K^+]$. Finally, since the tips of the electrodes may be many times larger than the inter-fibre space (Hník et al. 1976; Juel 1986), their locations cannot be properly described as extracellular or intracellular. It is with these reservations in mind that such mean maximum values as 8.8 mM (Hník et al. 1976), and 10 mM (Juel 1986), must be considered.

The study by Vyskočil et al. (1983), though subject to the same sources of uncertainty, is particularly interesting since it was carried out in the intact human brachioradialis; the ion-sensitive electrode was introduced through a stainless steel trocar, before being advanced into the muscle tissue. Although the average $[K^+]_e$ rose from 4.5 mM to 9.5 mM, these authors showed a value of at least 15 mM being reached during a maximal contraction (see Fig. 2B of Vyskočil et al. 1983).

Theoretical determination of interstitial K^+ concentration.
The theoretical estimation of $[K^+]_e$ during a maximal voluntary contraction depends on surprisingly few assumptions. Thus, the K^+ efflux per impulse is known (see above), as are the dimensions of the muscle fibres and interstitial spaces and the motor unit firing rates during maximal voluntary contractions. Since the contraction is maximal, the intramuscular pressure will be well above that required to occlude the arterial inflow (Barcroft & Millen 1939; Sjøgaard 1990); hence the interstitial spaces and intramuscular capillaries can be considered as a closed compartment with diffusion of ions between one and the other. The

only major uncertainty is the extent of Na^+ and K^+ pumping between the interstitial spaces and the muscle fibres; in order to calculate the maximum possible rise in interstitial $[K^+]$, we shall ignore the effect of the pump.

Regarding fibre dimensions, an average value for the diameters of type I and type II fibres in the human biceps brachii muscle would be 50 μm (Brooke & Engel 1969), while transverse sections of the same muscle suggest that the inter-fibre distance is no more than 1 μm. If the fibres are considered as closely packed, 4-6 sided columns, then the interstitial space would be approximately 4% of the muscle volume, with the T-tubules adding a further 0.3% (Peachey 1965) and the capillaries 1-2%. A value of 6% for the extracellular space in skeletal muscles is much smaller than most biochemical estimates, for which chloride, inulin, and sucrose have been the most frequently used markers; it is likely that such estimates would be inflated by the connective tissue separating muscle fascicles and that surrounding the neurovascular bundles. More recently, however, Lindinger & Heigenhauser (1987) obtained mean values as low as 6.5% for some of the fast-twitch muscles in the rat, using mannitol as a marker. Taking these various considerations into account, it can be calculated by simple geometry that a single impulse will raise the interstitial $[K^+]$ by 0.2 mM.

How quickly will $[K^+]_e$ rise during a voluntary contraction? Firing rates greater than 100 Hz have been observed at the onset of maximal voluntary contractions of human hand muscles (Marsden et al. 1971), and a conservative value for the first few seconds of a muscle such as biceps brachii might be 25 Hz; such a value is obviously a global one, in the sense that some motor units have higher maximum firing rates than others (Freund et al. 1975). In a maximal contraction, all motor units will be recruited and, in one second, the interstitial $[K^+]$ could reach 5 mM (*i.e.* 25 x 0.2 mM). The amount of depolarization this rise might cause can be calculated from the following equation (Hodgkin 1958):

$$E_m = \frac{RT}{F} \log_e \frac{[K^+]_e + b\,[Na^+]_e}{[K^+]_i + b\,[Na^+]_i}$$

in which E_m is the membrane potential, and e and i denote extracellular and intracellular values; b is the ratio of the Na^+ and K^+ permeabilities of the muscle fibre membrane and is approximately 0.04 (see Hicks & McComas 1989). If allowances are made for changes in intracellular $[Na^+]$ and $[K^+]$ during contractile activity (see, for example, Sreter 1963), a rise of interstitial $[K^+]$ from 4 mM to 9 mM would depolarize a muscle fibre by approximately 14 mV, more than enough to cause impulse block.

