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# INCREASED SODIUM PUMP ACTIVITY FOLLOWING REPETITIVE STIMULATION OF RAT SOLEUS MUSCLES

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SUMMARY

1. Soleus muscles of anaesthetized rats were stimulated tetanically (4 s at 20 Hz every 5 s for 5 min), following which the resting and action potentials were measured in surface fibres.

2. At the end of the stimulation period, the mean resting potential was found to have increased from a control value of $-79{\cdot}5 \pm 4{\cdot}8$ mV (mean $\pm$ S.D.) to $-90{\cdot}5 \pm 6{\cdot}3$ mV. The hyperpolarization started to decline after 9 min but was still present at 15 min.

3. Associated with the membrane hyperpolarization was an increase in the mean amplitude of the muscle fibre action potential, from $82{\cdot}2 \pm 10{\cdot}8$ to $96{\cdot}8 \pm 10{\cdot}0$ mV.

4. Both the hyperpolarization and the enlargement of the muscle fibre action potential were abolished by $1{\cdot}25 \times 10^{-4}$ M-ouabain, cooling the bathing fluid to 19 °C or removing $K^+$ from the bathing fluid.

5. The results are explained in terms of an increase in electrogenic sodium pump activity resulting from tetanic stimulation. When the bathing fluid contained 20 mM-$K^+$, the mean resting potential of stimulated fibres was approximately $-30$ mV greater than that calculated from the Goldman–Hodgkin–Katz equation.

6. The increase in sodium pumping not only acts to restore the concentrations of $Na^+$ and $K^+$ on either side of the muscle fibre membrane, but, through its electrogenic effect, enables fibres to remain excitable during continuous contractile activity.

## INTRODUCTION

The muscle compound action potential, or M-wave, is commonly used in human fatigue experiments as an index of the effectiveness of neuromuscular transmission and impulse propagation in muscle fibres. While repetitive stimulation will ultimately cause the M-wave to decline and broaden (Bigland-Ritchie, Jones & Woods, 1979) there is often an initial increase in peak-to-peak amplitude (Fitch & McComas, 1985). One explanation for this early enhancement of the M-wave would be that the resting and action potentials of individual muscle fibres increase during muscle activity but this suggestion has not been supported by the results of previous studies in animal muscles. Thus, it has been a general finding that the resting and action potentials of mammalian skeletal muscle fibres *decrease* following repetitive

stimulation (for example, Locke & Solomon, 1967; Hanson, 1974; Juel, 1986), presumably as a result of the rise in interstitial [$K^+$] associated with impulse activity (Hnik, Holas, Krekule, Kriz, Mejsnar, Smiesko, Ujec & Vyskocil, 1976; Juel, 1986). These results are not conclusive, however, since most of the animal experiments have been conducted *in vitro*, using bathing media likely to cause partial depolarization of muscle fibres with a concomitant rise in intracellular [$Na^+$] (Creese & Northover, 1961; Kernan, 1963).

In view of the above consideration we have examined the *in vivo* effects of repetitive stimulation on fibre membrane potentials of the rat soleus, a muscle which has been frequently employed for *in vitro* studies (see above). Contrary to previous reports, we have found that the intracellularly recorded action potential does, in fact, undergo significant enlargement and that this is accompanied by hyperpolarization of the resting membrane. These changes, in turn, are shown to result from enhanced activity of the electrogenic sodium pump. The implications of these results for an understanding of membrane excitability during muscle activity are discussed. Some of these results have been presented elsewhere (Garner, Hicks & McComas, 1986).

## METHODS

### *Animal preparation*

Experiments were conducted on female Wistar rats weighing 220–280 g. Under intraperitoneal sodium pentabarbitone anaesthesia (35 mg/kg body weight) the soleus muscle of the right leg was exposed, its nerve twig identified, and its tendon freed; care was taken to preserve the blood supply to the muscle. The rat was placed prone on a warm brass plate and the limb was put in a Plexiglas chamber which contained approximately 150 ml of Liley's solution (Liley, 1956; mM: NaCl, 140; KCl, 4·5; $NaH_2PO_4$, 1; $MgCl_2$, 1; glucose, 11; $NaHCO_3$, 12; $CaCl_2$, 2) plus albumin (16 g/l). The bathing fluid was maintained at 36–38 °C by circulating water and radiant heat. The limb was fixed to the base of the bath by Plexiglas clamps at the ankle and knee. The tendon of the soleus was attached by 5 cm of 3·0 gauge silk thread to a force transducer (Grass Type FTO36), the compliance of the entire system being 180 $\mu$m/N; the muscle was set to its optimal length for twitch tension.

