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ACTIVATION AND INACTIVATION OF EXCITATION–CONTRACTION COUPLING IN RAT SOLEUS MUSCLE

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SUMMARY

1. Potassium (K^+) contractures have been used to characterize the processes of activation and inactivation of excitation–contraction coupling during prolonged depolarization of fibres in small bundles dissected from rat soleus muscles at 23 °C.

2. The smallest measurable K^+ contracture tension was recorded with depolarization to −40 mV in 30 mM-K^+ and maximum tension was achieved between −26 mV in 80 mM-K^+ and −19 mV in 120 mM-K^+.

3. The rate of inactivation of K^+ contracture tension was voltage dependent. Tension decayed from 80 to 20% of the peak amplitude within 44·0 ± 2·2 s at −26 mV (in 80 mM-K^+), compared with 66·7 ± 4·8 s at −35 mV (in 40 mM-K^+). Results are given as mean ± 1 S.E.M.

4. The effect of inactivation on maximum tension was determined using a two pulse protocol in which a 'conditioning' depolarization in solutions containing 20–120 mM-K^+ was applied for 0·5–10 min before a 'test' depolarization to −8 mV in 200 mM-K^+. The amplitude of the test contracture was compared with the mean amplitude of 'control' 200 mM-K^+ contractures elicited in normally polarized fibres immediately before and after the two pulse protocol. Conditioning depolarization to −47 mV (in 20 mM-K^+) did not reduce test 200 mM-K^+ contracture tension. Significant inactivation was seen with further conditioning depolarization to more positive potentials: after 10 min at −40 mV (in 30 mM-K^+), or −35 mV (in 40 mM-K^+), test 200 mM-K^+ contracture tension was reduced by 33 and 70% respectively.

5. In contrast to amphibian muscle, where maximum tension falls to zero within a few minutes of depolarization to potentials positive to −50 mV, test 200 mM-K^+ contracture tension in rat soleus fibres fell initially rapidly and then slowly, but was not reduced to zero, even after 10 min at −19 mV in 120 mM-K^+.

6. The fast phase of inactivation of test 200 mM-K^+ contracture tension occurred during the decay of the conditioning K^+ contracture. The slow phase of inactivation reached completion after 10 min of conditioning depolarization and occurred during the period when conditioning tension was reduced to zero or to a plateau level. Both phases of inactivation in rat soleus fibres are slow compared with fast and slow inactivation times of 5–100 s respectively reported for amphibian muscle.

7. When repolarized after prolonged depolarization, the muscle fibres were initially refractory, i.e. unable to produce tension in response to electrical

stimulation. Repriming was observed after the refractory period: twitch and tetanic tension slowly recovered to final values that were within 90–100% of the peak tension recorded before the two pulse depolarization. The rate of repriming was measured as the time taken for tetanic tension to reach 90% of its final value and depended (a) on the depolarizing potassium concentration and (b) on the period of exposure to the high-potassium solution. Repriming times were 3–5 min after brief (30–50 s) exposures to solutions containing 30–60 mM-K^+. The longest repriming times recorded were 15–20 min. Repriming slowed to this rate after 3 min at −26 mV in 80 mM-K^+ and did not slow further with depolarization to more positive membrane potentials, or with prolonged depolarization.

8. The biphasic time course of inactivation and repriming suggest that, as in amphibia, there are two inactivated states in the excitation–contraction coupling process in mammalian skeletal muscle. These may be located in the 'voltage-sensor', or in the coupling mechanism between the voltage sensor and the calcium release channel.

INTRODUCTION

The aim of this investigation was to characterize the voltage dependence of excitation–contraction (EC) coupling in intact, slow-twitch, mammalian skeletal muscle fibres. EC coupling in skeletal muscle depends on depolarization of the transverse (T) tubule membrane inducing an unknown secondary process which opens calcium release channels in the sarcoplasmic reticulum (SR). The basic response of the EC coupling to depolarization is seen in a potassium (K^+) contracture where a rapid, voltage-dependent, increase in tension slowly decays under the influence of an inactivation process (Hodgkin & Horowicz, 1960; Luttgau, 1963; Heistracher & Hunt, 1969*a*; Caputo, 1972, 1976). These changes in tension depend on different states of a voltage-sensitive molecule in the T-tubule membrane (Chandler, Rakowski & Schneider, 1976; Rakowski, 1981) and, therefore, reflect the way in which the 'voltage sensor' responds to depolarization to regulate calcium release from the SR.

The characteristics of EC coupling have been studied in detail in amphibian muscle, but not in mammals. EC coupling is important in determining many of the contractile properties of mammalian muscle fibres (Close, 1972; Close & Luff, 1974; Luff, 1981; Dulhunty & Gage, 1983, 1985; Dulhunty, Gage & Lamb, 1987). The voltage dependence of tension and asymmetric charge movement (Dulhunty & Gage, 1983, 1985) and calcium release from the SR, measured with calcium-sensitive dyes (Eusebi, Miledi & Takahashi, 1980, 1985), differ in fast- and slow-twitch mammalian fibres and it is likely that these and other aspects of EC coupling contribute to the unique contractile properties of different types of mammalian muscle.

Soleus is one of the few mammalian muscles that contain predominantly slow-twitch fibres (see e.g. Ariano, Armstrong & Edgerton, 1973) but the fibres are too long (1–1·5 cm), relative to their space constant, 0·08 cm (Dulhunty, Carter & Hinrichsen, 1984), for point voltage clamping with microelectrodes: changing the extracellular potassium concentration is the most effective way to control membrane potential in intact fibres. The K^+ contracture technique has been used in elegant studies of EC coupling in amphibian muscle (Hodgkin & Horowicz, 1960; Luttgau,

1963; Caputo, 1972, 1976; Luttgau & Spiecker, 1979; Nagai, Takauji, Kosaka & Tsutsu-Ura, 1979) and has yielded results that are comparable to those obtained under voltage clamp conditions (Heistracher & Hunt, 1969*a*, *b*; Caputo & De Bolanos, 1979; Luttgau, Gottschalk & Berwe, 1986). The voltage and time dependence (a) of inactivation of tension during depolarization and (b) repriming of EC coupling upon repolarization, are examined in this study. The results show that there are some similarities, and some important differences, in both inactivation and repriming, between rat soleus fibres and amphibian or reptilian twitch fibres.

METHODS

Biological preparation

Soleus muscles were removed from adult male Wistar rats weighing 250–350 g. Rats were killed by asphyxiation in CO_2. The muscles were pinned out in a Petri dish lined with Sylgard (Dow Corning Corp., Midland, MI, USA), bathed in a control Krebs solution (Table 1) and dissected into small bundles containing 5–10 fibres.

Solutions

Solutions containing low chloride concentrations were used for all experiments. This was done to allow a rapid change in membrane potential when potassium concentration was changed (Hodgkin & Horowicz, 1960; Dulhunty, 1979; Dulhunty & Gage, 1985). The anion used to substitute for chloride was sulphate so that the results could be compared with results of previous experiments in which sulphate was also used as the main anion (Chua & Dulhunty, 1988, 1989). The solutions used are listed in Table 1.

Standard abbreviations are shown in Table 1. The solutions are referred to by the concentration of potassium (in mM); for example, contractures induced by exposure to 20 mM-K^+ or 200 mM-K^+ are referred to as 20 mM-K^+ or 200 mM-K^+ contractures.

Stimulation and isometric tension recording

Bundles of fibres were mounted in a small volume (0·5 ml), rapid flow (2 ml/s) bath with an equilibration time of less than 0·5 s. Massive platinum electrodes extended along either side of the preparation for simultaneous electrical stimulation of the entire fibre length. Twitches were elicited at 0·1 Hz with supramaximal 0·5 ms pulses. Tetanic contractions were elicited with trains of stimuli at 50 Hz for the time necessary to establish a clear tension plateau, usually 0·8–1·5 s. K^+ contractures were elicited by rapidly changing from the control solution to solutions containing elevated potassium concentrations (20–200 mM). The high potassium solutions were allowed to flow at maximum velocity to ensure a rapid rate of depolarization. Tension was recorded continuously via an Akers semiconductor transducer (model AE875, SensoNor a.s. Horten, Norway) on a chart recorder (7402A, Hewlett-Packard Co., Palo Alto, CA, USA) and selected twitches, tetanic contractions and potassium contractures were stored on magnetic disc for later computer analysis. The frequency response of the chart recorder was 55 Hz for full-scale deflections and 125 Hz for 10 mm deflections (the usual recorded amplitude of twitch contractions). The experiments were done at $22·5 \pm 0·5$ °C.