On theoretical grounds, then, as little as one second of maximal effort would be expected to induce muscle paralysis. This surprising conclusion flies in the face of everyday experience, in which force can be maintained reasonably well for a minute or more; equally significant is the preservation of the muscle compound action potential (Bigland-Ritchie et al. 1982). Why are theory and observation so different? One reason is that the Cl^- conductance of the muscle fibre will tend to moderate the effects of a changing K^+ concentration gradient across the surface membrane, as reflected in the permeability and concentration terms for Cl^- in the Goldman-Hodgkin-Katz equation for membrane potential (cf. Goldman 1943). However, as shown by Hodgkin & Horowicz (1959b), the stabilizing effect of Cl^- is short-lived, due to redistribution of water and solute across the fibre membrane. Instead, it would appear that a second factor is more important in preventing K^+-induced paralysis; this factor is the electrogenic Na^+,K^+-pump.

Evidence for increased Na^+,K^+-pumping in muscle activity

Everts et al. (1988) showed, using ^{86}Rb, that there was a doubling of pump activity during stimulation of the isolated rat soleus at 2 Hz. The authors pointed out, however, that this increase was still far from the theoretical maximal rate of Na^+ and K^+ transport (cf. Clausen et al. 1987). A much earlier study, carried out on single frog semitendinosus muscle fibres, also showed a doubling of ^{24}Na extrusion during modest rates of stimulation (Hodgkin & Horowicz 1959a).

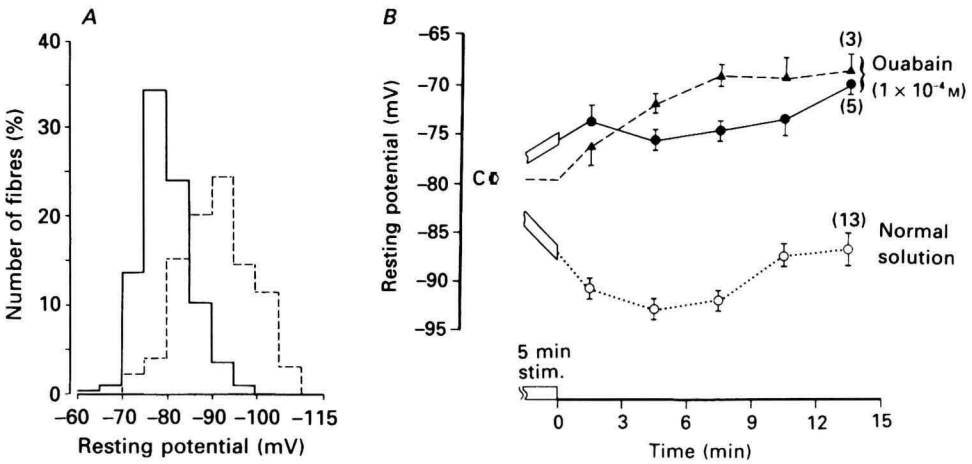

Figure 1,A. Effect of intermittent tetanic stimulation on rat muscle fibre membrane potentials; results in control and stimulated fibres shown by continuous and interrupted lines respectively. **B.** Abolition of tetanus-induced fibre hyperpolarization by ouabain (continuous line; stimulation period indicated by box). Other curves show effects of ouabain without stimulation (dashed line) and of stimulation without ouabain (dotted line). See text and Hicks & McComas 1989.

Physiological evidence for increased Na^+,K^+-pumping during muscle activity has been obtained in our own laboratory. In rat soleus muscles examined *in vivo*, Hicks & McComas (1989) observed that repeated tetani at 20 Hz increased the mean resting potential from -79.5 mV to -90.5 mV (Fig. 1,A). That this was due to the electrogenic effect of the Na^+,K^+-pump was shown by the absence of hyperpolarization if the experiments were repeated in the presence of ouabain or in the absence of extracellular K^+ (Fig. 1,B); cooling the muscle to 19°C produced a similarly negative result. Since the resting potentials of tetanized, but otherwise untreated, fibres were increased, the fibre action potentials enlarged by similar amounts and this, in turn, caused potentiation of the M-wave (muscle compound action potential). In surface fibres Hicks & McComas calculated that the Na^+,K^+-pump must be

contributing -20 mV to the resting potential; when stimulated fibres were challenged with 20 mM K^+ in the bathing fluid, the electrogenic component increased to -30 mV.

It is surprising that muscle fibre hyperpolarization had not been observed previously in investigations of muscle fatigue. The simplest explanation is that other workers have omitted protein in the bathing fluid, despite the earlier admonitions of Creese & Northover (1961) and Kernan (1963). As these last authors showed, the absence of protein leads to an increase in Na^+ permeability of the muscle fibre membrane *in vitro*, and to a depolarization of 10 mV or so.