### *Stimulation and recording system*

Intracellular recordings were made from the first and occasionally the second layers of rat soleus muscle fibres with glass microelectrodes filled with 3 M-KCl solution; the electrodes had DC tip resistances of 8–15 M$\Omega$ and tip potentials of 5 mV or less (cf. Adrian, 1956). The reference electrode was a Ag:AgCl wire in agar bathing solution. Resting potentials were recorded with a high-input-impedance amplifier (WP1 Model M-707) and displayed on a storage oscilloscope (Model 141 B, Hewlett–Packard Ltd). Single action potentials were evoked by 20 $\mu$s voltage pulses applied to the soleus nerve twig through fine silver wire electrodes. M-wave recordings were also made, using a silver wire electrode twisted around the end of the muscle belly at its tendon of insertion. After the resting and action potentials had been recorded in six to ten fibres, the soleus was stimulated at 20 Hz for 4 out of every 5 s, this fatiguing sequence being repeated for 5 min. As soon as possible afterwards a further set of resting and action potential measurements was made. No attempts were made to carry out continuous recordings from single fibres during the recovery period. In those experiments in which the activity of the sodium pump was to be modified, the composition of the bathing fluid was altered a few seconds before the termination of the fatiguing sequence. In the case of the ouabain experiments, 5 ml of bathing fluid containing 0·128 mg ouabain were added to the bathing medium which was then stirred, the final concentration of ouabain being $1{\cdot}25 \times 10^{-4}$ M. For those experiments in which external [$K^+$] was to be altered or the muscle cooled (see Results), the bathing solution was drained and then replaced, the procedure taking 15–20 s. At the end of each

experiment, the animal was killed by an intraperitoneal injection of sodium pentobarbitone solution.

Throughout the text mean values have been given with their standard deviations; significance between means was determined by Student's *t* test.

## RESULTS

### *Effect of tetanic stimulation on resting and action potentials*

Under control conditions the mean resting potential of 400 soleus muscle fibres, studied in sixty-eight animals, was $-79{\cdot}5 \pm 4{\cdot}8$ (mean $\pm$ S.D.) mV, while the mean action potential amplitude was $82{\cdot}2 \pm 10{\cdot}8$ mV. Since not all the impaled muscle

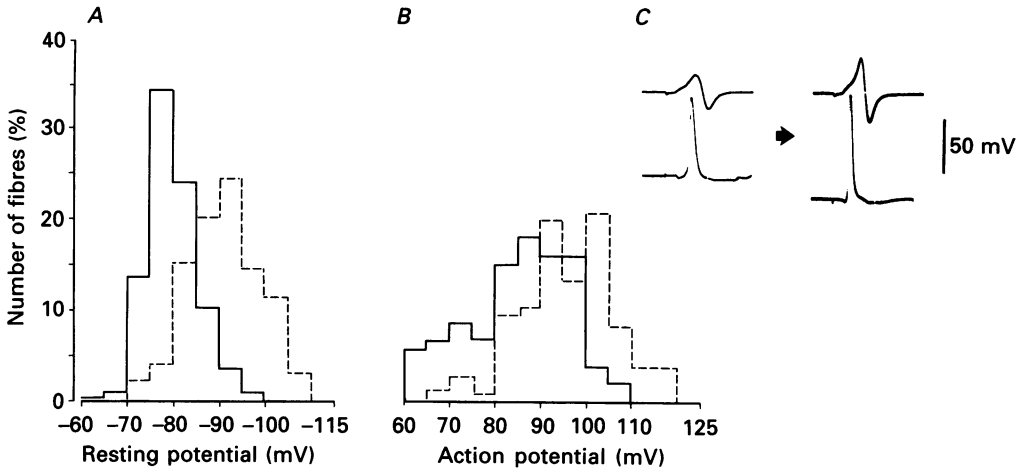


Fig. 1. Effects of intermittent tetanic stimulation on resting membrane potentials (*A*) and action potentials (*B*) of rat soleus muscle fibres. Results in control (resting) and stimulated fibres shown in histograms by continuous and interrupted lines, respectively. Mean values ($\pm$S.D.) for resting potentials, $-79{\cdot}5 \pm 4{\cdot}8$ mV (control fibres) and $-91{\cdot}6 \pm 4{\cdot}4$ mV (stimulated fibres) and for action potentials, $82{\cdot}2 \pm 10{\cdot}8$ mV (control fibres) and $97{\cdot}8 \pm 8{\cdot}9$ mV (stimulated fibres). *C* shows typical resting and action potentials of two fibres examined in control period (left) and post-stimulation period (right), respectively; the larger resting and action potentials in the stimulated fibres are associated with enlargement of the M-wave (top traces).

