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SLOW CHANGES IN POTASSIUM PERMEABILITY IN SKELETAL MUSCLE

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SUMMARY

1. Voltage clamp experiments on sartorius muscle fibres at 3° C showed that the potassium current is divisible into three components, namely:

(*a*) Current in the delayed rectifier channel, which reached a maximum in about 0·1 sec at −30 mV, and declined with a time constant of about 4 msec when the fibre was repolarized to −100 mV; this component had an approximately linear instantaneous current–voltage relation and an equilibrium potential E_1 at 10–15 mV positive to the resting potential.

(*b*) A slow component which reached a maximum in about 3 sec at −30 mV, and declined with a time constant of about 0·5 sec when the fibre was repolarized to −100 mV; this component had an approximately linear instantaneous current–voltage relation and a mean equilibrium potential E_2 at −83 mV in fibres where E_1 averaged −75 mV.

(*c*) Current in the inward rectifier channel which decreased with a time constant of about 0·25 sec when the fibre was hyperpolarized to −150 mV. This component had an equilibrium potential close to the resting potential and an instantaneous current–voltage relation which was that of an inward rectifier.

2. The general characteristics of the late after-potential in muscles in hypertonic solutions at 3° C are consistent with those of the slow conductance change. The sign of the late after-potentials was reversed by depolarizing below −80 mV.

3. The decline of current during a maintained hyperpolarization cannot be attributed solely to a decrease in tubular potassium concentration, since there may be a large decrease in current without much alteration of equilibrium potential. The negative slope conductance often seen at −150 mV is also difficult to reconcile with the tubular depletion hypothesis.

4. Replacement of 10 mM-K by 10 mM-Rb abolished inward rectification but had less effect on the fast and slow components of the potassium conductance.

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INTRODUCTION

In a previous article we described the changes in sodium and potassium conductance which are responsible for the action potential of skeletal muscle (Adrian, Chandler & Hodgkin, 1970). While investigating these relatively rapid changes we obtained evidence that depolarization caused a second type of potassium conductance change, which was characterized by a much longer time constant and a slightly different equilibrium potential. These results are given in the first part of the present paper, together with an account of the late after-potentials, for which the slow conductance changes appear to be largely responsible under the conditions of our experiments.

The second part of the paper is concerned with a slow decrease in permeability associated with hyperpolarization. Adrian & Freygang (1962) showed that when a constant inward current is applied to a muscle fibre in sulphate Ringer solution, the initial increase in potential is followed by a slow hyperpolarization, which they attributed to depletion of tubular potassium. In their model the tubular potassium concentration fell because the transport number of potassium in the tubular wall exceeded that in a hypothetical membrane at the mouth of the tubule. The finding that large molecules like ferritin can penetrate the tubule (Huxley, 1964; Page, 1964) makes the assumption of a selective membrane at the mouth of the tubule untenable, but the same type of hypothesis is still applicable since the transport number of potassium in the wall of the tubule should exceed that in the tubule. The slow changes described by Adrian & Freygang (1962) are conspicuous with the voltage-clamp method. If the potential is switched from -100 mV to -150 mV the ionic current falls to about one quarter of its initial amplitude with a time constant of 0·25 sec. This time constant is similar to that of the slow conductance change and some experimental analysis was required to decide whether there are one or two slow systems.

The tentative conclusion of the paper is that the potassium current in muscle is divisible into three components with the properties summarized below

$$I_K = I_1 + I_2 + I_3, \tag{1}$$

$$I_1 = (V - E_1) g_1, \tag{1·1}$$

$$I_2 = (V - E_2) g_2, \tag{1·2}$$

$$I_3 = f(V - E_3) P_3 \tag{1·3}$$

where I_1 is the fast component with an equilibrium potential E_1 and conductance g_1; I_2, g_2 and E_2 are similar quantities for the slow system; I_3 is the current in the inward rectifier which has an instantaneous current–voltage relation given by $f(V - E_3)$ and a time-dependent permeability

factor, or scaling factor, denoted by P_3 (cf. Noble & Tsien, 1968 who use the symbol s for a similar quantity). The symbols τ_1, τ_2 and τ_3 are used for the time constants of the three systems. In general it might be better to use symbols such as I_{K_1}, g_{K_1}, etc., but in the present context there is no risk of confusion with currents carried by other ions and the more elaborate symbols are unnecessarily cumbersome.

METHODS

The technique was the same as that used by Adrian *et al.* (1970) and needs only a brief description. Three electrodes were inserted at distances of l, $2l$ and $2l + l'$ from the pelvic end of a fibre in the frog's sartorius muscle. The spacing usually employed was 0·25, 0·5 and 0·55 mm but l was sometimes increased from 0·25 to 0·5 or 1·0 mm in order to obtain greater sensitivity. In these cases, the control voltage was taken from the middle electrode instead of from the end electrode. However, the potential plotted as the abscissa in graphs is always the voltage V_1 at $x = l$.

The membrane current was calculated from the voltage difference $V_2 - V_1$ by

$$i_m = \frac{2}{3l^2 r_i}(V_2 - V_1), \tag{2}$$

$$I_m = \frac{a}{3l^2 R_i}(V_2 - V_1), \tag{3}$$

$$j_m = \frac{2}{3l^2 R_i}(V_2 - V_1), \tag{4}$$

where i_m is the membrane current per unit length, I_m is the membrane current per unit area of surface, j_m is the membrane current per unit volume of muscle; r_i is the internal resistance per unit length; R_i is the internal resistivity which was taken from S. Nakajima's unpublished results as 180 Ω cm at 18·5° C or 303 Ω cm at 2° C for fibres in isotonic solutions, and 400 Ω cm at 2° C for fibres in hypertonic solutions (Ringer plus 350 mM sucrose).

RESULTS

Distinction between rapid and slow changes in potassium conductance

The first clear evidence that muscle undergoes changes in conductance which are much slower than those responsible for delayed rectification came from experiments of the kind illustrated in Fig. 1. The left-hand family are the usual low-gain medium-speed records obtained in determining the equilibrium potential of the delayed rectifier. When the fibre was repolarized, the currents decreased with a time constant of 4–20 msec and had an equilibrium potential at −80 mV. These currents belong to the ordinary delayed rectifying system and will be referred to as the fast component. The right-hand family was obtained on the same fibre, but with a higher gain and slower time base. The fast decrease in current which appears as an almost vertical line was followed by a much slower decline. In this experiment the time constant of the slow component was

300 msec at −60 mV and 250 msec at −114 mV, whereas that of the fast system was 18 msec at −60 mV and about 4 msec at −100 mV. Other measurements of the slow time constant indicate that it decreased by a factor of about 2·5 when the repolarizing potential was changed from −120 to −180 mV. At 2° C and −115 mV the time constant of the slow system varied between 0·25 and 1·2 sec with a mean of 0·6 sec. At 20° C and −115 mV it varied between 0·16 and 0·5 sec with a mean of 0·3 sec. A comparison of the time constant of the slow system with that of the inward rectifier is given in Table 3.