Data analysis and normalization procedures

All results were obtained using the protocol outlined in Fig. 1. The sequence of tetanic stimulation and K^+ contractures was as follows: a tetanus (of amplitude $P1$) immediately preceded a test 200 mM-K^+ contracture (of amplitude t_c1, where t represents directly measured amplitude and c a control tension parameter) in fibres equilibrated in the control (3·5 mM-K^+) solution; a recovery period of at least 10 min was allowed in the control solution and then a second tetanus (of amplitude $P2$) was recorded before a conditioning K^+ contracture (of amplitude t_a, where a represents an activation tension parameter); after several minutes in the conditioning solution a test 200 mM-K^+ contracture (of amplitude t_i, where i represents an inactivation tension parameter) was recorded; when recovery from the conditioning and test contractures was achieved in the

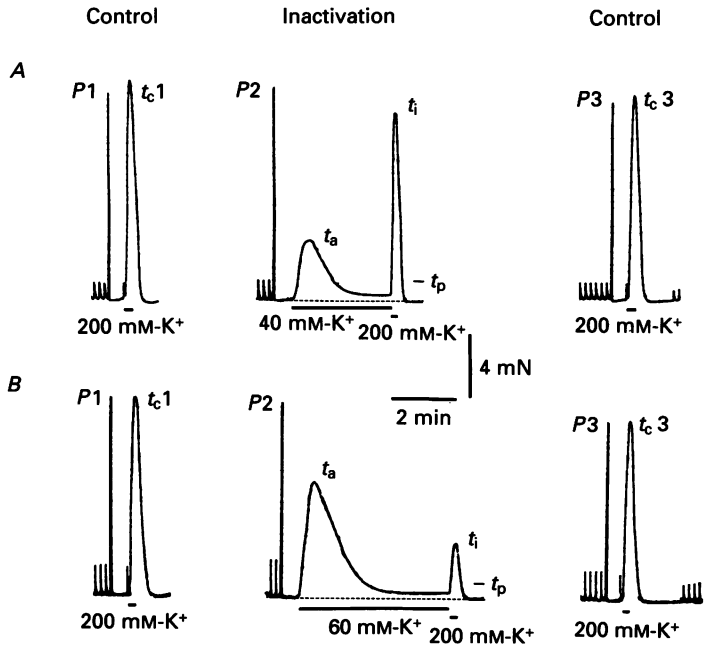

Fig. 1. Illustration of the protocol used to measure inactivation of K^+ contracture tension. *A*, chart records of tension as a function of time taken from an experiment designed to measure inactivation of test 200 mM-K^+ contracture tension during conditioning depolarization in 40 mM-K^+. *B*, a similar experiment examining inactivation in a conditioning 60 mM-K^+ solution. The small vertical deflections are twitches, the larger deflections (*P*1, *P*2 and *P*3) are tetanic contractions and the slower tension changes (t_c1, t_a, t_i and t_c3) are K^+ contractures. The symbols are defined in the text. Potassium concentration is shown beneath each contracture and the duration of exposure to high potassium is indicated by the continuous lines. The interrupted lines in the centre column show baseline tension with which to compare pedestal tension (t_p). The first column contains control records; the central column shows exposure to conditioning high-potassium solutions with test 200 mM-K^+ contractures after 3 min in 40 mM-K^+ (*A*) and 5 min in 60 mM-K^+ (*B*). The records in the third column were obtained following recovery from inactivation.

TABLE 1. Composition of solutions (mM)

Solution	Na_2SO_4	NaCl	K_2SO_4	KCl	Sucrose
Control	32·25	16	1·75	0	170
20 mM-K^+	88	0	2	16	0
30 mM-K^+	85	0	7	16	0
40 mM-K^+	80	0	12	16	0
50 mM-K^+	75	0	17	16	0
60 mM-K^+	70	0	22	16	0
80 mM-K^+	60	0	32	16	0
120 mM-K^+	40	0	52	16	0
200 mM-K^+	0	0	92	16	0

All solutions contained 7·6 mM-$CaSO_4$, 1 mM-$MgSO_4$, 11 mM-glucose and 2 mM-*N*-tris-(hydroxymethyl)-methyl-2-amino-ethanesulphonic acid (TES) buffer adjusted to pH 7·4 with NaOH.

control solution a final tetanus (of amplitude $P3$) and test 200 mM-K^+ contracture (of amplitude t_c3) were recorded. Average normalized amplitudes of t_a and t_i (T_a and T_i, where T represents normalized amplitude) were used respectively to construct activation and inactivation curves.

A dual normalization procedure was used: firstly the amplitude of all K^+ contracture (t_c1, t_a, t_i and t_c3) was divided by the amplitude of the immediately preceding tetanus ($P1$, $P2$ or $P3$). The mean amplitude of control 200 mM-K^+ contractures (relative to peak tetanic tension) was called T_{max}, since 200 mM-K^+ elicits maximum tension in soleus fibres (Chua & Dulhunty, 1988, 1989), i.e.

$$T_{max} = (t_c1/P1 + t_c3/P3)/2. \quad (1)$$

TABLE 2. Membrane potentials measured in control and high-potassium solutions

Potassium concentration (mM)	Membrane potential (mV)	n
3·5	−81·8±0·3	183
20·0	−46·4±0·5	22
30·0	−39·8±0·2	47
40·0	−34·7±0·4	58
60·0	−30·1±0·5	40
80·0	−25·6±0·2	36
120·0	−18·9±0·4	26
200·0	−6·5±0·4	39

Potentials were measured with intracellular glass microelectrodes, containing 2·5 M-KCl, with resistances of 5–10 MΩ. Measurements were made in ten preparations. In each preparation, five fibres were impaled in the control solution. Ten minutes was allowed for equilibration in the high-potassium solution before measurements were made in eight to ten fibres. The high-potassium solution was washed out for 20 min before a further set of control readings were obtained. Results are shown ±1 S.E.M.

Secondly, tension was normalized to T_{max}. Therefore, the amplitude of contractures induced by exposure to conditioning potassium solutions, T_a, was given by

$$T_a = (t_a/P2)/T_{max}, \quad (2)$$

and the amplitude of test 200 mM-K^+ contractures, T_i, was given by

$$T_i = [(t_i - t_p)/P2]/T_{max}. \quad (3)$$

Note that the pedestal tension, t_p, is subtracted from t_i in order to calculate the tension produced by the additional depolarization in 200 mM-K^+.

Construction of activation and inactivation curves

Peak K^+ contracture tension in conditioning and test solutions was assumed to reflect a steady-state level of activation. To obtain steady-state activation curves, average T_a was plotted against membrane potential in the conditioning solutions (measured in a separate series of experiments, see Table 2) and fitted with a Boltzmann equation of the form:

$$T_a = T_{max}/[1 + \exp(V_a - V_m)/k_a], \quad (4)$$

where T_a is the normalized conditioning K^+ contracture amplitude at membrane potential V_m (Fig. 1), V_a is the potential at which $T_a = 0{\cdot}5\ T_{max}$ and k_a is a slope factor.

An activation curve, measured after a depolarization duration of d min, was obtained from average normalized test 200 mM-K^+ contracture tension, $T_{i(d)}$, plotted against membrane potential in conditioning potassium solutions. The data were fitted with a Boltzmann equation of the form:

$$T_{i(d)} = T_{max}/[1 + \exp(V_c - V_{i(d)})/k_{i(d)}], \quad (5)$$

where $T_{i(d)}$ is the relative test contracture amplitude, after depolarization for d min, at a conditioning potential V_c (Fig. 1), $V_{i(d)}$ is the potential at which $T_{i(d)} = 0{\cdot}5\ T_{max}$ and $k_{i(d)}$ is a slope factor.

Run-down effects in mammalian muscle fibres

A slow 'run-down' in twitch, tetanus and K^+ contracture tension characteristically occurs in isolated mammalian muscle fibres. The amplitude of twitch and tetanic contractions and K^+ contractures fell progressively during the present experiments, by 10–50 % over 2–3 h. Despite this progressive run-down, the ratio of maximum K^+ contracture tension (in 200 mM-K^+) to peak

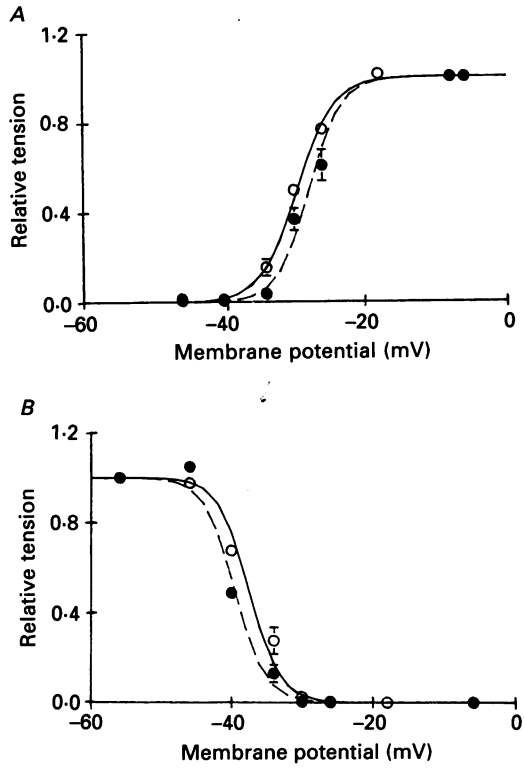

Fig. 2. A comparison of the amplitude of K^+ contractures recorded at the beginning and end of an experiment. Relative tension is plotted against membrane potential, measured in high-potassium solutions in separate experiments (Table 2). The open circles (○) show average relative tension at the beginning of an experiment and the filled circles (●) show average data obtained about 1 h later, in a third series of K^+ contractures. Each point shows average data from five preparations. Here and in subsequent figures, vertical bars show ±1 S.E.M. where this is greater than the dimensions of the symbols. The normalized amplitude of conditioning K^+ contractures, used to construct activation curves, is plotted in *A*. The normalized amplitude of 200 mM-K^+ contractures, used to construct inactivation curves after 10 min in conditioning high-potassium solutions, is shown in *B*. Tension normalization procedures are described in the methods. The curves show the best fit (by eye) of Boltzmann equations (eqn (4) in *A* and eqn (5) in *B*) to the data. In *A*, the Boltzmann constants were $V_a = -29{\cdot}5$ mV and $k_a = 2{\cdot}6$ mV for the initial data (continuous line) and $V_a = -27{\cdot}8$ mV and $k_a = 2{\cdot}3$ mV for the final observations (interrupted line). The constants in *B* were $V_i = -37{\cdot}5$ mV and $k_i = 2{\cdot}2$ mV for the initial results (continuous line) and $V_i = -39{\cdot}5$ mV and $k_i = 2{\cdot}2$ mV for the final data (interrupted line).

tetanic tension remained remarkably constant (Fig. 1) and was used to normalize contracture tension.

