Inactivation of Excitation-Contraction Coupling in Rat Extensor Digitorum Longus and Soleus Muscles

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ABSTRACT K contractures and two-microelectrode voltage-clamp techniques were used to measure inactivation of excitation-contraction coupling in small bundles of fibers from rat extensor digitorum longus (e.d.l.) and soleus muscles at 21°C. The rate of spontaneous relaxation was faster in e.d.l. fibers: the time for 120 mM K contractures to decay to 50% of maximum tension was 9.8 ± 0.5 s (mean ± SEM) in e.d.l. and 16.8 ± 1.7 s in soleus. The rate of decay depended on membrane potential: in e.d.l., the 50% decay time was 14.3 ± 0.7 s for contractures in 80 mM K (V_m = 25 mV) and 4.9 ± 0.4 s in 160 mM K (V_m = −3 mV). In contrast to activation, which occurred with less depolarization in soleus fibers, steady state inactivation required more depolarization: after 3 min at −40 mV in 40 mM K, the 200 mM K contracture amplitude in e.d.l. fell to 28 ± 10% (n = 5) of control, but remained at 85 ± 2% (n = 6) of control in soleus. These different inactivation properties in e.d.l. and soleus fibers were not influenced by the fact that the 200 mM K solution used to test for steady state inactivation produced contractures that were maximal in soleus fibers but submaximal in e.d.l.: a relatively similar depression was recorded in maximal (200 mM K) and submaximal (60 and 80 mM K) contracture tension. A steady state "pedestal" of tension was observed with maintained depolarization after K contracture relaxation and was larger in soleus than in e.d.l. fibers. The pedestal tension was attributed to the overlap between the activation and inactivation curves for tension vs. membrane potential, which was greater in soleus than in e.d.l. fibers. The K contracture results were confirmed with the two-microelectrode voltage clamp: the contraction threshold increased to more positive potentials at holding potentials of −50 mV in e.d.l. or −40 mV in soleus. At holding potentials of −30 mV in e.d.l. or 0 mV in soleus, contraction could not be evoked by 15-ms pulses to +20 mV. Both K contracture and voltage-clamp experiments revealed that activation in soleus fibers occurred with a smaller transient depolarization and was maintained with greater steady state depolarization than in e.d.l. fibers. The K contracture and voltage-clamp results are described by a model in which contraction depends on the formation of a threshold concentration of activator from a

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voltage-sensitive molecule that can exist in the precursor, activator, or inactive states.

INTRODUCTION

During prolonged depolarization, tension in skeletal muscle fibers first increases (activation of contraction) and then spontaneously decays. The decay of tension is called inactivation (Heistracher and Hunt, 1969) and occurs as a result of changes in one of the steps in excitation-contraction coupling that link T-tubule depolarization with Ca release from the terminal cisternae, rather than a change in the ability of the contractile proteins to generate tension. Ca transients measured with Ca indicators show inactivation kinetics during tetanic stimulation (Allen et al., 1984) and during long depolarizing voltage-clamp pulses (Kovacs et al., 1979; Baylor et al., 1983; Eusebi et al., 1985). In addition, rapid inactivation of Ca release from the sarcoplasmic reticulum has been described (Melzer et al., 1984). The contractile proteins are not involved in inactivation since the myofilament response is maintained for as long as the Ca concentration is high (see, e.g., Stephenson and Williams, 1981). Activation and inactivation of contraction have been studied in amphibia (Hodgkin and Horowicz, 1960; Luttgau, 1963; Caputo, 1972; Caputo and Bolanos, 1979; Caputo et al., 1984) and in reptiles (Heistracher and Hunt, 1969). Activation of contraction has been studied in detail in mammals, in which slow-twitch fibers develop tension with less depolarization than fast-twitch fibers (Dulhunty and Gage, 1983, 1985). The characteristics of inactivation have not been investigated in detail in mammalian muscles with different contraction speeds.

In this study, the inactivation of contraction has been examined in mammalian muscles using K contracture and voltage-clamp techniques under conditions designed to achieve a low T-tubule Cl conductance for optimal control of the transverse-tubule membrane potential. The characteristics of inactivation were very different in fibers from fast- and slow-twitch muscles. Inactivation was relatively slow and incomplete in soleus, and, in contrast to the activation process, significantly more depolarization was required to inactivate the slower fibers. The fact that activation is more sensitive to depolarization in soleus and inactivation is less sensitive makes it unlikely that differences between extensor digitorum longus (e.d.l.) and soleus fibers in both processes can be attributed to differences between the electrical properties of the T-system (Leoty and Leaute, 1982). The results suggest that the characteristics of the voltage-dependent processes in excitation-contraction coupling, like those of the contractile proteins, are appropriate for the functional demands placed upon different types of mammalian muscle fibers.

METHODS

Biological Preparations and Solutions

Soleus and e.d.l. muscles were removed from male Wistar rats (300–400 g) and dissected in a dish lined with Sylgard (Dow Corning Corp., Midland, MI) into bundles of 5–10 fibers for K contractures or into layers, 2–5 fibers thick, for microelectrode studies. Muscles were dissected in a low-Cl solution containing 3.5 mM K and 16 mM Cl. The composition of the Krebs, low-Cl, and high-K solutions are given in Dulhunty and Gage (1985). The solutions for voltage-clamp

experiments contained tetrodotoxin (2×10^{-7} M) to eliminate action potential activity. Experiments were done at 21 ± 1°C.

Use of Low-Cl Concentrations for K-Contracture Experiments

Fibers were equilibrated in low-Cl solution to avoid the effects of the high Cl permeability in mammalian T-tubule membranes (Dulhunty, 1979). K contractures in preparations equilibrated in low-Cl solutions are larger and faster than those normal solutions (Dulhunty and Gage, 1985), even when the [K] × [Cl] product of the K solution (Hodgkin and Horowicz, 1960) is the same as that of the control solution. One explanation for this observation is that the cable properties of the T-system influence the K-contracture amplitude in mammals. K-contracture tension in mammalian muscle must increase in response to passive spread of surface depolarization along the T-tubule membranes rather than direct depolarization of the T-tubule membrane by the high-K solution, because the time to peak tension in 200 mM K (3–5 s) is much faster than the time necessary for K to diffuse into the T-tubules (i.e., ~40 s; Dulhunty, 1979). The use of low-Cl solutions improves the passive spread of surface depolarization because the T-tubule length constant is increased (Dulhunty et al., 1984) and, as a result, activation is more synchronous and the K-contracture amplitude is increased. Maintaining a constant [K] × [Cl] product, by reducing Cl in high-K solutions (Hodgkin and Horowicz, 1960), does not increase the length constant (Dulhunty and Gage, 1985) because, like K, Cl in the T-tubules cannot equilibrate sufficiently rapidly with the external solution and remains high during the early part of the contracture.

