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Potassium Contractures and Mechanical Activation in Mammalian Skeletal Muscles

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Summary. Potassium (K-) contractures were recorded from slow-twitch (mouse soleus) and fast-twitch (mouse extensor digitorum longus (EDL) and rat sternomastoid) muscles. The mouse limb muscles responded to a maintained increase in external potassium concentration with a rapid increase in tension (fast contracture) which inactivated and was followed by a slow contracture. Rat sternomastoid muscles responded with fast contractures only. The threshold potassium concentration for contraction was higher in fast-twitch muscles than in soleus muscles, at 22 and at 37 °C. After corrections had been made for the more rapid depolarization of soleus fibers, the threshold potential for soleus fiber contraction was 15 mV closer to the resting membrane potential than the threshold for fast-twitch fiber contraction. The K-contracture results were confirmed by two microelectrode voltage-clamp experiments. Activation of fast twitch fibers required depolarizing pulses that were 15 to 20 mV greater than the pulses required to activate soleus fibers. When the time courses of K-contractures were compared it was evident that inactivation with prolonged depolarization was much faster in the fast-twitch muscles than in the soleus muscles. The results suggest that the voltage dependence and kinetics of the process coupling T-tubule depolarization with calcium release from the sarcoplasmic reticulum may depend on fiber type in mammalian skeletal muscle.

With few exceptions mammalian skeletal muscles are twitch muscles and their subdivision into fast or slow-twitch depends on isometric twitch contraction time and resistance to fatigue. There are well documented morphological and biochemical differences between fast and slow-twitch muscle fibers (Close, 1972; Burke & Edgerton, 1975), and the different contractile properties have been correlated with different myosin isoenzymes and with rates of calcium accumulation by the sarcoplasmic reticulum. In addition, it has been suggested that there are differences in the kinetics of excitation-contraction coupling and activation of fast and slow-twitch muscle (Close, 1972). The aim of this study has been to compare excitation-contraction coupling in fast and slow-twitch muscles by examining the relation between membrane potential and tension. Two techniques have been successfully used in similar studies in amphibian preparations. Potassium (K-) contractures (Hodgkin & Horowicz, 1960; Caputo, 1972*a, b*; Costantin, 1972) have been used to determine the kinetics of mechanical activation during prolonged depolarization, and a two-microelectrode voltage-clamp technique (Adrian, Chandler & Hodgkin, 1969; Costantin, 1974; Almers & Best, 1976) has been used to determine the threshold for mechanical activation by brief depolarizing pulses.

Previous experiments (Lorkovic, 1971) on red and white rat muscle have shown that a lower concentration of external potassium is needed to produce tension in red muscles than in most white muscles, although the threshold membrane potential, determined by current pulse techniques, was similar in all muscles. The results presented below show that the threshold membrane potential for contraction, determined in K-contracture and voltage-clamp experiments, is 15 mV closer to the resting membrane potential in slow-twitch (red) fibers than in fast-twitch (red or white) fibers. In addition, mechanical inactivation with prolonged depolarization proceeds 3–6 times more rapidly in fast-twitch (red or white) fibers than in slow-twitch (red) fibers. Some of these results have been reported briefly elsewhere (Dulhunty, 1978, 1979*a*).

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Table 1. Table of Solutions

Solution	Ion concentration (mM)								Sucrose (mM)
	Na	K	Ca	Mn	Cl	SO_4	HCO_3	Hepes	
A[a]	145	3.5	2.5	–	130.5	–	25	–	–
B	150	3.5	2.5	–	158.5	–	–	1.0	–
C	0	224	5.0	–	2.4	117	–	1.0	–
D[b]	150	3.5	2.5	20	159.5	–	–	1.0	500
E[b]	0	224	5.0	20	2.4	117	–	1.0	500
F[a]	145	3.5	2.5	–	130.5	–	25	–	44

NB: In addition, all solutions contained 1 mM $MgCl_2$ and 11 mM glucose.
[a] Solutions bubbled with carbogen (5% CO_2, 95% O_2) to pH = 7.4.
[b] Sodium dantrolene = 10^{-6} g/ml and TTX = 5×10^{-7} g/ml (some dantrolene remained undissolved and the exact concentration in solution was not known).

Methods

The experiments were performed on mouse (C57BL) soleus (typical slow-twitch muscles, Luff & Atwood, 1972) and extensor digitorum longus muscles (EDL) and dissected bundles of rat (wistar) red and white sternomastoid muscles (typical fast-twitch muscles; Luff & Atwood, 1972; Dulhunty & Dlutowski, 1979). The preparations were mounted in a perspex bath which was surrounded by a temperature controlled water jacket. Solutions at the same temperature flowed continuously over the preparation. The volume of the bath was small and the solution could be changed in 1 sec. Isometric tension was recorded with an RCA 5734 transducer. Preparations were stimulated electrically with two electrodes situated on either side of the muscle. The stimulating pulse parameters were adjusted to supramaximal values (with the pulse duration < 1 msec). Muscle length was adjusted to give maximum tetanic tension. Mouse EDL and soleus muscles recovered slowly, and often incompletely, from a K-contracture (Lorkovic, 1971, Dulhunty, 1981), and a second contracture was always smaller and had a faster time course than the first contracture. For this reason only one contracture was recorded from each mouse muscle.

Solutions

The solutions most commonly used are shown in Table 1. K-contractures were normally induced by changing the external solution from a control solution containing 3.5 mM potassium with Cl as the main anion (solution *A* or *B*) to a test solution containing 224 mM potassium with SO_4 as the main anion (solution *C*). In some experiments the potassium concentration of the test solution was varied but the [K]·[Cl] product was kept constant by appropriate replacement of Cl with SO_4. These solutions were kept isosmotic with solution *C* by appropriate replacement of K_2SO_4 with Na_2SO_4 or NaCl.