fibres were stimulated, there was a small discrepancy between the mean amplitude of the observed action potential overshoots ($4{\cdot}0 \pm 12{\cdot}9$ mV) and the difference between the mean resting and action potentials for the entire muscle fibre population (2·7 mV). After the control measurements had been made in each experiment, the muscle was subjected to intermittent tetanic stimulation for 5 min; earlier experiments showed that this pattern of simulation caused the mean twitch tension to decline to $40{\cdot}2 \pm 17{\cdot}9$% of its initial value. Further determinations of resting and action potential amplitude were then made over a 15 min period; the distributions of the measurements, together with those of the control period, are shown in Fig. 1*A* and *B*. It can be seen that the resting potentials were increased by the period of

stimulation, the new mean value being $-91{\cdot}6 \pm 4{\cdot}4$ mV; this change was associated with a corresponding rise in the mean action potential amplitude, the post-stimulation value being $97{\cdot}8 \pm 8{\cdot}9$ mV. The changes in mean resting potential and action potential amplitudes were both statistically significant ($P < 0{\cdot}001$). Figure 1*C* shows typical recordings of resting and action potentials in two different fibres

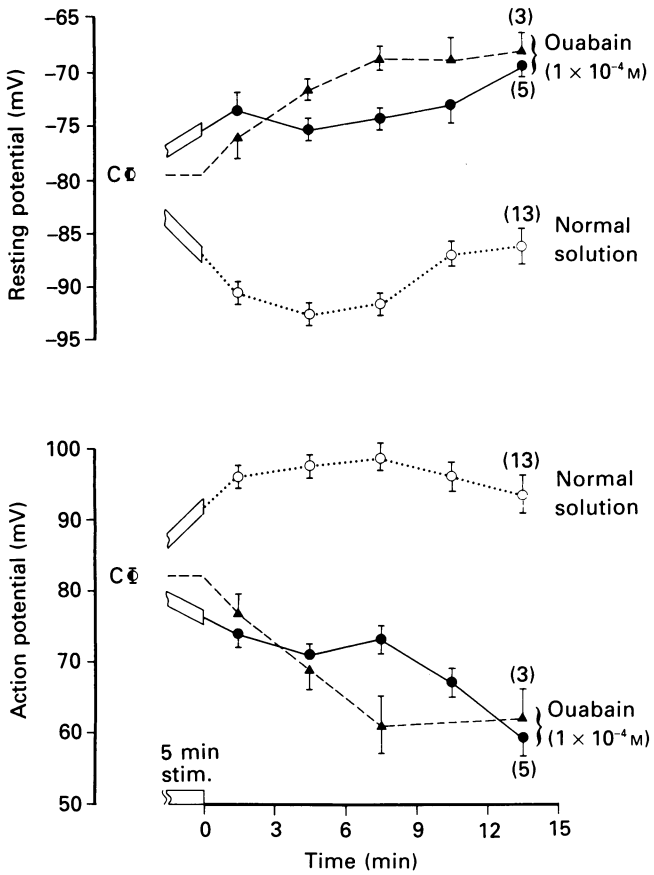


Fig. 2. Effects of bath-applied ouabain ($1 \times 10^{-4}$ M) on resting and action potentials of soleus muscle fibres which had either been stimulated tetanically for 5 min or left in resting conditions (curves with and without 'boxes' respectively). Mean control values indicated at left (C); other points are means ($\pm$standard errors of the mean) for each 3 min epoch following treatment (see Methods). Values in parentheses denote numbers of animals used in each type of experiment.

during the control and recovery periods respectively of the same experiment; this figure also displays the prominent enlargement of the M-wave (muscle compound action potential) following repetitive stimulation. This enlargement could sometimes be observed during the period of intermittent tetanic stimulation; more often, the M-wave amplitude was depressed immediately after the end of the intermittent tetani but regained its control value within the first minute of recovery and reached a

mean value of $149 \pm 25\%$ by 9 min. Enlargement of the M-wave was found in all animals not subjected to inhibition of the sodium pump (see below).

In Fig. 2 (top) the time course of the change in resting potential has been plotted. It was found that even the first fibre impalements, performed at the end of the tetanus, showed elevations of resting potential; thus, the mean value in the first 3 min period of recovery was $-11$ mV greater than that of the control period. Figure 2 also shows that, although the hyperpolarization began to diminish 9 min after the end of tetanic stimulation, it was still evident at 15 min.