Evidence for increased Na^+,K^+-pump activity in contracting human muscles.

There has long been evidence of augmented Na^+,K^+-pump activity during human muscle contractions in the phenomenon of 'pseudofacilitation', a term which describes the gradual increase in the amplitude of the M-wave during stimulated or voluntary activity. That the enlargement of the M-wave is not an artefact of tetanic stimulation, or of ischaemia, is demonstrated by similar findings during intermittent voluntary contractions of the intrinsic muscles of the hand, with the circulation intact (Hicks et al. 1989). In the past 'pseudofacilitation' has been attributed to greater synchronization of the individual muscle fibre action potentials, but there are good reasons for rejecting this explanation, certainly as a major cause (Hicks et al. 1989). Not infrequently, enlargement of the M-wave during stimulated or voluntary contraction has been recorded but without any comment as to its physiological significance or cellular mechanism; a recent example is the striking potentiation noted in single FF and F(int) motor units of the cat tibialis posterior muscle (Enoka et al. 1992).

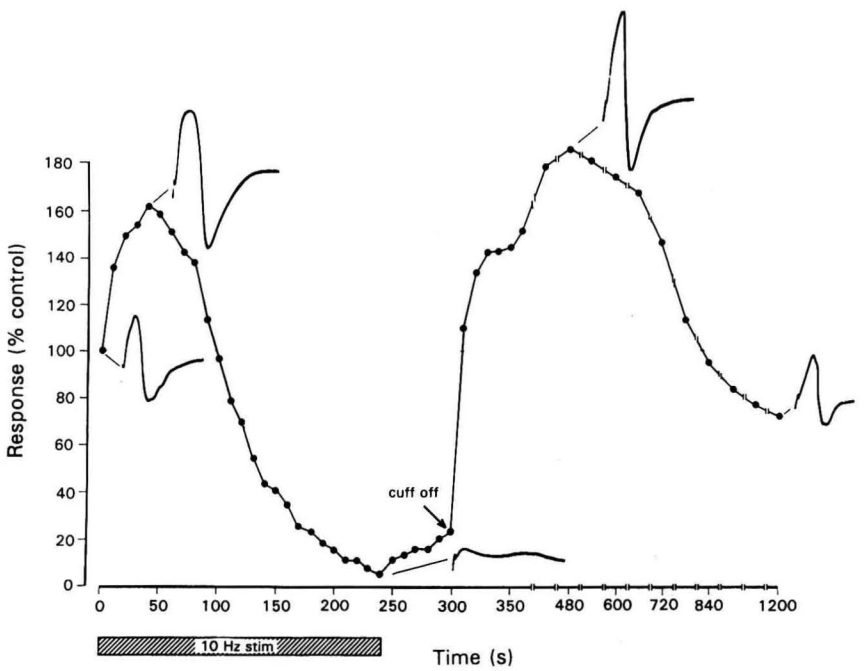

Figure 2. Changes in M-wave amplitude in biceps brachii muscle of a 20 yr old man, during 10 Hz stimulation, performed under ischaemic conditions; arterial cuff released at arrow (Galea, McComas & Einhorn, unpublished observations).

On the basis of the animal studies, described above, it now seems certain that M-wave potentiation is largely due to the increased amplitudes of the muscle fibre action potentials, following pump-induced hyperpolarization. We have pursued M-wave studies in man, having discovered that the biceps brachii is a particularly favourable preparation for demonstrating facilitation (Fig. 2). By using arterial occlusion during this type of study, it has also been possible to document the time-course of recovery. It can be seen in Fig. 2 that the M-wave enlarges only slightly when tetanization is discontinued, provided ischaemia is maintained. As soon as the cuff is deflated, however, the M-wave amplitude rapidly increases; we attribute this change to the flushing out of K^+ from the interstitial spaces of the muscle, causing an instantaneous increase in the K^+ equilibrium potential, and hence in the muscle fibre resting potentials. There is then a slower potentiation which is maximal approximately three minutes after termination of ischaemia, and is probably due to increased Na^+,K^+-pumping (Galea & McComas 1991).

What happens during submaximal contractions?

