J. Physiol. (1980), **307**, *pp.* 43–57
With 11 *text-figures*
Printed in Great Britain

COMPARISON BETWEEN THE DELAYED OUTWARD CURRENT IN SLOW AND FAST TWITCH SKELETAL MUSCLE IN THE RAT

BY A. DUVAL AND C. LÉOTY

From the Laboratory of General Physiology (L.A.: C.N.R.S. no. 290), University of Poitiers, Poitiers 86022, France

(*Received 22 June 1979*)

SUMMARY

1. A comparison of the delayed outward current of isolated fibres from rat soleus and iliacus muscle has been made using a double sucrose-gap voltage–clamp method.

2. The fast and slow components of the outward current were separated using time constants of the tail currents. The results indicate that in both iliacus and soleus fibres there is a shift in reversal potential which depends on the quantity of current that flows during depolarization.

3. The shift is larger in iliacus than in soleus; it is absent in glycerol-treated muscles.

4. The results obtained in normal and in detubulated fibres show that the shift is due to an accumulation process of potassium ions in the lumen of the T-tubules.

5. In detubulated soleus fibres the outward current is composed of a fast and a slow component, each with the same reversal potential; in detubulated iliacus the slow component is absent.

6. In both types of muscles TEA produces a dose-dependent block of the total outward current. 4-aminopyridine has different effects; it inhibits the total outward current in iliacus fibres and only the fast component in soleus fibres.

7. These results show that in soleus fibres a fast and a slow component participate in the potassium outward current, while only a fast component is present in iliacus muscle.

INTRODUCTION

In the previous paper (Duval & Léoty, 1980) the properties of the sodium and potassium conductances in rat slow muscle fibres have been described and compared to those of fast fibres (Duval & Léoty, 1978). The main electrical differences between these two muscle types lies in the characteristics of the delayed outward current. The existence, in rat muscle fibres, of two equilibrium potentials for outward current which are associated with two time constants of deactivation has led us to propose that the outward current is composed of two components, as in frog skeletal muscle fibres (Adrian, Chandler & Hodgkin, 1970). The differences in time course of the outward current in the two mammalian fibres suggest that in slow fibres there is a larger participation of the slow component. Although a similarity between frog and rat muscle is found, the equilibrium potential of the slow component is always more positive in rat fibres. This observation could be related to a real difference between

0022-3751/80/1800-0305 $07.50

muscles or to errors in the determination of the equilibrium potential. For example, the equilibrium potential was determined after the development of a large outward potassium current which could have raised the potassium concentration in the T-system, thereby altering the potassium equilibrium potential.

The aim of the present paper was to study the extent to which changes in external potassium concentration in the T-system can affect the reversal potential of the outward current and also to determine the relative contribution of the two components to the delayed outward current in each type of muscle. A preliminary report of some of the work has been already published (Duval & Léoty, 1979).

Methods

The experiments were carried out on isolated fibers from rat soleus and iliacus muscles using the same technique as that described in the previous paper. The solutions have the same composition as those already mentioned.

Results

1. Delayed outward current in fast and slow muscle

Fig. 1 shows typical records of delayed outward currents in iliacus (A) and soleus (B) fibres. In iliacus fibres, the outward current always shows a single peak (Fig. $1A_1$) and then decreases with time (Fig. $1A_2$). In soleus fibres the outward current shows two peaks (Fig. $1B_1$) after which the current decreases with time in a roughly exponential manner (Fig. $1B_2$). The time constant of this decrease is about ten times larger than that found for the fast fibres (Fig. $1A_2$ and B_2).

In an attempt to resolve the total potassium current into its two components, a series of experiments similar to those described by Adrian *et al.* (1970) was carried out. Fast tail currents were measured by repolarizing the fibre to a potential close to the reversal potential of the slow component, whereas slow tail currents were measured by repolarizing to a potential near the reversal potential of the fast component. Tail currents so obtained are illustrated in Fig. 2.

The fast tail currents obtained in both types of preparation (Fig. $2A_1$ and B_1) are outward and deactivate with a time constant of about 10 msec. The duration of the first pulse necessary for the peak tail current to reach maximum amplitude corresponds to the time to peak of the delayed outward current. The amplitude of the tail current then decreases as the duration of the first pulse is increased.

By contrast, the amplitude of slow tail currents in both fibre types (Fig. $2A_2$ and B_2) increases with the duration of the depolarizing step. These tail currents decrease with a time constant of about 150 msec for depolarizations no longer than 250 msec.

The tail currents have been analysed in the following way. The fast and slow components of current were assumed to obey Ohm's law and the reversal potentials were assumed to remain constant during each pulse. The value of the reversal potential for each component was then obtained by the double-step method as previously described (Duval & Léoty, 1980). This analysis proves unsatisfactory because the sum of the two reconstructed curves is different from the recorded current, especially during the first 100 msec when the fast component is decreasing quickly (Fig. $2A_1$ and B_1) and the slow component is developing slowly (Fig. $2A_2$

and B_2). Moreover, for depolarizing steps larger than 100 msec, the amplitude of the slow component increases to become larger than the total current recorded and fails to show inactivation (Fig. $2A_2$ and B_2).