Isometric Tension Recording and K Contractures

The methods for stimulating and recording isometric twitches, tetani, and K contractures are given in Dulhunty and Gage (1983). A rapid-flow bath with a solution-change time of 500 ms was used. Tension was recorded via a semiconductor force transducer (Akers, Aksjeselskapet Mikro-Elektronikk, Horten, Norway) and stored digitally on magnetic media for later analysis.

Contracture tension was normalized to maximum tetanic tension to eliminate variability in tension due to differences in bundle size and to give an index of contracture tension relative to the cross-sectional area: maximum tetanic tension in e.d.l. and soleus fibers is 19.6×10^{-5} mN/cm^2 at 21°C (Dulhunty and Gage, 1985). Each preparation was subjected to only one series of solution changes (see Fig. 1 *A*) because many preparations (particularly e.d.l.) deteriorated after multiple exposures to high K. Inactivation of K contractures was measured as shown in Fig. 1 *A*. The fiber was depolarized in a conditioning solution (step *b*) for 3 min. The 3-min period was chosen because it was sufficiently long for the contraction threshold to stabilize at a new value after a change in the holding potential in voltage-clamp experiments (see below). No further change in K-contracture inactivation was seen when the conditioning period was extended to 5 min. Therefore, any slow phase of inactivation (Nagai et al., 1979) was either absent in the mammalian fibers or occurred within the 3-min conditioning period. Test K contractures were recorded before (*a*), during (*c*), and after (*d*) exposure to the conditioning solution. Results were rejected if the test K-contracture tension did not recover to within 5% of its original value.

Microelectrodes and Two-Microelectrode-Point Voltage Clamp

Microelectrodes were filled with 3 M KCl and had resistances of 2–5 MΩ. Membrane potential was measured as the potential difference between an intracellular electrode and a reference electrode in the external solution, <80 μm away. Holding current was monitored as an indicator of the condition of the fiber. The voltage and current electrodes were inserted into

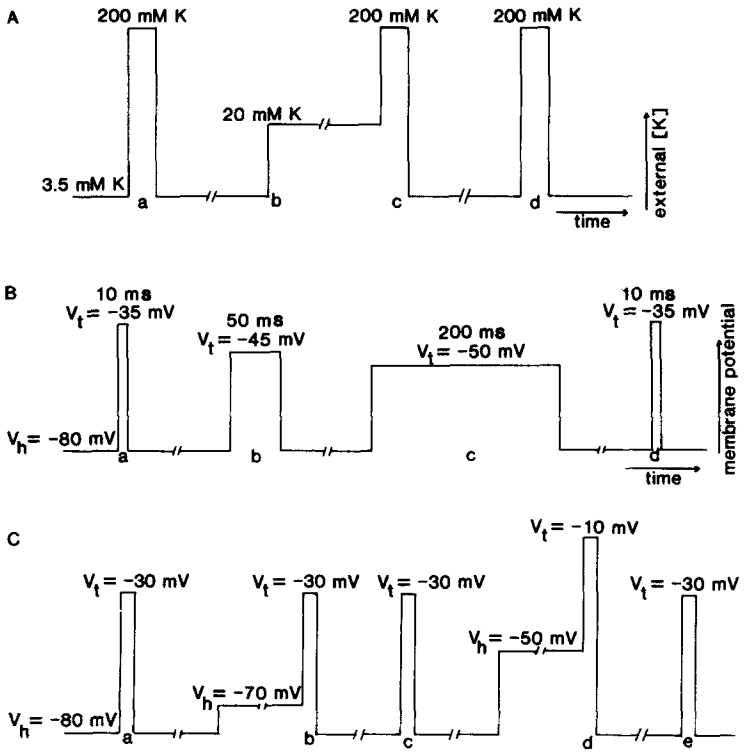

FIGURE 1. Methods for K contracture and voltage-clamp experiments (see text for full description). (*A*) Measurement of steady state inactivation after depolarization in high K: steps *a* and *d* are changes from control (3.5 mM K) to a test solution containing 200 mM K; step *b* is a change from control to a conditioning solution containing 20 mM K; step *c* is the 200 mM K test used to assess inactivation after 3 min in 20 mM K. Preparations were allowed to recover for at least 20 min after each contracture. Recovery was assessed from the amplitude of twitches and tetani. (*B*) Examples of voltage steps of different thresholds (V_t) and durations (shown above each pulse), which elicited just threshold contractions and described the strength-duration relationship for the contraction threshold. (*C*) A sequence of pulses used to measure effects of inactivation on threshold: steps *a*, *c*, and *e* are control 15-ms test pulses from a holding potential (V_h) of −80 mV; steps *b* and *d* are test pulses from depolarized holding potentials of −70 and −50 mV. The contraction threshold increased for the test pulse from V_h = −50 mV as a result of steady state inactivation.

opposite edges of the fibers, 50–100 μm apart, to minimize the effects of three-dimensional current spread (Eisenberg and Johnson, 1970).

Determination of Contraction Threshold

The surface sarcomeres between the points of entry of the current and voltage electrodes were observed with a Zeiss compound microscope using an Olympus 6.0-mm-working distance 32× air objective and a 16× Zeiss eyepiece. A high-resolution (800 lines/in.) video camera was attached to the phototube and images were displayed on a TV monitor with a total magnification of 1,000: Z lines and M lines could be clearly seen. The microscope was focused on the edges of fibers so that contraction of surface myofibrils could be easily observed.

The strength-duration relationship for pulses giving threshold contractions was determined (Fig. 1 *B*) in fibers held at a potential (V_h) of −80 mV. A test pulse was delivered at 5-s intervals (*a–d*) and its amplitude was increased in 2-mV steps until sarcomere shortening was detected. Then the pulse was reduced in 0.2-mV steps until contraction was no longer visible. The membrane potential of the pulse to just below threshold (threshold potential, V_t) usually remained constant, within 0.5 mV, for ≥30 min. Fibers were rejected if the threshold varied by more than ±1 mV during a series of tests. Inactivation was measured as described in Fig. 1 *C*. The threshold took up to 3 min to reach a new steady value after the new holding potential was established. Large depolarizing changes in the holding potential were applied slowly (up to 2 min for a depolarization to 0 mV) to avoid contracture damage around the microelectrodes. Results were rejected if the threshold at $V_h = -80$ mV did not recover after each run at a more depolarized holding potential.

Fibers were considered to be mechanically inactive when the contraction threshold exceeded +20 mV since further increases in the pulse amplitude (when the membrane potential could be controlled) did not elicit contraction. A 15-ms test pulse was used to test the contraction threshold. This width was chosen because it elicited a sharp, easily detectable contraction that caused minimal damage to the fiber. Longer pulses that may have better mimicked a K depolarization produced long slow contractures that caused damage around the current microelectrode. Since a clear rheobase was not achieved in these fibers (see Fig. 2), there seemed little point in using a test pulse longer than 15 ms.