Electrical Measurements

Intracellular glass microelectrodes filled with 3 M KCl were used to measure membrane potential and to pass current in voltage clamp and current clamp experiments. The space constant and input resistance of fibers were determined by standard cable analysis techniques (Fatt & Katz, 1952). The size of at least 20 voltage transients (in response to a constant current pulse) were sampled by a microprocessor system at each electrode separation along the fiber, and the average data were used to calculate the cable constants.

A two-microelectrode voltage-clamp technique was used to determine the mechanical activation threshold (Adrian et al., 1969; Costantin, 1974; Almers & Best, 1976). The two microelectrodes were inserted into the fiber between 50 and 100 μm apart. The membrane between the microelectrodes was observed through a dissecting microscope (× 100). Pulse duration was set and pulse amplitude increased until contraction of surface myofibrils was just observed and then decreased in 0.5 mV steps until contraction was no longer visible. The membrane potential during the step to just below threshold was recorded. There was some hysteresis in this method in that the threshold value finally recorded at the end of a run was several millivolts closer to the holding potential than the potential at which contraction was first observed. Similar hysteresis has been described in frog fibers (Costantin, 1974). The reason for the hysteresis is not clear, but it is independent of repetition rate between 0.1 and 2.0 Hz. The accuracy of the threshold determination was such that, for a particular pulse duration, threshold could be determined to within ±0.5 mV in repeated trials. The accuracy was less (±2 mV) for brief (0.1 to 1.0 msec) pulses. The membrane potential in a voltage step was established in less than 20 μsec.

Results

EDL and Soleus K-Contractures

K-contractures recorded from mouse EDL and soleus muscles are shown in Fig. 1. The external potassium concentration was increased at a time indicated by the step in the black bar above the records. The muscles were maintained in the high potassium solution for the duration of the contracture. The EDL muscle at 22 °C (upper trace; Fig. 1 *A*) developed a fast K-contracture which quickly inactivated to about 60% of its peak value and was then followed by a slow contracture which also inactivated with time. Most EDL muscles did not have a slow contracture at higher temperatures (middle and lower traces; Fig. 1 *A*). Soleus muscles also responded to an increase in external potassium concentration with a fast and a slow contracture. The two contractures are clear in Fig. 1 *D* where the potassium concentration was increased to 60 mM. When the potassium concentration was in-

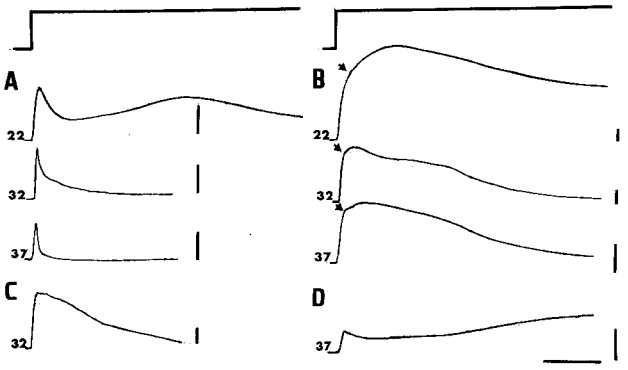

Fig. 1. K-contractures recorded from mouse EDL and soleus muscles. The K-contracture records were taken at 22, 32, and 37 °C as shown at the beginning of each trace. The muscles were equilibrated in a 3.5 mM potassium solution (solution *B*, Table 1) before being exposed to the high potassium solution at times indicated by the step in the line above the record. (*A*): EDL K-contractures in 224 mM-potassium; (*B*): soleus K-contractures in 224 mM-potassium; (*C*): fast soleus K-contractures in 224 mM-potassium at 32 °C; (*D*): soleus K-contracture in 80 mM-potassium at 32 °C. The arrows in *B* indicate the inflection in the tension curve which corresponds in time to the peak of fast contractures in *C* and *D*. The horizontal calibration is 24 sec, and the vertical calibration is 2.0 g

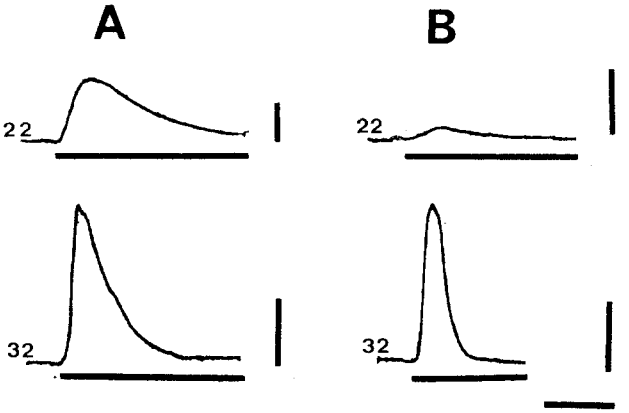

Fig. 2. K-contractures recorded from red and white rat sternomastoid muscles when the external potassium concentration was changed from 3.5 mM (solution *B*, Table 1) to 224 mM (solution *C*, Table 1) for the period indicated by the line under each record. Records were taken at 22 and 32 °C. Temperature, in °C, is shown on the left-hand side of each record. (*A*): Records from red sternomastoid bundles. (*B*): Records from white sternomastoid bundles. The horizontal calibration is 12 sec and the vertical calibration is 1.0 g

creased to 224 mM (Fig. 1 *B*), the two contractures overlapped. There was always an inflection in the tension profile (*see* arrows in Fig. 1 *B*) at a similar time to the peak of the fast contracture seen in Fig. 1 *D*. The slow contracture was sometimes absent from soleus muscles at higher temperatures (Fig. 1 *C*), and the resulting fast contracture was compared with the fast EDL contractures. The time course, temperature sensitivity, and voltage dependence (*see* below) of the fast contracture were qualitatively similar to properties of amphibian muscle K-contractures (Hodgkin & Horowicz, 1960; Costantin, 1972; Caputo, 1972*a*, *b*), suggesting that it is generated by the activation process involved in twitch and tetanic contraction. The properties of the slow contracture are described elsewhere (Dulhunty, 1981).