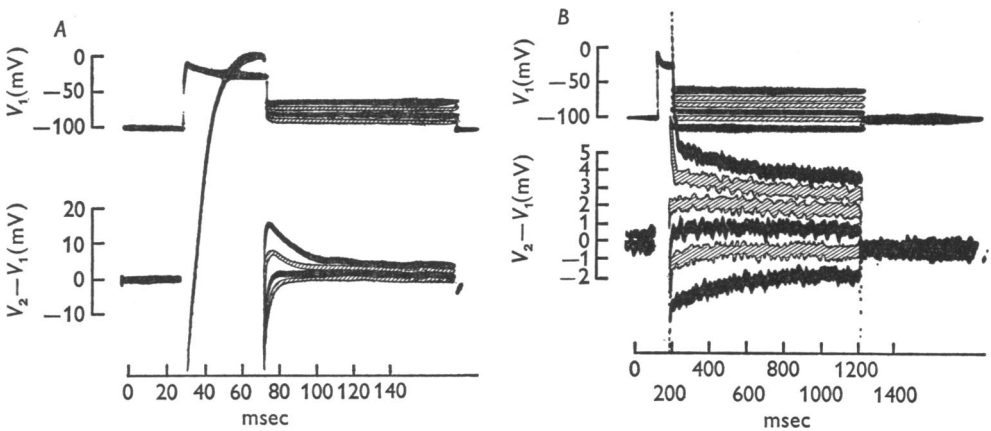

Fig. 1. Records and tracings illustrating fast and slow decline of conductance when a muscle fibre is repolarized. Fibre diameter 63 μ; temperature 3° C; chloride Ringer fluid with 2·5 mM-K and 350 mM sucrose. Recording distance 250 μ. The ordinate scale for I_m is 1 mV ≡ 4·2 μA/cm². Tetrodotoxin was not used in this experiment. The voltages in the second step are (*A*) −60, −70, −80, −90 mV and (*B*) −60, −70, −80, −90, −100, −114 mV.

As can be seen from Fig. 1 the slow system had a definite equilibrium potential which was about 10 mV more negative than that of the fast system. Other experiments gave similar results, the average slow equilibrium potential with hypertonic Ringer at 3° C being −83 mV in three muscles where the fast equilibrium potential averaged −75 mV (Table 1). The difference in equilibrium potential is explained by supposing that the slow system is more selective towards potassium than the fast system. The slow equilibrium potential varied with external potassium concentration but not with holding potential or with chloride concentration.

Extrapolation of the slow component to the beginning of the second pulse indicated that the current–voltage relation in the slow system was approximately linear (Fig. 2).

TABLE 1. Equilibrium potentials in muscle

Fibre no.	Solution	V_R	V_H	E_1 (mV)	E_2	E_3
11·9	2·5-K, Cl, S	−90	−90	−81	−91	—
			−110	−81	−91	—
			−80	−82	−91	—
			−100	−81	−90	—
			−100	−80	—	—
			−100	−76	−87	—
12·6		−89	−100	−71	−76	—
12·7		−96	−100	−72	−81	—
19·8		−81	−100	—	−83	—
Mean	2·5, K, Cl, S	−89	−99	−75	−83	—
31·3	2·5-K, SO_4, S	−78	−100	—	−78	—
31·5		−84	−100	—	−86	—
47·7		−73	−100	—	−78	—
Mean	2·5-K, SO_4, S	−78	−100	—	−81	—
48·2	10-K, SO_4, S	−71	−71	—	−54	—
48·3		−75	−75	—	−64	—
51·4	2·5-K, SO_4	−88	−88	—	−77	−80
52·1		−88	−88	—	−88	−88
53·1		−89	−89	—	−91	−87
53·2		−91	−91	—	−84	−90
Mean	2·5-K, SO_4, S	−89	−89	—	−85	−85
33·7	5-K, SO_4	−77	−77	—	—	−70
54·1	10-K, SO_4	−67	−67	—	—	−66
54·4	10-K, SO_4	−68	−68	—	—	−59
54·5	10-K, SO_4	−66	−66	—	—	−59
Mean	10-K, SO_4	−67	−67	—	—	−61

Under solution, Cl or SO_4 indicates chloride or sulphate Ringer, S indicates hypertonic solution with 350 mM sucrose added. V_R is the initial resting potential, V_H the holding potential. E_1, E_2 and E_3 are the equilibrium potentials of (1) the fast component of potassium conductance (2) the slow component of potassium conductance and (3) the inward rectifier. All the experiments were carried out at 1–4° C. Tetrodotoxin (10^{-6} g/ml.) was present in all experiments from 31 onwards. The Table does not include experiments in which only E_1 was measured; these results were given in Adrian *et al.* (1970, Table 4) under V'_K.

The time course of the slow component with long depolarizing pulses

The simplest way of measuring the extent to which the slow channel had been turned on was to determine the current on repolarizing the fibre after pulses of different duration. When the potential was switched to values more negative than −100 mV, the fast component declined so rapidly that it merged into the capacitative transient, but the slow system gave a tail

of inward current lasting several hundred milliseconds (Fig. 3*B* and *C*). The amplitude of this tail varied with the duration of the pulse and gave the approximate time course of the slow component. At 20° C the slow component reached a maximum in 2 sec and then declined to a steady level in about 10 sec.

It was difficult to measure both fast and slow components simultaneously, since the former was best studied by repolarizing the membrane to −60 mV and the latter by repolarizing it to −100 or −150 mV. A further complication is that large and prolonged depolarizations may shift

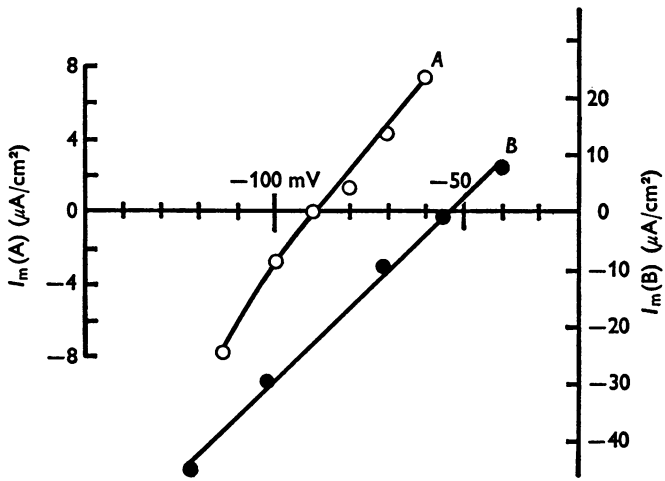

Fig. 2. Abscissa, membrane potential during second pulse. Ordinate, component of current which declined slowly extrapolated to beginning of second pulse. *A*, from the experiment of Fig. 1*B* with a 2·5 mM-K, hypertonic chloride solution containing no tetrodotoxin. *B*, from a similar experiment with a 10 mM-K hypertonic sulphate solution containing tetrodotoxin, 10^{-6} g/ml. The ordinate in *A* is the difference between the 'initial' and final currents in the second pulse, and in *B* it is the difference between the 'initial' current with prepulse and the initial current without prepulse; in both cases the fast component was excluded from the initial current. The first pulse was to −28 mV in *A* and to −24 mV in *B*; it lasted 0·1 sec in *A* and 2 sec in *B*.

the equilibrium potential of both fast and slow systems. These shifts were hard to measure since fibres deteriorated when subjected to large and prolonged depolarizations.

The right hand graphs in Fig. 3 are an attempt to divide the total potassium current into its two components using either fast tails (3*D*) or slow tails (3*E* and *F*) as a basis for the analysis. Curve 1 is the total current; curve 2 is the fast component obtained directly in *D*, or by subtraction in