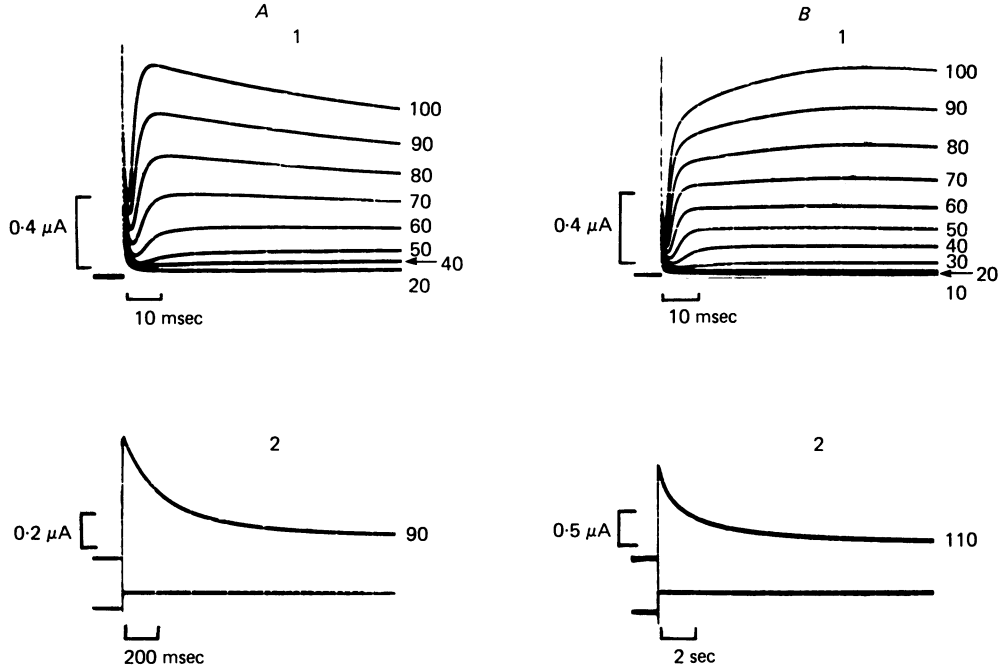

Fig. 1. Records of outward potassium current obtained in rat muscle fibres in Ringer solution at 20 °C. A, in iliacus and B, in soleus fibres. A_1 and B_1, time course of the outward current for short depolarizing steps of different amplitude in the presence of TTX (5×10^{-7} g/ml.). A_2 and B_2, records, on slower time scale, showing the decrease of potassium current; note the different scales in A_2 and B_2.

2. Shift in the reversal potential of the outward current

The reversal potential of the outward current obtained in a soleus fibre for a depolarizing step of +100 mV, was measured for different durations of conditioning pulse (Fig. $3A$). The reversal potential, which depends on the step duration, was found to vary from +10 mV at 7 msec (Fig. $3A_1$) to +40 mV at 270 msec (Fig. $3A_4$). A semilogarithmic plot of the 'off' currents was used to distinguish between the fast and the slow component in this sort of experiment. The net tail currents were obtained by subtracting the leak current from the total current. The leak current was assumed to correspond to the steady outward current recorded for low value of potential and was considered to be a linear function of the voltage. Since the rate constants of the tail currents were much slower than the capacitative transient the records were not corrected for the capacitative currents. Fig. $3B$ gives an example of such an analysis of the tail current when the membrane was repolarized to 0 mV. The 'off' current traces show two phases of decay which correspond to the deactivation of the fast and of the slow component. For depolarizing steps longer than 7 msec and at different levels of repolarization the 'off' current always shows two phases of decay which correspond to the deactivation of the fast and the slow

components respectively. The faster phase has a time constant of 10–15 msec and the slower one of 100–250 msec which depends on the step duration (Fig. 3*B*). The instantaneous currents of the fast and of the slow component were estimated by extrapolating both the fast and the slow phases of the tail currents to the beginning of the second pulse. In this way the instantaneous current–voltage relations for the

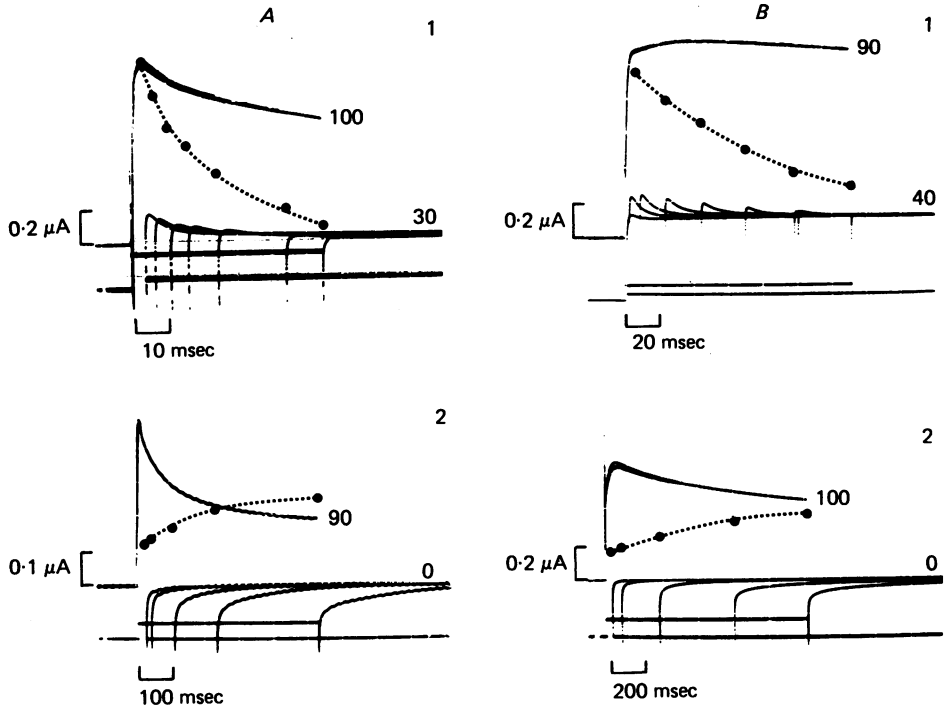

Fig. 2. Separation of the delayed outward current into a fast and a slow component. *A* in iliacus, *B* in soleus fibres at 20 °C. In A_1 and B_1, the fast component (dotted lines) was calculated from the amplitude of the instantaneous net outward tail currents measured at +30 mV (A_1) and +40 mV (B_1) sssuming that this component obeys Ohm's law. The measured reversal of the fast component was found to be 14 mV positive to the holding potential in iliacus and +16 mV in soleus fibres. In two other fibres, A_2 and B_2, the slow component (dotted lines) was calculated, in a similar way as for the fast component, for a repolarization to 0 mV. The measured reversal potential was +36 mV for iliacus and +32 mV for soleus.

fast and slow components (Fig. 4) were estimated from records of Fig. 3*A*. The curves are linear and cross the voltage axis at a potential which corresponds to the reversal potential. For the different depolarizing steps the reversal potential of the slow component, when present, is identical to the reversal potential of the fast component. Both are shifted in a similar manner to less negative values when the duration of the depolarization is increased, Similar behaviour was also encountered in fast fibres.