Sternomastoid K-Contractures

K-contractures recorded from red and white sternomastoid bundles at 22 and 32 °C are shown in Fig. 2. The sternomastoid bundles did not have a slow potassium contracture. The time to peak and decay time of the K-contractures decreased at higher temperatures (*see* Fig. 2), as did the fast EDL and soleus contractures (*see* Fig. 1). However, unlike the EDL and soleus contractures, the peak K-contracture tension in sternomastoid bundles was greater at higher temperatures. This temperature effect is particularly obvious in white sternomastoid bundles. The contracture at 22 °C, shown in Fig. 2 *B*, was the largest K-contracture recorded from a white sternomastoid bundle at that temperature, more commonly, there was no recordable tension. The record at 32 °C in Fig. 2 *B* is a typical white sternomastoid contracture at a higher temperature.

Comparison of K-Contractures at 32 °C

The fast component of soleus and EDL K-contractures is compared with red and white sternomastoid K-contractures in Table 2. Average values for the amplitude, time to peak, and 50% decay time of K-contractures recorded at 32 °C are listed. K-contracture tension is expressed as a fraction of maximum tetanic tension. Specific tetanic tension was calculated from measured cross-sectional areas of frozen sections of several experimental preparations, and average values are listed in the first line of Table 2. The values obtained for each muscle were constant and K-contracture tension was generally normalized to tetanic tension. The average results in Table 2 show that the soleus K-contracture was larger and slower than the K-contracture recorded from the fast twitch muscles. The different rates of K-contracture inactivation must mean that soleus fibers have a slower rate of mechanical inactivation with prolonged depolarization than the fast-twitch fibers. It would be most

Table 2. Average parameters of fast K-contractures recorded at 32 °C when the external potassium concentration was increased from 3.5 mM to 224 mM[a]

	Specific tetanic tension (kg/cm^2)[b]	K-contracture tension (as a fraction of tetanic tension)	Time to peak tension (sec)	50% decay time (sec)
Soleus	1.63 ±0.04(8)	0.23 ±0.01(5)	8.2 ±1.1(5)	55.6 ±8.6(5)
EDL	1.55 ±0.09(8)	0.13 ±0.03(5)	3.4 ±0.24(5)	10 ±0.9(5)
R.S.[c]	2.67 ±0.24(8)	0.12 ±0.03(8)	9.8 ±0.8(8)	22.6 ±2.6(8)
W.S.[c]	2.50 ±0.23(8)	0.08 ±0.04(6)	4.8 ±0.48(6)	11.0 ±0.45(6)

[a] Average parameters are shown with ±1 SEM and the number of muscles in parentheses.
[b] Cross-sectional area was measured from frozen sections of 3 preparations of each muscle, and was calculated from two measured dimensions of the remaining 5 preparations (assuming an elliptical outline). Areas determined by the two techniques were similar.
[c] Red sternomastoid and white sternomastoid abbreviated to R.S. and W.S., respectively.

Table 3. Cross-sectional areas of preparations and fibers in the preparation[a]

		Soleus	Red SM[b]	EDL	White SM[b]
Preparation area	Mean (cm^2)	0.013	0.007	0.015	0.008
	±SE	±0.001	±0.001	±0.002	±0.002
	n	8	8	8	8
Fibre area (*A*)	Mean (μm^2)	4351	2104	4049	5109
	±SE	±90	±67	±100	±224
	n	621	201	474	207
Equivalent diameter	$D=\frac{\sqrt{4A}}{\pi}$	74.4	51.75	71.8	80.65

[a] Muscle cross-sectional areas measured as described in Table 2. Fiber cross-sectional areas were calculated from two measured axes of fibers in transverse frozen sections (Dulhunty & Dlutowski, 1979). An eliptical outline was assumed to facilitate the calculation.
[b] Sternomastoid has been abbreviated to SM.

Table 4. Rate of depolarization of fibers on the surface (1) and next two layers (2 and 3) below the surface of three soleus and EDL muscles following a change in $[K]_o$ from 3.5 to 224 mM, as illustrated in Fig. 2.

Layer	Soleus depolarization rate (mV/sec)	EDL depolarization rate (mV/sec)
1	22.73±1.4 (33)	24.0±1.3 (35)
2	11.55±1.2 (18)	11.65±0.9 (32)
3	7.25±1.04 (32)	7.37±0.5 (22)

[a] Results are expressed as mean ±SE and the number of fibers is given in brackets

useful to interpret the different K-contracture sizes in terms of the mechanical responses of individual slow-twitch and fast-twitch fibers. However, geometrical factors could have altered the characteristics of the K-contracture and must be considered before such an interpretation is made.

Diffusion Distance: Cross-Sectional Area Measurements

The results of cross-sectional area measurements in Table 3 indicate that the difference between soleus and fast-twitch muscle K-contractures cannot be accounted for in terms of simple geometrical factors. EDL and soleus muscles had similar cross-sectional areas (line 1, Table 3) and similar fiber diameters (line 3, Table 3). Sternomastoid bundles also had similar cross-sectional areas (line 1, Table 3) but very different fiber diameters (line 3, Table 3).

Diffusion Rates

The rate of potassium diffusion through the muscle is reflected in the initial rates of depolarization of fibers at different distances from the surface of the muscle. Membrane potential records from EDL and soleus fibers are shown in Fig. 3*B* and *C* and the position of the fibers in the muscle shown schematically in Fig. 3*A*. The average rates of depolarization, measured during the first two seconds of depolarization, are given in Table 4. The rate of depolarization of deep fibers was slower as predicted by the longer diffusion distances. There is, however, no significant difference between results from EDL and soleus muscles.

The lack of correlation between preparation geometry and K-contracture size suggests that the K-contractures depend on intrinsic properties of individual muscle fibers. These properties include T-tubule geometry (which could alter the rate of potassium equilibration in the T-system and rate of T-tubule membrane depolarization) and the characteristics of the mechanical activation process.