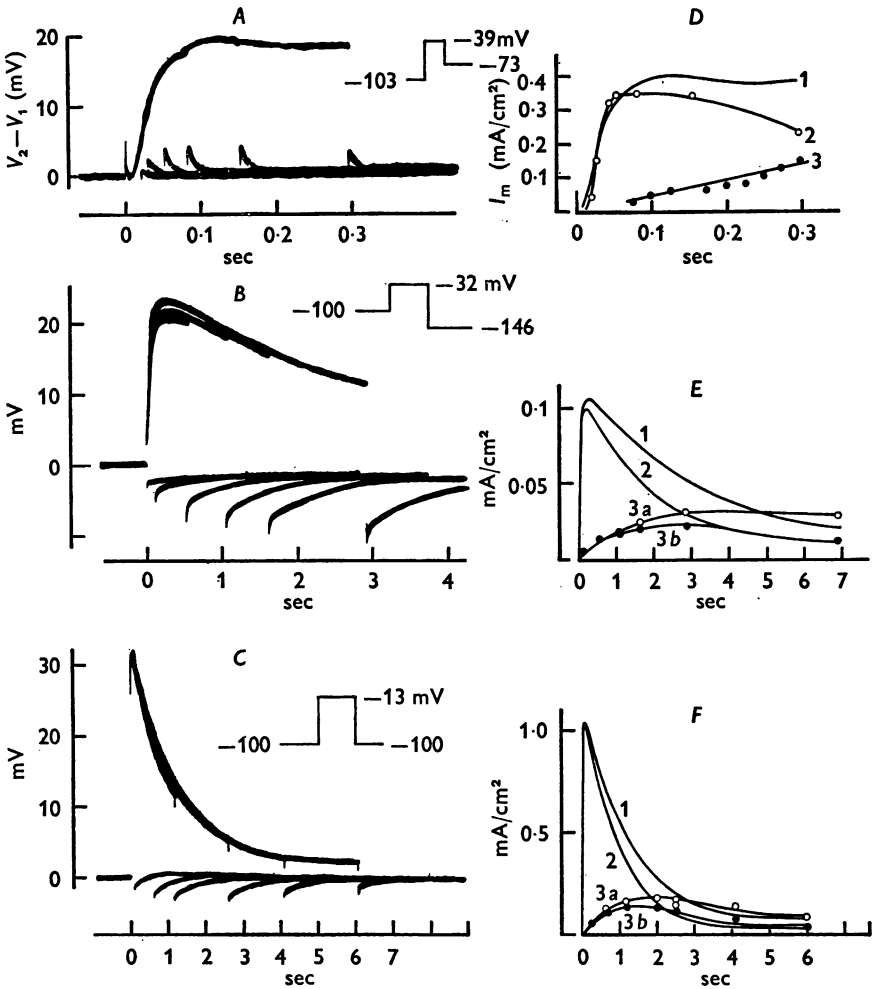

Fig. 3. Division of potassium current into fast and slow components. Tracings on left; derived curves on right. The outward current tails in *A* show the relaxation of the rapid component at − 73 mV. The inward current tails in *B* and *C* show the relaxation of the slow component at − 146 mV (*B*) or − 100 mV (*C*). Temperatures *A*, 3° C; *B*, 1° C; *C*, 19·5° C. Fibre diameters 57, 55 and 75 μ. Hypertonic solutions with 350 mM sucrose and tetrodotoxin, 10^{-6} g/ml.; sulphate Ringer in *B*; others with chloride Ringer. Electrode spacing 125 μ in *A* and *C*, 250 μ in *B*. Derived curves (*D*, *E*, *F*) curve 1; total current. Curve 2; rapidly relaxing component obtained directly in *D* or by subtracting curve 3*b* from curve 1 in *E* and *F*. Curves 3, 3*a*, 3*b*; slowly relaxing component obtained directly in *E* and *F* or by subtraction in *D*. Equilibrium potentials were taken as (*D*) $E_1 = -82$ mV, (*E*) $E_2 = -86$ mV throughout in curve 3*a* and with linear change to − 66 mV in 6 sec in curve 3*b*, (*F*) $E_2 = -70$ mV throughout in 3*a* and with linear change to − 50 mV at 6 sec in 3*b*.

E and *F*; curve 3 is the slow component obtained by subtraction in *D*, or from the slow tails in *E* and *F*. For curve 3*a* the equilibrium potential of the slow system was taken as constant and for curve 3*b* it was assumed to shift by the amount found in an experiment similar to that in Fig. 3*c*, − 20 mV in 6 sec. This is rather arbitrary but it is necessary to assume a shift of this magnitude, since subtracting curve 3*a* (no shift) from curve 1 makes the fast component reverse in sign at long times.

Although there is doubt about quantitative detail the qualitative conclusions from Fig. 3 are reasonably clear. The slow system contributes relatively little at first and can be ignored during the first 50 msec. It reaches a maximum when the fast component has fallen to about one third and is then about one half the fast component in amplitude. Both fast and slow components are inactivated, the former nearly completely and the latter to perhaps one third.

Presence of slow system in fibres in isotonic solutions

The records described in the previous sections were obtained in solutions with a tonicity which was 2·4 times that of Ringer fluid. The powerful contractions associated with large depolarizing pulses made it impossible to carry out similar experiments with isotonic solutions. Evidence for the presence of the slow system under normal conditions was provided by the time course of the small currents produced by depolarizations near the contraction threshold. In order to observe these small currents it was necessary to increase the recording distance. In the experiment of Fig. 4, electrode 1 was at 1 mm and electrode 2 at 2 mm from the end of the fibre;

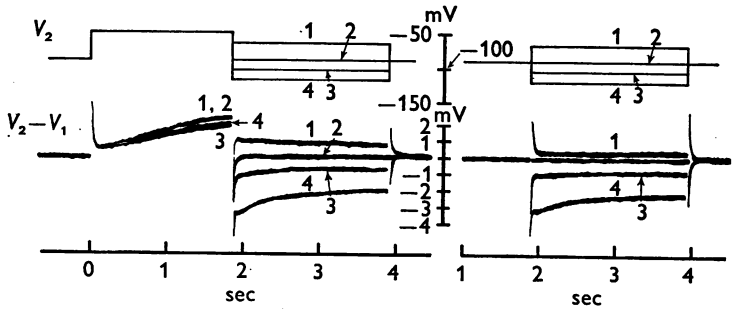

Fig. 4. Slow changes in current in isotonic 2·5 mM-K sulphate containing tetrodotoxin. Upper trace membrane potential (V_2). Lower trace membrane current, 1 mV ≡ 0·28 μA/cm². Left with prepulse to − 47 mV; right without prepulse. Recording distance 1 mm with control from V_2. Temperature 4° C. Initial resting potential = holding potential = − 88 mV. Fibre diameter = 50 μ.

the control voltage was taken from electrode 2 rather than from electrode 1. The Figure shows that the conductance rose along an S-shaped curve, and fell with a time constant of roughly 0·5 sec, and that the equilibrium potential of the slow system was near the resting potential. However in this type of experiment it is difficult to distinguish currents carried in the slow conductance system from those in the inward rectifier (see p. 655).

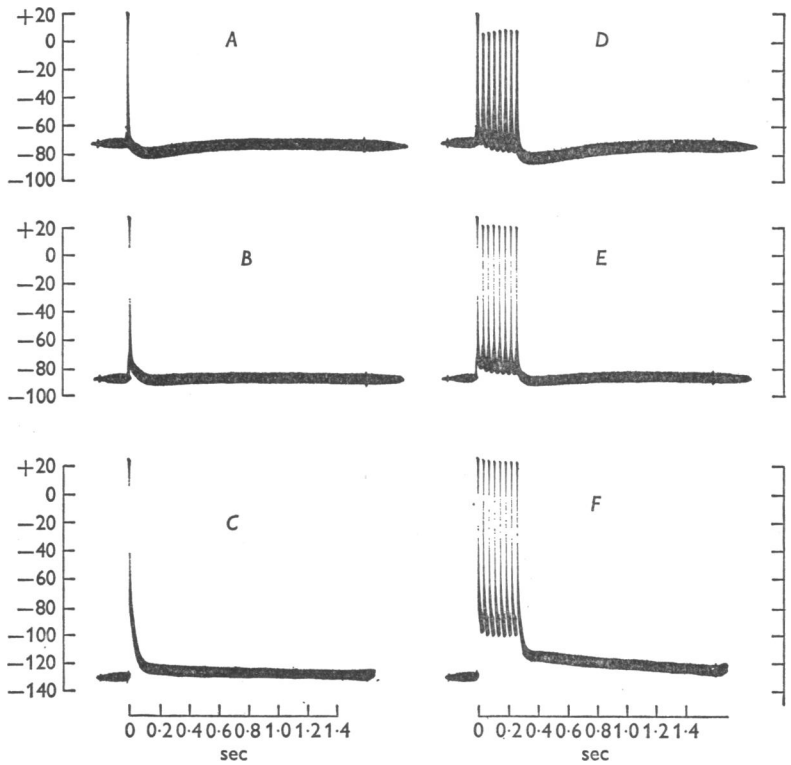

Fig. 5. Late after-potentials after one or eight action potentials. The initial resting potential was −103 mV. Hypertonic chloride Ringer solution at 3° C.