RESULTS

Membrane Potentials in High-K Concentrations

Membrane potentials were measured in fibers equilibrated in 3.5 mM K and then exposed to a high external K solution (Table I). Potentials were also measured in the 200 mM K solution after equilibration in 20 mM K (Table II), mimicking the sequence of solution changes similar to that in Fig. 1 *A* routinely used with conditioning and test depolarizations in the inactivation experiments. The average depolarization in 200 mM K was not influenced by the K concentration in the previous bathing solution (compare data in Tables I and II).

Contractile Activation

The activation properties of fibers used in inactivation experiments are shown in Fig. 2. The average amplitudes of K contractures in solutions with different K concentrations (Fig. 2 *A*) were similar to results obtained in previous studies (continuous curves, Fig. 2 *A*; Dulhunty et al., 1987). Tension was evoked with less depolarization in soleus fibers than in e.d.l.: the potential at which fibers generated half their maximum tension was 14 mV closer to the resting membrane potential in soleus, being −28 mV in soleus and −14 mV in e.d.l.

The contraction threshold for long voltage-clamp pulses (0.5–2 s) in soleus fibers was similarly 15–20 mV closer to the resting membrane potential than in e.d.l. (Fig. 2 *B*): the threshold for contraction with a 1.9-s pulse was −64 mV in soleus and −46 mV in e.d.l. However, the difference between the contraction thresholds in the two types of fiber was less (~10 mV) with shorter-duration pulses (<200 ms). The strength-duration curve for contraction threshold in the low-Cl solution used in these experiments (see Methods) was the same as in normal Krebs (Dulhunty and Gage, 1983), which suggests that, as expected, depolarization of the T-tubule membrane

TABLE I

Average Membrane Potentials Recorded from e.d.l. and Soleus Fibers in Solutions with Different K Concentrations

K concentration (*mM*)	3.5	10	20	40	80	120	160	200
e.d.l.								
Mean (*mV*)	−83.0	−67.6	−54.9	−37.9	−24.8	−17.4	−9.4	−2.7
± SD	2.2	4.4	3.3	3.8	2.3	2.3	3.8	2.9
n	125	24	22	22	22	22	23	23
Soleus								
Mean (*mV*)	−85.9	−69.5	−54.8	−40.3	−27.2	−16.0	−8.0	−1.6
± SD	3.2	2.8	3.0	2.7	1.6	2.2	3.6	1.8
n	115	22	18	20	16	29	20	21

Membrane potentials in high K were recorded within 3 min of the solution change and fibers were returned to 3.5 mM K for at least 20 min before control measurements were taken to bracket each exposure to high K. Membrane potentials are shown as means ± SD, with the number of fibers (*n*).

close to the fiber surface in the region in which sarcomere shortening was observed was not significantly influenced by the length constant of the T-tubules, which was larger in the low-Cl solution (see Methods).

The Time Course of Spontaneous Relaxation

The decay of K contractures during prolonged depolarization in 40–200 mM K solutions was faster in e.d.l. fibers than in soleus (Fig. 3). For example, the average

TABLE II

Average Membrane Potentials Recorded in Different K Concentrations during a Series of Solution Changes Used in Inactivation Experiments

K concentration (*mM*)	3.5	20	200	3.5
e.d.l.				
Mean (*mV*)	−83.0	−56.0	−3.8	−84.3
± SD	2.4	3.2	1.4	4.1
n	12	10	13	10
Soleus				
Mean (*mV*)	−84.4	−54.6	−4.1	−83.1
± SD	2.4	3.8	3.2	1.6
n	12	10	13	10

The fibers were equilibrated in the 20 mM K solution for 3 min before the membrane potentials were recorded and measurements in 200 mM K were taken as soon as the solution was changed. Membrane potentials are means ± SD, with the number of fibers (*n*).

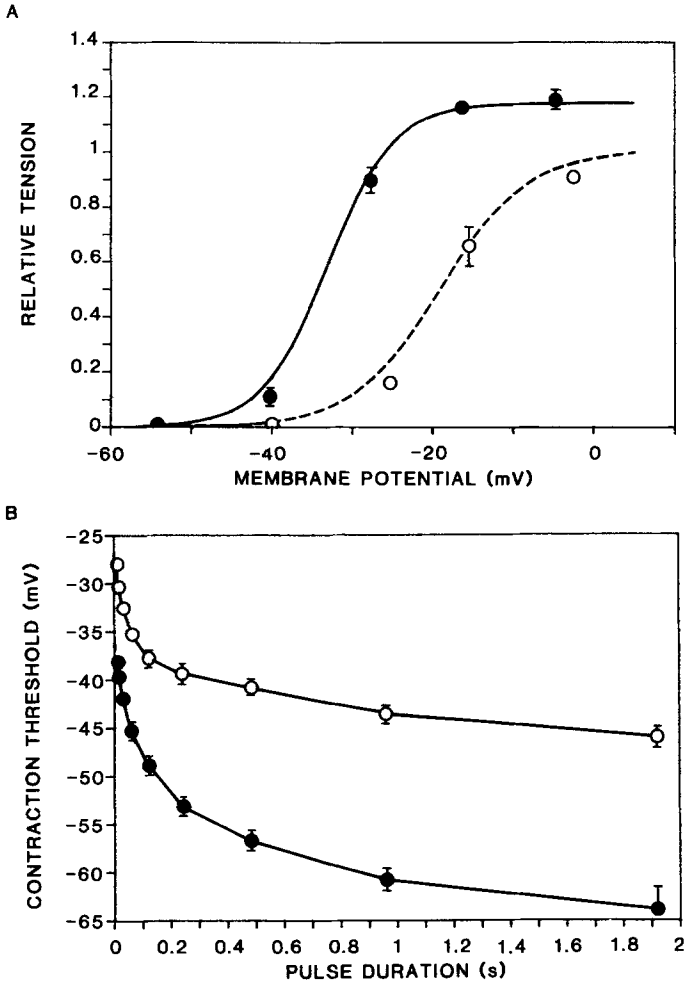

FIGURE 2. Activation properties of e.d.l. and soleus muscle fibers. Panel *A* shows relative tension (the ratio of peak K contracture tension to tetanic tension) as a function of membrane potential (measured separately in the same K solutions; Table I). The data were obtained from the preparations used in inactivation experiments (see Fig. 5 below). Average values were taken from at least five bundles of soleus (filled circles) and e.d.l. fibers (open circles). The continuous lines, taken from Dulhunty et al. (1987) and included for comparison, are average curves fitted to sets of data from individual preparations. The curves were obtained by fitting a Boltzmann equation (text, Eq. 1) to the data. The constants for soleus were $T_{max} = 1.18$, $V' = -28$ mV, and $k = 4.0$ mV, and for e.d.l. they were $T_{max} = 1.01$, $V' = -14$ mV, and $k = 5.4$ mV. *B* shows strength-duration curves for the contraction threshold in 21 e.d.l. (open circles) and 20 soleus (filled circles) fibers. The threshold membrane potential (vertical axis) in millivolts is plotted against pulse duration (horizontal axis) in seconds. The vertical bars denote ±1 SEM where this was larger than the dimensions of the symbol.