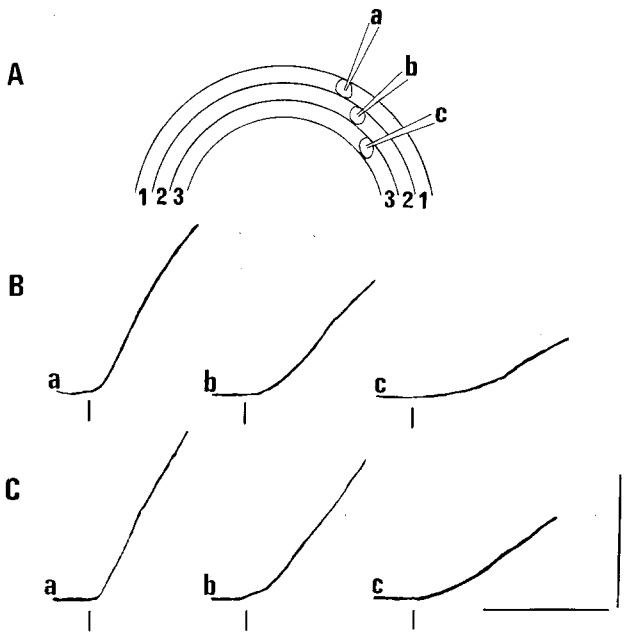

Fig. 3. Intracellular membrane potential recorded from EDL and soleus muscles when the external potassium concentration was changed from 3.5 mM (solution *B*; Table 1) to 224 mM (solution *C*; Table 1) at a time indicated by the vertical line beneath each record. TTX, 5×10^{-7} g/ml and Na dantrolene (*see* Methods) were added to the solution. (*A*): A schematic illustration of part of a cross-section of an EDL or soleus muscle, showing the concentric arrangement of fibers, into layers which have been labeled *1* (surface fibers), *2* and *3*. Records labeled *a* were obtained from layer *1*. Records labeled *b* were obtained by gently pushing the electrode through a fiber in layer *1* and into the fiber beneath it. Records labeled *c* were obtained by gently pushing the electrode through the fiber in layer *2* and into a fiber in layer *3*. The increase in diffusion distance with each step was roughly equal to the diameter of the fiber through which the electrode had been pushed. (*B*): Records from soleus fibers; (*C*): records from EDL fibers. The preparations were exposed to 224 mM potassium for 3 to 5 sec, and complete recovery of membrane potential in 3.5 mM potassium was allowed between subsequent exposures to 224 mM potassium. The vertical calibration is 25 mV, and the horizontal calibration is 2 sec. Temperature = 22 °C

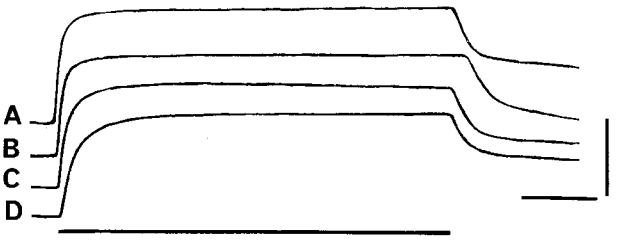

Fig. 4. Intracellular membrane potential records showing depolarization to a new steady-state value when the external potassium concentration was changed from 3.5 mM (solution *D*, Table 1) to 224 mM (solution *E*, Table 1) for the period indicated by the line under the records. All records were from fibers on the surface of the muscles or bundles (layer 1; Fig. 3*C*). (*A*): A record from a soleus fiber; (*B*): a record from a red sternomastoid fiber; (*C*): a record from an EDL fiber; (*D*): a record from a white sternomastoid fiber. The horizontal calibration is 24 sec, and the vertical calibration is 40 mV. Temp. = 22 °C

Potassium Equilibration in the T-System

The time course of potassium equilibration in the T-system of individual fibers can be estimated from the time course of depolarization, when this is measured over a longer time than that shown in Fig. 3 (Nakajima, Nakajima & Peachey, 1973; Dulhunty, 1979*b*). Records from preparations equilibrated in a hypertonic solution (solution *D*, Table 1), to eliminate movement, and then exposed to a 224 mM potassium solution (solution *E*, Table 1) are shown in Fig. 4. The initial rate of depolarization of soleus and EDL fibers (Fig. 4*A* and *C*) was the same as that recorded under isotonic conditions (*see* Fig. 3*Ba* and *Ca*). The steady-state membrane potential in 224 mM-potassium was −20 mV in each record in Fig. 4. The membrane potential approached its steady-state value most quickly in soleus fibers (Fig. 4*A*) and most slowly in white sternomastoid fibers (Fig. 4*D*). The average decay of membrane potential to its steady-state value has been plotted in Fig. 5, using a logarithmic scale to display small slow changes in potential. The decay of membrane potential has a fast component, with a time course which reflects the rate of K-diffusion to the surface membrane, and a slow component, with a time course which reflects the rate of potassium diffusion into the T-system. There is of course significant overlap between surface and T-tubule membrane depolarization (for an analysis of the time course of depolarization in red sternomastoid fibers, *see* Fig. 2, Dulhunty, 1979*b*). Slow T-tubule depolarization in white sternomastoid fibers, for example, is reflected in the slopes of both components (open circles, Fig. 5). The similarity between initial depolarization rates in EDL and soleus fibers (Table 3) is true for only 1 or 2 sec after the solution change.