The amplitudes of the single-fibre action potentials followed a similar time-course to that of the resting potentials (Fig. 2, bottom). In the first 3 min period after stimulation the mean amplitude had risen to $96{\cdot}8 \pm 10{\cdot}0$ mV, reaching a maximum value of $98{\cdot}3 \pm 10{\cdot}1$ mV 6–9 min after stimulation; the action potential amplitudes then began to decline but, like the resting potentials, were still elevated at 15 min (Fig. 2).

*Effects of ouabain*

In view of the strong likelihood that the post-tetanic hyperpolarization was due to the electrogenic effect of increased sodium pump activity, an attempt was made to block pump sites with the specific inhibitor, ouabain. Earlier experiments had revealed a difficulty in that intraperitoneal injection of this drug in an effective dose caused a progressive depolarization of muscle fibres (cf. Locke & Solomon, 1967) and eventually a loss of excitability. Under these conditions repetitive stimulation would have had uncertain effects on the muscle since an increasing proportion of the fibres would have become unresponsive. We therefore stimulated the muscles repetitively first, so as to induce any impulse-mediated ionic changes, and applied ouabain to the bathing fluid a few seconds before the end of the tetanus. Any immediate effect of the drug should have been evident in the surface fibres used for microelectrode impalement, even if deeper fibres were affected more slowly. The second, related, problem was to find a bath concentration of ouabain which would only alter the resting potential of unstimulated fibres to a minimal extent but would inhibit the sodium pump sufficiently so that the latter was unable to deal with the ionic perturbations of tetanized soleus muscles. The optimum dose of ouabain proved to be $1{\cdot}25 \times 10^{-4}$ M; in unstimulated muscles this concentration caused an immediate depolarization of 4 mV but thereafter the decline was acceptably slow (1 mV/min).

When ouabain was added to the bathing medium at the end of the stimulation period, the fibre membranes no longer exhibited a hyperpolarization in the immediate recovery period, nor were the action potentials enlarged. Thus, following tetanic stimulation and in the presence of $1{\cdot}25 \times 10^{-4}$ M-ouabain, the mean resting potential ranged from $-74{\cdot}1 \pm 4{\cdot}6$ mV in the first 3 min, to $-70{\cdot}1 \pm 2{\cdot}5$ mV at 12–15 min (Fig. 2). During this time the mean amplitude of the action potentials fell by a greater amount, from the control value of $82{\cdot}2 \pm 10{\cdot}8$ to $61{\cdot}8 \pm 6{\cdot}7$ mV.

*Effects of cooling*

A second approach to investigating the putative activity of the sodium pump in stimulated muscle was to study the effect of cooling, since active transport should have been diminished (cf. Hodgkin & Keynes, 1955). As in the experiments with

ouabain, the intervention was delayed until the end of the 5 min of intermittent tetani, so as not to diminish the ionic changes resulting from impulse propagation in the muscle fibres. The temperature of the bathing fluid was reduced from 37 to 19 °C by replacing one solution with another, this procedure taking approximately 15 s (see Methods).

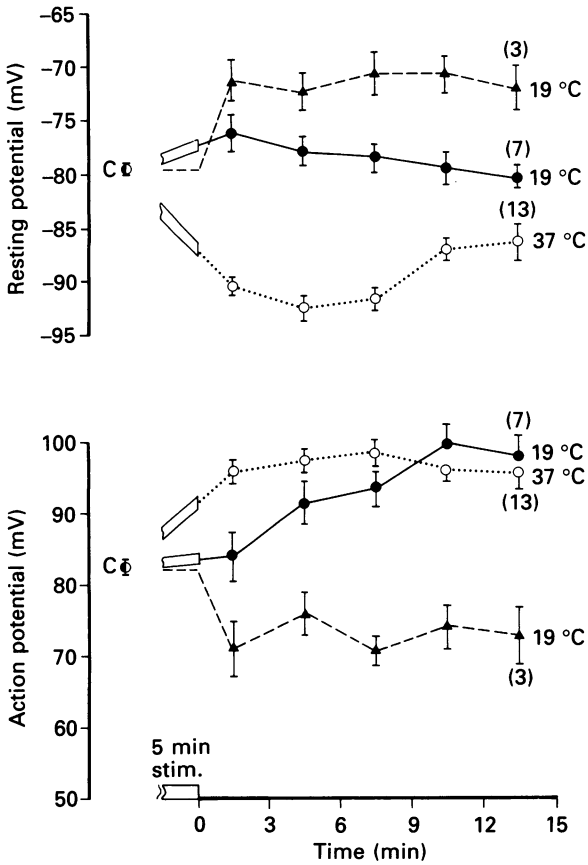


Fig. 3. Effects of cooling bathing fluid to 19 °C on resting and action potentials of soleus muscle fibres which had either been stimulated tetanically for 5 min or left in the resting state (curves with and without 'boxes' respectively). 'C' indicates mean control values while other points are means for each 3 min epoch following treatment (see Methods); all values given with standard errors. Numbers of animals used in each type of experiment given in parentheses.