Late after-potentials

The late after-potentials of skeletal muscle were described by Freygang, Goldstein & Hellam (1964) who attributed them to an increase in the concentration of potassium in the transverse tubules (see also Freygang, Goldstein, Hellam & Peachey, 1964). An alternative explanation is that the potassium equilibrium potential does not change and that the late after-potentials arise from the slow relaxation of a component of the potassium conductance. Such after-potentials should be in the depolarizing

direction if the internal potential is more negative than the equilibrium potential and in the hyperpolarizing direction if it is more positive. From the results described in the first part of the paper one would expect the after-potentials to last about 0·5 sec and to increase with the number of impulses in trains lasting less than about a second.

Fig. 5 shows that the late after-potentials recorded from fibres in hypertonic Ringer at 3° C have properties which are consistent with the voltage-clamp results. The membrane potential was altered by applying a steady current through a stimulating electrode at a distance of about 1 mm from

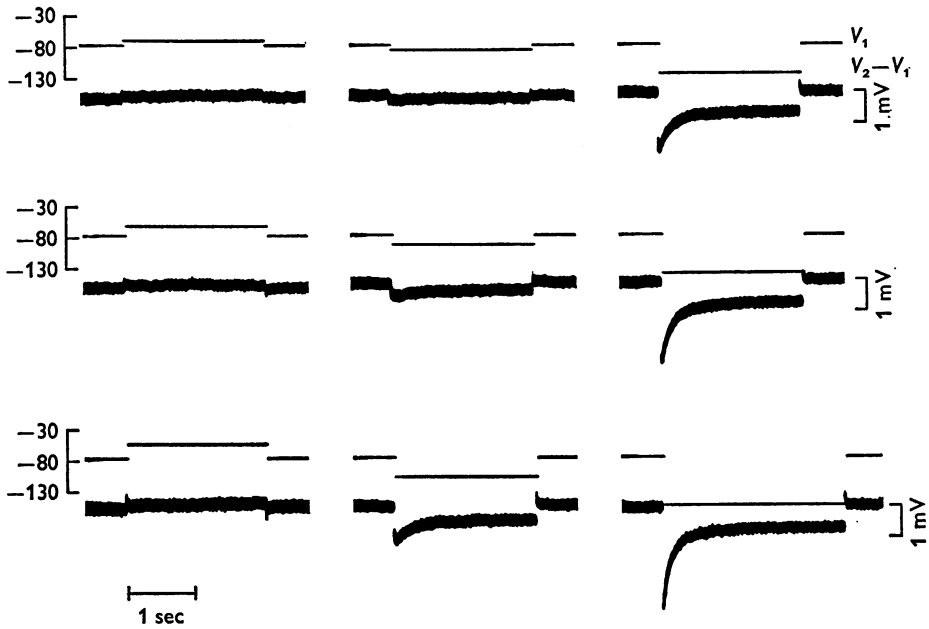

Fig. 6. Membrane currents at different voltages between −154 and −54 mV, for fibre in isotonic 5 mM-K sulphate Ringer at 1·5° C. Holding potential = resting potential = −77 mV, fibre diameter 75 μ. Electrodes at 250, 500, 550 μ from end of fibre; 1 mV ≡ 6·7 μA/cm².

the recording electrode. As can be seen from Fig. 5, the sign of the late after-potentials depended on the steady level of potential, and there was an equilibrium potential at about −88 mV. The time constant of the late after-potential was about 0·5 sec in record *D* and about 1 sec in record *F*. It is not clear why hyperpolarization should increase the duration, but, apart from this possible discrepancy, the general characteristics of the after-potentials seem consistent with those of the slow conductance change. The potential at which the late after-potentials changed sign averaged −89 mV in nine fibres with a mean resting potential of −96 mV; the

equilibrium potential of the early after-potential was −79 mV in these fibres. The voltage-clamp method gave the slow equilibrium potential as −83 mV in four fibres with a resting potential of −80 mV and a fast equilibrium potential of −75 mV.

In Fig. 5 the potential at which the after-potential changed sign was the same after eight impulses as after one impulse. With longer trains, there was evidence of a change of equilibrium potential of the kind expected from potassium accumulation, but the effect was difficult to investigate since the resting potential often failed to return to its original value after a long train of spikes.

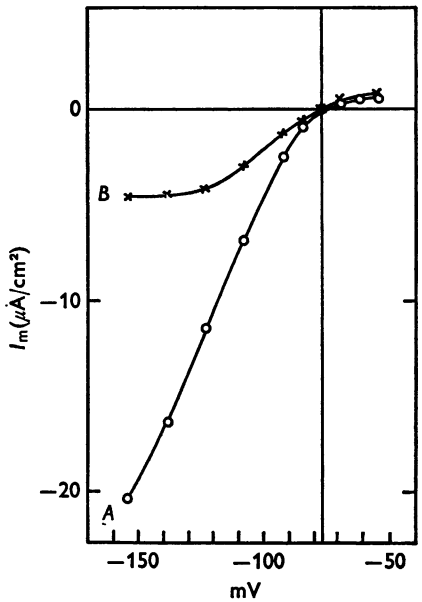

Fig. 7. Current–voltage relation between −154 and −54 mV for fibre in isotonic 5 mM-K. Sulphate Ringer at 1·5° C, from experiment of Fig. 6. *A* (○) Instantaneous currents; *B* (×) current at 2·1 sec after applying voltage.

The time dependence of the inward rectifier

The slow changes described by Adrian & Freygang (1962) show up in a striking way with the voltage-clamp technique. In Fig. 6, a hyperpolarization of 60 mV gave an inward current which declined to one quarter of its initial amplitude with a time constant of 0·17 sec; this decline occurred long after the initial capacitative surge was complete. Curve *A* in Fig. 7 gives the initial current–voltage relation and curve *B* the steady-state relation. Both show inward rectification but the rectification when the voltage was first applied was greater than in the steady state. In Fig. 7,

the steady inward current reached a constant limiting value at large negative potentials, but in about half the experiments the current–voltage relation had a negative slope for $V < -150$ mV (Fig. 8). This is difficult to reconcile with the Adrian–Freygang hypothesis but there are other facts which fit rather well with the hypothesis.

If depletion of tubular potassium is responsible for the decrease in current seen on hyperpolarizing the fibre, one would expect the total quantity of current which flows in the transient to be less than the charge on the tubular potassium ions. If

$$Q = \int_0^\infty [j_m(t) - j_m(\infty)]\, dt, \tag{5}$$

where $j_m(t)$ is the current per unit volume of muscle at time t, which can be calculated from the recording voltage by eqn. (4), then on the tubular depletion hypothesis one would expect

$$Q < F[K]_{T0}\rho \tag{6}$$

where F is the Faraday, $[K]_{T0}$ is the initial tubular potassium concentration, which is taken to be identical with the external potassium concentration $[K]_0$, and ρ is the fraction of the muscle volume occupied by the tubules, which Peachey (1965) estimated as 0·003. When the initial current $j_m(0)$ is much greater than the steady current $j_m(\infty)$ one can probably replace the inequality in (6) by

$$Q \doteqdot F[K]_0\rho. \tag{7}$$

Table 2 gives estimates of ρ at different temperatures (muscle 30) and at potassium concentrations varying between 2·5 and 80 mM, at 2–4° C. The constancy of ρ and its absolute value of about 0·002 are clearly consistent with the Adrian–Freygang hypothesis and with Peachey's estimate of the tubular volume.