The effect of increasing the duration of the depolarizing step on the reversal potential of the delayed outward current illustrates the extent to which accumulation occurs under voltage-clamp conditions. In an attempt to investigate this process further a quantitative analysis was undertaken. As the reversal potential of

each component appears to be identical whatever the step duration, the analysis was simplified by only taking the variation of the reversal potential of the total outward current into account. In both slow and fast fibres, the shift in the reversal potential has been measured from records like those illustrated in Fig. 2A_1 and B_1). For fixed durations, the amplitude of both the net outward current and the net instantaneous tail current was measured. Using a similar method to that proposed by Noble (1976), these two values when plotted against the amplitude of depolarization yield straight lines which cross the voltage axis at a value corresponding to the reversal potential

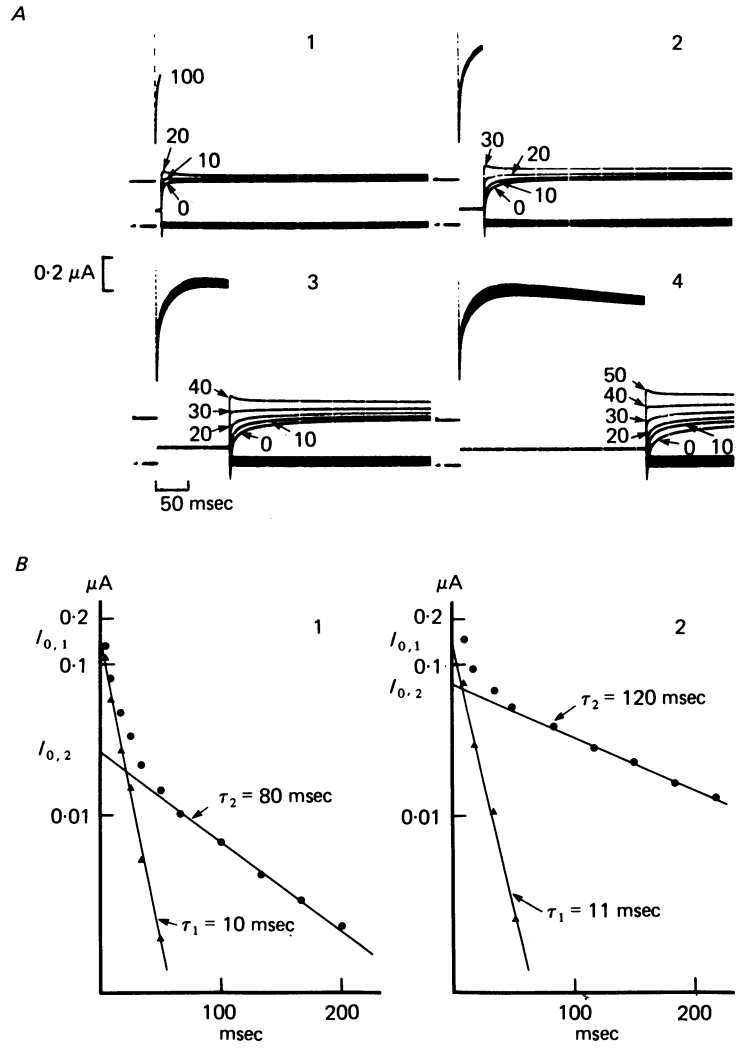

Fig. 3. A, effect of duration of the 100 mV depolarization on the reversal potential of the total outward current in soleus fibres. Records obtained in presence of TTX (5×10^{-7} g/ml.) at 19 °C. Numerical values indicate the level of repolarization in mV. B, semilogarithmic plot of the net tail current showing two exponential phases B_1 and B_2 respectively, from the records of A_2 and A_3 on repolarizing the membrane at 0 mV. I_0 and τ are the instantaneous current and the time constant of deactivation respectively of the fast (1) and of the slow (2) components.

of the total outward current. Such an analysis from the current recorded in Fig. $2A_1$ and B_1 are illustrated in Fig. $5A_1$ and B_1.

The variation of reversal potential following a +90 or +100 mV pulse in four iliacus and four soleus fibres is shown in Fig. $5A_2$ and B_2. A depolarizing pulse of 100 msec causes a large change in the reversal potential of about 20 mV in both

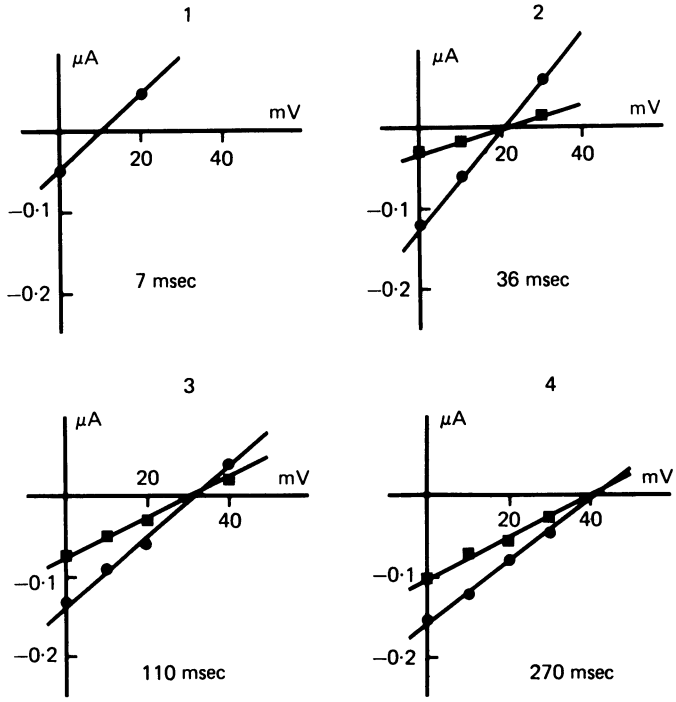

Fig. 4. Instantaneous current–voltage relations for the fast (circles) and for the slow (squares) components at the end of depolarizations (100 mV) of different durations. The results in 1, 2, 3 and 4 are from the experiment illustrated Fig. $3A_1$ A_2, A_3 and A_3 and analysed according to the method shown in Fig. $3B$.

iliacus and soleus fibres. These changes which are time-dependent are also affected by the amplitude of the current. Similar measurements made for different amplitudes of current induced by different depolarizing steps in an iliacus fibre (Fig. $6A_1$) and in a soleus fibre (Fig. $6B_1$) show that the larger the depolarization applied, and consequently the larger the amplitude of the current, the larger is the shift that occurs.

These results demonstrate that the change in the equilibrium potential is affected by both changes in the duration and the amplitude of the delayed outward current.

In Fig. $5A_3$ and B_3, and in Fig. $6A_2$ and B_2, the time dependence of the potassium flux calculated from the net outward currents (IK) is plotted. The potassium flux MK (t) over the interval from 0 to t was obtained from the relationship given by eqn. (1) (Frankenhaeuser & Hodgkin, 1956).

$$M\mathrm{K}\ (t) = \frac{1}{F}\int_0^t (I\mathrm{K})\ \mathrm{d}t, \tag{1}$$

where F is the Faraday.