It is interesting to note that the data in Fig. 5 is consistent with known properties of the T-system in each muscle. The volume fraction of T-system is smallest in soleus fibers (Luff & Atwood, 1972; Eisenberg & Kuda, 1976) and the consequently smaller area of T-tubule membrane would minimize the influence of T-tubule membrane potential on the recorded depolarization, provided the relative potassium permeability of the T-tubule membranes were similar. The volume fraction of the T-system is similar in red and white sternomastoid fibers (C.F., Franzini-Armstrong & L.D. Peachey, *personal communication*). However, the total area of T-tubule membrane must be greater in white sternomastoid fibers because of their greater cross-sectional area (Hodgkin & Nakajima,

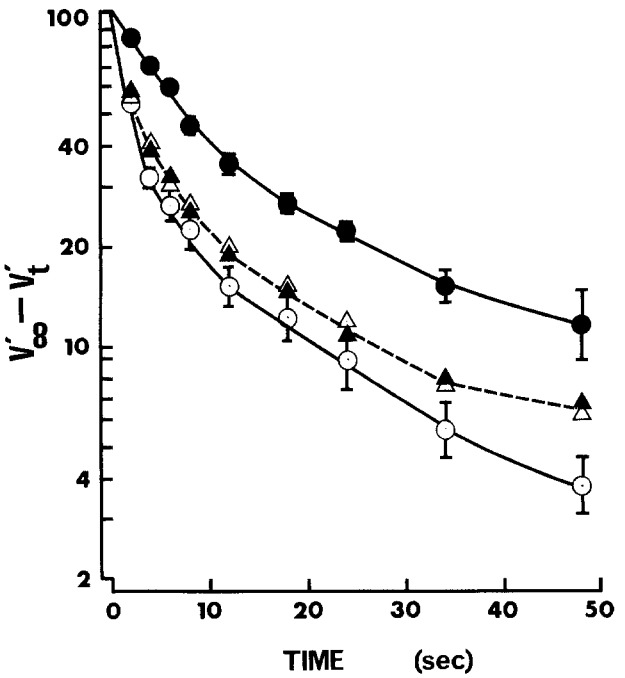

Fig. 5. Decay of membrane potential from a steady-state value in the 3.5 mM potassium solution to a steady-state value in the 224 mM potassium solution. The points were obtained from records similar to, and including, the records in Fig. 4. The difference between the steady-state depolarization, $V_m\infty$, and the depolarization at time t, $V_m(t)$, and the normalized values (normalized to $V_m\infty$), $V'_\infty - V'_t$ are shown on the vertical axis. Time, in seconds, is shown on the horizontal axis. Open circles – average values from 4 soleus fibers; open triangles – average values from 3 EDL fibers; filled triangles – average values from 3 red sternomastoid fibers; filled circles – average values from 4 white sternomastoid fibers. Only those fibers which repolarized to their original steady-state value when returned to 3.5 mM potassium were used for measurement of membrane potential. The solid lines were drawn by eye through data from white sternomastoid and soleus fibers. The broken line was drawn by eye through data from EDL and white sternomastoid fibers. The vertical bars show ±SEM where this is larger than the symbol

1972), and T-tubule membrane potential would have a greater influence on the recorded depolarization, provided potassium permeability is evenly distributed over all membranes. In addition, potassium equilibration through the longer length of white sternomastoid T-system would be slower than potassium equilibration in the T-system of smaller diameter fibers.

There is a correlation between the rate of depolarization (Fig. 5) and K-contracture amplitude (Table 2). Soleus fibers depolarize most rapidly and have the largest K-contracture. White sternomastoid fibers demonstrate the slowest depolarization and have the smallest K-contracture. Since tension depends on membrane potential, it is important to know the membrane potential of fibers at the peak of the K-contracture in order to compare K-contractures in different muscles.

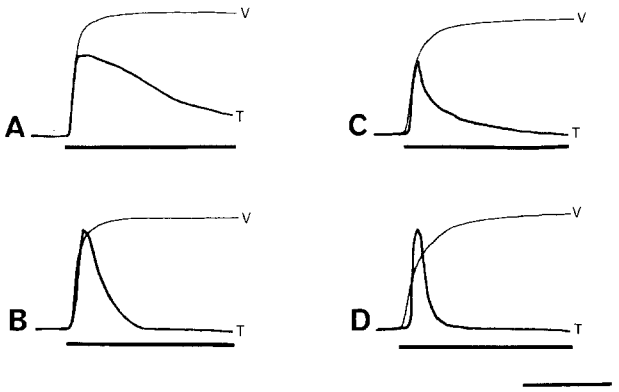

Fig. 6. Comparison of the time course of depolarization and tension following a change in the external potassium concentration from 3.5 to 224 mM, indicated by the line under each pair of records. The thin lines, V, were traced from depolarization records in Fig. 4 and the thicker lines, T, were traced from tension records in Figs. 1 and 2. (*A*): Soleus; (*B*): red sternomastoid; (*C*): EDL; (*D*): white sternomastoid. Horizontal calibration, 24 sec

Comparison of the Time Course of K-Contractures and Depolarization

Traced records of membrane potential and tension have been superimposed in Fig. 6. In each case the peak K-contracture tension was recorded during the rapid phase of depolarization, well before significant potassium diffusion into the T-system. The membrane potential records were taken from surface fibers. The comparisons shown in Fig. 6 imply that the maximum K-contracture tension was generated by fibers close to the surface of the muscle. Fibers deep in the muscle would not be significantly depolarized at times corresponding to peak of the K-contracture because of the longer diffusion distance for potassium ions. The membrane potential generating the peak K-contracture tension can be assumed to be close to the membrane potential of the surface fibers at times corresponding to the peak of the K-contracture. The average times to peak tension of EDL and soleus K-contractures are listed in Table 5 together with the fraction of the steady-state depolarization achieved by surface fibers at the time of the peak (the latter values were read from curves shown in Fig. 5). It is clear that tension in soleus muscles develops in response to a greater depolarization than it does in EDL fibers, and so it is not surprising that soleus muscles have a greater K-contracture amplitude. The results do not exclude the possibility that the voltage dependence of contraction is different in fast- and slow-twitch muscles and this possibility is examined in later sections of the paper.