time for 120 mM K contractures to decay to 50% was 9.8 ± 0.5 s (mean ± SEM) in e.d.l. and 16.8 ± 1.7 s in soleus. The time for relaxation from the peak tension to 50% of the peak depended on the membrane potential (Fig. 3 *A*) and therefore on the peak contracture tension (Fig. 3 *B*) in both muscles. In e.d.l., for example, the

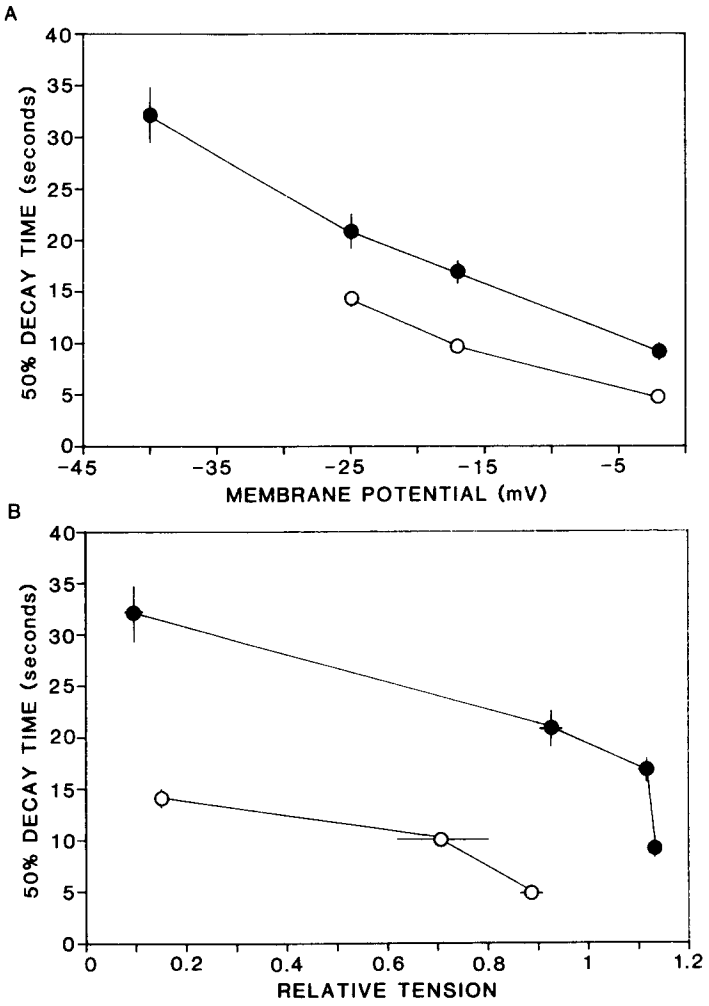

FIGURE 3. Comparison of the rate of decay of K contracture tension in e.d.l. (open symbols) and soleus (filled symbols) fibers. The 50% decay time of contractures recorded during prolonged exposure to high-K solutions has been plotted in *A* as a function of membrane potential (measured separately in the same K solutions; Table I) and in *B* as a function of relative tension (K contracture/tetanus). The symbols show average values obtained from at least five preparations and the vertical or horizontal bars on the symbols denote ±1 SEM where this was larger than the dimensions of the symbol.

average 50% decay time was 14.3 ± 0.7 s for contractures in 80 mM K (V_m = −25 mV), with a peak tension of ~20% of the maximum tetanic tension (Fig. 2 *A*), whereas a contracture to 65% of the tetanic tension (Fig. 2 *A*) in 160 mM K (V_m = −3 mV) decayed to 50% in 4.9 ± 0.4 s. Since the relative tension at each membrane potential

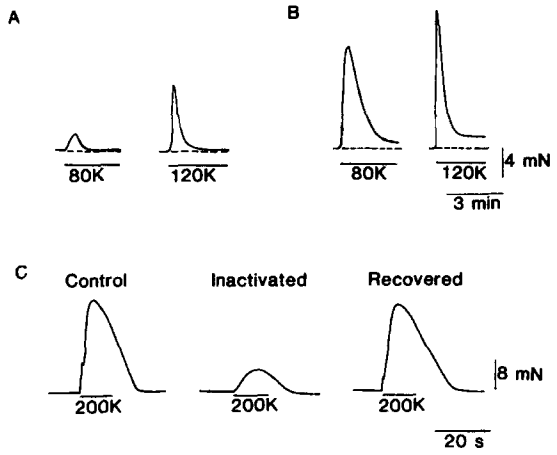

FIGURE 4. Records of contractures in high-K solutions. (*A*) Records from bundles of e.d.l. fibers exposed to 80 and 120 mM K, showing that tension decayed to levels close to the baseline (broken line). (*B*) Records from bundles of soleus fibers exposed to 80 and 120 mM K. A clear pedestal of tension was maintained during exposure to high K, which indicates that a component of the tension did not inactivate. (*C*) K contractures recorded from soleus fibers during brief exposures to the 200 mM K test (the duration of exposure is indicated by the horizontal bar under each record) in a steady state inactivation experiment. The control and recovered contractures were recorded after equilibration for 20 min in 3.5 mM K and the inactivated contracture after equilibration for 3 min in 80 mM K.

was less in e.d.l. than in soleus (Fig. 2), the data plotted as a function of membrane potential (Fig. 3 *A*) give an underestimate of the true difference between the two types of muscle. The results have been replotted as decay time against relative contracture tension in Fig. 3 *B*. When plotted in this way, inactivation in e.d.l. was 2.5 times faster than in soleus at the 50% activation level.

The time course of relaxation of 80 mM and 120 mM K contractures in e.d.l. and soleus fibers can be compared in the records shown in Fig. 4. K contractures inactivated more fully in e.d.l. fibers. Although tension fell to a level close to the baseline at most potassium concentrations, careful examination of the records in Fig. 4 *A* show that tension did not reach the baseline during the 3-min exposure to 80 or 120 mM K. A larger pedestal of tension was maintained in soleus while external K was elevated (Fig. 4 *B*). The 120 mM K contracture in Fig. 4 *B* provides a good example of a pedestal, but the pedestal tension was an unusually large fraction of the

TABLE III

Amplitude of the Pedestal of Tension Remaining after K Contracture Inactivation in e.d.l. and Soleus Fibers after Contractures in 40, 80, and 120 mM K

K concentration	Membrane Potential	Relative pedestal tension	
		e.d.l.	Soleus
mM	*mV*		
40	−39	0±0 (4)	0.033±0.005 (8)
80	−26	0.008±0.003 (4)	0.053±0.008 (7)
120	−17	0.012±0.003 (4)	0.015±0.006 (6)

contracture tension in 120 mM K (see Table III). The absolute pedestal tension was greatest in 80 and 120 mM K, but obviously represented a greater fraction of the K-contracture tension in 40 mM K. It seems possible that this pedestal is due to a "voltage window" effect (see Discussion), rather than to a subpopulation of fibers that did not inactivate: at each K concentration, the amplitude of the pedestal represented a similar fraction of contracture tension in the smallest (2–3 fibers) and largest (up to 10 fibers) preparations. Unfortunately, we were unable to test these possibilities unequivocally in single fibers because of the difficulty of intact single fiber dissection in rat soleus muscles.

