J. Membrane Biol. 135, 245–252 (1993)

The Journal of
Membrane
Biology

Do Independent Processes Control the Activation and Inactivation of Potassium Contracture Tension in Rat Skeletal Muscle?

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Received: 24 November 1992/Revised: 10 March 1993

Abstract. Potassium (K^+) contracture tension, measured in small bundles of rat soleus muscle fibers during maintained depolarization, increases to a peak value and then decays either to the baseline or to a pedestal level. We have tested the hypothesis that the rise and fall of tension are determined by independent activation and inactivation processes. If the "Independence" hypothesis is correct, tension during the decay of K^+ contractures should equal tension predicted from the product of the activation and inactivation parameters determined from the same K^+ contractures. Both the measured and predicted tensions decayed to a pedestal level that was increased in amplitude in the presence of perchlorate ions. However, the measured tensions in normal solutions and in the presence of perchlorate were three to five times smaller than the predicted tensions. This result indicates that the activation and inactivation of processes controlling the rise and decay of K^+ contracture tension are not independent.

Key words: Rat skeletal muscle — Potassium contractures — Excitation-contraction coupling — Activation — Inactivation

Introduction

When muscle fibers are depolarized for long times in high potassium solutions, tension rapidly increases and then slowly decays, either to the baseline or to a pedestal level (Hodgkin & Horowicz, 1960; Chua & Dulhunty, 1988). It has been variously suggested (i) that there are "parallel" independent activation and inactivation reactions that control tension (Independence hypothesis: Luttgau & Spiecker, 1979; Caputo, 1981; Graf & Schatzmann, 1984), or (ii) that the process undergoes sequential conformational changes from a resting state, to an active and then inactive states during prolonged depolarization (Sequence hypothesis). Sequence hypotheses vary from simple schemes in which the process becomes active and then inactive (Luttgau, Gottschalk & Berwe, 1986; Dulhunty & Gage, 1988) to more complex schemes in which it can also pass directly from a resting state to an inactive state (Rios & Pizarro, 1991). Preliminary observations on pedestal tensions in rat muscle seemed consistent with the Independence hypothesis (Chua & Dulhunty, 1988, 1989).

Independence hypotheses are not unique to excitation-contraction (EC) coupling. Hodgkin & Huxley (1952) proposed that the activation and inactivation of voltage-dependent sodium channels depend on the movement of independent particles, and it was later suggested that the overlap of these independent activation and inactivation processes could account for a noninactivating component of sodium currents (window current) in cardiac muscle (Attwell et al., 1979; Colatsky, 1982). However, it has now been demonstrated that persistent noninactivating sodium currents in neurons (French & Gage, 1985), skeletal muscle (Gage, Lamb & Wakefield, 1989) and cardiac muscle (Saint, Ju & Gage, 1992) are not window currents. The failure of the Independence hypothesis to explain the behavior of sodium channels led us to look more closely at the Independence hypothesis for control of skeletal muscle contraction.

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We have tested the Independence hypothesis by comparing the amount of noninactivating tension remaining after the decay of K^+ contractures, with tension predicted using the assumption that the activation and inactivation parameters for K^+ contractures, measured in the same experiments, are independent processes. If the Independence hypothesis is correct, the measured pedestal or noninactivating tension should be given by the product of the activation and inactivation parameters (*see* Materials and Methods and Chua & Dulhunty, 1989). If, on the other hand, contraction is controlled by a process that passes sequentially from a resting to an active and then an inactive state, the amplitude of the noninactivating tension will depend on the rate constants linking the resting, active and inactive states and would not necessarily be equal to the product of the activation and inactivation parameters.

We assume (i) that slow changes in tension during depolarizations lasting for minutes reflect changes in myoplasmic calcium concentration and (ii) that myoplasmic calcium concentration is determined by the state of the voltage sensor. That these assumptions are reasonable for tension generation during prolonged depolarizations in mammalian muscle is illustrated by the close relationship between the voltage dependence of asymmetric charge movement (i.e., the response of the voltage sensor) and tension in different types of normal fibers and with chronic changes in fiber-type (Dulhunty & Gage, 1983, 1985). The assumptions are made with the necessary reservations (a) that tension is produced only after a threshold concentration of calcium has been achieved and (b) that, as in amphibian muscle, the contractile response may saturate before the calcium release process. In the latter case, the tension response would not reflect the activation and calcium release processes because increases in calcium release may occur with depolarization to potentials beyond the potential at which tension reaches its maximum value. The second reservation is probably not important in mammalian muscle since charge movement and tension activation curves, measured in the same population of rats, both saturate between -10 and 0 mV in slow-twitch fibers and between $+10$ and $+20$ mV in fast-twitch fibers (Dulhunty & Gage, 1983, 1985). If calcium release is proportional to charge movement, tension in mammalian muscle does not saturate at potentials negative to those at which calcium release is maximal. Therefore, it is reasonable to use tension as an indicator of the processes activating calcium release in rat muscle.