Suppose only half the muscle fibres are contracting; what happens to the resting potentials of the other half? On *a priori* grounds, one might expect the inactive fibres to depolarize, due to the accumulation of K^+, released by the contracting fibres, in the interstitial fluid. To explore this possibility, Kuiack & McComas (1990, 1992) stimulated half of the ventral root axons innervating the rat soleus muscle and compared the resting potentials in the quiescent and previously-tetanized muscle fibres. They found that the quiescent fibres exhibited hyperpolarizations as large as those in the contracting fibres. This response is functionally advantageous, for it enables the quiescent fibres to be called into action as fatigue sets in, or if a stronger contraction is required.

Mechanism of increased Na^+,K^+-pump activity

It is probable that several mechanisms stimulate the Na^+,K^+-pump during muscle contraction. One factor will be the rise in *intracellular [Na^+]*, in keeping with the effects of direct stimulation of single muscle fibres (Hodgkin & Horowicz 1959a), and of Na^+ injection into neurones (Thomas 1972). However, in rat soleus muscles stimulated at 2 Hz, Everts et al. (1988) found a 63% increase in pumping, without any measurable increase in intracellular [Na^+]. The results of Kuiack & McComas (1990, 1992), using half-maximal contractions, also exclude intracellular Na^+ as the only factor stimulating the pump, since the non-tetanized fibres were also hyperpolarized. Similarly, a rise in interstitial [K^+] cannot be a major stimulus, since the effect of increasing $[K^+]_e$ in resting muscle is to *depolarize* the fibres (see, for example, Hicks & McComas 1989).

In contrast, there is evidence that *noradrenaline* may be a potent stimulus, since the contraction-induced hyperpolarization is abolished in the presence of propranolol, a ß-adrenergic blocker (Kuiack & McComas 1992; see, however, Everts et al. 1988). The source of noradrenaline in the muscle is presumably the sympathetic nerve fibres; in addition to those in the walls of the intramuscular arteries and arterioles (Fuxe & Sedvall 1965), there are others which end directly on the muscle fibres (Barker & Saito 1981) and might respond to action currents in the latter, as well as to efferent sympathetic drive. During voluntary contractions *adrenaline* may also stimulate the Na^+,K^+-pump but is clearly not essential, since M-wave enlargement in man is equally prominent in ischaemic as in non-ischaemic

conditions (Galea & McComas 1991). Another possible pump stimulant is *CGRP* (calcitonin gene-related peptide) since this peptide is released from motor nerve endings (Uchida et al. 1990) and has recently been shown to enhance Na^+,K^+-transport in rat skeletal muscle (Clausen & Andersen 1991).

Summary of roles of the Na^+,K^+-pump in muscle contraction

In the paper we have concentrated on the electrogenic action of the Na^+,K^+-pump during muscle contraction. By contributing up to -30 mV to the resting membrane potentials, the pump is able to overcome the depolarizing tendency of the raised interstitial $[K^+]$ and to keep all the fibres in the muscle excitable. Further, the amount of pump activation appears to match the excitation frequency of the muscle fibres. Thus, even under ischaemic conditions, a full minute of non-decremental excitation of the fibres is guaranteed, at least, for frequencies up to 30 Hz (Galea & McComas, unpublished observations). The preservation of muscle fibre excitability is continued until force begins to decline, after which there is no longer any benefit in maintaining the resting membrane potential.

It is additionally possible that, by having a normal-sized or enlarged action potential at the surface membrane, effective excitation-contraction coupling is assured; thus, even if impulse conduction were to fail in the T-tubules, inward electrotonic spread of the surface signal might still be adequate to activate myofibrils in the centre of the fibre (Adrian et al. 1969).

The role of the Na^+,K^+-pump in restoring ionic equilibrium in the intracellular and extracellular compartments is equally important. Because of the limited pumping capacity, however, the exercise-induced changes in $[Na^+]$ and $[K^+]$ cannot be corrected as rapidly as the membrane potential; therefore the interstitial $[K^+]$ remains high at a time when the resting potential is normal or elevated.

Finally, by creating a membrane potential which is substantially above the K^+ equilibrium potential, the pump allows K^+ to diffuse passively down the transmembrane electrical gradient and into the fibre. It is to the advantage of the muscle fibre that the K^+ permeability should be high; it is possible that those K^+ channels which are opened by raised intracellular $[Ca^{2+}]$ (Pallotta et al. 1981) and by ATP deficiency (Spruce et al. 1987) have important roles in mediating the passive influx of K^+ during fatigue. This view is diametrically opposed to the one which proposes that such channels would further disrupt ionic homeostasis in fatigue, by promoting K^+ efflux (Sjøgaard 1990; Juel 1988).

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