Inactivation after 3-min Conditioning Depolarization Measured with 200 mM K Contractures

K contractures recorded from soleus fibers during an inactivation experiment are shown in Fig. 4 *C*. The reduced amplitude of the 200 mM K contracture in the central panel of Fig. 4 *C* (compared with the control and recovered contractures) resulted from inactivation with depolarization to −27 mV for 3 min in a conditioning solution containing 80 mM K. Increasing the time in the conditioning K solution to 4 or 5 min did not increase the amount of inactivation. The time to peak tension of the inactivated K contracture in Fig. 4 *C* is slower than in the control contractures. This slowing of partially inactivated test contractures was commonly observed. In five soleus preparations, the time to peak tension of the test contracture was 39.4 ± 8.6% slower than normal after 3 min in the 80 mM K conditioning solution.

The average amplitude of 200 mM K contractures in each conditioning solution has been plotted against membrane potential in the conditioning solution in Fig. 5. Membrane potentials were measured in separate experiments and are given in Table I. There was an average 30% depression of 200 mM K contracture tension in e.d.l. fibers after depolarization to −70 mV, in marked contrast to soleus, where 85% of maximum tension could still be generated in fibers depolarized to −38 mV (Fig. 5 *A*). The potential at which fibers could generate only 50% of their maximum tension was ~22 mV closer to the resting membrane potential in e.d.l. (approximately −54 mV) than in soleus (approximately −32 mV). Inactivation, in contrast to activation, was seen with smaller depolarizations in the e.d.l. fibers.

Inactivation of Submaximal K Contractures after a 3-min Conditioning Depolarization

We were concerned that the difference between the voltage dependence of inactivation in e.d.l. and soleus fibers might have been due to the fact that the test 200 mM K contracture generated maximal tension and was located on the saturated part of the activation curve in soleus fibers, but generated submaximal tension and was located on the steep part of the curve in e.d.l. (see Fig. 2 *A*) and therefore would be more depressed if the curve were shifted to more positive potentials as a result of inactivation. To test this possibility, we examined inactivation using 60 and 80 mM K tests, which produced contractures on the steep part of the activation curve in soleus (see Fig. 2 *A*). As with 200 mM K, the 60 or 80 mM K contractures were only minimally depressed by a steady state depolarization to −38 mV (Fig. 5 *B*).

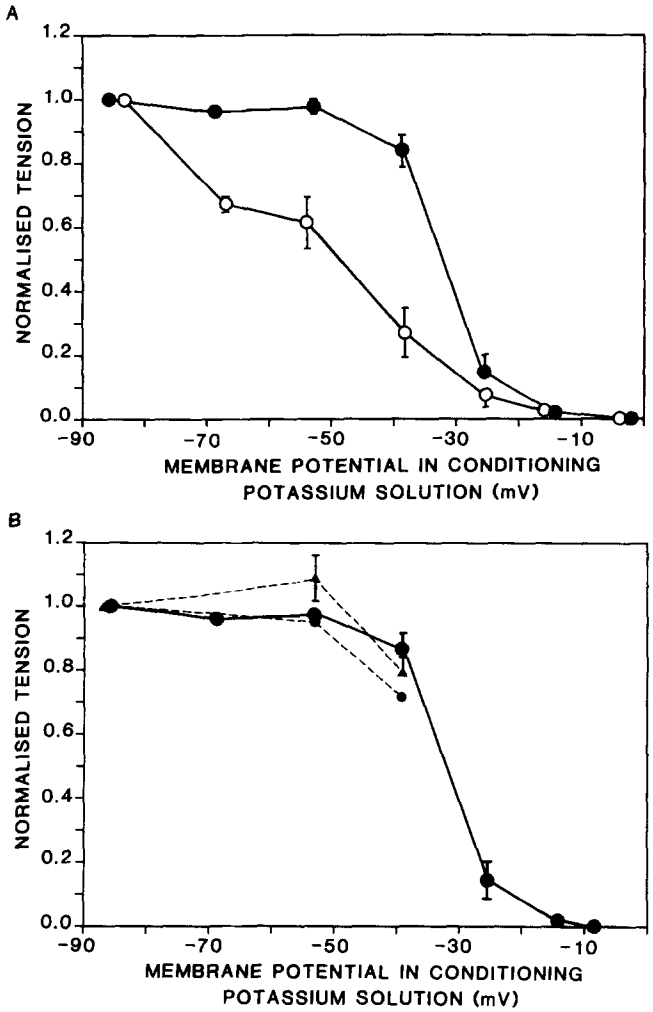

FIGURE 5. Steady state inactivation of K contracture tension. Each point shows average normalized tension, i.e., the test K contracture tension recorded after 3 min in the conditioning K solution, normalized to the mean of the control and recovered contracture tensions (see Fig. 4 *C*). Normalized tension is plotted against membrane potential (measured separately in the same K solutions; Table I). (*A*) Average values obtained using a 200 mM K test concentration in at least five bundles of e.d.l. (open circles) and soleus (filled circles) fibers. (*B*) Data obtained from soleus fibers using 60 mM K (small filled circles) and 80 mM K (filled triangles) test concentrations with the 200 mM K test data (large filled circles, as in *A*) are included for comparison. The symbols indicate the average values and the vertical bars denote ±1 SEM where this was larger than the dimensions of the symbol.

Therefore, the difference between inactivation in e.d.l. and soleus was not significantly influenced by the location of the test K contracture on the tension vs. membrane potential curve, i.e., by the degree of activation during the test K contracture.