At 19 °C it was found that the mean resting potential of unstimulated surface fibres fell by 8 mV over 15 min and the action potentials also diminished in addition to becoming broader (Fig. 3). At the same reduced temperature the mean resting potential of stimulated fibres remained between $-77{\cdot}0 \pm 5{\cdot}3$ and $-79{\cdot}9 \pm 4{\cdot}7$ mV over the 15 min recovery period, neither value being significantly different from the control mean of $-79{\cdot}5 \pm 4{\cdot}8$ mV. The fibre action potentials behaved rather differently, in that their amplitudes began to rise within 3–6 min of recovery, reaching

a maximum mean value of $100{\cdot}1 \pm 9{\cdot}8$ mV at 9–12 min and still being markedly elevated at 15 min (Fig. 3). The mean overshoot of the action potential at 9–12 min was $21{\cdot}0 \pm 6{\cdot}6$ mV and was significantly larger than those in control and stimulated muscles at 37 °C ($4{\cdot}0 \pm 12{\cdot}9$ and $8{\cdot}4 \pm 12{\cdot}2$ mV respectively; $P < 0{\cdot}001$). An increased overshoot following cooling was also found by Nastuk & Hodgkin (1950) in single amphibian muscle fibres and by Ward & Thesleff (1974) in rat muscle; the phenomenon may be attributed to the longer open time of the sodium channels and slowing of the delayed $K^+$ rectifying current (cf. Hodgkin & Huxley, 1952).

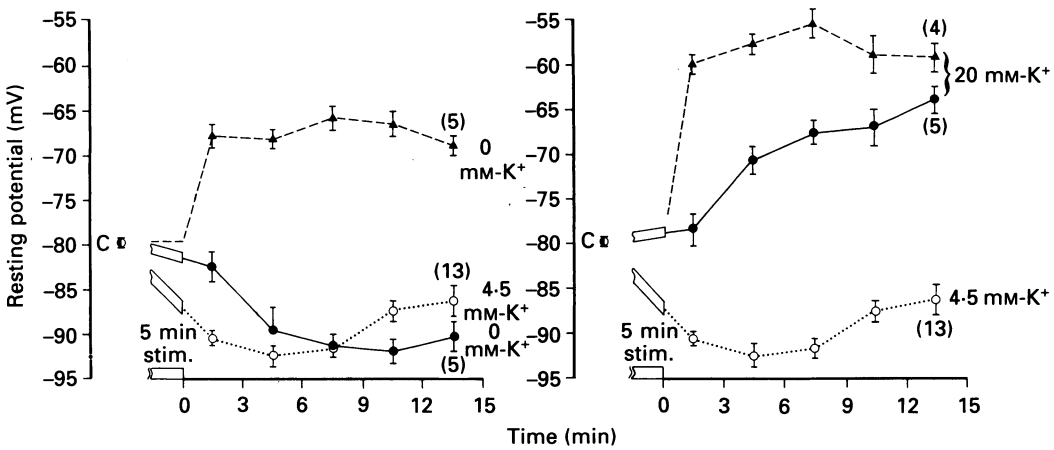


Fig. 4. Effects of altering potassium concentrations in bathing fluid on resting potentials of tetanically stimulated or quiescent muscle fibres (curves with and without 'boxes' respectively). All values are means ± S.E.M. and represent control observations (C) or potentials measured in 3 min epochs following treatment (see Methods). Numbers of animals used in each type of experiment given in parentheses.

*Effect of reduced* $[K^+]_o$

The final experiment, aimed at inhibiting sodium pump activity, was to replace the bathing solution with one which was $K^+$-free (Hodgkin & Keynes, 1959); as in the cooling and ouabain experiments, this intervention was carried out just before the end of the tetanic stimulation.