Difficulties with the tubular depletion hypothesis

Many of the curves relating membrane current to voltage in the steady state had a negative slope when the membrane potential was less than -150 mV. At still more negative potentials (-180 mV), the inward current increased again, often somewhat abruptly and after a delay of about a second; this is attributed to membrane break-down and is clearly a separate phenomenon which we did not investigate further. At first we thought that the negative slope conductance might result from lack of differentiality in the recording electrodes. However, the phenomenon was equally conspicuous in experiments in which the recording length was increased from 0·25 to 1·0 mm; in these experiments the possible error in $V_2 - V_1$ was negligible in comparison with the observed negative slope.

A steady-state curve with a region of negative slope is difficult to reconcile

TABLE 2. Properties of inward rectifier

Fibre	Diameter (μ)	Temp. (°C)	$[K]_0$ (mM)	V_R	$(V_1)_0$ (mV)	$(V_1)_\infty$	$(I_m)_0$ (μA/cm²)	$(I_m)_\infty$ (μA/cm²)	τ_3 (sec)	ρ
51·2	68	3	2·5	−89	−151	−151	7·8	2·5	0·13	0·0016
					−167	−167	9·9	1·4	0·12	0·0025
					−182	−182	10·6	0·7	0·08	0·0020
51·4*	75	4	2·5	−88	−156	−175	10·9	2·7	0·29	0·0052
52·1*	50	4	2·5	−88	−143	−147	2·1	0·87	0·52	0·0018
					−157	−163	2·6	0·75	0·40	0·0022
53·1*	60	3	2·5	−89	−148	−160	6·2	2·4	0·21	0·0022
53·2*	63	3	2·5	−91	−153	−164	5·4	1·8	0·27	0·0026
Mean	63	3	2·5	−89	−157	−164	6·9	1·6	0·25	0·0025
33·4	81	2	5	−81	−143	−143	33·7	16·0	0·2	0·0048
33·7	75	2	5	−77	−154	−154	20·3	4·5	0·14	0·0025
30·3	70	2	5	−84	−147	−147	12·7	6·2	0·31	0·0024
30·4	75	2	5	−70	−147	−147	9·0	1·8	0·25	0·0020
30·5	90	3	5	−72	−147	−147	13·8	5·6	0·21	0·0016
Mean	78	2	5	−77	−148	−148	17·9	6·8	0·22	0·0027
Mean†	78†	2†	5†	−75†	−147†	−147†	11·8†	4·5†	0·25†	0·0020†
30·1	95	21	5	−70	−147	−147	42·5	18·3	0·08	0·0027
30·2	95	21	5	−80	−147	−147	30·4	9·9	0·09	0·0016
30·3	70	21	5	−79	−147	−147	31·1	8·9	0·08	0·0020
Mean	87	21	5	−76	−147	−147	34·7	12·4	0·08	0·0021
54·1‡	(71)	4	10	−67	−134	−141	16·7	5·4	0·32	0·0024
54·5‡	55	4	10	−66	−132	−137	12·3	5·9	0·35	0·0020
55·6	70	4	10	−65	−142	−142	20·4	7·3	0·27	0·0021
Mean	63	4	10	−66	−136	−140	16·5	6·2	0·31	0·0022
34·1	50	2	80	−16	−167	−167	139·4	72·8	0·13–0·5	0·0014

V_R is the initial resting potential; $(V_1)_0$ and $(V_1)_\infty$ are the membrane potentials at $x = l$ at the beginning and end of the pulse. $(I_m)_0$ and $(I_m)_\infty$ are the initial and final membrane current densities; τ_3 is the time constant with which the current declined; ρ is the fraction of the muscle volume calculated by eqn. (7). The holding potential was equal to the initial resting potential except in muscle 30 where it was −100 mV. The recording distance was 0·25 mm except for * where it was 1 mm and ‡ where it was 0·5 mm. *‡ Feedback from V_2.† Mean of muscle 30 for comparison with values from same muscle at 21° C. Fibre 54·1 was assumed to have the average diameter of 71 μ. The solutions were isotonic sulphate solutions.

with a hypothesis in which tubular potassium is regarded as the variable controlling the potassium current through the membrane of the tubules. For example, in Fig. 8 the steady current at -163 mV is 10% less than the steady current at -147 mV. If the mean tubular potassium concentration $[\hat{K}]_T$ is the controlling variable we must assume that $[\hat{K}]_T$ at -163 mV is less than at -147 mV. However, this is not consistent with the requirement that the steady potassium current is proportional to $[K]_o - [\hat{K}]_T$. The first consideration requires that $[\hat{K}]_T$ should fall on switching V from -147 to -163 mV and the second that it should rise. The difficulty disappears if one abandons the idea that tubular potassium concentration is the controlling variable, or that potassium ions carry all the current.

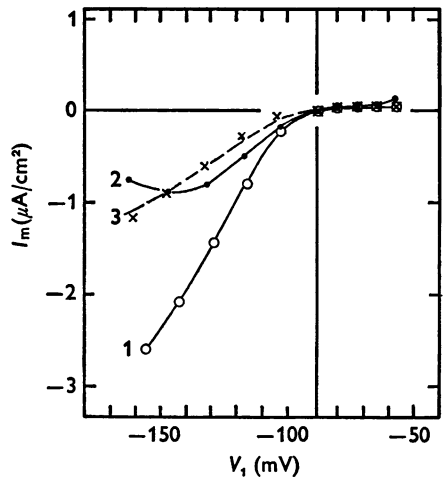

Fig. 8. Current relation between -160 and -56 mV for fibre in isotonic 2·5 mM-K sulphate Ringer at 4° C. Holding potential = resting potential = -88 mV; fibre diameter 50 μ; electrodes at 1, 2 and 2·05 mm from end. 1 μA/cm² ≡ 3·6 mV; feed-back from V_2.

○ Instantaneous current–voltage relation about resting potential, -88 mV.

● 'Steady-state' current–voltage relation measured after 2 sec.

× Instantaneous current–voltage relation after 2 sec pulse to -147 mV.

Curves 1 and 2 were drawn through the points by eye.

Curve 3 is curve 1 × 0·39.

A further difficulty appeared when the three curves in Fig. 8 were analysed. The open circles, curve 1, give the instantaneous current–voltage relation with the membrane potential initially at -88 mV; curve 2 (●) is the steady-state current–voltage relation and the crosses on curve 3 are the instantaneous current–voltage relation with the potential initially at -147 mV. Curve 3 itself, which is clearly a good fit to the experimental

points, was calculated by a hypothesis of the kind used by Noble & Tsien (1968) in their work on Purkinje fibres. It is assumed that the potassium current in the inward rectifier is given by

$$I_3 = P_3 f(V - E_3), \tag{8}$$

where $f(V-E_3)$ is the instantaneous current–voltage relation, which is that of an inward rectifier, and P_3 is a variable which decreases slowly when the fibre is hyperpolarized. The variable P_3 might represent the number of rectifier channels available or the total amount of a potassium carrier. In applying eqn. (8) to Fig. 8 we assumed that switching from −88 mV to

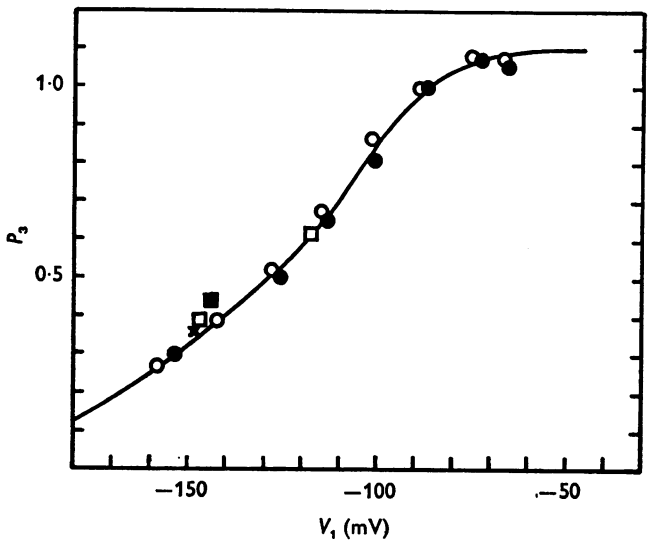

Fig. 9. Steady-state values of the factor P_3 as a function of membrane potential.