The results showed that tension during the decay and pedestal phases of the K^+ contracture was always less than the tension predicted from the K^+ contracture activation and inactivation curves and the Independence assumption. Therefore, it is unlikely that activation and inactivation are independent processes, and we suggest that a sequential model may provide a more accurate description of the response of the voltage sensor for EC coupling during prolonged depolarization.

Materials and Methods

Biological Preparation and Solutions

Soleus muscles, removed from adult male Wistar rats, were bathed in a low chloride Krebs solution and dissected into bundles of 5–10 fibers. The solutions are tabulated in previous publications (Dulhunty, 1991; Dulhunty et al., 1992). Chloride was replaced as the major anion in the external solution to allow a rapid change in membrane potential when K^+ concentration was changed (Hodgkin & Horowicz, 1960; Dulhunty, 1979). The experiments were performed at 22.5 ± 0.5°C.

Electrical Stimulation and Isometric Tension Recording

The methods for stimulation and recording have been described (Chua & Dulhunty, 1988, 1989). Figure 1 shows a typical experiment in which a tetanus (50 Hz for 0.8–1.5 sec) and control 200 mM K^+ contracture were recorded and followed, after a recovery period (of about 10 min), by a tetanus, conditioning 60 mM K^+ contracture of amplitude t_{an} (where "an" is used as an abbreviation for "activation," *see below*) and test 200 mM K^+ contracture of amplitude t_{in} (where "in" is used as an abbreviation for "inactivation," *see below*). A "recovered" tetanus and 200 mM K^+ contracture (*not shown*) were finally recorded. Full sets of records with analysis and normalization procedures have also been given previously (Dulhunty, 1991; Dulhunty et al., 1992). All peak K^+ contracture tensions were normalized to tetanic tension and then t_{an} and t_{in} were expressed relative to the mean normalized amplitude of control 200 mM K^+ contractures obtained before and after the test contracture to obtain the relative tensions T_{an} and T_{in}. Preparations in which the ratio of the amplitudes of the control 200 mM K^+ contracture and the tetanus was less than 0.8 (indicating an excessively large bundle), or greater than 1.2 (indicating depolarized fibers), were rejected.

Construction of Activation and Inactivation Curves

For reasons outlined in the Introduction, we considered that tension provided a reasonable indicator of the processes activating calcium release under our experimental conditions. Therefore, the voltage dependences of tension activation and inactivation were used to obtain activation and inactivation parameters for EC coupling.

To obtain activation curves, average normalized peak K^+ contracture tension, T_{an}, was plotted against membrane potential,

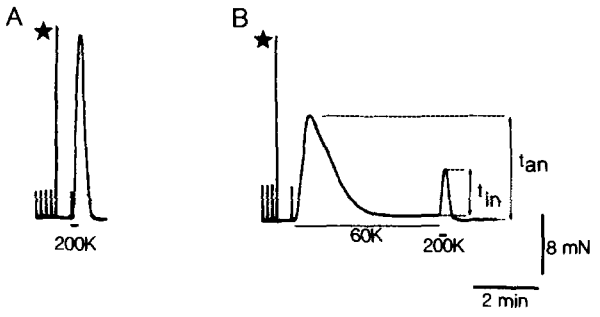

Fig. 1. Typical tension records. Small deflections are twitches, larger deflections (★) are tetanii and the slow transients are K^+ contractures. (*A*) Control tetanus and 200 mM K^+ contracture. (*B*) Recovered tetanus, conditioning contracture in 60 mM K^+, and test 200 mM K^+ contracture. Horizontal lines and vertical arrows indicate (from the top down): conditioning K^+ contracture (t_{an}), test 200 mM K^+ contracture (t_{in}), pedestal and baseline tension.