The fact that the peak of the K-contracture occurs before significant potassium diffusion into the T-sys-

Table 5. Membrane potential of surface EDL and soleus fibers at times corresponding to the peak of the K-contructure[a]

		Time to peak tension (sec)		Depolarization	
		20 °C	37 °C	20 °C	37 °C
EDL	Mean	6.57	3.40	0.72	0.62
	± SEM	±0.36	±0.25		
Soleus	Mean	7.00	4.55	0.78	0.72
	± SEM	±0.48	±0.31		
	n	7	10		

[a] Depolarization is expressed as a fraction of the steady-state membrane potential in 224 mM external potassium

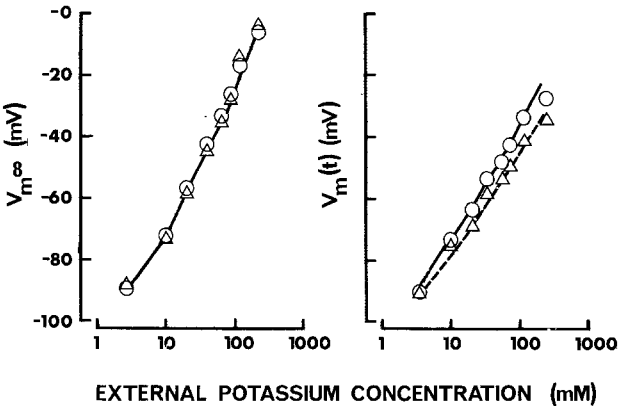

Fig. 7. Intracellular membrane potential in EDL and soleus fibers exposed to different external potassium concentrations. External potassium concentration, in mM, is shown on the horizontal axes. (*A*): Steady-state membrane potential recorded between 5 and 15 min after a change in external potassium concentration. Preparations were allowed to recover in 3.5 mM potassium (solution *B*) between each exposure to a higher potassium concentration. Open circles show average results for soleus fibers and open triangles show average results for EDL fibers. Data from at least 10 fibers were used for each point, and the standard errors were smaller than the symbols. (*B*): Calculated membrane potential, $V_m(t)$, for fibers on the surface of the muscles at times corresponding to the peak of the K-contracture at 37 °C. $V_m(t)$ was calculated from the steady-state membrane potential in *A* using data given in Table 4

tem implies that K-contracture tension develops in response to the passive spread of surface membrane depolarization through the T-system. Inactivation, also turned on by depolarization, inhibits tension development before potassium equilibration in the T-system. The peak K-contracture tension is probably later in soleus muscles than in EDL muscles because of the slower onset of inactivation in soleus fibers.

The Dependence of Tension on Surface Membrane Potential

The voltage dependence of contraction in EDL and soleus muscles can be determined from the peak K-contracture tension recorded with different potassium concentrations in the test solution (*see* Methods). The steady-state membrane potential in each test solution is shown in Fig. 7*A*. The membrane potential corresponding to the peak of the EDL and soleus K-contracture was calculated from the steady-state membrane potential values using the fractions of steady-state depolarization listed in Table 4. Calculated values for EDL and soleus fibers at 37 °C are shown in Fig. 7*B*.

K-contractures recorded following exposure to the different test solutions are shown in Fig. 8. The potassium concentration in the test solution is shown on the left-hand side of each record. There is a clear difference between EDL and soleus muscles in the threshold potassium concentration for recordable tension. There was no EDL contracture when the test solution potassium concentration was less than 40 mM (Fig. 8*C* and *D*). On the other hand, soleus muscles developed K-contractures when exposed to 10 mM potassium of 22 °C (Fig. 8*A*). The 10 mM potassium

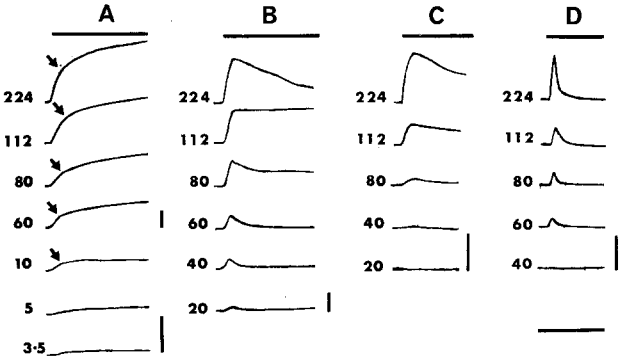

Fig. 8. Tension records obtained from EDL and soleus muscles when the external potassium concentration was changed from 3.5 mM (solution *A* or *B*, Table 1) to the concentration shown on the left-hand side of each record. The [K]·[Cl] product of each test solution was 538 and Cl was replaced by SO_4. Each test solution was isotonic with solution *C* (Table 1). (*A*): Soleus muscles at 22 °C; (*B*): soleus muscles at 37 °C; (*C*): EDL muscles at 22 °C; (*D*): EDL muscles at 37 °C. In *A*, the peak of the early phase was masked by the slow phase of contraction when potassium concentrations of 60 mM or more were used. An inflection in the rising phase, marked with an arrow, corresponded in time with the peak of contractures initiated by lower potassium concentrations and tension was measured at the inflection. Vertical calibration, 2 g; horizontal calibration, 24 sec

contracture in Fig. 1*A* is superimposed on a small hypertonic contracture which persisted in 5 and 3.5 mM potassium test solutions at 22 °C (Fig. 8*A*). Note the change in recorder gain for the last 3 records

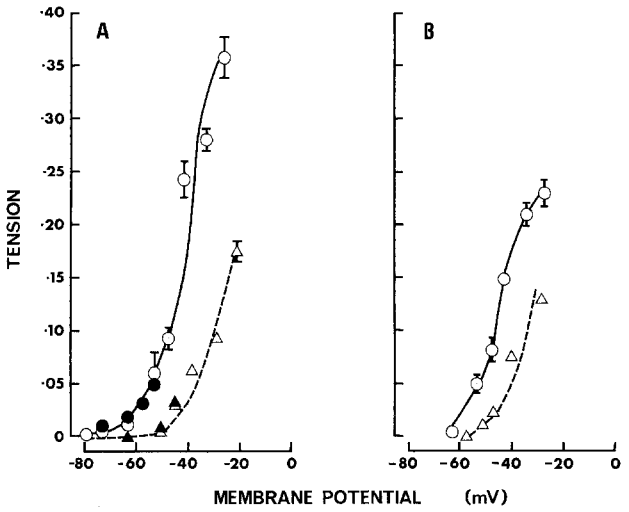

Fig. 9. Peak K-contracture tension as a function of the membrane potential at the peak of the contracture. K-contracture tension, normalized to maximum tetanic tension, is shown on the vertical axis, and membrane potential, calculated as described in the legend to Fig. 7, is shown on the horizontal axis. Open circles – average values for at least 5 soleus contractures at each potential; open triangles – average values for at least 5 EDL contractures at each potential. Vertical bars indicate 1 SEM, where this is greater than the symbol. Filled symbols – show contracture tension recorded from preparations that had been equilibrated in a hypertonic solution (solution *F*; Table 1) prior to exposure to the high potassium solutions. (*A*): Results obtained at 22 °C; (*B*): results obtained at 37 °C