○, ●, obtained with $V(S_2)$ constant (≒ −140 mV) and $V(S_1)$ variable; P_3 is proportional to the current at the beginning of S_2 and is unity when there is no prepulse, i.e. when $V(S_1)$ = resting potential.

□, ■, ×, from experiments similar to that in Fig. 8, P_3 is equal to the scaling factor, e.g. $P_3 = 0{\cdot}39$ at $V = -147$ mV in Fig. 8.

Data obtained from five fibres in isotonic 2·5 mM-K sulphate Ringer fluid. The resting potential varied between −87 and −91 mV.

−147 mV reduced P_3 in the ratio 1:0·39, the factor of 0·39 being the ratio of final to initial current at −147 mV. If there is no change in equilibrium potential, curve 3 can be obtained by multiplying curve *A* by 0·39. The fact that this procedure fits the experimental points is evidence that the apparent equilibrium potential did not shift by more than 1 or 2 mV. This makes it difficult to attribute the large change in P_3 to an alternation of tubular potassium concentration. Similar results were obtained in other

experiments with 2·5 mM-K in the external solution. However, in experiments with 10 mM or 80 mM-K there was evidence of a shift of equilibrium potential of about 5 mV in the direction required by the Adrian–Freygang hypothesis.

Fig. 9 illustrates a more complete test of eqn. (8). Here the symbols □, ■, × denote the steady-state values of P_3 obtained by the method just described and the symbols ○, ● are steady-state values obtained by using a second pulse of constant amplitude and varying the initial level with a prepulse. The value plotted is the ratio of the initial current at −150 mV with a prepulse to the same current without prepulse. Both methods show that the steady-state relation between P_3 and V is an S-shaped curve which was half maximal at about −120 mV. Changing the external potassium concentration from 2·5 to 10 mM did not seem to alter the position or shape of the P_3–V curve.

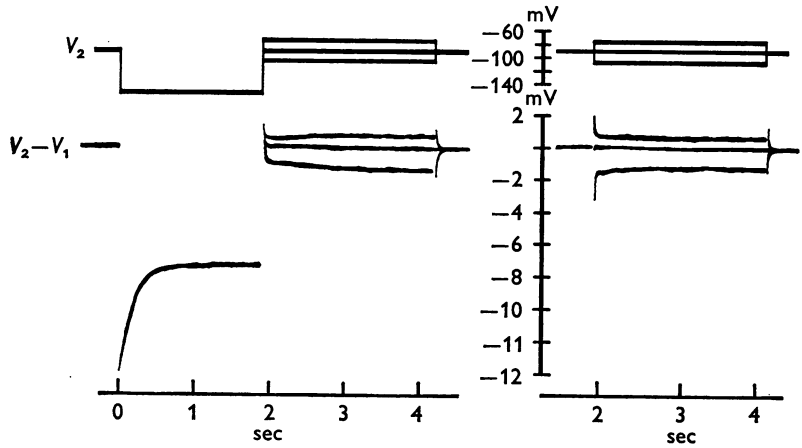

Fig. 10. Tracing illustrating method of estimating E_3, the equilibrium potential of the rectifier system, for fibre in isotonic 2·5 mM-K sulphate Ringer at 4° C. The electrodes were at 1·0, 2·0 and 2·05 mm from the end of the fibre with voltage control from the middle electrode V_2. Resting potential, −89 mV; fibre diameter, 60 μ; 1 mV ≡ 0·33 μA/cm². The equilibrium potential was estimated as −87 mV, i.e. slightly positive to the middle record.

Measurement of equilibrium potential of the inward rectifier

Outward currents through the rectifier were so small that it was difficult to measure the equilibrium potential of the system in a convincing manner. The method employed was to determine the potential at which the membrane current remained constant under conditions in which the permeability was increasing. In the experiment of Fig. 10, P_3 was first reduced by a hyperpolarizing pulse to −151 mV; its return to the resting

level is indicated by the slowly increasing separation of the three current traces at -74, -89 and -104 mV. At -74 mV, dI_m/dt was positive, whereas at -104 mV and probably at -89 mV it was negative. From these and other records, the equilibrium potential was estimated as -87 mV. Values obtained by this method are summarized in Table 1; they are usually slightly positive to the initial resting potential, but this may be because the measurement of the resting potential preceded the measurement of E_3 by 5–10 min.

The effect of membrane potential and other factors on the time constant of the inward rectifier

For large negative potentials, the time constant of the inward rectifier could be obtained from the rate at which the current declined from its initial value. This method was used for most of the points in Fig. 11. An

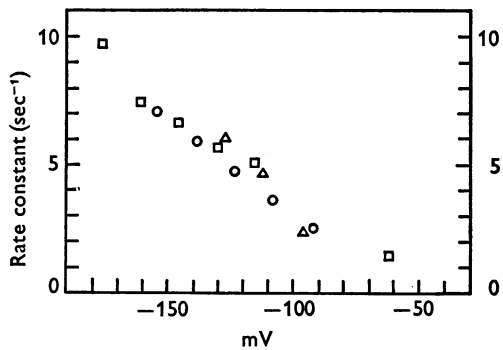

Fig. 11. Effect of membrane potential on rate constant of rectifier system. For $V < -90$ mV the rate constants were obtained from the rate of decline of current on switching the potential from the resting potential to the potential shown on the abscissa. For $V = -62{\cdot}5$ mV the rate constant was obtained by plotting the initial current at -130 mV as a function of the duration of a prepulse to $V = -62{\cdot}5$ mV. The data were obtained on three fibres from the same muscle with isotonic 5 mM-K sulphate Ringer at 1° C.

alternative method, which was used for the point at $-62{\cdot}5$ mV, was to measure the amplitude of the initial current in a hyperpolarizing test pulse as a function of the time at $-62{\cdot}5$ mV. This avoided the difficulty that the outward current at $-62{\cdot}5$ mV was so small that it could not be measured reliably. It can be seen from Fig. 11 that the rate constant τ_3^- varies with potential in an approximately linear manner from 1·4 sec^{-1} at -60 mV to 10 sec^{-1} at -175 mV.

The external potassium concentration did not have any large effect on the time constant of the rectifier at a fixed membrane potential. When

fibres in 80 mM-K sulphate were hyperpolarized to -170 mV the current declined with an initial time constant of 0·13 sec which is about the same as that for fibres in 2·5 or 5 mM-K. However, the 80 mM curves were not exponential and the initial rapid fall of current was followed by a slower decline.

Noble & Tsien (1968) found that the Q_{10} of the rate constant of their time-dependent rectifier was 6, an exceptionally high value which fits with the striking effects of temperature on the frequency of spontaneous rhythm in Purkinje fibres. The Q_{10} of the rate constant of the inward rectifier in skeletal muscle was certainly not as high as this, the value obtained by comparing different fibres in one muscle being about 1·8. The difference makes it likely that the resemblance between the behaviour of the inward rectifier in skeletal muscle and Purkinje fibres is superficial. As can be seen from muscle 30 in Table 2, the Q_{10} of the absolute magnitude of the initial inward currents at -150 mV is about the same as the Q_{10} of the rate constant τ_3^{-1}. This fits with the tubular-depletion hypothesis, but is not strong evidence in favour of the hypothesis.