In the immediate recovery period, the mean resting potential was significantly lower in surface fibres which had been exposed to the $K^+$-free medium rather than to the normal bathing solution ($P < 0{\cdot}05$; Fig. 4, left). By 3–6 min, however, both the resting and action potential amplitudes had increased, the mean resting potential reaching $-91{\cdot}7 \pm 4{\cdot}6$ mV at 6–9 min ($P < 0{\cdot}001$). The elevations in resting and action potential amplitude were sustained for the remainder of the 15 min observation period. The increases in resting and action potential amplitudes were probably due to renewed activity of the sodium pump, following diffusion of $K^+$ from deeper parts of the muscle into the vicinity of the surface fibres.

In control experiments without tetanic stimulation, the $K^+$-free solution caused the muscle fibres to depolarize by approximately 12 mV (Fig. 4, left); this result was

interpreted as evidence of the contribution of the electrogenic sodium pump to the normal resting potential (see Discussion).

*Effect of 20 mM* $[K^+]_o$

Having shown, by the application of ouabain, cooling and low external $[K^+]$, that the enhancement of the resting and action potentials was due to the sodium pump, in the final part of the study an attempt was made to estimate the possible magnitude of the electrogenic component in the presence of a high external potassium concentration. Soleus muscles were again tetanized and the normal bathing fluid was rapidly replaced by one containing 20 mM-$K^+$ (and 124·5 mM-$Na^+$). This concentration was rather higher than the estimates of interstitial $[K^+]$ made during fatigue in other laboratories (see Discussion) but it served to reduce the theoretical potassium equilibrium potential, $E_K$, to a level well below the control resting potential, so that the size of the electrogenically derived potential would be more readily apparent.

It was found that, in the first 3 min recovery period, the mean resting potential, $-79{\cdot}5 \pm 8{\cdot}6$ mV, was identical to the control value (Fig. 4, right) but remarkably different to that of unstimulated fibres exposed to the same high-$K^+$ medium. The latter fibres underwent an immediate depolarization, the mean resting potential falling to $-59{\cdot}9 \pm 3{\cdot}5$ mV in the first 3 min.

The increase in $[K^+]_o$ had a significant effect on the action potential of stimulated muscle fibres, the mean value declining from $82{\cdot}2 \pm 10{\cdot}8$ to $59{\cdot}7 \pm 8{\cdot}3$ mV. By 6 min after the end of tetanic stimulation no action potentials could be evoked by indirect stimulation and by this time the mean resting potential had fallen to $-68{\cdot}1 \pm 5{\cdot}0$ mV. In unstimulated fibres the high-$K^+$ medium depolarized the surface muscle fibres to render them inexcitable.

## DISCUSSION

There are three issues to be addressed. First, how are the changes in resting and action potentials, observed in the present study, to be explained? Secondly, why were elevations of resting and action potential amplitude not seen in previous studies of tetanized mammalian muscle? Finally, what is the significance of the mechanisms underlying the changes in membrane potential for an understanding of muscle fibre excitability during stimulated or voluntary activity?

In relation to the first question, there can be little doubt that the rise in resting potential, detectable immediately after stimulation had ceased, was caused by heightened activity of the electrogenic sodium pump. The results of all three experimental interventions point to this conclusion. It should be added that, in the ouabain experiments, an attempt was made to inhibit the sodium pump only partially, so the resting potentials in the stimulated fibres remained at approximately normal values. Had higher concentrations of ouabain been used, the resulting depolarization block would not have allowed the specificity of the ouabain effect on the stimulus-induced hyperpolarization to be demonstrated.

In the control experiments the rapid depolarization of surface fibres, following the application of ouabain, cooling or a low-$K^+$ solution, suggested that the sodium

pump also made an electrogenic contribution to the resting potentials of unstimulated fibres; the amounts were −4 mV (ouabain), −8 mV (cooling) and −11 mV ($K^+$-free solution). These values may be compared with that calculated on the basis of the mean control resting potential and the ionic concentrations in the rat soleus. Using the equation of Mullins & Noda (1963):

$$E_m = \frac{RT}{F} \ln \frac{r[K^+]_o + b[Na^+]_o}{r[K^+]_i + b[Na^+]_i},$$

where $E_m$ is the resting potential, $r$ is 1·5 and is the $Na^+:K^+$ ion exchange ratio for the sodium pump, and $b$ is the ratio of the membrane permeabilities for $Na^+$ and $K^+$,