The relative amplitude of submaximal K^+ contractures was depressed when fibres were repeatedly exposed at high-potassium solutions. In general, preparations tolerated at least three sequences of the four K^+ contractures used to measure inactivation (see Fig. 1) with little effect of run-down on the activation and inactivation curves. Average results from five preparations are

shown in Fig. 2, where data from the first sequence of K^+ contractures recorded from the preparations is compared with data obtained between 1 and 2 h later, in a third sequence of K^+ contractures. The activation curve was shifted by 1·5 mV to the right and the inactivation curve shifted by 2 mV to the left in the aged preparation. These run-down effects became significant in preparations maintained for longer than 2 h and analysis was confined to data obtained from 'fresh' preparations.

RESULTS

Small bundles of mammalian fibres, equilibrated in low-chloride Krebs solutions, depolarize rapidly when suddenly exposed to high-potassium solutions also containing low chloride (Dulhunty, 1979). Since depolarization is 90–95% complete after 20–30 s, the changes in tension seen after this time are considered to occur in response to a steady-state depolarization.

Development of K^+ contracture tension

Tension did not develop in response to depolarization to −46 mV in 20 mM-K^+. Small contractures developed at −40 mV in 30 mM-K^+ and tension increased to maximum values between −26 mV (in 80 mM-K^+) and −19 mV (in 120 mM-K^+). The concentrations of calcium ions required for threshold and maximum calcium activation of the contractile proteins in soleus fibres are 3×10^{-7} M and 3×10^{-6} M respectively (Stephenson & Williams, 1981). Thus depolarization to −40 mV is required to increase the myoplasmic calcium concentration to 3×10^{-7} M in soleus fibres and depolarization to −20 mV required to increase the calcium concentration to 3×10^{-6} M.

The voltage dependence of peak K^+-contracture tension is shown in Fig. 3 where the average normalized amplitude of conditioning K^+ contractures (see Fig. 1, Methods) is plotted against membrane potential in the conditioning potassium solution. Three sets of data are shown to illustrate the consistency of the results between different preparations. Contracture tension was normalized to the test 200 mM-K^+ contracture tension at −8 mV. The curves show the best fit of a Boltzmann equation (eqn (4)) to the data. The values listed for V_a and k_a in Table 3 are also similar to Boltzmann constants obtained in previous experiments on normal rat soleus fibres (Dulhunty & Gage, 1983, 1985; Chua & Dulhunty, 1988).

It is interesting to note that, in each of the three sets of data shown in Fig. 3, the average tension in 120 mM-K^+ was slightly (2–5%) greater than the average tension in 200 mM-K^+. An apparent depression of peak tension might occur if the maximum contracture amplitude was not achieved because of the rapid inactivation in 200 mM-K^+.

Time course of K^+ contracture decay

The decay of K^+ contracture tension in high-potassium solutions followed a simple monophasic time course lasting 2–3 min (Fig. 4*A*). This fall in tension was significantly slower than the 10–20 s required for tension to decay to the resting level when contractures were interrupted by repolarization in 3·5 mM-K^+ (Fig. 4*B*). The rapid repolarization-induced decay can also be seen in the 200 mM-K^+ contractures in Fig. 1, where the potassium concentration was suddenly lowered to 3·5 mM at the peak of the contractures.

The slow inactivation of tension in soleus fibres was surprising since inactivation

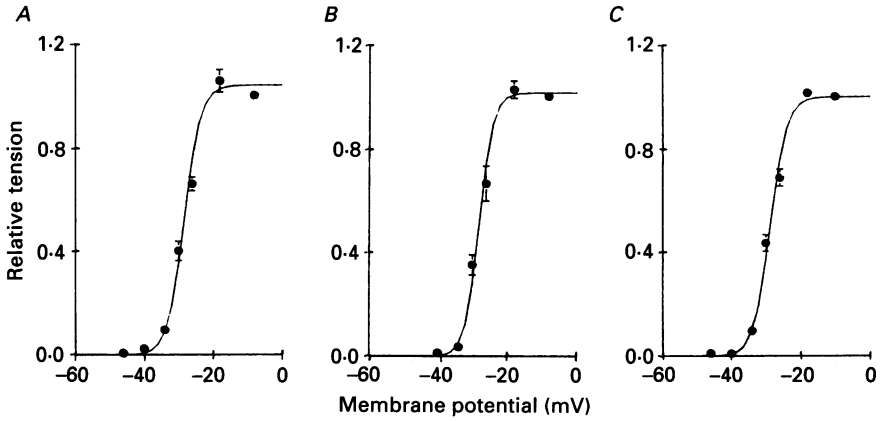

Fig. 3. The voltage dependence of K^+ contracture tension. The normalized amplitude of contractures in conditioning high-potassium solutions is plotted against the membrane potential in conditioning solutions (Table 2). Data obtained during experiments measuring inactivation after 3, 5 and 10 min in conditioning solutions are show in *A*, *B* and *C* respectively. The curves through the data are the best fit (by eye) of a Boltzmann equation, (4), to the data. The constants used for each curve are listed in Table 3.

TABLE 3. Constants used to fit Boltzmann equations (eqns (4) and (5)) to tension data obtained in activation and inactivation experiments

Time in conditioning high-potassium solution (min)	Activation curve		Inactivation curve	
	V_a (mV)	k_a (mV)	V_i (mV)	k_i (mV)
3·0	−28·3	2·4	−33·8	1·9
5·0	−28·0	2·0	−36·2	2·6
10·0	−28·7	2·4	−37·5	2·5

Inactivation of test 200 mM-K^+ contracture tension was measured at the indicated times after exposure to conditioning high-potassium solutions. A separate set of activation data was obtained for each set of inactivation data. Values for the membrane potential for 50 % tension for activation, V_a, and inactivation, V_i, and the slopes, k_a and k_i, are given.

of EC coupling in amphibian muscle fibres causes tension to decay to zero within a few seconds after the peak of the K^+ contracture (Hodgkin & Horowicz, 1960). Inactivation of the voltage sensor in rat soleus may be intrinsically slow. Alternatively, the calcium release channel could be kept open by a slow secondary process, for example inositol 1,4,5-trisphosphate (Vergara, Tsien & Delay, 1985) or calcium (Lamb & Stephenson, 1990). The repolarization-induced decay of tension should also be slowed after longer depolarizations if a slow voltage-independent secondary process was acting on the calcium release channel. The 80 mM-K^+ contractures were interrupted at the peak (after depolarization for 14·6 s; Fig. 4*B*) and then at various times during the decay (after depolarization for 26–74 s; Fig. 4*C–G*). The time taken for tension to fall to zero increased from 20·4 s, after the 14·6 s depolarization, to 25·7 s after the 70 s depolarization. This lengthening of decay was attributed to the slower repolarization recorded after longer exposures to high potassium (potassium equilibration in mammalian T-tubules takes 30–60 s, therefore the time required to reduce the tubular potassium to concentrations close to 3·5 mM

during wash-out is longer after 70 s than after 15 s (Dulhunty, 1979)). The 4 s increase in decay time was not consistent with a secondary process holding the calcium channels open for minutes after rapid inactivation of the voltage sensor.

A third possibility was that calcium influx across the surface membrane provided sufficient calcium to directly activate the contractile proteins or the calcium release

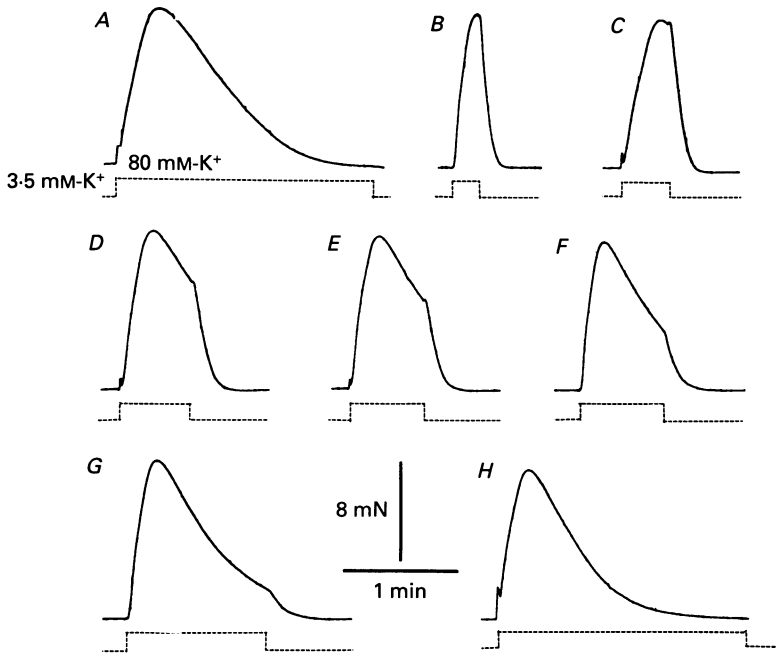

Fig. 4. The decay of K^+ contracture tension. The decay of 80 mM-K^+ contracture tension during prolonged depolarization (*A*) is compared with the decay of tension induced by repolarization in the control solution containing 3·5 mM-K^+ (*B–G*). Repolarization was induced by rapidly flowing the 3·5 mM-K^+ solution past the preparation. Contractures were interrupted at the following times after depolarization: 14·6 s (*B*); 25·6 s (*C*); 36 s (*D*); 39·3 s (*E*); 44·5 s (*F*); 74 s (*G*). The 80 mM-K^+ contracture in *H* was obtained after the preparation had been equilibrated for 5 min in a low-calcium solution; $CaSO_4$ in the control and 80 mM-K^+ solutions (Table 1) was replaced by 20 mM-Ca–EGTA and an extra 9 mM-Mg^{2+} was added to the solutions. The interrupted line under each record indicates the times at which the external potassium concentration was changed from 3·5 to 80 mM. All records were obtained from one preparation.