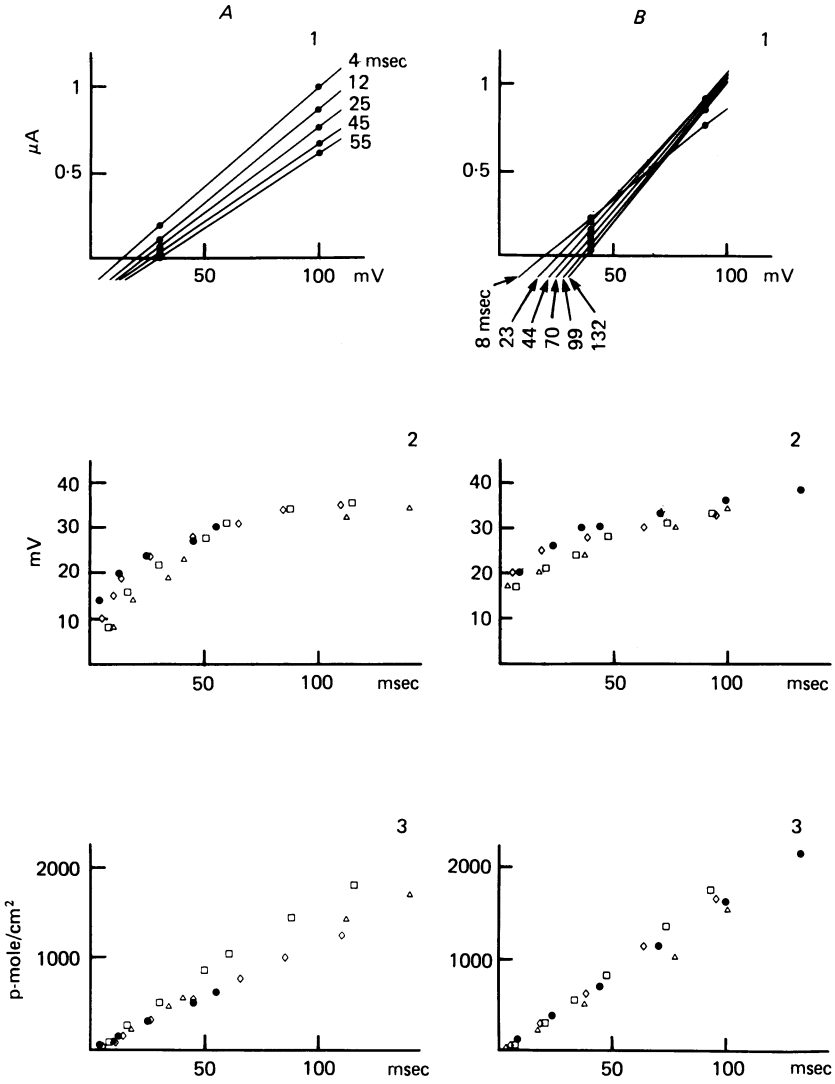

Fig. 5. The effect of the duration of the depolarizing step on the reversal potential of the total outward current in iliacus (A) and in soleus (B) fibres. A_1 and B_1 are, for each duration of depolarization, the reversal potentials of the outward currents illustrated in Fig. 2 A_1 and B_1; they were estimated according to the method described in the text. Ordinate, amplitude of the net outward current. Abscissa, amplitude of the depolarizing step. In A_2, A_3 and B_2, B_3; pooled results obtained with four iliacus and in four soleus fibres. Filled circles correspond to the experiments in Fig. 2 A_1 and B_1. A_2 and B_2: the relationship between the reversal potential (ordinate) and the depolarizing steps duration (abscissa). A_3 and B_3: relationship between the quantity of ions carried by the outward potassium currents (ordinate) and the duration of the depolarizing steps (abscissa).

The comparison between the curves obtained for different amplitude of depolarization Fig. 6*A* and *B* and for the same amplitude but in different fibres Fig. 5*A* and *B* show a good correlation between the shift recorded and the quantity of potassium ions calculated. The quantity of potassium ions carried is roughly proportional to the duration of the depolarization (Fig. 6A_2 and B_2) while there is an exponential varia-