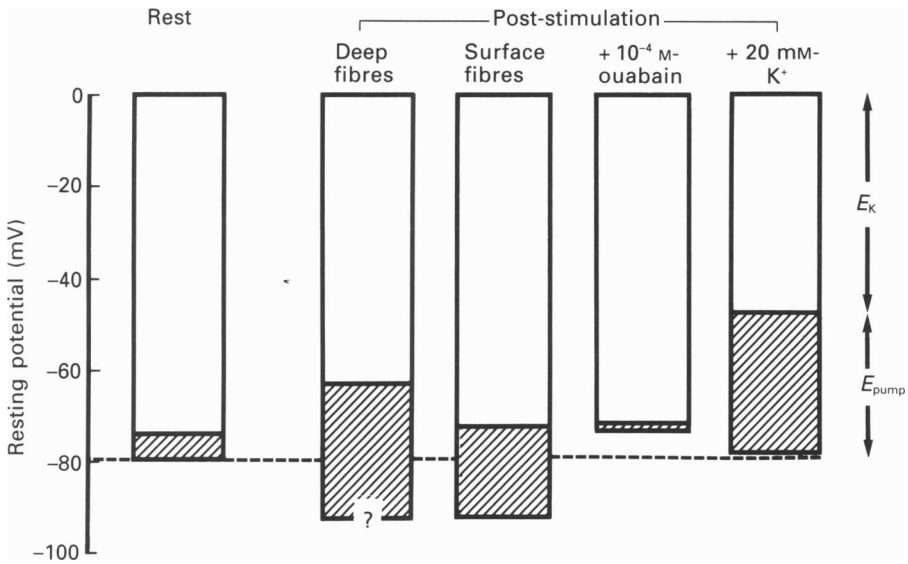


Fig. 5. Theoretical contributions of potassium equilibrium potential ($E_K$) and electrogenic sodium pump ($E_{pump}$) to resting membrane potentials of resting (Rest) and tetanically stimulated soleus muscle fibres situated deeply or superficially and treated with $10^{-4}$ M-ouabain or 20 mM $[K^+]_o$. Pump contributions shown by hatched columns (see text).

respectively; steady-state conditions are assumed. In the present experiments the observed value of $E_m$ was −79·5 mV, $[K^+]_o$ was 4·5 mM and $[Na^+]_o$ was 140 mM. If values of 163 and 13 mM are taken for $[K^+]_i$ and $[Na^+]_i$ respectively in the resting rat soleus (Sreter, 1963) and activity coefficients of unity are assumed, then $b$ must be 0·04. When this value is used in the Goldman–Hodgkin–Katz equation (Hodgkin & Katz, 1949), the calculated value for $E_m$ becomes −74·4 mV and the electrogenic contribution of the sodium pump would be −(79·5–74·4) = −5·1 mV (see Fig. 5). Although this value is smaller than those derived by Locke & Solomon (1967) and by Williams, Withrow & Woodbury (1971), it is difficult in such studies to distinguish between the immediate effect of ouabain, in abolishing the electrogenic component of the resting potential, and the more gradual effect of the running down of the ionic concentration differences across the muscle fibre membrane.

In fibres which had been stimulated the size of the electrogenic component of the resting potential probably varied, depending on the positions of the fibres within the muscle belly. Thus, for deeper fibres $[K^+]_o$ would be higher because of poor diffusion of $K^+$ released by impulse activity (cf. Creese, Hashish & Scholes, 1958). Studies with ion-sensitive microelectrodes have yielded values of 7–10 mM for the concentration of $K^+$ in the interstitial spaces of muscles following repetitive stimulation (Hnik *et al.* 1976; Hirche, Schumacher & Hagermann, 1980; Juel, 1986), although higher concentrations have been found during voluntary contraction of human forearm muscles (see Fig. 2*B* of Vyskocil, Hnik, Rehfeldt, Vejspada & Ujec, 1983). If, in our experiments, the deeper fibres had similar resting potentials to the surface fibres after stimulation, with $[K^+]_i = 152$ mM, $[Na^+]_i = 19$ mM (see Sreter, 1963), $[K^+]_o = 9$ mM and $[Na^+]_o = 135{\cdot}5$ mM, then the electrogenic contribution of the pump might have been as much as $-29{\cdot}5$ mV (Fig. 5). For surface fibres, however, exposed to 4·5 mM-$K^+$ and 140 mM-$Na^+$ in the bathing solution, the electrogenic potential would be $-20$ mV. To see how high the electrogenic potential might become in surface fibres, the latter were bathed in 20 mM-$K^+$; under these circumstances the electrogenic component of the resting potential increased to $-30$ mV after repetitive stimulation (Fig. 5). This value may appear large but is commensurate with those found in other circumstances. Thus, Creese, Head & Jenkinson (1987) have recently shown that the sodium pump can contribute at least $-33$ mV to the membrane potential in the presence of depolarizing drugs. Also, Kernan (1968) found that rat muscle fibres, which had been loaded with sodium following exposure to a cold $K^+$-free bathing solution, developed electrogenic potentials of $-22$ mV on exposure to external $K^+$. It is also pertinent that, in dogs which had undergone endurance training, Knochel, Blachley, Johnson & Carter (1985) found that the electrogenic component of the muscle fibre resting potential had increased from $-9$ to $-30$ mV due to increased sodium pump activity. In our experiments with 20 mM-$K^+$ we have attributed the fall in resting potential later in the recovery period to a reduction in electrogenic sodium pump activity following restitution of $[Na^+]_i$ to normal values; the correction of $[Na^+]_i$ would have been hastened by the high $[K^+]_o$ driving the pump (Clausen, Everts & Kjeldsen, 1987).