channel. However, contractures were not significantly abbreviated in solutions containing 20 mM-Ca–EGTA and 10 mM-Mg^{2+} (Fig. 4*H*). Taken together, the records in Fig. 4 suggest that the slow decay of K^+-contracture tension during maintained depolarization is due to slow inactivation of the voltage sensor or the coupling process between the voltage sensor and the calcium release channel.

The rate of decay of contracture tension in high potassium depended on potassium concentration and was faster in 80 than 40 mM-K^+ (Fig. 5*A*). Unexpectedly, contractures in 120 mM-K^+ were not always faster than 80-mM-K^+ contractures: the decay of some 120 mM-K^+ contractures developed a very slow component which continued for several minutes (Fig. 5*B*). The nature of the slow component was not examined further.

The tail of monophasic contractures, from 70% of peak tension to the pedestal level, could be fitted with a single exponential time constant (Fig. 5*C*). The decay from 80 to 20% of the peak contracture tension minus the pedestal tension was used as a more general index of relaxation times and is plotted against membrane potential in Fig. 6*A*.

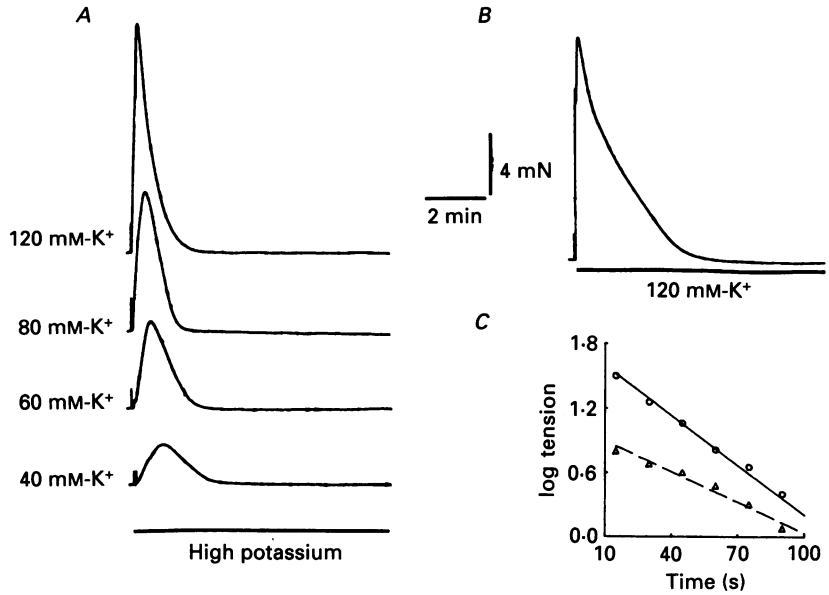

Fig. 5. The decay of K^+ contracture tension depends on potassium concentration. *A*, typical records obtained in 40, 60, 80 and 120 mM-K^+. Fibres were maintained in high potassium for the duration of the record (indicated by the lower horizontal line). *B*, a contracture in 120 mM-K^+ with a pronounced slow phase. *C*, a graph showing the exponential decay of the 40 mM-K^+ (△) and the monophasic 120 mM-K^+ (○) contractures shown in *A*. Tension was measured between 15 and 90 s after the peak of the contracture. Log relative tension is plotted against time. The lines through the data have regression coefficients of 0·985 for the 40 mM-K^+ contracture and 0·995 for the 80 mM-K^+ contracture. The tension and time calibration apply to both *A* and *B*.

The average decay time at −26 mV (80 mM-K^+) was 44·03 ± 2·23 s compared with 66·7 ± 4·81 s at −35 mV (in 40 mM-K^+). Monophasic contractures in 120 mM-K^+ had an average 80–20% decay time of 43·5 ± 2·8 s in thirteen preparations at −19 mV (○).

The inactivation of maximum tension

A general model for EC coupling suggests (a) that depolarization converts a fraction of the resting voltage sensors to an active form and then to an inactive form and (b) that calcium release (and tension) is proportional to the concentration of voltage sensors in the active state (Hodgkin & Horowicz, 1960; Caputo, 1972; Chandler *et al.* 1976; Rakowski, 1981; Luttgau, Gottschalk & Berwe, 1986; Chua & Dulhunty, 1988, 1989; Dulhunty & Gage, 1988). The concentration of resting activator available for conversion to the active state is reduced by inactivation. The

available activator concentration was determined from the amplitude of 'test' 200 mM-K^+ contractures (see Fig. 1, Methods), which reflect maximal activation in rat soleus fibres (Dulhunty & Gage, 1983, 1985; Chua & Dulhunty, 1988, 1989).

The time course of inactivation of test 200 mM-K^+ contracture tension during conditioning depolarization in different potassium solutions is shown in Fig. 6*B*.

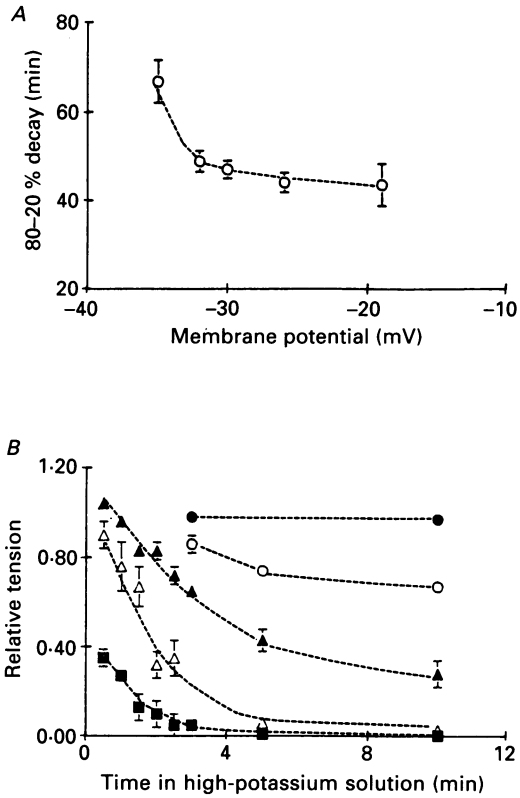

Fig. 6. Decay of conditioning K^+ contracture tension (*A*) and peak test 200 mM-K^+ contracture tension (*B*) as a result of inactivation. *A*, the time taken for conditioning K^+ contracture tension to decay from 80 to 20% of the tension between the peak of the contracture and the final pedestal level (vertical axis), plotted against membrane potential in high-potassium solutions (measured in separate experiments and listed in Table 2). The open circles (○) show average data for contractures with a monophasic decay: $n = 21$ at 40 mM-K^+ and -35 mV; $n = 13$ at 50 mM-K^+ and -33 mV; $n = 24$ at 60 mM-K^+ and -30 mV; $n = 31$ at 80 mM-K^+ and -26 mV; and $n = 15$ at 120 mM-K^+ and -19 mV. *B*, the rate of decay of test 200 mM-K^+ contracture amplitude during depolarization in high-potassium solutions. The symbols show the average amplitude of test 200 mM-K^+ contractures, in five to seven preparations, at different times (horizontal axis) during conditioning depolarization in 20 (●), 30 (○), 40 (▲), 50 (△) and 80 mM-K^+ (■). The lines have been drawn by eye through the data points for clarity.