in Fig. 8*A*. The peak K-contracture tension was read simply from EDL and soleus K-contractures at 37 °C and from EDL K-contractures at 22 °C. Tension was read at the inflection of the soleus K-contractures (*see* arrows in Figs. 1*A* and 8*A*) at 22 °C when higher potassium concentrations were used. A component of this tension must have been generated by the slow contracture, and thus the measured tension was an overestimate of the true fast-contracture tension.

Average peak tension is plotted against appropriate membrane potential values in Fig. 9 (*see* legend to Fig. 9). The threshold membrane potential for contraction is closer to the resting membrane potential in soleus muscles (open circles) than in EDL muscles (open triangles) at 22 °C (Fig. 9*A*) and at 37 °C (Fig. 9*B*). The peak tension values of 37 °C (Fig. 9B) were uncontaminated by slow contracture tension or by hypertonic tension and are therefore more accurate than the values measured at 22 °C (Fig. 9*A*). To exclude the possibility of a selective hypertonic effect on the soleus K-contracture, some fibers were equilibrated in a hypertonic solution (Solution *F*, Table 1) before exposure to the high K solutions. The results (filled symbols, Fig. 9*A*) were identical to those obtained from preparations equilibrated in normal Krebs (open symbols, Fig. 9*A*).

The different thresholds for EDL and soleus contraction may be indicative of a difference in the voltage dependence of activation. On the other hand, it could be explained by a more uniform spread of surface membrane potential through the T-system of soleus fibers. A two-microelectrode voltage-clamp technique was used to measure the threshold membrane potential for mechanical activation of surface myofibrils (Adrian et al., 1969). The technique depends on depolarization of areas of T-tubule membrane close to the (exterior) surface membrane. The results may be influenced by the "access" resistance to the T-tubules (Valdiosera, Claussen & Eisenberg, 1974) but are independent of overall T-tubule geometry.

Activation Threshold at 37 °C

Strength-duration curves for activation threshold were obtained for EDL, soleus, and red and white sternomastoid fibers using the two-microelectrode voltage-clamp technique described in the Methods section. Average strength-duration curves are shown in Fig. 10. The triangles are average values for EDL, red and white sternomastoid fibers. The fast-twitch fibers are shown collectively because their individual curves were not significantly different from each other. The circles are average values for soleus fibers. Soleus fibers were activated at potentials significantly closer to the holding potential (resting membrane potential) for all pulse durations tested.

There was a remote possibility that calcium influx through the slow calcium channel in the surface membrane (Sanchez & Stefani, 1978; Potreau & Raymond, 1979) might have influenced the value of the rheobase potential required to activate the surface myofibrils. However, it can be shown that the brief pulse (0.5 to 5 msec) threshold potential is more sensitive to withdrawal of calcium from the external solution (using solutions with zero added calcium, 1 mM Mg and 10 mM EGTA) than the rheobase potential (A.F. Dulhunty, *unpublished observations*). Thus it is unlikely that the surface membrane calcium current makes a significant contribution to activation.

The possible effect of fiber cable properties on the measurement of contraction threshold should be considered. The microelectrodes were inserted into the fiber 50 to 100 μm apart and contraction was observed between the electrodes. The two electrodes and the point of contraction were thus located with a length of the fiber that was equivalent to its diameter and very much shorter than its long pulse space constant (*see* Table 6). Thus the membrane potential between the current and voltage electrodes was essential-

ly uniform for long pulses. It might be argued that the clamp potential would spread for a greater longitudinal distance from the voltage electrode in fibers with longer space constants. As a result, more sarcomeres would be activated in a longitudinal direction; movement would therefore be more visible and contraction threshold apparently lower. The space constants listed in Table 5 do not bear any obvious relationship to the strength-duration curves shown in Fig. 10, suggesting that the effect of space constant on measured contraction threshold is not significant.

Discussion

The results from voltage-clamp and K-contracture experiments on EDL and soleus preparations indicate that the threshold membrane potential for contraction is 15 to 20 mV closer to the resting membrane potential in soleus fibers. The contraction thresholds determined with voltage-clamp techniques (Fig. 10) were 10 mV more positive than the K-contracture thresholds (Fig. 9). However, the voltage-clamp steps were brief compared with the time course of the K-contracture,and it is apparent (Fig. 10) that a constant rheobase potential was not reached with the longest pulse (1000 msec). The threshold potentials would have approached the K-contracture threshold potentials if longer pulses had been used.

The different K-contracture thresholds may have been due to more effective spread of surface membrane depolarization through the soleus fiber T-system. An "access" resistance at the mouth of T-system of fast-twitch fibers could account for the K-contracture results and could influence the results of the voltage-clamp experiments. The simple effect of an access resistance would be to reduce the rate of T-tubule membrane depolarization and, to a degree depending on the exact value of the access resistance, reduce the amplitude of brief pulses in the T-system. This would appear as an excessive increase in the apparent threshold for brief pulses in fast-twitch fibers. The fact that the activation curves for soleus and fast-twitch fibers are parallel for pulse durations from 0.5 to 1000 msec (*see* Fig. 10) argues against a significant access resistance to the fast-twitch T-system. It is likely that the contraction threshold data reveals a true difference between the voltage dependence of activation in fast and slow-twitch fibers.