Comparison of the Voltage Dependence of Activation and Inactivation

The voltage dependence of activation and inactivation in e.d.l. and soleus fibers was compared (Fig. 6) by fitting a Boltzmann-type equation (Dulhunty and Gage, 1983) to the data in Figs. 2 *A* and 5 *A*. The Boltzmann equation is derived specifically for

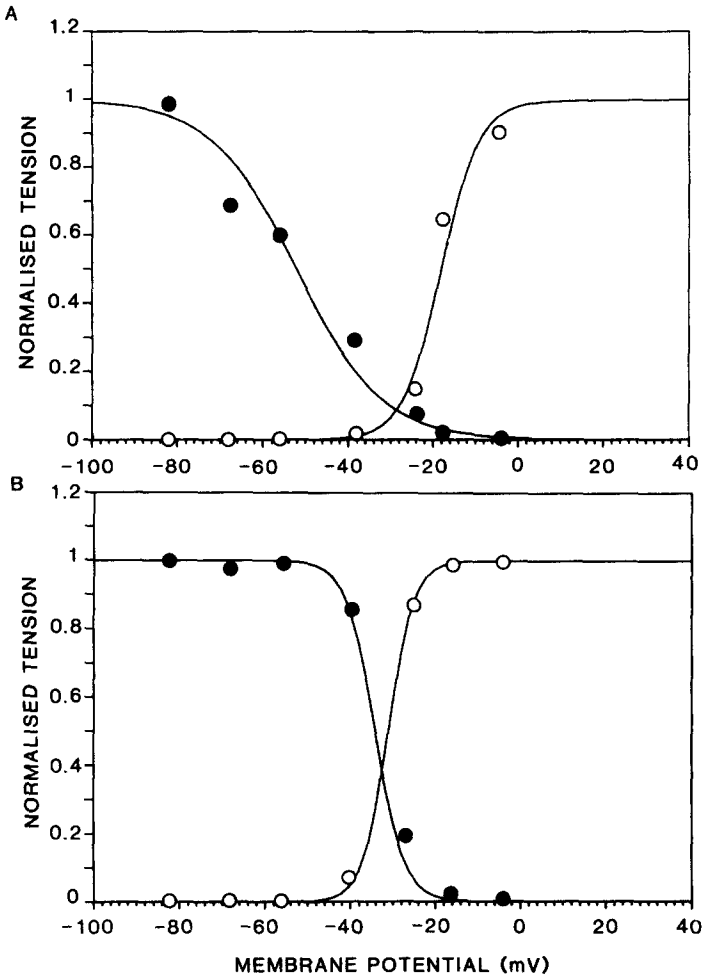

FIGURE 6. Comparison of activation (open symbols) and inactivation (filled symbols) data in e.d.l. (*A*) and soleus (*B*) fibers. The curves through the data show fits of Boltzmann-like equations (Eqs. 1 and 2) to the data using constants given in the text. Note the relatively large voltage window between the activation and inactivation curves in soleus (*B*).

equilibrium situations and is therefore not strictly applicable to the present situation. However, with this reservation in mind, it provides a useful basis for a comparison of the data. The equation for activation was:

$$T = T_{max}/[1 + \exp(V' - V_m)/k], \quad (1)$$

where T_{max} is maximum tension, V_m is membrane potential, V' is the potential at which

tension is equal to 50% of T_{max}, and k is a slope factor. The inactivation data were described by the similar equation (see, e.g., Rakowski, 1981):

$$T = T_{max}/[1 + \exp(V_c - V'')/k], \quad (2)$$

where V_c is the conditioning membrane potential and V'' is the conditioning potential at which tension is reduced to 50% of T_{max}. T_{max} was set at 1.00 for ease of comparison (and the data points were scaled appropriately). V', V'', and k were determined by the fit of Eqs. 1 and 2 to the data. For activation, the respective values for V' and k were -18 and 4.5 mV (e.d.l., Fig. 6 *A*) or -31 and 3.0 mV (soleus, Fig. 6 *B*). For inactivation, the values for V'' and k were -52 and 10 mV (e.d.l., Fig. 6 *A*) or -34 and 3.4 mV (soleus, Fig. 6 *B*). These values provided the best fit to the data shown in Fig. 6 and to the pedestal tension data given in Table III and shown in Fig. 9 (see Discussion).

In summary, both the activation and inactivation curves were steeper in soleus fibers (Fig. 6 *B*) than in e.d.l. (Fig. 6 *A*) and the activation curve in each muscle was steeper than the inactivation curve. The voltages for half-maximum tension were 34 mV more negative for activation ($V' = -18$ mV) than inactivation ($V'' = -52$ mV) in e.d.l. and 3 mV more positive in soleus (-31 mV compared with -34 mV). Therefore, there was a relatively large overlap or "voltage window" between the activation and inactivation curves in soleus fibers (Fig. 6 *B*), and this could account for the pedestal of tension seen in fibers after a K contracture (see Discussion). The overlap of the curves in Fig. 6 *B* suggests that the pedestal tension should be greatest with depolarization to between -40 and -10 mV as was seen in 40–120 mM K (Table III). The overlap between activation and inactivation curves in e.d.l. was less than in soleus, which suggests that, as shown in Fig. 4 *A* and Table III, there would be less of a pedestal at most K concentrations. The possibility that a "window" tension can be predicted from the curves in Fig. 6, using steady state Hodgkin-Huxley activation and inactivation parameters, is considered in the Discussion.

Inactivation Measured under Voltage-Clamp Conditions

The depolarization required to initiate contraction with a 15-ms test pulse was reduced and the contraction threshold was shifted toward the resting membrane potential when the holding potential was reduced from -80 to -60 mV. A further reduction in holding potential to more positive potentials produced a sharp increase in the contraction threshold and significantly larger depolarizations were required to elicit sarcomere shortening (Fig. 7). The increase in threshold, indicative of inactivation, was observed with steady state depolarization to between -50 and -40 mV in e.d.l. (*A*) or -40 to -30 mV in soleus (*B*).

The average contraction threshold could not be calculated over a range of membrane potentials because threshold values did not exist for fully inactive fibers (i.e., threshold exceeded $+20$ mV) and fibers became inactive at different holding potentials. Therefore, the data in Fig. 7 are shown for groups of fibers that became inactive at a common holding potential. Most soleus fibers could still contract when the membrane was depolarized beyond -40 mV (Fig. 7 *B*), in contrast to e.d.l., where all fibers were inactive at potentials more positive than -40 mV (Fig. 7 *A*).

The possibility that the K contracture results in Fig. 6 and the contraction

threshold results in Fig. 7 can be explained by one model for inactivation is explored in the Discussion and a model is developed in which it is assumed that (*a*) tension depends on the formation of an activator that can be immobilized with maintained depolarization and (*b*) that the contraction threshold is reached after the formation of a constant amount of activator.