V_m, in conditioning solutions and fitted with an equation of the form:

$$T_{an} = T_{max}/[1 + \exp\{(V_{an} - V_m)/k_{an}\}] \quad (1)$$

where V_{an} is the potential at which $T_{an} = 0.5\ T_{max}$ and k_{an} is a slope factor. A steady-state activation variable, AN(∞), was defined as:

$$AN(\infty) = 1/[1 + \exp\{V_{an} - V_m)/k_{an}\}] \quad (2)$$

T_{an} is equal to AN(∞) T_{max}. Since T_{max} is a normalized value and is equal to 1.0, the parameters used to fit Eq. (1) to the data also define the activation variable.

Inactivation curves were obtained after conditioning depolarizatons for time, x, from average normalized test 200 mM K^+ contracture tension, $T_{in}(x)$, plotted against V_m. The data were fitted with an equal of the form:

$$T_{in}(x) = T_{max}/[1 + \exp\{(V_m - V_{in}(x))/k_{in}(x)\}] \quad (3)$$

where $V_{in}(x)$ is the potential at which $T_{in}(x) = 0.5\ T_{max}$ and $k_{in}(x)$ is a slope factor. Inactivation variables at time x, IN(x), were defined by:

$$IN(x) = 1/[1 + \exp\{(V_m - V_{in}(x))/k_{in}(x)\}] \quad (4)$$

and $T_{in}(x)$ is equal to IN(x) T_{max}. In practice, since $T_{max} = 1$, the parameters fitted to the inactivation data also define IN(x).

Prediction of Tension

Tension during the decay and pedestal phases of K^+ contractures was predicted from activation and inactivation variables. If activation and inactivation are independent processes, the AN(∞) curve expresses the probability of activation, and the IN(x) curve expresses 1—the probability of inactivation. Therefore, the probability of tension at time, x, is given by AN(∞)IN(x) and the predicted tension, $T_p(x)$, is given by:

$$T_p(x) = AN(\infty)IN(x)T_{max} \quad (5)$$

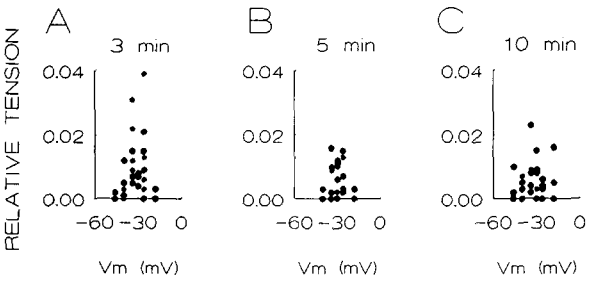

Fig. 2. Individual pedestal tensions (filled circles) plotted against membrane potential, V_m (in mV), in high K^+ solutions. Tension was measured after depolarizations lasting 3 (*A*), 5 (*B*) and 10 (*C*) min.

Table. Average pedestal tension measured after depolarizations lasting 3 min (column 2), 5 min (column 3) and 10 min (column 4) in the highest potassium solutions with K^+ concentrations shown in the first column

$[K^+]$ (mM)	3 min	5 min	10 min
20	0.0005 ± 0.0004 (4)		0.003 ± 0.001 (6)
30	0.003 ± 0.002 (7)	0.001 ± 0.001 (5)	0.004 ± 0.001 (5)
40	0.012 ± 0.008 (10)	0.007 ± 0.003 (5)	0.009 ± 0.003 (5)
60	0.006 ± 0.0007 (5)	0.007 ± 0.002 (6)	0.004 ± 0.003 (5)
80	0.011 ± 0.003 (12)	0.008 ± 0.002 (6)	0.004 ± 0.001 (5)
120	0.0006 ± 0.0005 (5)	0.0006 ± 0.0004 (5)	0.004 ± 0.003 (5)

The results are expressed as a fraction of T_{max} and are shown as mean ± 1 sem with the numbers of observations in parentheses.

Results

K^+ Contracture and Pedestal Tension in Soleus Muscle Fibers

K^+ contracture tension reaches a peak in 10 to 20 sec and then decays to the baseline or to a pedestal level within 90 to 120 sec, the time to peak and decay time being faster at higher K^+ concentrations (Dulhunty, 1991). Pedestal tensions were observed and were measured immediately before the test 200 mM K^+ contracture was elicited, after depolariza-

tions lasting 3, 5 and 10 min. The amplitude of pedestal tension in Fig. 1*B*, is 0.03 T_{max}. Pedestal tensions were seen in 73% of preparations with depolarizations to potentials between −46 mV (40 mM K^+) and −19 mV (120 mM K^+). K^+ contractures in 27% of preparations decayed to the baseline. The curves for the voltage dependence of pedestal tension, peak tension and test 200 K^+ contractures were obtained for average data rather than in individual preparations because run-down effects, seen with the long recovery times needed in mammalian preparations, prevented a full set of data being obtained from any one preparation (Dulhunty, 1991). Individual pedestal tensions varied between 0 and 0.04 T_{max} (Fig. 2).