Regarding the second question, we think it likely that the finding of depolarization, rather than hyperpolarization, in previous mammalian studies may have been due, in *in vitro* experiments (e.g. Hanson, 1974; Juel, 1986), to an absence of protein in the artificial bathing media. It was shown by Kernan (1963) that the resting potentials of rat muscle fibres *in vitro* corresponded to $E_K$ if plasma proteins were present in the bathing fluid; if they were absent the mean resting potential was 12·4 mV lower. In related studies Creese and colleagues (Creese, 1954; Creese & Northover, 1961) demonstrated that, without plasma protein, there was a rapid rise in $[Na^+]_i$ in mammalian muscles *in vitro*. This rise was attributed to an increase in $P_{Na}$; however, the contrasting effects of stimulation on the resting potential in the *in vitro* experiments cited above, and in our own *in vivo* preparation, raise the possibility that plasma proteins may be necessary for the normal operation of the sodium pump.

Even in *in vivo* experiments, stimulus-induced depolarization has been reported by Locke & Solomon (1967). In those experiments it is probable that the impulse-

mediated $K^+$ efflux caused a fall in $E_K$ which could not be compensated for by the electrogenic sodium pump (see below). Thus, the stimulus frequency of 300 Hz employed by these authors was far higher than the maximal motoneurone firing frequency (Marsden, Meadows & Merton, 1971) and also the stimulus frequency used in the present study (20 Hz). Our findings are in accord with the recent results of Fong, Atwood & Charlton (1986) who observed hyperpolarization after stimulating rat soleus extensor digitorum longus muscles, and with those of Brodal, Eeg-Larsen, Iversen, Jebens & Roed (1975), who found a 28% increase in $Na^+$-$K^+$-ATPase activity following indirect stimulation of rat hindlimb muscle. Our results are also qualitatively similar to those in other excitable tissues in which hyperpolarization was induced by activity; those preparations include unmyelinated nerve fibres (Ritchie & Straub, 1957), snail neurones (Thomas, 1969) and hippocampal pyramidal neurones (Gustafsson & Wigström, 1983).

What is the functional significance of the changes in membrane potential found in the present study? It should first be stressed that, although intracellular recordings could not be made during the stimulation period, it is probable that hyperpolarization was present between successive action potentials, at least in superficial fibres. This was suggested by the enlargement of the M-wave sometimes observed in the course of the tetanus, similar to that invariably seen in human experiments (Fitch & McComas, 1985). The significance of the change in resting potential becomes apparent if a calculation is made of the membrane potential after tetanization in the absence of pump activity. If Sreter's (1963) *in vivo* results are used, 5 Hz stimulation for 4 min would cause $[K^+]_i$ in rat soleus to fall from 163 to 152 mM and $[Na^+]_i$ to rise from 13 to 19 mM. Suppose, on the basis of ion-sensitive electrode measurements (see above), that $[K^+]_o$ rose to 9 mM and, also, that the $Na^+$:$K^+$ permeability ratio was 0·04; the predicted resting potential would then be $-63$ mV and sufficiently low to cause depolarization block (see Results). The effect of the electrogenic sodium pump is evidently to prevent such a block from occurring; by raising the membrane potential it renders the $Na^+$ channels available for excitation while at the same time ensuring that the size of the action potential will be sufficient for onward propagation. This function of the sodium pump during stimulated or voluntary muscle contraction is additional to its role in restoring the intracellular concentrations of $Na^+$ and $K^+$ during the recovery period. Finally, the present results provide a satisfactory explanation for the enlargement of the M-wave in human studies (Fitch & McComas, 1985). Indeed, if this last interpretation is correct, observations of M-wave amplitude would provide a simple, non-invasive method for monitoring sodium pump activity in human subjects.

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