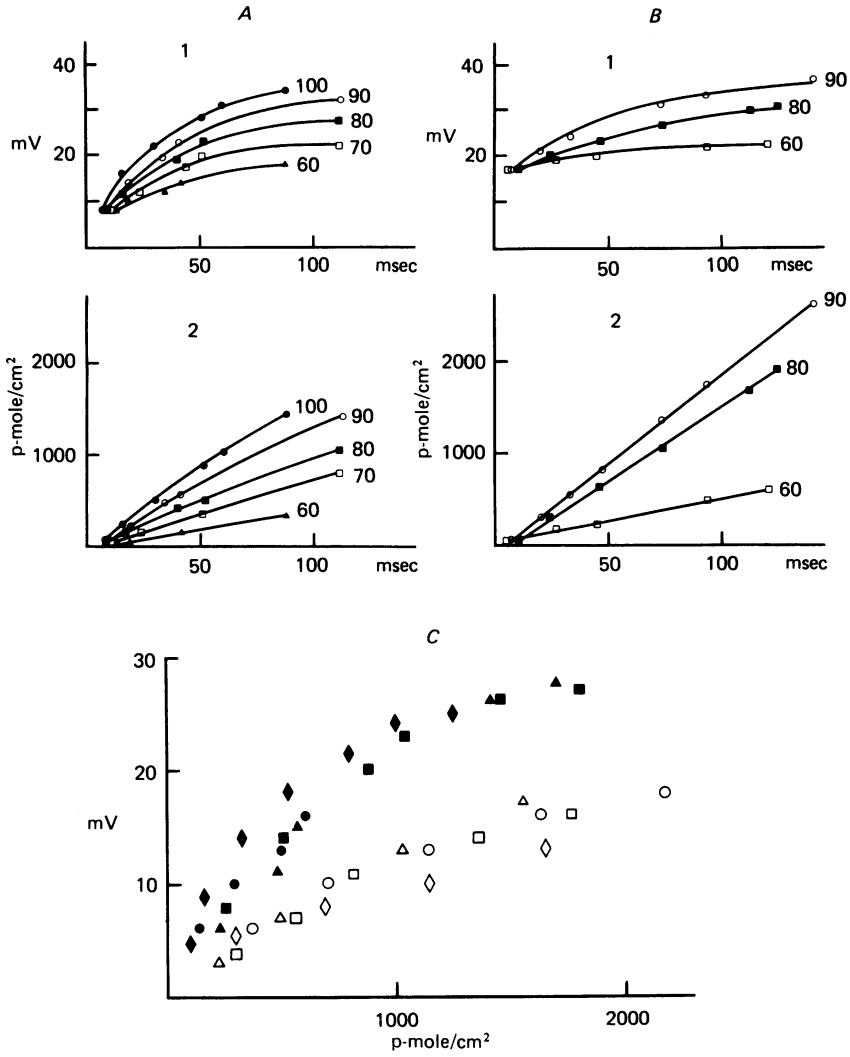

Fig. 6. A_1 and B_1: in normal Ringer at 20 °C, effect on the reversal potential of the total outward current (ordinate) of the duration of the depolarizing steps (abscissa) for different amplitudes of depolarizations, in iliacus (A_1) and in soleus (B_1). A_2 and B_2: relationship between the quantity of ions (ordinate) carried by the outward potassium current and the duration of the depolarizing step (abscissa), in iliacus (A_2) and in soleus (B_2) fibres. The values are from the outward currents analysed in A_1 and B_1. *C*: the relationship between the variation in the equilibrium potential of the total outward current (ordinate) and the quantity of potassium ions carried by the outward current (abscissa). Filled symbols are from outward currents generated by + 90 or + 100 mV depolarizing steps in four iliacus fibres and open symbols from four soleus fibres.

tion in the resulting shift in the reversal potential *vs.* the duration of the depolarization (Fig. 6A_1 and B_1). This observation which could be evidence for a saturation process, is in favour of a dependence of the equilibrium potential upon an accumulation process. The variation in the reversal potential in iliacus and soleus fibres was plotted as a function of the quantity of ions carried by the outward current (Fig. 6*C*). The results show that for an equivalent quantity of potassium ions the change in the reversal potential in iliacus fibres is about twice more important as compared to soleus fibres.

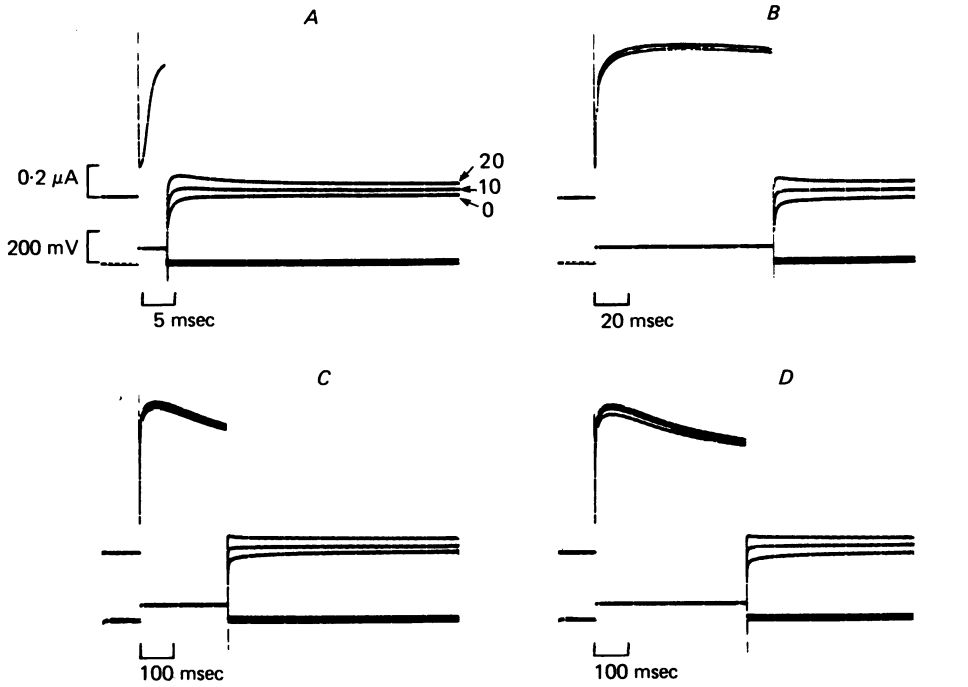

Fig. 7. Measurement of reversal potential of the delayed outward current in a glycerol-treated soleus fibre. The levels of repolarization (0, +10, +20 mV) are identical for all the records. Normal Ringer, with TTX (5×10^{-7} g/ml.), 20 °C.

3. Outward current in glycerol-treated preparations

The application of a depolarizing step induces the development of an outward current in detubulated fibres from iliacus and soleus muscles. Although the amplitude of the outward current was always smaller than in normal fibres the time course of this current is unchanged.

Fig. 7 shows currents obtained in a glycerol-treated soleus fibre, where the effect of pulse duration on the reversal potential is investigated. The result shows that the reversal potential of the outward current for a short depolarizing pulse (4 msec) is between 0 and +10 mV positive to the holding potential (Fig. 7*A*), a value identical to that found for the fast component in normal fibres. For longer depolarizations the reversal potential becomes slightly more positive and reaches a maximum value of about +10 mV (Fig. 7*B*, *C* and *D*). Similarly detubulation of iliacus fibres supresses the large change in the reversal potential of the total outward current.

Detubulated soleus fibres, similar to normal soleus fibres show two components of outward current as indicated by the existence of two time constants of deactivation of tail currents (Fig. 8*C*). As the total outward current at +90 mV could be reconstructed from the tail current obtained at +40 mV (Fig. 8*A* and *B*), the reversal potential for the outward current appears to be relatively insensitive to pulse duration.