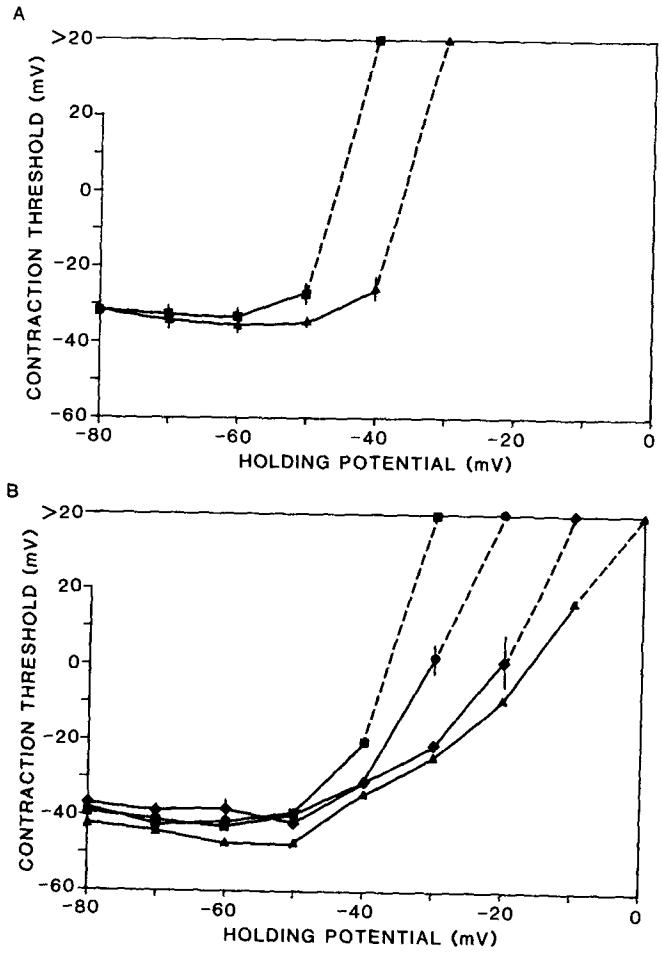

FIGURE 7. Effect of steady state inactivation on contraction threshold measured under two-microelectrode voltage clamp in e.d.l. (*A*) and soleus (*B*) fibers. Contraction threshold (vertical axis) has been plotted against the holding potential (horizontal axis). Measurements were taken 3 min after the new holding potential had been established. The data were collected into groups of fibers in which the threshold exceeded +20 mV at a common holding potential, i.e., −40 (squares, $n = 4$) and −30 mV (triangles, $n = 13$) in e.d.l. (*A*) or −30 (squares, $n = 6$), −20 (circles, $n = 4$), −10 (diamonds, $n = 3$) and 0 mV (triangles, $n = 1$) in soleus (*B*).

The number of fibers that could be activated at each holding potential (Fig. 8) fell as depolarization increased and the fall produced a curve that was similar to the inactivation of 200 mM K contractures (Fig. 5 *A*) in that more depolarization was required for inactivation of soleus fibers than e.d.l. fibers. Therefore, the contraction

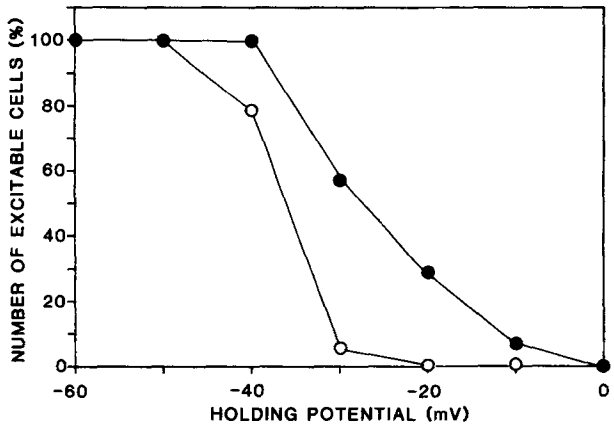

FIGURE 8. The percentage of fibers that could be activated with a 15-ms test pulse (i.e., the threshold was less than +20 mV) plotted as function of holding potential in e.d.l. (open circles) and soleus (filled circles) fibers under two-microelectrode voltage clamp. Measurements were taken 3 min after the new holding potential had been established. The contraction threshold in all fibers recovered to the control value when the holding potential was reduced to −80 mV.

threshold responded to prolonged depolarization in much the same way as the maximum K contracture tension. The major discrepancy between the two techniques occurred in e.d.l. with depolarization to potentials between −70 and −50 mV, where K contractures were partly inactivated (Fig. 5 *A*), but the contraction threshold fell closer to the resting membrane potential (Fig. 7 *A*). This discrepancy can be explained in terms of "window" effects in which a steady state concentration of activator is added to the activator released by a test pulse so that the total activator concentration after the test pulse is not reduced, even though the maximum available activator concentration is reduced by inactivation (see Discussion).

DISCUSSION

General Comments

Soleus fibers, in contrast to e.d.l., develop tension with less depolarization, inactivate more slowly, and generate maximum tension after maintained depolarization to more positive potentials. These properties may reflect an adaptation for the sustained activity in soleus (Lomo et al., 1974; Hennig and Lomo, 1985). E.d.l. fibers are more susceptible to fatigue (Burke and Edgerton, 1975) and, although the mechanism of fatigue is not understood (Edwards, 1981), changes in action potentials (Hanson, 1974) cannot explain the tension depression (Metzger and Fitts, 1986). Inactivation of excitation-contraction coupling probably contributes to fatigue (Grabowski et al., 1972). Indeed, the rate of inactivation in fast-twitch fibers is similar to the decay of tension during high-frequency stimulation (Dulhunty and Dlutowski, 1979).

Potentiation of K contractures with steady state depolarization (Anwyl et al., 1984) was not observed with maximal 200 mM K test contractures (Fig. 5 *A*), but it was seen with submaximal tension in this (Fig. 5 *B*) and many other studies (e.g., Sandow and

Khan, 1952). Although Anwyl et al. (1984) used 180 mM K test contractures, tension may have been submaximal because of diffusion effects and the use of Cl solutions, which cause slow depolarization of the surface and T-tubule membranes (see Methods) and inactivation without an increase in tension (Caputo and Bolanos, 1986). The amplitude of contractures in whole muscles is a complex function of activation and inactivation of fibers at different depths in the muscle.

Inactivation could arise in: (*a*) the voltage-sensitive process in the T-tubular membrane, (*b*) the communication between the T-tubule and the terminal cisternae, (*c*) the Ca release mechanism, or (*d*) the releasable Ca store. The last two processes can be eliminated. Ca channels in the sarcoplasmic reticulum do not inactivate (Smith et al., 1985; Suarez-Isla et al., 1986), in contrast to some types of Ca channel in neurons and cardiac muscle (Nowycky et al., 1985; Nilius et al., 1985). Ca stores are not depleted since full caffeine contractures can be recorded in inactivated fibers (Luttgau and Oetliker, 1968). Either the voltage-sensitive process in the T-system or the link between the T-system and the terminal cisternae may be subject to inactivation. Asymmetric charge movement (Schneider and Chandler, 1973) can be immobilized (Chandler et al., 1976; Rakowski, 1981) and may reflect the voltage-dependent step in contractile activation. The communication between T-tubules and terminal cisternae could also be inactivated, perhaps by depletion of a precursor, as suggested by Hodgkin and Horowicz (1960).

An attempt has been made in the following sections to develop a model that explains the pedestal tension and inactivation data in terms of a voltage-dependent molecule in the T-system, which can assume precursor, activator, or inactivated conformations (Dulhunty and Gage, 1988).