Conditioning depolarization to -47 mV in 20 mM-K^+ had very little effect on test 200 mM-K^+ contracture tension (●), whereas 10 min depolarizations to -40 mV (in 30 mM-K^+) or -35 mV (in 40 mM-K^+) produced, respectively, 25% (○) and 70% (▲) reductions in the test tension. The reduction in test 200 mM-K^+ contracture

tension was biphasic during conditioning depolarization to membrane potentials between −35 and −19 mV: the greatest reduction in test tension occurred during the first 3 min of depolarization. For example, at −32 mV in 50 mM-K^+ (△), test tension fell rapidly to 25% of control after 3 min and then fell slowly by a further

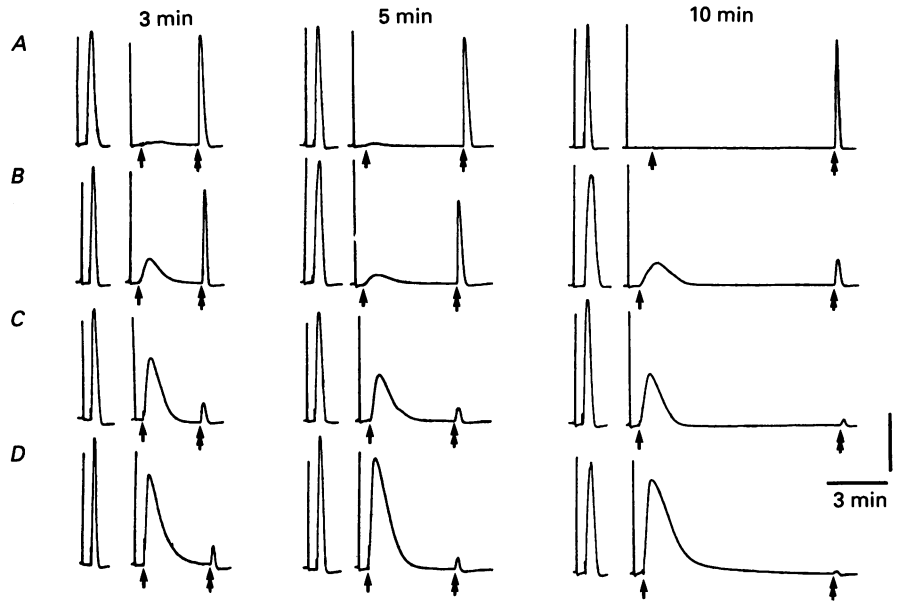

Fig. 7. Inactivation of test 200 mM-K^+ contracture tension during submaximal depolarization for 3, 5 and 10 min. The records are examples of those analysed to obtain the average results shown in Fig. 6. Separate panels are shown for 3, 5 and 10 minute conditioning depolarization. Each panel contains four sets of records showing conditioning and test contractures obtained with conditioning solutions containing 30 (*A*), 40 (*B*), 60 (*C*), and 80 mM-K^+ (*D*) conditioning solutions. Each set of records shows five tension transients which are, in order of appearance: a tetanus, a control 200 mM-K^+ contracture, a recovered tetanus, a conditioning K^+ contracture and a test 200 mM-K^+ contracture. The single arrow indicates the addition of the conditioning high-potassium solution and the double arrow indicates the time at which the test 200 mM-K^+ solution was applied to the preparation. The vertical calibration differed for each set of records and varied between 4 and 6 mN.

22%, to 3% of control, during the following 7 min. In general, the rapid phase of inactivation of test 200 mM-K^+ contracture tension occurred at the same time as the decay of the conditioning K^+ contracture. The slow phase of inactivation occurred after the contracture had decayed either fully or to a pedestal tension.

Pedestal tensions and the slow phase of inactivation

The average pedestal tension after depolarization for 5 min in 40 and 60 mM-K^+ were, respectively, $0{\cdot}7 \pm 0{\cdot}3$% and $0{\cdot}7 \pm 0{\cdot}2$% of maximum tension. Tensions of less than 1% of the maximum tension were not measured. The pedestal tension was significant in some preparations (see Fig. 1) and zero in others (Fig. 7), yielding average values of less than 1% of maximum tension.

The records in Fig. 7, showing some of the original data evaluated for Fig. 6*B* (above), were selected to show the continuing decline of test 200 mM-K^+ contracture tension during depolarization lasting for longer than 3 min in preparations in which the conditioning contracture tension had essentially decayed to zero. There are two interpretations of this observation. The first is that a significant fraction of the voltage-sensitive molecules turn from the resting to the inactivated state between 3 and 10 min without passing through the active state. If this is the case, activation and inactivation must be independent processes and the pedestal tension presumably arises from overlap of activation and inactivation variables (Chua & Dulhunty, 1988, 1989). The alternative hypothesis is that there is a sequential transition from the active to inactive state and the pedestal tension reflects a slow shift of the voltage sensor through the active state. Slow inactivation in the absence of a pedestal tension in some fibres (Fig. 7) could occur if the concentration of voltage sensors in the active state was not sufficient to raise the myoplasmic calcium to concentrations required for the fibres to generate measurable tension.

Average test 200 mM-K^+ contracture tension was not reduced to zero after 10 min conditioning depolarization in the 20–120 mM-K^+ solutions. The average test 200 mM-K^+ contracture tension (following subtraction of the pedestal tension) after 10 min at −26 mV in 80 mM-K^+ was $0{\cdot}4 \pm 0{\cdot}2$ % (■, Fig. 6*B* above) and a smaller average 200 mM-K^+ contracture tension of $0{\cdot}08 \pm 0{\cdot}07$ % maximum was recorded after 10 min at −19 mV in 120 mM-K^+. Once again, the unmeasurably small average tension at −19 mV is due to the inclusion of some individual values of zero tension: test 200 mM-K^+ contracture tension was abolished in one out of five preparations after 10 min at −26 mV and in three out of five preparations after 10 min at −19 mV.

The voltage dependence of inactivation in rat soleus fibres differs significantly from that described in frog fibres (Nagai *et al.* 1979) where maximum tension falls to zero after several minutes in solutions containing more than 15 mM-K^+. The biphasic nature of inactivation of test 200 mM-K^+ contracture tension in soleus fibres was also seen in amphibian muscle (Nagai *et al.* 1979). Caputo & de Bolanos (1990) used a repriming protocol to demonstrate 'ultraslow' inactivation in frog fibres which reached completion in more than 100 s. This inactivation can be compared with the 'hyperslow' inactivation in rat soleus fibres which continued for 600 s.

Steady-state inactivation and the contribution of run-down effects to the slow phase of inactivation.

The slow phase of inactivation continued up until 10 min; test 200 mM-K^+ contracture tension measured at 10 min was always smaller than tension measured at 5 min. To ascertain whether inactivation had reached a steady-state equilibrium after 10 min, a conditioning depolarization to −47 mV (in 20 mM-K^+) was maintained for 25 min. The average test 200 mM-K^+ contracture at 25 min was $0{\cdot}81 \pm 0{\cdot}019$, i.e. considerably less than the $0{\cdot}97 \pm 0{\cdot}016$ recorded after a 10 min conditioning depolarization. This result initially suggested that inactivation continued during depolarization periods of up to 25 min and was far from a steady-state situation at 10 min. However, run-down could have contributed to the decline in tension between 10 and 25 min since the test 200 mM-K^+ contracture was

normalized to a tetanus, $P2$, recorded some 25 min beforehand (Fig. 1 and eqn (3), Methods).

To overcome the problem of run-down, the tetanus recorded following recovery from the conditioning and test depolarizations, $P3$ (Fig. 1, above), was included in the calculation of relative tension; $P3$ was usually recorded 20–25 min after the test

TABLE 4. Comparison of relative test 200 mM-K^+ contracture tensions

	30 mM-K^+			40 mM-K^+			80 mM-K^+		
	T_i	T_{i*}	$T_{i,c}$	T_i	T_{i*}	$T_{i,c}$	T_i	T_{i*}	$T_{i,c}$
3 min	0·909	0·950	0·920	0·655	0·890	0·715	0·046	0·076	0·053
S.E.M.	±0·022	±0·036		±0·033	±0·061		±0·013	±0·021	
(n)	(6)	(6)		(10)	(10)		(12)	(12)	
5 min	0·735	0·928	0·812	0·430	0·633	0·511	0·009	0·028	0·016
S.E.M.	±0·029	±0·022		±0·050	±0·047		±0·004	±0·011	
(n)	(5)	(5)		(6)	(6)		(6)	(6)	
10 min	0·675	0·921	0·839	0·280	0·414	0·369	0·004	0·015	0·011
S.E.M.	±0·029	±0·052		±0·060	±0·070		±0·002	±0·004	
(n)	(5)	(5)		(5)	(5)		(5)	(5)	

Comparison of relative test 200 mM-K^+ contracture tensions T_i (calculated using eqn (3)), T_{i*} (calculated from eqn (6)) and $T_{i,c}$ (tension corrected for the temporal separation between the contracture, and the preceding and following tetanic contractions, $P2$ and $P3$: see text). Test tensions are shown after 3, 5 and 10 min conditioning depolarizations in 30, 40 and 80 mM-K^+. Average values are shown as mean ± 1 S.E.M. with the number of observations in parentheses.

200 mM-K^+ contracture. Therefore, for a 25 min conditioning depolarization, the test 200 mM-K^+ contracture was equally separated from $P2$ and $P3$. The data was re-evaluated using the mean of $P2$ and $P3$ to normalize the test 200 mM-K^+ contracture tension, i.e.

$$T_{i*} = [2(t_i - t_p)/(P2+P3)]/T_{max}. \quad (6)$$

The average test 200 mM-K^+ contracture tension at 30 min, calculated using eqn (6), was 0·94 ± 0·016 and was not significantly different (Student's t test) from the test tension of 0·97 ± 0·016 recorded after the 10 min conditioning depolarization. It was concluded that inactivation probably approached a steady-state level during 5–10 min conditioning depolarization.