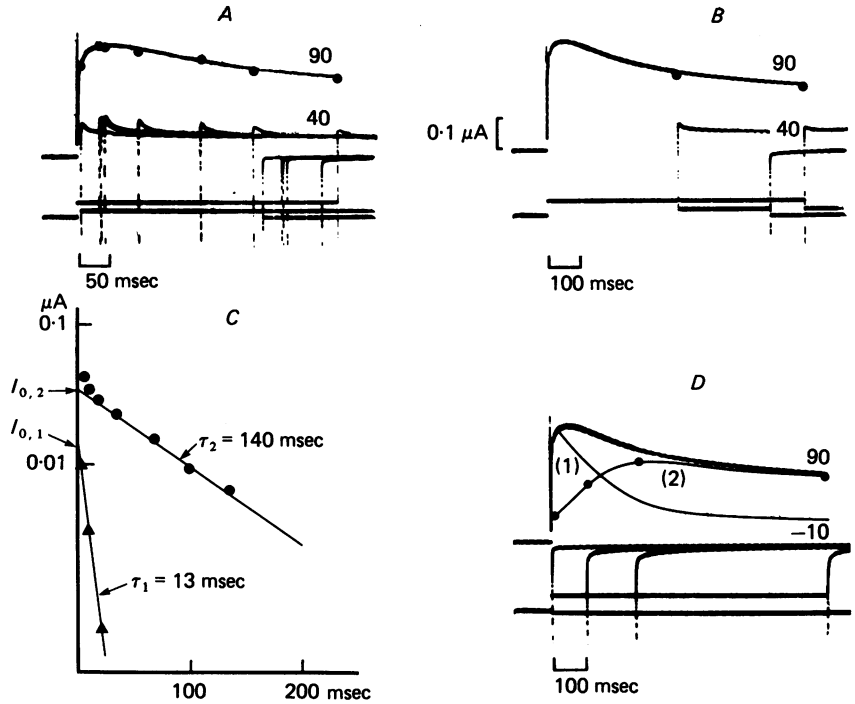

Fig. 8. Current records from a detubulated soleus fibre. The duration of the first pulse (+90 mV) was varied; repolarization was always to +40 mV (*A* and *B*) or to −10 mV (*D*). Filled circles in *A* and *B* correspond to an extrapolation of the straight line connecting the tail current at +40 mV and a constant reversal potential at +10 mV. *C*, semilogarithmic plot of the net tail current, obtained in *B* when the membrane is repolarized at +40 mV after +90 mV depolarizing step of 400 msec. I_0 and τ, respectively, instantaneous current and time constant of deactivation of the fast (1) and of the slow (2) components. *D*, division of potassium current into fast (1) and slow (2) component. The slow component was calculated from the amplitude of the instantaneous net inward slow tail currents measured at −10 mV, assuming that this component obeys Ohm's law and that the reversal potential is constant at +10 mV. The fast component was obtained by subtracting the slow component from the total outward current.

In a similar experiment when the membrane was repolarized to −10 mV, the slow component has been reconstructed (Fig. 8*D*) from the analysis of the slow tail currents. This component reaches a maximum in about 250 msec and then inactivates slowly. When it is at a maximum this component corresponds to about 90% of the total outward current. Moreover, this analysis shows that in the first 50 msec, the outward current is mainly due to the fast component but its participation becomes negligible for depolarizing steps longer than 300 msec. In contrast to the results

obtained from normal fibres, in detubulated soleus muscle the time constants of the slow tail currents (50–100 msec), obtained when the membrane is repolarized at a potential more negative than the holding potential, are independent of the conditioning step duration (Fig. 8*D*).

In glycerol-treated iliacus fibres a similar analysis (not shown) shows that the slow tail currents are virtually absent, indicating that the slow component is greatly reduced.

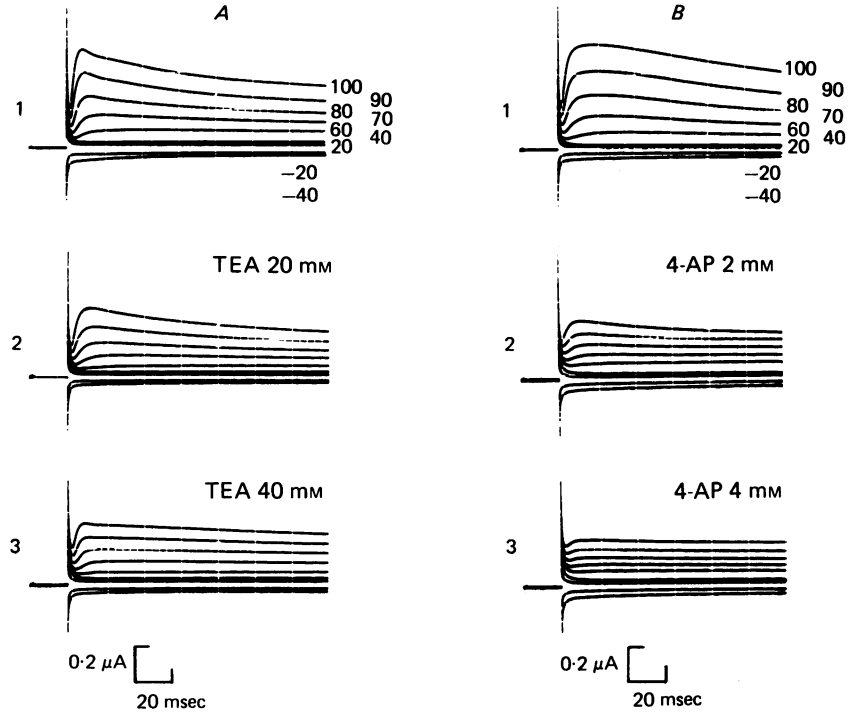

Fig. 9. Effects of TEA (*A*) and of 4-AP (*B*) on delayed outward currents in iliacus fibres. A_1, Ringer solution (TTX 5×10^{-7} g/ml.); A_2 20 mM-TEA added to the Ringer solution, A_3 40 mM-TEA. B_1, Ringer solution (with TTX 5×10^{-7} g/ml.) B_2, 2 mM-4-AP added to the Ringer solution; B_3 4-mM-4-AP. Temperature 18–20 °C.

4. Effect of the inhibitors of the potassium permeability

Previous experiments have shown that in skeletal muscle potassium permeability can be modified by substance like tetraethylammonium (TEA) (Stanfield, 1970) and 4-amino-pyridine (4-AP) (Gillespie & Hutter, 1975; Gillespie, 1977) It was therefore of interest to study the effect of these compounds on the delayed outward current in iliacus and soleus fibres.

Fig. 9 illustrates the effects of different concentrations of TEA and of 4-AP on the delayed outward current developed in iliacus fibres.

Fig. 9*A* shows that the addition of TEA to the normal Ringer solution reduces the amplitude of the delayed outward current. This effect, which is fully reversible, is more pronounced with 40 mM-TEA (A_3) than with 20 mM-TEA (A_2); however, even at the higher concentration there remains a detectable outward current. On the other

hand the amplitude and the time course of the inward current produced by hyperpolarization are unchanged.