"Window" Tensions

The overlap between the voltage dependence of activation and inactivation of tension (Fig. 6) suggested that the pedestal tensions seen after K-contracture inactivation (Table III) may be produced by a phenomenon analogous to the "window" currents that can be predicted from steady state Hodgkin-Huxley activation and inactivation parameters for Na conductance (Attwell et al., 1979; Colatsky, 1982). A similar type of analysis can be applied to the tension data. A steady state activation parameter, a_∞, can be expressed as

$$a_\infty = 1/[1 + \exp(V'_a - V_m)/k_a], \tag{3}$$

where V_m is the membrane potential, V'_a is the potential at which $a_\infty = 0.5$, and k_a is a slope factor. An inactivation variable, i_∞, is defined by

$$i_\infty = 1/[1 + \exp(V_c - V'_i)/k_i], \tag{4}$$

where V_c is the conditioning potential, V'_i is the conditioning potential at which $i_\infty = 0.5$, and k_i is the slope factor. The steady state tension, T_∞, is given by

$$T_\infty = a_\infty i_\infty T_{max}, \tag{5}$$

where T_{max} is the maximum tension. The steady state tensions in Fig. 9 (curves) were calculated using constants obtained from fitting Eqs. 1 and 2 to the K contracture data (Results). Pedestal tensions from Table III are plotted on the same graph

(symbols) and are close to the predicted curves in both e.d.l. (dashed line, open symbols) and soleus (continuous line, filled symbols) fibers. The maximum calculated steady state tensions were not recorded experimentally, probably because K concentrations that would have yielded maximum tensions, i.e., between 40 and 80 mM, were not used.

Changes in the Contraction Threshold with Steady State Depolarization

The sharp increase in membrane potentials for the contraction threshold at steady state potentials between −60 and −20 mV can be explained in terms of a three-state (precursor-activator-inactivated) model with a constant value for the threshold activator concentration.

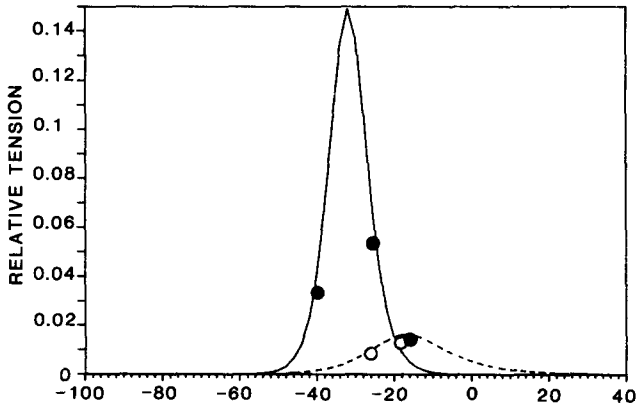

FIGURE 9. Curves for steady state "window" tensions in e.d.l. (dashed line) and soleus (continuous line) fibers predicted by Eq. 5, using values for a and i calculated using V', V'', and k values, determined from fitting Eqs. 1 and 2 to the activation and inactivation of tension (Fig. 6), for V'_a, V'_i, k_a, and k_i. Values for the constants are given in the Results section. The average pedestal tensions, indicated by open circles for e.d.l. or filled circles for soleus, were taken from Table III.

Steady state activator concentrations, A_∞, are given by

$$A_\infty = r_\infty h_\infty A_{max}, \tag{6}$$

where A_{max} is the maximum activator concentration, r_∞ is a steady state activation term, and h_∞ is a steady state inactivation term.

The contraction threshold requires a constant amount of activator, A_T, which is a fraction, x, of the maximum activator concentration,

$$A_T = xA_{max}. \tag{7}$$

A test pulse set to the membrane potential for the contraction threshold from a holding potential of −80 mV releases the threshold concentration of activator. A test

depolarization to the same membrane potential releases less activator, i.e., $xh_\infty A_{max}$, after steady state inactivation at more positive holding potentials. Therefore, the amplitude of the test pulse must be increased to achieve A_T and contraction. The threshold potential for contraction increased at holding potentials more positive than −50 mV in e.d.l. or −40 mV in soleus (Fig. 7). Similarly, depression of K contractures occurred at more negative conditioning potentials in e.d.l. (−70 mV) than in soleus (−50 mV).

Two unexpected observations were: (*a*) the increase in the contraction threshold required a larger conditioning depolarization than the reduction in maximum tension (200 mM K contractures), as outlined in the previous paragraph, and (*b*) the membrane potential for contraction threshold in fact fell at holding potentials between −80 and −60 mV. Both these observations can be explained by the addition of a steady state concentration of activator to the activator released by the test depolarization if it is assumed that there are subthreshold steady state activator concentrations between −80 and −60 mV. This assumption is reasonable since the relationship between tension and membrane potential is probably steeper than the relationship between the activator concentration and the membrane potential because of the threshold and saturation characteristics of tension. In addition, the relationship between asymmetric charge movement and membrane potential has slope factors of 13 mV in e.d.l. and 11 mV in soleus (Dulhunty and Gage, 1983) compared with the slope factors for tension of 4.5 mV in e.d.l. and 3.0 mV in soleus.

The effect of a steady state activator concentration on contraction depends on the fraction, x, of activator released by a test depolarization and the relative values of r_∞ and h_∞. This is illustrated in two hypothetical situations in which (*a*) the fraction of activator released is small, e.g., $x = 0.05$, or (*b*) the fraction of activator is large, e.g., $x = 0.9$. The voltage dependence of r_∞ and h_∞ is assumed to be such that, at a holding potential of −80 mV, $r_\infty = 0$, $h_\infty = 1$, and $A_\infty = 0$, and at a holding potential of −70 mV, $r_\infty = 0.02$, $h_\infty = 0.9$, and $A_\infty = 0.018$. In the first case, the total activator concentration ($A_\infty + xh_\infty A_{max}$) would increase from $0.05A_{max}$ at −80 mV to $0.065A_{max}$ at −70 mV and tension would increase. In the second case, the total activator concentration would fall from $0.9A_{max}$ at −80 mV to $0.83A_{max}$ at −70 mV and tension would decrease. Example *a* is analogous to the threshold situation, where the membrane potential for contraction threshold decreased between holding potentials of −80 and −70 mV (Fig. 7), and the second example is analogous to the 200 mM K contracture situation in e.d.l., where tension was depressed at −70 mV (Figs. 5 and 6). Differences between e.d.l. and soleus presumably occur as a result of differences in the fraction of activator released by the test pulse and the voltage dependence of a_∞ and h_∞ in the two types of muscle.

The threshold potential for contraction would clearly fall over any range of membrane potentials in which the sum of the activator released by the test pulse plus the steady state activator concentration exceeded the amount of activator formed by the test pulse at a holding potential of −80 mV. The same arguments can be used to explain the potentiation of submaximal K contracture tension with small conditioning depolarizations (Fig. 5).

The model does not include a description of the relationship between the activator

and myoplasmic concentration of free Ca ions. Indeed, the available data probably do not justify the development of such a complex model. It is sufficient to assume that Ca release occurs as a result of activator formation and that the relationship between activator concentration and tension includes a Ca release function as well as a function for the Ca sensitivity of the contractile proteins.

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