At a fixed membrane potential the time constant with which P_3 increased after a previous hyperpolarization was usually about 50 % longer than the time constant with which it decreased after a previous depolarization. Thus the transition -77 mV $\rightarrow -107$ mV gave a time constant of 0·27 sec whereas $-162 \rightarrow -107$ mV gave one of 0·40 sec. A difference of this kind is to be expected on the Adrian–Freygang hypothesis since movement of potassium from tubules to sarcoplasm is easier than movement in the opposite direction.

Experiments involving both inward rectification and the slow conductance mechanism

In the previous sections the time dependence of the inward rectifier was investigated with membrane potentials varying between -180 and -60 mV. In this region the slow conductance channel is shut off and currents through it are small compared with those in the inward rectifier. Depolarizing the fibre beyond about -60 mV brings in the slow conductance mechanism and in that case both channels contribute to the tail of inward current which follows repolarization.

Fig. 12 is from an experiment with a fibre in a hypertonic sulphate solution containing 2·5 mM-K and tetrodotoxin, 10^{-6} g/ml. The membrane potential was switched to -155 mV after 1·25 sec at a potential V_I which varied between -190 and -11 mV. The fast component of the potassium current declined so rapidly at -155 mV that it was indistinguishable from the capacitative surge; g_2 and P_3 relaxed much more slowly and both contribute to the long tail of inward current. All the tails look rather similar

and in the absence of other information one might think that only a single process is involved.

The abscissa in Fig. 13, which is derived from Fig. 12, is the potential during the first pulse. The ordinate for curve *A* is the current at the

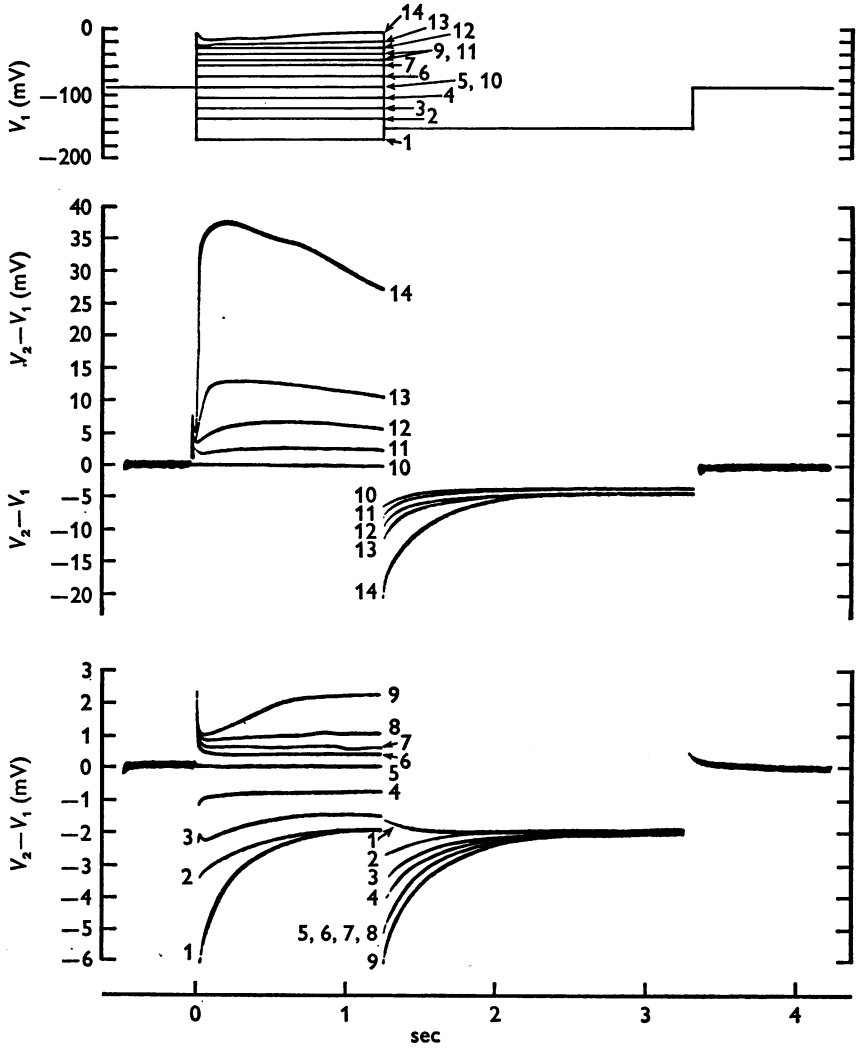

Fig. 12. Effect of prepulse on membrane current at −155 mV. Top, voltage V_1; middle, membrane current (V_2-V_1) at low gain; bottom, membrane current (V_2-V_1) at high gain. Fibre in 2·5 mM-K sulphate Ringer plus 350 mM sucrose with tetrodotoxin, 10^{-6} g/ml. at 2·5° C. Holding potential = resting potential = −91 mV. Fibre diameter, 50 μ; electrodes at 250, 500 and 550 μ from end of fibre with voltage control from V_1; 1 mV ≡ 3·3 μA/cm^2.

beginning of the first pulse; as expected this has the characteristics of an inward rectifier. Curve B is the current at the end of the first pulse and C is the current at the beginning of the second pulse. Both B and C are somewhat similar in shape, but it is easier to discuss curve C since the potential was constant and the ordinate provides a better indication of permeability.

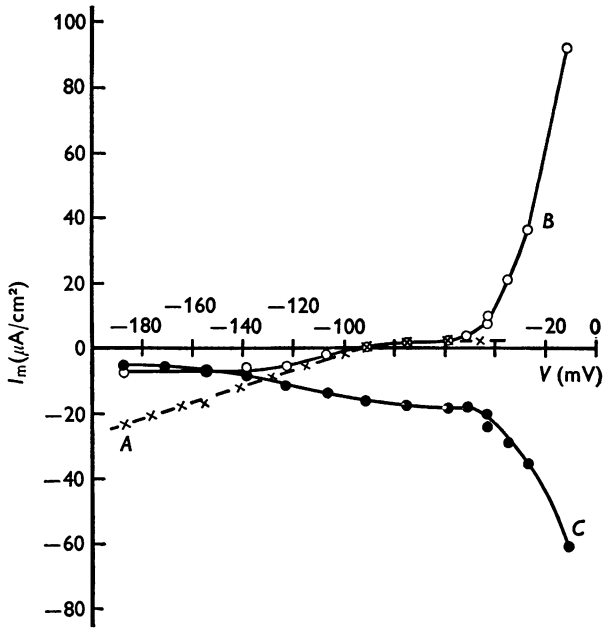

Fig. 13. Current–voltage relations in the experiment of Fig. 12. The abscissa is the potential (V_I) during the prepulse S_1. The ordinate for curve A is the membrane current at the beginning of S_1; for B it is the current at the end of S_1; for C it is the current at the beginning of S_2, the potential during S_2 being -155 mV in all cases.

It can be seen that the initial inward current at -155 mV (curve C) increases between $V_I = -190$ and -90 mV and then remains constant; this change reflects alterations in P_3 and here the instantaneous current–voltage relation is that of an inward rectifier. Between $V_I = -50$ and -11 mV the initial inward current at -155 mV increases sharply as g_2 increases. From other experiments (Fig. 2) it is known that the instantaneous current–voltage relation of the additional component is approximately linear. The experiment does not give information about the behaviour of P_3 beyond -50 mV, but the simplest assumption is that it remains constant. On that basis it turns out that in record 14 of Fig. 12, where $V_I = -11$ mV, about one third of the initial current at -155 mV is in the inward rectifier and about two thirds in the slow conductance channel.