Pedestal tensions were greatest between −35 mV (60 mM K^+) and −25 mV (80 mM K^+) at 3, 5 and 10 min. The largest individual tensions were recorded after 3 min (Fig. 2*A*); tensions were smaller after 5 min (Fig. 2*B*) and 10 min (Fig. 2*C*). The largest average pedestal tensions (Table) were 0.012 ± 0.008 T_{max} at −35 mV after 3 min, 0.008 ± 0.002 T_{max} at −25 mV after 5 min and 0.009 ± 0.003 T_{max} at −35 mV after 10 min. The average values were small because of the inclusion of zero tensions recorded from preparations in which tension declined to the baseline.

Activation (AN(∞)) and inactivation (IN(x)) parameters were obtained in the same experiments. The overlap of the parameters (Fig. 3*A*, *B* and *C*) was greater at 3 min (Fig. 3*A*), than at 5 or 10 min (Fig. 3*C* and 3*B*). Pedestal tensions (unbroken lines, Fig. 3*D*, *E* and *F*) predicted from the activation and inactivation parameters were five times greater than the measured tensions at 3 min and three times greater at 5 and 10 min.

The necessary assumption was made that the level of activation remained the same as that at the peak of the conditioning K^+ contracture for the period of depolarization. A small shift in the activation curve to more positive membrane potentials with time could explain the difference between the measured and predicted tensions in Fig. 3*D*, *E* and *F*. Tensions similar to the measured tension could be calculated (broken lines, Fig. 3*D*, *E* and *F*) using the experimentally derived activation parameters and a hypothetical activation parameter in which V_{an} was shifted by +3 to +4 mV to more positive membrane potentials (*see legend* to Fig. 3). Measured pedestal tensions could not be predicted if the inactivation parameter was varied while the activation parameter had their experimentally determined values.