The extent to which run-down might contribute to the slow phase of inactivation during conditioning depolarizations of 3, 5 or 10 min was also considered (Table 4) for conditioning depolarizations in 30, 40 and 80 mM-K^+ by using the average test tensions calculated with eqn (3) and (6) (T_i and T_{i*} respectively). Equation (3) assumes that the test 200 mM-K^+ contracture immediately follows $P2$ and that there is no run-down between the two contractions. Equation (6) assumes (a) that run-down is linear and (b) that there are equal time intervals between $P2$ and t_i and between t_i and $P3$. The second assumption is not true when the test tension is recorded 3, 5 or 10 min after $P2$ and 20 min before $P3$. The true tension lies between T_i and T_{i*} and a corrected tension, $T_{i,c}$, was estimated (a) using the known time intervals between $P2$, t_i and $P3$ and (b) again assuming that run-down is linear.

The slow phase of inactivation remained obvious in the corrected tensions. $T_{i,c}$ was

smaller after 5 min than after 3 min and, with the exception of the 30 mM-K^+ conditioning depolarization, smaller after 10 min than after 5 min. Therefore the slow phase of inactivation is not due to run-down of soleus fibres.

Inactivation curves obtained from test 200 mM-K^+ contracture amplitude

Average normalized test 200 mM-K^+ contracture tension (calculated using eqn (3)) is plotted against membrane potential in conditioning high potassium solutions in

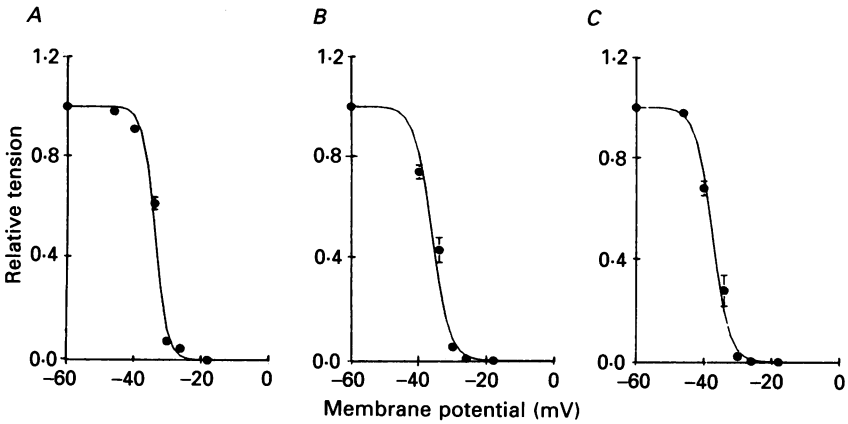

Fig. 8. The voltage dependence of inactivation of test 200 mM-K^+ contracture tension measured at different times during exposure to conditioning high-potassium solutions. Average test 200 mM-K^+ contracture amplitude is plotted against membrane potential in the conditioning high-potassium solutions (Table 2). Data was obtained after 3 (*A*), 5 (*B*) and 10 min (*C*). The curves are the best fit by eye of a Boltzmann equation (eqn (5)) to the data. The Boltzmann constants for the curves are listed in Table 3.

Fig. 8. Inactivation was measured after 3 min (*A*), 5 min (*B*) and 10 min (*C*) in conditioning potassium solutions. The continuous curves show the best fit of a Boltzmann equation (eqn (5)) to the data. These curves again illustrate the fact that inactivation slowly approaches a steady-state level as there is a progressive shift to the left in the membrane potential for 50% inactivation (V_i) during depolarization (Table 3). However the V_i at 10 min is only 1·3 mV more negative than the V_i at 5 min, suggesting once again that inactivation may be close to a steady-state equilibrium by 10 min. The results in Table 4 show that the use of the correction procedure for run-down would result in a small positive shift in all three curves but would not alter the fact that the curves shifted to more negative potentials after longer depolarizations.

Recovery from inactivation: repriming of tension

Twitch and tetanic contractions were recorded continuously in 3·5 mM-K^+ during recovery from depolarization (Fig. 9) and usually achieved 90–100% of the peak tension recorded before depolarization. The time taken for the amplitude of the

tetanus to recover to 90% of its final value was used as an index of the rate of repriming. These experiments examine the repriming of tetanic tension after long (0·5–10 min) exposures to a conditioning high-potassium solution plus a brief (10–20 s) exposure to the test 200 mM-K^+ solution.

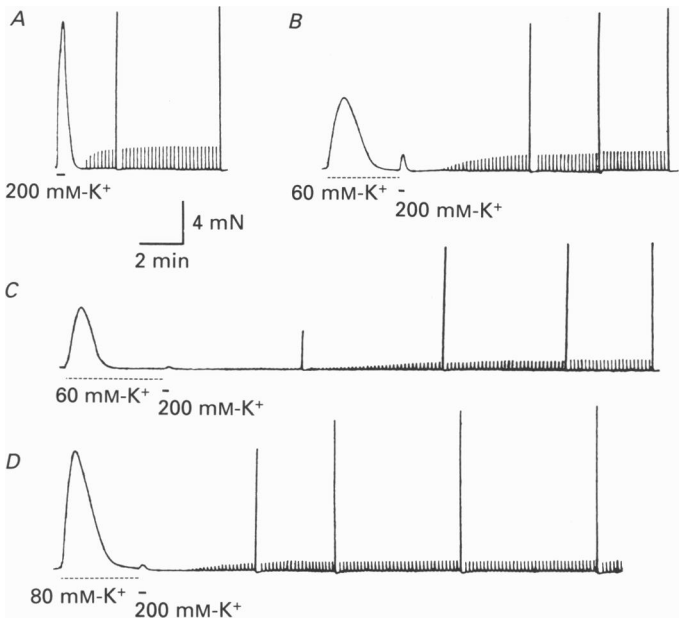

Fig. 9. Repriming of twitch and tetanic tension during recovery from inactivation. The chart records show twitch (small vertical deflections), tetanic (large vertical deflections) and K^+ contracture (slow tension changes) tension. Potassium concentrations are shown beneath each contracture, the continuous lines indicate the period of exposure to 200 mM-K^+ and the interrupted lines indicate the period of exposure to conditioning high-potassium solutions. *A*, recovery from a brief (15 s) exposure to 200 mM-K^+ alone. *B*, 3 min in 60 mM-K^+ plus 15 s in 200 mM-K^+. *C*, 5 min in 60 mM-K^+ and 15 s in 200 mM-K^+. *D*, 3 min in 80 mM-K^+ and 15 s in 200 mM-K^+.

The brief exposure to 200 mM-K^+ was terminated before significant inactivation had occurred and the recovery of twitch and tetanic tension (Fig. 9*A*) presumably reflects the time course of repolarization which depends on the time taken for 200 mM-K^+ to wash away from the fibres and out of the T-tubules. The 1–2 min recovery times (Fig. 9*A*) were similar to repolarization times recorded in single mammalian fibres (Dulhunty, 1979). Similar rapid recovery was recorded after fibres had been exposed to conditioning solutions containing 20–60 mM-K^+ for less than 20 s. The slower recovery after longer exposures to conditioning high-potassium solutions is thought to reflect the time taken for the voltage sensors to return from the inactivated state to the resting state where they are available for activation with depolarization.

The rate of recovery of tetanic tension depended on the period of exposure to the conditioning high-potassium solution and on the conditioning potassium concentration. Recovery after 3 min in 60 mM-K^+ (Fig. 9*B*) was faster than recovery

after 5 min in 60 mM-K^+ (Fig. 9*C*) or 3 min in 80 mM-K^+ (Fig. 9*D*). The length of the silent period immediately after repolarization, in which no twitch or tetanic tension was recorded, also depended on the amplitude and duration of the conditioning depolarization.

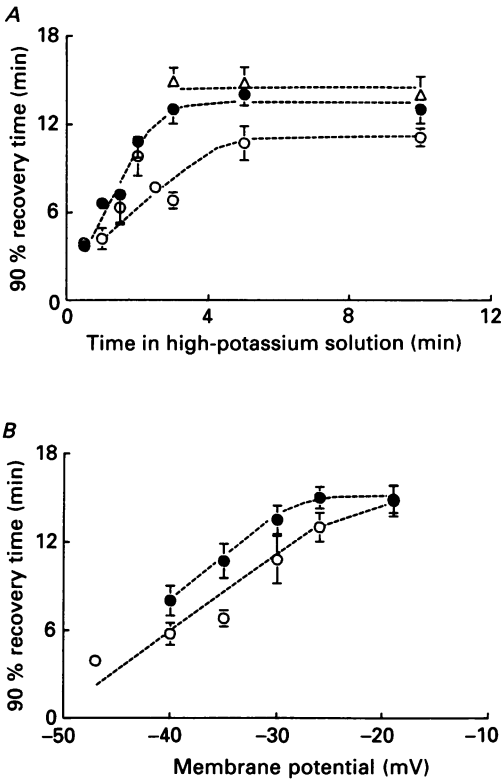

Fig. 10. The repriming of tetanic tension (90% recovery time) upon repolarization depends on the duration of depolarization in conditioning high-potassium solutions (*A*) and membrane potential in the conditioning high-potassium solutions (*B*). *A*, average 90% repriming time during recovery from depolarization in 40 mM-K^+ ($n = 3$–6, ○), 80 mM-K^+ ($n = 3$–6, ●) and 120 mM-K^+ ($n = 4$–7, △) for periods shown on the horizontal axis. *B*, average 90% repriming time is plotted against membrane potential in the conditioning high-potassium solution (Table 2), for 3 min (○) and 5 min (●) depolarizations ($n = 4$–7). The dashed lines are drawn by eye through the data for clarity.