Fig. 9*B* illustrates the effect of 4-AP. This inhibitor induces changes in the delayed outward current similar to those observed with TEA, except that the effect is not reversible. At concentrations higher than 4 mM the outward current is completely inhibited but a large background current develops which is associated with the deterioration of the fibres.

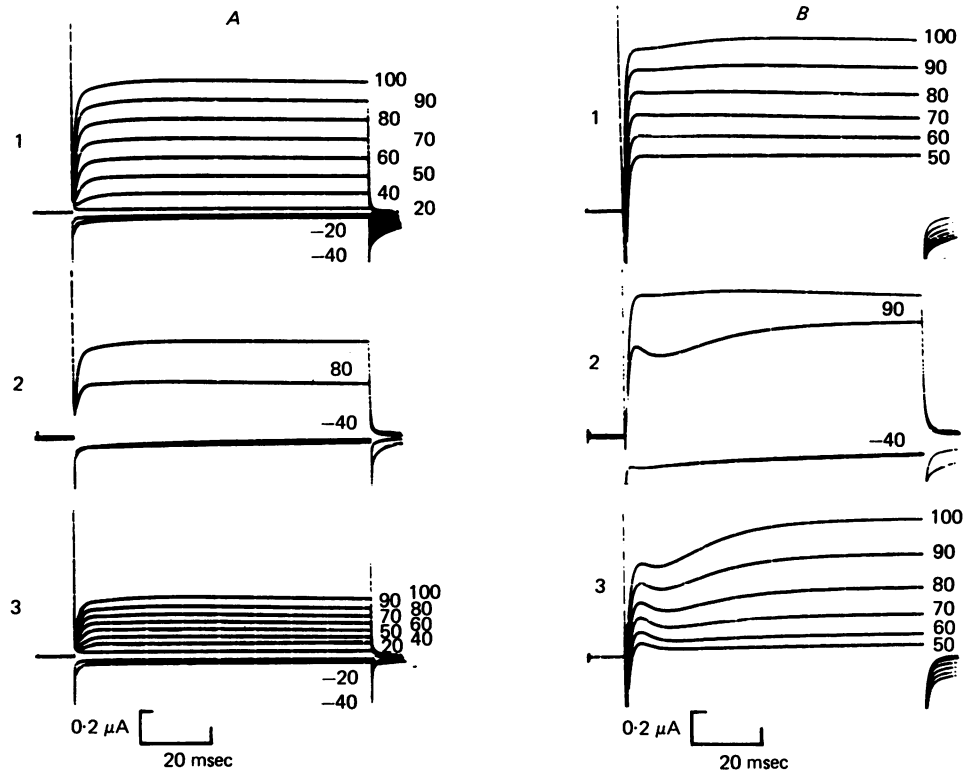

Fig. 10. Effects of TEA (*A*) and of 4-AP (*B*) on delayed outward currents in soleus fibres. A_1, control experiments in Ringer solution (TTX 5×10^{-7} g/ml.); A_2, superimposed traces obtained at +80 mV and −40 mV in normal Ringer and in Ringer plus 40 mM-TEA; A_3, Ringer plus 40 mM-TEA. B_1, control experiments in Ringer solution; B_2 superimposed traces obtained at +90 and −40 mV in normal Ringer and in Ringer plus 4 mM-4-AP; B_3, Ringer plus 4 mM-4-AP. Temperature 18–20 °C.

Fig. 10 illustrates a similar series of experiments on soleus fibres. The action of TEA (Fig. 10*A*) is similar to that observed in iliacus but the effects of 4-AP (4 mM) are different (Fig. 10*B*). Fig. 10*B* shows that the amplitude of the first peak is strongly reduced and a large, slowly developing outward current is seen. The modifications in the time course suggest that 4-AP may preferentially inhibit the fast component. The superimposed traces in Fig. 10B_2, show that 4-AP does not affect the inward current associated with hyperpolarization. At concentrations higher than 4 mM the total current is strongly depressed and often a deterioration of the preparation occurs.

In soleus fibres, in the presence of 2 mM-4-AP, the tail currents obtained at +40 mV show, associated with the first peak in the outward current, that the tail current has a time constant of deactivation of about 10 msec. For depolarizations longer than 50 msec, the 'off' currents show only one time constant of about 60 msec (Fig. 11*A*).

With 4-AP the participation of the fast component in the outward current is less pronounced; this therefore allows an estimate of the reversal potential of the slow