TABLE 3. Comparison of time constants

Fibre no.	V_P (mV)	τ_3 (sec)	τ_2 (sec)
50·1	−123	0·48	0·58
	−155	0·32	0·36
	−187	0·10	0·21
31·3	−131	0·40	0·54
31·4	−161	0·49	0·89
31·5	−161	0·62	0·68

τ_3 is the time constant with which the inward current declined when the potential was switched from the holding potential (−90 to −100 mV) to V_P. τ_2 is the time constant with which the additional current I^* declined at V_P. I^* = (current with prepulse to −20 mV)−(current without prepulse). Temperature 1–3° C; 2·5 mM-K sulphate plus 350 mM sucrose.

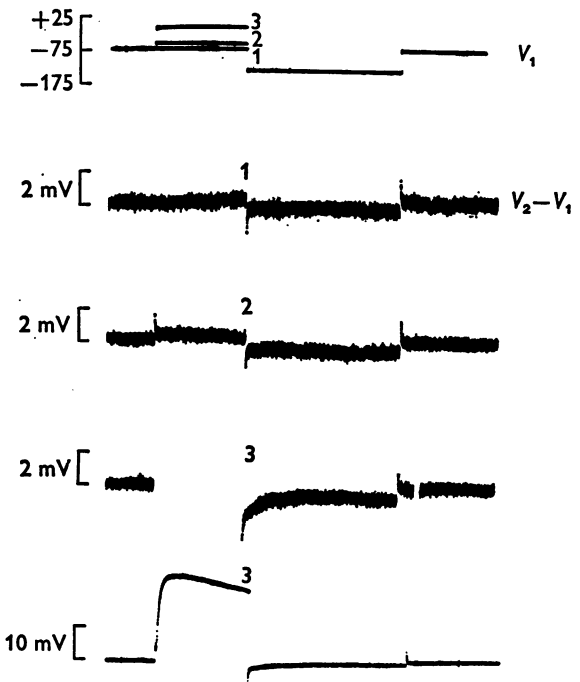

Fig. 14. Membrane currents in 10 mM-Rb sulphate + 350 mM sucrose + tetrodotoxin, 10^{-6} g/ml. at 1° C. Top, voltage V_1; middle, membrane current ($V_2 - V_1$) at high gain; bottom, membrane current at low gain (record 3). Inward rectification and the associated decline in current are absent. Delayed rectification is present and there is some slowly declining component though much less than with K in the external solution. Holding potential = resting potential = −75 mV. Electrodes at 250, 500 and 550 μ from end of fibre; fibre diameter 50 μ. 1 mV ≡ 3·3 μA/cm^2.

Although the inward tails in Fig. 12 all have the same general appearance they do not have identical time constants. Table 3 summarizes an analysis of four experiments and shows that the time constant for the relaxation of the linear system is about 0·1 sec longer than that of the rectifier.

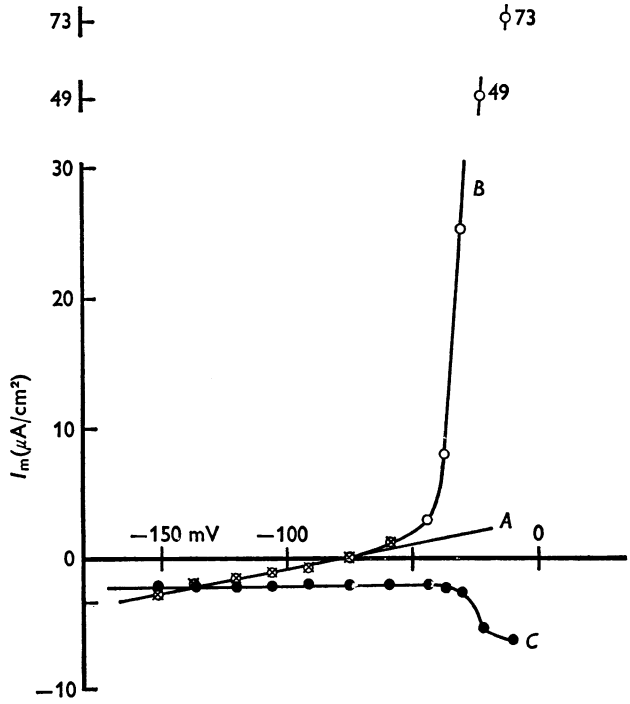

Fig. 15. Current–voltage relations with 10 mM-Rb sulphate + 350 mM sucrose in the external solution, from the experiment of Fig. 14. The abscissa is the potential during the prepulse S_1; the ordinate for curve A is the membrane current at the beginning of S_1; for B it is the current at the end of S_1; for C it is the current at the beginning of S_2, the potential during S_2 being −137 mV in all cases.

Replacement of external potassium by rubidium

Adrian (1964) showed that external rubidium does not carry appreciable current through the inward rectifier. This was confirmed by the present method. Figs. 14 and 15 are from an experiment with a 10 mM-Rb sulphate solution to which 350 mM sucrose had been added. Between −140 and −50 mV, the currents were very small, time-independent and proportional to the applied voltage. Depolarizing to −11 mV gave a large outward current and there was some slowly declining current at −137 mV. However, the tail was smaller than with 10 mM-K in the external solution,

and the instantaneous current–voltage relation was not linear but rectified in the outward direction. The conclusion is that replacing 10 mM-K by 10 mM-Rb eliminates the inward rectifier, but has less effect on the slow conductance mechanism. However, it is possible that rubidium cannot move through either channel and that the small tail of inward current at -137 mV was carried by potassium ions which had moved outward during the depolarization and returned when the internal potential was switched to -137 mV.

DISCUSSION

The experiments described here provide some basis for identifying the following pathways in skeletal muscle: (1) a delayed rectifying channel, similar to that in nerve, but inactivating more rapidly and completely; (2) a channel in which depolarization increases potassium conductance but at a rate that is one or two orders of magnitude slower than in the delayed rectifier; and (3) an inward rectifier in which current declines with a time constant of 0·25 sec at -150 mV. For completeness the presence of a path for active uptake of potassium ions should also be mentioned. The first channel is responsible for rapid repolarization during the falling phase of the action potential, and for the early after-potential; the second can give rise to late after-potentials and the third is responsible for the slow hyperpolarization seen by Adrian & Freygang (1962). At present there is no certain way of localizing ionic channels in muscle, but indirect evidence suggests that the delayed rectifier may be mainly in the surface membrane and the other two channels in the tubular system. Thus the absence of late after-potentials and slow hyperpolarization from detubulated muscle make it probable that these effects arise in the tubules (Gage & Eisenberg, 1969). The position of the delayed rectifier is less clear but there must be some delayed rectification in the surface, since detubulated fibres give normal spikes, which are followed by an early after-potential when polarized away from the equilibrium potential (Gage & Eisenberg, 1969). This does not exclude delayed rectification from the tubules but the constancy of the equilibrium potential makes it rather unlikely that large delayed currents flow through the tubular membrane (Adrian *et al.* 1970).

Although our experiments do not conflict with the view that late after-potentials and slow hyperpolarization arise in the tubules they bring out serious objections to the idea that either effect can be explained solely by changes in tubular potassium concentration. If the late after-potentials were caused by potassium accumulation one would not expect their sign to reverse when the membrane is depolarized below an apparent equilibrium potential. This effect, which was conspicuous at 3° C, is explained by the slow conductance changes seen with the voltage-clamp method.

In some respects, the time dependence of the inward rectifier fits rather well with the potassium depletion hypothesis of Adrian & Freygang (1962). Thus there is a satisfactory equivalence between the time integral of the transient part of the current and the total quantity of tubular potassium ions. But the hypothesis does not account for the apparent constancy of the equilibrium potential or for the negative slope conductance at -150 mV. Both effects can be explained if one supposes that potassium ions do not carry all the current or that some variable other than membrane potential is the factor which controls potassium permeability.

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