The average 90% repriming times were 4–6 min after brief (1–2 min) depolarizations to potentials between −35 and −25 mV (40–80 mM-K^+) and the recovery time increased to average values of 15 min after 3 min depolarizations to −25 mV or more positive potentials (80–120 mM-K^+). It is interesting that the recovery time did not increase further after longer exposures, up to 10 min (Fig. 10*A*) or depolarization to more positive potentials (Fig. 10*B*). In other words, the graph of repriming time as a function of depolarization times, or conditioning membrane potential, tended to 'saturate' at a maximum value of 15 min.

The short and long repriming times can be explained in terms of the voltage sensor assuming two inactivated states. It could be argued that the voltage sensors require

4–6 min to return to the resting state from an inactivated state achieved during the rapid phase of inactivation. A longer recovery time, of 15 min, is required for the voltage sensors to recover from a second inactivated state achieved during the slow phase of inactivation.

The long repriming times of 15 min are in contrast to recovery times of a few seconds after brief exposure to high potassium (see 200 mM-K^+ contractures in Fig. 1 and Hodgkin & Horowicz, 1960; Heistracher & Hunt, 1969*b*) and are reminiscent of the slow recovery from the 'paralysed' state described by Luttgau *et al.* (1986) in amphibian muscle.

DISCUSSION

Inactivation of EC coupling is thought to arise in the voltage sensor because asymmetric charge movement is inactivated during depolarization, with a time course similar to that of tension inactivation (Chandler *et al.* 1976; Rakowski, 1981). Changes in the voltage dependence of charge movement in depolarized fibres (Schneider & Chandler, 1976; Adrian, Chandler & Rakowski, 1976) are also well correlated with inactivation of contraction (Brum & Rios, 1987). Inactivation of EC coupling is not caused by calcium depletion from the SR or inactivation of the calcium release channel or contractile proteins: low concentrations of caffeine, which release calcium from the SR (Delay, Ribalet & Vergara, 1986; Fryer & Neering, 1989) without affecting the myofilaments (Wendt & Stephenson, 1983), produce a non-inactivating contracture (Caputo, 1976) which is greater than normal following inactivation in 190 mM-K^+ for 30 min (Axelsson & Thesleff, 1958).

Mammalian skeletal muscle fibres are resistant to inactivation

Several observations suggest that the voltage sensor for EC coupling in mammals is more resistant to inactivation than in amphibia. The decay of K^+ contracture tension, at 24 °C, is slower in mammals than in amphibia, taking 60–120 s (see Fig. 4) compared with 2–5 s in frog fibres (Hodgkin & Horowicz, 1960). Diffusion delays cannot explain the difference in time course: if diffusion were rate limiting (and inactivation complete within 5 s), (a) the decay of tension would not be voltage dependent and (b) maximum K^+ contractures would not have amplitudes equivalent to tetanic tension since tension in superficial fibres would inactivate before deeper fibres were activated.

Inactivation of test 200 mM-K^+ contracture tension was also slower in rat soleus than in amphibia: both fast and slow phases of inactivation were up to six times slower than in frog fibres (Luttgau *et al.* 1986; Caputo & de Bolanos, 1990). Finally, test tension could be recorded in some soleus preparations after 10 min at −19 mV (120 mM-K^+) in contrast to the frog where maximum tension is reduced to zero after 6 min in 30 mM-K^+ (Nagai *et al.* 1979). This difference cannot be attributed to a greater depolarization in high potassium in the frog (amphibian fibres depolarize to −48 mV in 30 mM-K^+ (Hodgkin & Horowicz, 1960)), or in fibre types (fast-twitch mammalian fibres also inactivate at relatively positive membrane potentials (Chua & Dulhunty, 1988)).

The resistance to inactivation of tension probably reflects a property of the voltage-sensitive molecule. It is not clear whether the different time course and

voltage sensitivity of inactivation in different kinds of muscle fibres depends on (a) the amino acid sequence of the protein (b) modification of the molecules at a post-translational level or (c) the influence of regulatory factors (it has been shown in hippocampal cells for example that the voltage for 50% inactivation of transient potassium currents can be shifted 40 mV to more positive potentials by $GABA_B$ (γ-aminobutyric acid) agonists (Saint, Thomas & Gage, 1990)).

The two phases of inactivation and repriming of the voltage sensors for excitation–contraction coupling in skeletal muscle

The biphasic time course of inactivation and repriming cannot be attributed to the rates of depolarization and repolarization since the changes in membrane potential are complete within 30 and 60 s respectively (Dulhunty, 1979). Fatigue might contribute to the reduction in test 200 mM-K^+ contracture tension, but is unlikely to proceed for 5–10 min when the conditioning tension is reduced to low levels. The simplest interpretation of the observations is that there are two stages of inactivation in the EC coupling process. It has been suggested that these are two states of the voltage sensor in amphibian muscle (Luttgau *et al.* 1986). Nagai *et al.* (1979) define the states as 'inactivation 1' and 'inactivation 2', while Luttgau *et al.* (1986) use the terms 'refractory' and 'paralysed'. The refractory state is responsible for the decay of K^+ contracture tension and is rapidly reversed upon repolarization. The paralysed state occurs with longer periods of depolarization (Hodgkin & Horowicz, 1960), with deprivation of external calcium (Luttgau *et al.* 1986) and in the presence of the Ca^{2+} channel antagonist gallopomil (D600, Berwe, Gottschalk & Luttgau, 1987) and nifedipine (Rios & Brum, 1987; Dulhunty & Gage, 1988). Recovery from the paralysed state is slow, with repriming times lasting many minutes.

A sequential state model for activation and inactivation of the voltage sensor for excitation–contraction coupling

The two inactivated states can be described by a simple 'state' model which is an extension of previous models proposed by Luttgau *et al.* (1986) and Dulhunty & Gage (1988). The calcium channel in the terminal cisternae opens when the voltage-sensitive molecule in the T-tubules is converted from a precursor state, P, to an active state, A. The conversion to A requires the initial formation of an intermediate state, Q, followed by the rapid dissociation of calcium which converts Q to A. The two-step, calcium-dependent transition from P to A (Dulhunty & Gage, 1988) accounts for effects of low external-calcium concentration on contraction (Graf & Schatzmann, 1984; Luttgau *et al.* 1986; Brum, Fitts, Pizarro & Rios, 1988). During prolonged depolarization, A is slowly converted to an inactive state, I, and then to a second inactivated state Y. It is proposed that the conversion of I to Y involves the dissociation of additional Ca^{2+} since the paralysed state is achieved more readily (Luttgau *et al.* 1986; Dulhunty & Gage, 1988), in low calcium concentrations. These events can be represented by

$$\begin{array}{ccccccccc} P & \rightleftharpoons & Q & \rightleftharpoons & A + Ca^{2+} & \rightleftharpoons & I & \rightleftharpoons & Y + Ca^{2+} \\ & & & & \Downarrow & & & & \\ & & & & C & \rightleftharpoons & O. & & \end{array}$$

The formation of A switches the calcium release channel in the terminal cisternae from a closed (C) to an open (O) state.

After a 3 min conditioning depolarization V_i was $-33{\cdot}8$ mV and repriming upon repolarization was rapid. The model suggests that few of the voltage sensors were converted to Y at this time. Prolonging the conditioning depolarization caused (a) a further negative shift in V_i to $-36{\cdot}2$ mV after 5 min or $-37{\cdot}5$ mV after 10 min and (b) an increase in repriming time. The shift in V_i is due to the conversion of some of the voltage-sensitive molecules to state Y, thus reducing the probability of finding the molecules in state P. The longer repriming time can be explained if $k_{(YI)}$ is smaller than $k_{(IA)}$.

Submaximal contracture tension during the conditioning depolarization decays to the baseline when calcium release is insufficient to maintain the myoplasmic calcium concentration above threshold for contraction. Calcium concentrations might just exceed threshold in some preparations and produce a pedestal tension (Chua & Dulhunty, 1988, 1989).

Alternative models of inactivation of excitation–contraction coupling

It is interesting to consider models other than the sequential state model that might equally well explain the fast and slow inactivation of the EC coupling process. For example the results could also be explained if activation and inactivation acted in an independent, parallel manner (Chua & Dulhunty, 1989). In addition, the possibility that one component of inactivation occurs in the unknown coupling mechanism between the voltage sensor and the calcium release channel cannot be excluded.

Similarities between gating of excitation–contraction coupling and sodium channels

Fast and slow inactivation of the voltage-dependent sodium conductance have been observed. Slow inactivation of sodium channels in myelinated nerve (Brismar, 1977) and skeletal muscle (Almers, Stanfield & Stuhmer, 1983; Ruff, Simoncini & Stuhmer, 1987) is reminiscent of slow inactivation of EC coupling in that it acts over a time scale of minutes. The structural similarities and homology between the sodium channel and the dihydropyridine receptor calcium channel are striking (Tanabe, Takeshima, Mikami, Flockerzi, Takahashi, Kangawa, Kojima, Matsuo, Hirose & Numa, 1987) and raise the possibility that fast and slow inactivation depend on similar mechanisms in the two protein molecules. If this is the case the possibility raised above, that one component of the inactivation of EC coupling occurs in the coupling process between the voltage sensor and calcium release channel, can be excluded.