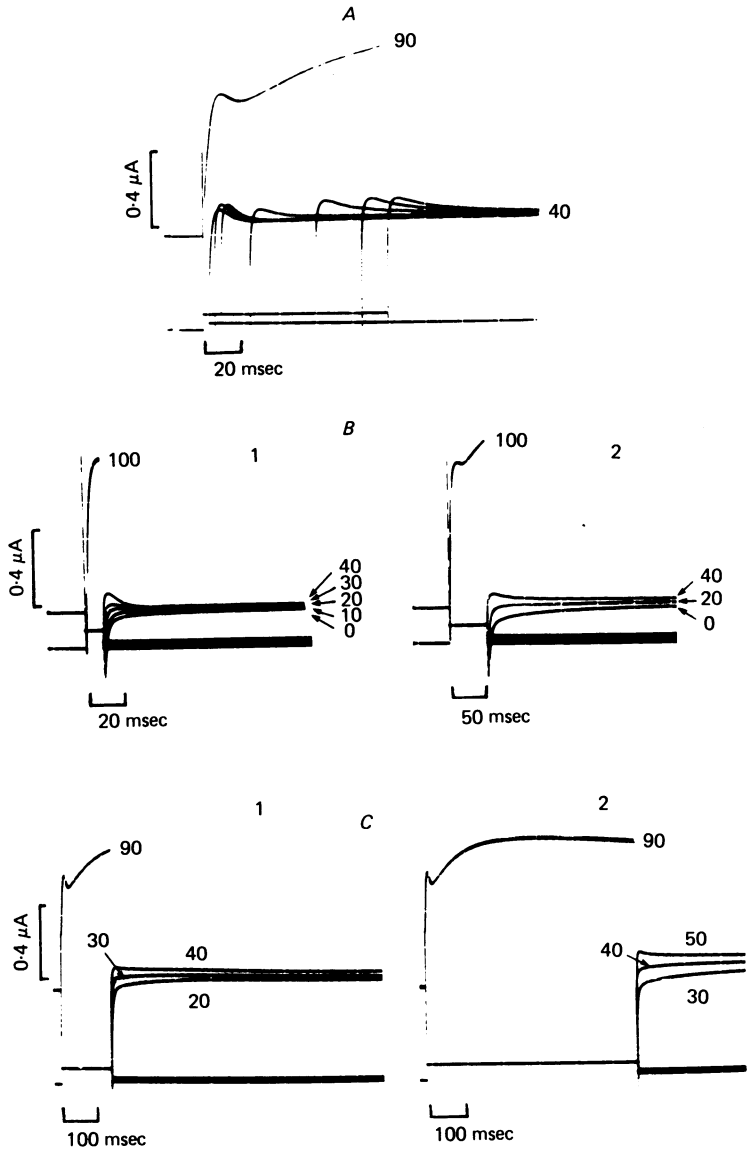

Fig. 11. Tail currents in soleus fibres in the presence of 2 mM-4-AP at 20 °C. *A*, tail currents at +40 mV following a +90 mV depolarization of different durations. *B*, measurement of the reversal potential of the fast component (B_1) and of the slow component (B_2). The values of potential refer to the level of repolarization. Same fibre as *B*; C_1 and C_2, measurements of the reversal potential of the slow component for two durations of depolarizing step.

component for depolarizing steps of short duration to be made. Fig. 11B_2 shows this reversal potential, measured with a 50 msec depolarizing step, to be about + 20 mV; a value not far from that found for the fast component (Fig. 11B_1). However, an increase in the duration of the depolarizations (Fig. 11C) is associated with a shift in reversal potential of the slow component similar to the shift observed with fibres not exposed to 4-AP.

DISCUSSION

The experiments show that the main differences in ionic currents recorded in soleus and iliacus fibres concern the characteristics of the potassium outward current. In iliacus muscle this current reaches a peak and then decreases rapidly, while in soleus two peaks are observed and the subsequent decrease is slower. Previous studies of outward current in fast and slow muscles have shown two reversal potentials associated with two time constants of deactivation. This observation has led to the proposal that both a fast and a slow component participate in the delayed outward current. However, in contrast to the results obtained in frog muscle (Adrian *et al.* 1970), in mammalian fibres the reversal potential of the slow component was always found to be positive to the reversal potential of the fast component.

The present results show that, for a given depolarization, the reversal potentials of the fast and slow components are similar and both are shifted identically by increasing the duration of the conditioning step. In glycerol-treated muscle, this shift is much reduced, suggesting that the variation in reversal potential in non-treated fibres is due to an accumulation of potassium ions in the T-system. Furthermore, the smaller shift found in soleus fibres could be related to the presence of a less well developed T-system in slow muscles (Eisenberg & Kuda, 1976). A variation in the potassium concentration at the T-system level has been already suggested for frog skeletal muscle (Adrian & Freygang, 1962; Adrian *et al.* 1970; Almers 1972; Barry & Adrian, 1973; Kirsch, Nichols & Nakajima, 1977), and was found to be responsible for the difficulty encountered in analysing the components of the outward current. However, from the results obtained on detubulated muscle it can be concluded that in both types of mammalian fibres there is a fast component in the outward current which develops at the surface membrane level. A large slower component is found only in soleus muscle. If these conclusions are extended to normal fibres a discrepancy still remains, because in normal iliacus fibres an apparent slow component was found, which can develop only at the level of the T-system. However, the time dependence of the time constant of the slow tail current on the depolarizing step duration occurring under normal conditions suggests that a secondary phenomenon may be present. A deaccumulation process could contribute to these slow tail currents, and in consequence the results obtained in iliacus fibres may be wrongly attributed to the presence of a slow component. The effect of TEA and 4-AP on the outward current from normal fibres supports this last suggestion. Indeed, if the sensitivity of the potassium permeability is identical in the two types of muscle these results show that the slow component should be virtually absent in iliacus fibres.

The authors are indebted to Dr R. A. Chapman for his help in writing the English and helpful criticism on this work. This work constitutes a part of Doctorat ès-Sciences of A. Duval.

REFERENCES

ADRIAN, R. H., CHANDLER, W. K. & HODGKIN, A. L. (1970). Slow changes in potassium permeability in skeletal muscle. *J. Physiol.* **208**, 645–668.

ADRIAN, R. H. & FREYGANG, W. H. (1962). The potassium and chloride conductance of frog muscle membrane. *J. Physiol.* **163**, 61–103.

ALMERS, W. (1972). Potassium conductance changes in skeletal muscle and the potassium concentration in the transverse tubules. *J. Physiol.* **225**, 33–56.

BARRY, P. H. & ADRIAN, R. H. (1973). Slow conductance changes due to potassium depletion in the transverse tubules of frog muscle fibres during hyperpolarizing pulses. *J. Membrane Biol.* **14**, 243–292.

DUVAL, A. & LÉOTY, C. (1978). Ionic currents in mammalian fast skeletal muscle. *J. Physiol.* **278**, 403–423.

DUVAL, A. & LÉOTY, C. (1979). Voltage clamp experiments in mammalian slow twitch skeletal muscle. *J. Physiol.* **289**, 37*P*.

DUVAL, A. & LÉOTY, C. (1980). Ionic currents in slow twitch skeletal muscle in the rat. *J. Physiol.* **307**, 23–41.

EISENBERG, B. A. & KUDA, A. M. (1976). Discrimination between fiber populations in mammalian skeletal muscle by using ultrastructural parameters. *J. Ultrastruct. Res.* **54**, 76–88.

FRANKENHAEUSER, B. & HODGKIN, A. L. (1956). The after-effects of impulses in the gaint nerve fibres of *Loligo*. *J. Physiol.* **131**, 341–376.

GILLESPIE, J. I. (1977). Voltage-dependent blockage of the delayed potassium current in skeletal muscle by 4-aminopyridine. *J. Physiol.* **273**, 64*P*.

GILLESPIE, J. I. & HUTTER, O. F. (1975). The actions of 4-aminopyridine on the delayed potassium current in skeletal muscle fibres. *J. Physiol.* **252**, 70*P*.

KIRSCH, G. E., NICHOLS, R. A. & NAKAJIMA, S. (1977). Delayed rectification in the transverse tubules. Origin of the late after-potential in frog skeletal muscle. *J. gen. Physiol.* **70**, 1–21.

NOBLE, S. J. (1976). Potassium accumulation and depletion in frog atrial muscle. *J. Physiol.* **258**, 579–613.

STANFIELD, P. R. (1970). The effect of the tetraethylammonium ion on the delayed currents of frog skeletal muscle. *J. Physiol.* **209**, 209–229.