The basis of the differences between fast and slow-twitch fibers is open to speculation. Experiments on skinned muscle fibers (Kerrick et al., 1976; Takagi & Endo, 1977; Stephenson & Forrest, 1979) show that slow-twitch fibers are more sensitive to Ca than fast-twitch fibers. However, the area of terminal

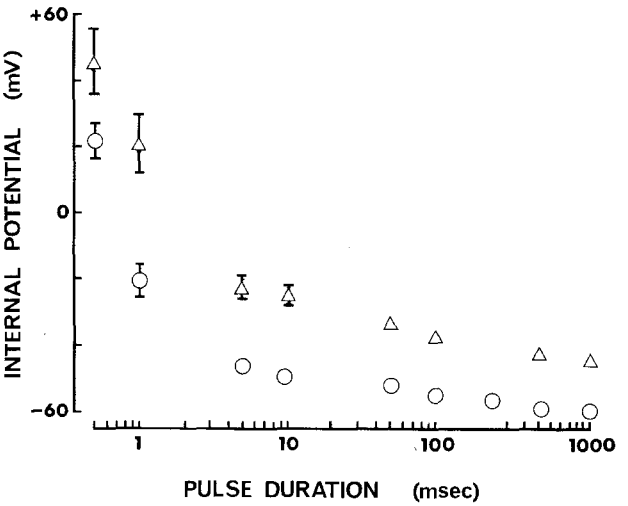

Fig. 10. Strength-duration curves for mechanical activation threshold at 37 °C. Membrane potential during the voltage step to just below contraction threshold (*see* Methods section) is shown on the vertical axis. Pulse duration is shown on the horizontal axis. The experiments were done in solution *A* (Table 1) with 5×10^{-7} g/ml TTX added to prevent action potentials. The fibers normally had a resting membrane potential of −70 to −80 mV, and a holding potential of −80 mV was routinely used. Open circles – average results from 20 soleus fibers. Open triangles – average results from 9 red sternomastoid, 18 EDL, and 3 white sternomastoid fibers. The vertical bars show ±1 SEM where this was greater than the symbol

Table 6. Cable constants of fibers from muscles listed in the table[a]

	Soleus	Red sterno-mastoid	EDL	White sterno-mastoid
Space constant (mm)	0.80 ±0.09	0.88 ±0.10	0.77 ±0.08	1.21 ±0.18
Input resistance (MΩ)	0.66 ±0.05	0.50 ±0.03	0.43 ±0.04	0.37 ±0.02
Number of fibers	14	13	10	14

[a] Values are expressed as mean ±SEM and the number of fibers is given in the last row

cisternae membrane in soleus fibers is about half that in EDL fibers (Eisenberg & Kuda, 1976; Wong & Davey, 1979) so that, if all else was equal, the release of free internal calcium would be signficantly less in soleus fibers following T-tubule depolarization. The low calcium pump activity (Feihn & Peter, 1971; Biggs, Poland & Solaro 1977) of the soleus sarcoplasmic reticulum would help maintain the free calcium ion concentration. It is not clear whether the low pump activity and increased contractile protein sensitivity in soleus fibers are sufficient to overcome the reduced calcium release to the extent that the fibers contract with less depolarization. There are some im-

portant differences between the calcium-activation curves and the membrane-potential-activation curves which suggest that activation in intact fibers is influenced by properties of the surface (including T-system) and sarcoplasmic reticulum membranes. For example, the calcium sensitivity of the contractile proteins is temperature dependent so that the threshold calcium concentration for contraction is similar in EDL and soleus fibers at 25 °C (Stephenson & Forest, 1979). However, the slope of the calcium activation curve is greater in soleus fibers and the saturation calcium concentration is thus lower than in EDL fibers. These trends are not evident in data shown in Fig. 9 (*see* Results) or in data presented by Lorkovic (1971). Calcium release at more negative potentials in soleus fibers would provide a useful offset to their small area of terminal cisternae membrane. It would be useful to study the voltage dependence of calcium release in mammalian fibers using a direct measure of internal calcium concentration.

The mechanism of mechanical inactivation during prolonged depolarization is also uncertain, but it is known that the rate of inactivation is not limited by the calcium accumulating abilities of the sacroplasmic reticulum because the decay of twitch and tetanic tension (which provide a lower limit for the rate of calcium accumulation by the sarcoplasmic reticulum; Caputo & Fernandez de Bolanos, 1979) are many times faster than K-contracture inactivation. Caputo (1972*a* and *b*) argued that inactivation is a property of the process coupling T-tubule depolarization with calcium release from the sarcoplasmic reticulum. This conclusion has been supported by studies of the kinetics of inactivation of asymmetry currents in skeletal muscle (Chandler, Rakowski & Schneider, 1976) and by studies of mechanical inactivation following very brief depolarizing pulses in mammalian fibers (Dulhunty, 1979*c*). Two additional observations support the idea that K-contracture inactivation is not calcium depletion. The first is that fibers below the surface of mammalian muscles do not contribute to the K-contracture, probably because slow depolarization effectively inactivates deep fibers before they can be signficantly activated. In this case inactivation is independent of calcium release. Secondly, the observation that inactivation in soleus fibers is slower than inactivation in EDL fibers suggests that inactivation is not calcium depletion. It might be expected that calcium depletion would proceed more rapidly in soleus fibers because, when compared with fast twitch fibers, the slow-twitch fibers have: a smaller area of terminal cisternae membrane (Eisenberg & Kuda, 1976; Wong & Davey, 1979); a lower rate of sarcoplasmic reticulum calcium accumulation (Feihn & Peter, 1971; Biggs et al., 1977); a lower sarcoplasmic reticulum capacity for steady-state calcium accumulation (Biggs et al., 1977); and a much greater K-contracture tension-time integral (10 to 100 times greater, *see* Table 2).

In conclusion, the results show that there are significant differences between the excitation-contraction coupling processes of fast-twitch and slow-twitch muscle fibers. A consideration of the results suggests that the differences may be in the process coupling T-tubule depolarization and calcium release from the sarcoplasmic reticulum.

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