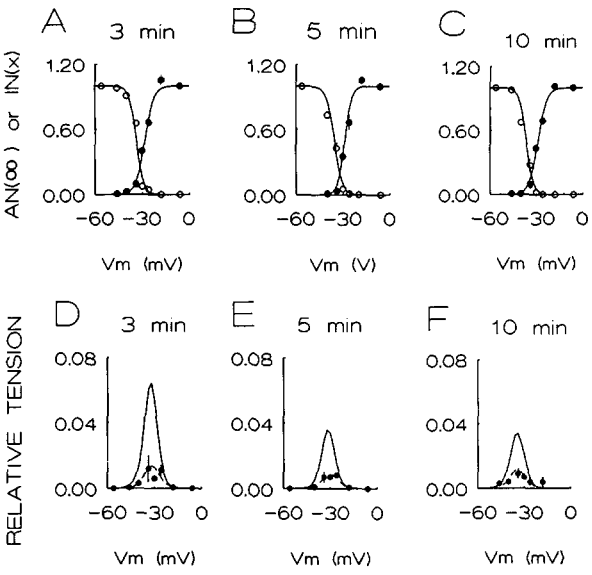

Fig. 3. Activation and inactivation parameters and pedestal tensions for depolarizations of 3 (*A* and *D*), 5 (*B* and *E*) and 10 (*C* and *F*) min. In *A*, *B* and *C*, AN(∞) and IN(x) obtained from least-squares fits of Eqs. (1) and (2) to K^+contracture data. Filled circles: average normalized conditioning K^+ contracture amplitudes. Open circles: average normalized test 200 mM K^+ contracture amplitude. Vertical bars: ± 1 SEM, where this exceeds the symbol. The data, from Dulhunty et al. (1992), are reproduced to show the origin of AN(∞) and IN(x). (*A*) V_{an} = −28.3 mV, k_{an} = 2.8 mV, V_{in} = −33.8 mV and k_{in} = 2.2 mV. (*B*) V_{an} = −28.8 mV, k_{an} = 2.2 mV, V_{in} = −35.5 mV and k_{in} = 2.4 mV. (*C*) V_{an} = −28.9 mV, k_{an} = 2.8 mV, V_{in} = −36.8 mV and k_{in} = 2.2 mV. In *D*, *E*, and *F*, the filled circles show average measured pedestal tensions (vertical bars show ±1 SEM where this exceeds the symbol). The unbroken lines show pedestal tensions predicted from AN(∞) and IN(x) using Eq. (5) and the broken lines show tension predicted from IN(x) and a hypothetical AN(∞) obtained by altering V_{an} and k_{an} until the predicted and measured tensions were similar. Final values for the hypothetical AN(∞) curve were: V_{an} = −23.0 mV and k_{an} = 3 mV in *D*; V_{an} = −24.5 mV and k_{an} = 2.4 mV in *E*; V_{an} = −25.0 mV and k_{an} = 3.0 mV in *F*. The data and curves in *F* have been published previously in review form in Dulhunty (1992) and are included to complete the set of data.

Tension in the Presence of Perchlorate Ions

Perchlorate was used to test the Independence hypothesis since it induces a −10 to −20 mV shift in the relationship between tension and membrane potential to more negative membrane potentials, with either no change (Luttgau et al., 1986) or a much smaller negative shift (Dulhunty et al., 1992) in inactivation. Therefore, the overlap between the activation and inactivation parameters should increase, and the Independence hypothesis would predict that the amplitude of the pedestal tension should also increase. Perchlorate is thought to act on the voltage sensor because it causes a similar negative shift in the voltage dependence of asymmetric charge movement (*see* Dulhunty, 1992, for review).

Perchlorate ions (2 or 10 mM) produced a significant increase in submaximal K^+ contracture tension; twitch tension and tension at −40 mV in 30 mM potassium were greater than normal (Fig. 4).

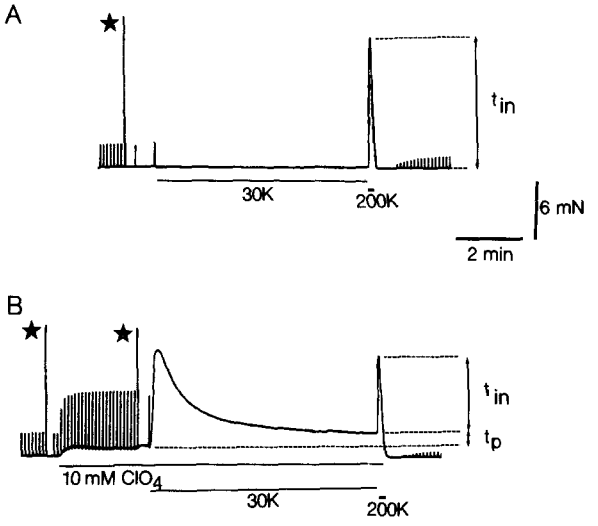

Fig. 4. Effect of 10 mM perchlorate on tension. (*A*) Control twitches and a tetanus (★), 10 min addition of 30 mM K^+ and test 200 mM K^+ contracture, followed by partial recovery of twitches on return to the control solution. (*B*) Records from the same preparation with addition of 10 mM perchlorate. Broken lines and vertical arrows indicate: *A*, test 200 mM K^+ contracture (t_{in}) and the baseline tension; *B*, the amplitude of t_{in} and the pedestal tension (t_p) and baseline tension (at the level of the perchlorate contracture). Unbroken horizontal lines show additions of perchlorate, 30 mM K^+ and 200 mM K^+. Calibration bars: vertical, 6 mN; horizontal, 2 min.