A second striking parallel between inactivation of sodium channels and the voltage sensor for EC coupling is that both processes occur at more negative membrane potentials in fast-twitch mammalian skeletal muscle fibres than in slow-twitch fibres (Ruff *et al.* 1987; Chua & Dulhunty, 1988, 1989). This suggests the voltage sensitivity of inactivation in the two different proteins might be modulated by a common cytosolic factor or by the membrane environment.

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REFERENCES

ADRIAN, R. H., CHANDLER, W. K. & RAKOWSKI, R. F. (1976). Charge movement and mechanical repriming in skeletal muscle. *Journal of Physiology* **254**, 361–388.

ALMERS, W., STANFIELD, P. R. & STUHMER, W. (1983). Slow changes in currents through sodium channels in frog muscle membrane. *Journal of Physiology* **339**, 253–271.

ARIANO, M. A., ARMSTRONG, R. B. & EDGERTON, V. R. (1973). Hindlimb muscle fiber populations of five mammals. *Journal of Histochemistry and Cytochemistry* **21**, 51–55.

AXELSSON, J. & THESLEFF, S. (1958). Activation of the contractile mechanism in striated muscle. *Acta Physiologica Scandinavica* **44**, 55–66.

BERWE, D., GOTTSCHALK, G. & LUTTGAU, H. CH. (1987). Effects of the calcium antagonist gallopamil (D600), upon excitation–contraction coupling in toe muscle fibres of the frog. *Journal of Physiology* **385**, 693–707.

BRISMAR, T. (1977). Slow mechanism for sodium permeability inactivation in myelinated nerve fibre of *Xenopus laevis*. *Journal of Physiology* **270**, 283–297.

BRUM, G., FITTS, R., PIZARRO, G. & RIOS, E. (1988). Voltage sensors of the frog skeletal muscle membrane require calcium to function in excitation–contraction coupling. *Journal of Physiology* **398**, 475–505.

BRUM, G. & RIOS, E. (1987). Intramembrane charge movement in frog skeletal muscle fibres. Properties of charge 2. *Journal of Physiology* **387**, 489–517.

CAPUTO, C. (1972). The time course of potassium contractures of single muscle fibres. *Journal of Physiology* **223**, 483–505.

CAPUTO, C. (1976). The effect of caffeine and tetracaine on the time course of potassium contractures of single muscle fibres. *Journal of Physiology* **255**, 191–207.

CAPUTO, C. & DE BOLANOS, P. F. (1979). Membrane potential, contractile activation and relaxation rates in voltage clamped short muscle fibres of the frog. *Journal of Physiology* **289**, 175–189.

CAPUTO, C. & DE BOLANOS, P. (1990). Ultraslow contractile inactivation in frog skeletal muscle fibers. *Journal of General Physiology* **96**, 47–56.

CHANDLER, W. K., RAKOWSKI, R. F. & SCHNEIDER, M. F. (1976). Effects of glycerol treatment and maintained depolarization on charge movement in skeletal muscle. *Journal of Physiology* **254**, 285–316.

CHUA, M. & DULHUNTY, A. F. (1988). Inactivation of excitation–contraction coupling in rat extensor digitorum longus and soleus muscles. *Journal of General Physiology* **91**, 737–757.

CHUA, M. & DULHUNTY, A. F. (1989). Noninactivating tension in rat skeletal muscle. Effects of thyroid hormone. *Journal of General Physiology* **94**, 183–203.

CLOSE, R. I. (1972). Dynamic properties of mammalian skeletal muscles. *Physiological Reviews* **52**, 129–197.

CLOSE, R. I. & LUFF, A. R. (1974). Dynamic properties of inferior rectus muscle of the rat. *Journal of Physiology* **236**, 259–270.

DELAY, M., RIBALET, B. & VERGARA, J. (1986). Caffeine potentiation of calcium release in frog skeletal muscle fibres. *Journal of Physiology* **375**, 535–559.

DULHUNTY, A. F. (1979). Distribution of potassium and chloride permeability over the surface and T-tubule membranes of mammalian skeletal muscle. *Journal of Membrane Biology* **45**, 293–310.

DULHUNTY, A. F., CARTER, G. & HINRICHSEN, C. (1984). The membrane capacity of mammalian skeletal muscle fibres. *Journal of Muscle Research and Cell Motility* **5**, 315–332.

DULHUNTY, A. F. & GAGE, P. W. (1983). Asymmetrical charge movement in slow- and fast-twitch mammalian muscle fibres in normal and paraplegic rats. *Journal of Physiology* **341**, 213–231.

DULHUNTY, A. F. & GAGE, P. W. (1985). Excitation–contraction coupling and charge movement in denervated rat extensor digitorum longus and soleus muscles. *Journal of Physiology* **358**, 75–89.

DULHUNTY, A. F. & GAGE, P. W. (1988). Effects of extracellular calcium concentration and dihydropyridines on contraction in mammalian skeletal muscle. *Journal of Physiology* **399**, 63–80.

DULHUNTY, A. F., GAGE, P. W. & LAMB, G. D. (1987). Potassium contractures and asymmetric charge movement in extensor digitorum longus and soleus muscles from thyrotoxic rats. *Journal of Muscle Research and Cell Motility* **8**, 289–296.

EUSEBI, F., MILEDI, R. & TAKAHASHI, T. (1980). Calcium transients in mammalian muscles. *Nature* **284**, 560–561.

EUSEBI, F., MILEDI, R. & TAKAHASHI, T. (1985). Aequorin-calcium transients in mammalian fast and slow muscle fibers. *Biomedical Research* **6**, 129–138.

FRYER, M. W. & NEERING, I. R. (1989). Actions of caffeine on fast- and slow-twitch muscles of the rat. *Journal of Physiology* **416**, 435–454.

GRAF, F. & SCHATZMANN, H. J. (1984). Some effects of removal of external calcium on pig striated muscle. *Journal of Physiology* **349**, 1–13.

HEISTRACHER, P. & HUNT, C. C. (1969*a*). The relation of membrane changes to contraction in twitch muscle fibres. *Journal of Physiology* **201**, 589–611.

HEISTRACHER, P. & HUNT, C. C. (1969*b*). Contractile repriming in snake twitch muscle fibres. *Journal of Physiology* **201**, 613–626.

HODGKIN, A. L. & HOROWICZ, P. (1960). Potassium contractures in single muscle fibres. *Journal of Physiology* **153**, 386–403.

LAMB, G. D. & STEPHENSON, D. G. (1990). Control of calcium release and the effect of ryanodine in skinned muscle fibres of the toad. *Journal of Physiology* **423**, 519–542.

LUFF, A. R. (1981). Dynamic properties of the inferior rectus, extensor digitorum longus, diaphragm and soleus muscles of the mouse. *Journal of Physiology* **313**, 161–171.

LUTTGAU, H. CH. (1963). The action of calcium ions on potassium contractures of single muscle fibres. *Journal of Physiology* **168**, 679–697.

LUTTGAU, H. CH., GOTTSCHALK, G. & BERWE, D. (1986). The role of Ca^{2+} in activation and paralysis of excitation–contraction coupling in skeletal muscle. *Fortschritte der Zoologie* **33**, 195–203.

LUTTGAU, H. CH. & SPIECKER, W. (1979). The effects of calcium deprivation upon mechanical and electrophysiological parameters in skeletal muscle fibres of the frog. *Journal of Physiology* **296**, 411–429.

NAGAI, T., TAKAUJI, M., KOSAKA, I. & TSUTSU-URA, M. (1979). Biphasic time course of inactivation of potassium contractures in single twitch muscle fibers of the frog. *Japanese Journal of Physiology* **29**, 539–549.

RAKOWSKI, R. F. (1981). Immobilization of membrane charge in frog skeletal muscle by prolonged depolarization. *Journal of Physiology* **317**, 129–148.

RIOS, E. & BRUM, G. (1987). Involvement of dihydropyridine receptors in excitation–contraction coupling in skeletal muscle. *Nature* **325**, 717–720.

RUFF, R. L., SIMONCINI, L. & STUHMER, W. (1987). Comparison between slow sodium channel inactivation in rat slow- and fast-twitch muscle. *Journal of Physiology* **383**, 339–348.

SAINT, D. A., THOMAS, T. & GAGE, P. W. (1990). $GABA_B$ agonists modulate a transient potassium current in cultured mammalian hippocampal neurons. *Neuroscience Letters* **118**, 9–13.

SCHNEIDER, M. F. & CHANDLER, W. K. (1976). Effects of membrane potential on the capacitance of skeletal muscle fibers. *Journal of General Physiology* **67**, 125–163.

STEPHENSON, D. G. & WILLIAMS, D. A. (1981). Calcium-activated force responses in fast- and slow-twitch skinned muscle fibres of the rat at different temperatures. *Journal of Physiology* **317**, 281–302.

TANABE, T., TAKESHIMA, H., MIKAMI, A., FLOCKERZI, V., TAKAHASHI, H., KANGAWA, K., KOJIMA, M., MATSUO, H., HIROSE, T. & NUMA, S. (1987). Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature* **328**, 313–318.

VERGARA, J., TSIEN, R. Y. & DELAY, M. (1985). Inositol 1,4,5-trisphosphate: A possible chemical link in excitation–contraction coupling in muscle. *Proceedings of the National Academy of Sciences of the USA* **82**, 6352–6356.

WENDT, I. R. & STEPHENSON, D. G. (1983). Effects of caffeine on Ca-activated force production in skinned cardiac and skeletal muscle fibres of the rat. *Pflügers Archiv* **398**, 210–216.