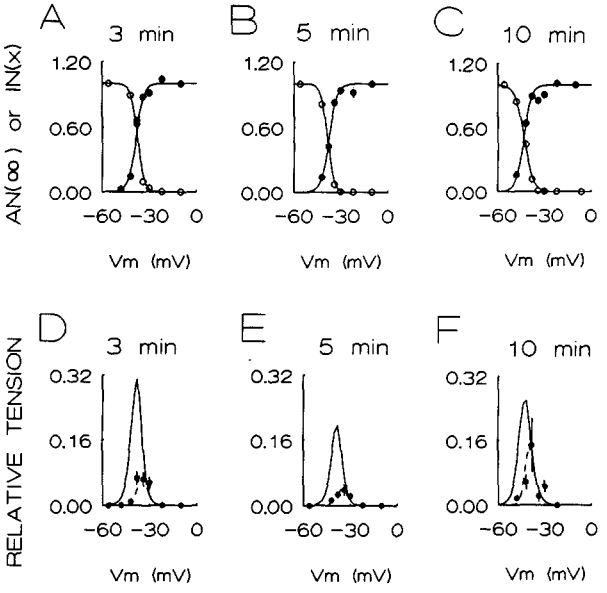

Fig. 5. Activation and inactivation and pedestal tension in perchlorate at 3, 5 and 10 min. In *A*, *B* and *C*, AN(∞) and IN(x) were obtained from least-squares fits of Eqs. (1) and (2) to K^+ contracture data obtained with 2 mM (*A* and *B*) or 10 mM (*C*) perchlorate. Filled circles: average normalized conditioning K^+ contracture tension. Open circles: average normalized amplitude of test 200 mM K^+ contractures after depolarizations for 3 (*A*), 5 (*B*) and 10 (*C*) min. Vertical bars: ±1 SEM where this exceeds the symbol. The data, from Dulhunty et al. (1992), are included to show the origin of AN(∞) and IN(x). *A*: $V_a = -38.5$ mV, $k_a = 2.5$ mV, $V_i = -37.5$ mV and $k_i = 2.0$ mV. *B*: $V_a = -37.5$ mV, $k_a = 2.4$ mV, $V_i = -38.5$ mV and $k_i = 2.4$ mV. In *C*, $V_a = -43.3$ mV, $k_a = 2.4$ mV, $V_i = -43.0$ mV and $k_i = 2.8$ mV. In *D*, *E* and *F* average measured (filled circles) and predicted (unbroken lines) pedestal tension after depolarizations of 3 and 5 min with 2 mM perchlorate (*D* and *E*) or 10 min with 10 mM perchlorate (*F*). Vertical bars: ±1 SEM where this exceeds the symbols. Tension is predicted from the AN(∞) and IN(x) curves. Broken lines: tension predicted from the IN(x) curves and a hypothetical AN(∞) obtained by altering V_{an} and k_{an}until the predicted and measured tensions were similar. Final values for the hypothetical AN(∞) were: $V_{an} = -34.2$ mV and $k_{an} = 1.5$ mV in *D*; $V_{an} = -33.2$ mV and $k_{an} = 1.8$ mV in *E*; $V_{an} = -40.5$ mV and $k_{an} = 1.4$ mV in *F*. The data and curves in *F* have been published previously in review form in Dulhunty (1992) and are included to complete the set of data.

Addition of perchlorate to the control solution (3.5 mM K^+) produced a "perchlorate contracture" (Fig. 4*B*) which has been attributed to a separate effect of the anions on a surface membrane calcium channel (Dulhunty et al., 1992). The decay of K^+ contractures recorded with perchlorate was biphasic: the slow phase of the decay continued for 10 min or longer (Dulhunty et al., 1992). For the purpose of this analysis, tension measured after depolarizations lasting 3 to 10 min is called a "pedestal" tension, although the pedestal is sloping. Pedestal tensions were measured as the difference between the perchlorate contracture tension and K^+ contracture tension (t_p, Fig. 4*B*). Of K^+ contractures recorded with 10 mM perchlorate, 37% decayed to the level of the perchlorate contracture (baseline) after 10 min depolarization.

The overlap between the tension-membrane potential curve and the inactivation curve in perchlorate-containing solutions was greater than normal: the negative shift in V_a was larger than that in V_i (compare values of V_a and V_i given in the legends to Figs. 3 and 5). The overlap after 3 min depolarization with 2 mM perchlorate (Fig. 5*A*) was larger than after 5 min (Fig. 5*B*). The greater overlap after 10 min depolarization (Fig. 5*C*) was due to an increase in perchlorate concentration to 10 mM. The largest average tensions, recorded at −38 mV in 10 mM perchlorate and 40 mM K^+, were 0.149 ± 0.067 T_{max}, i.e., 15 times greater than in control solutions.

Pedestal tensions (unbroken lines, Fig. 5*D*, *E* and *F*) calculated from the activation and inactivation variables obtained with perchlorate (*see* Fig. 5*A*, *B* and *C* above) were significantly greater than the measured tensions, and the calculated curve was at membrane potentials that were −8 to −10 mV more negative than the measured tensions. Once again, hypothetical pedestal tensions (broken lines, Fig. 5*D*, *E* and *F*) similar to the measured tension

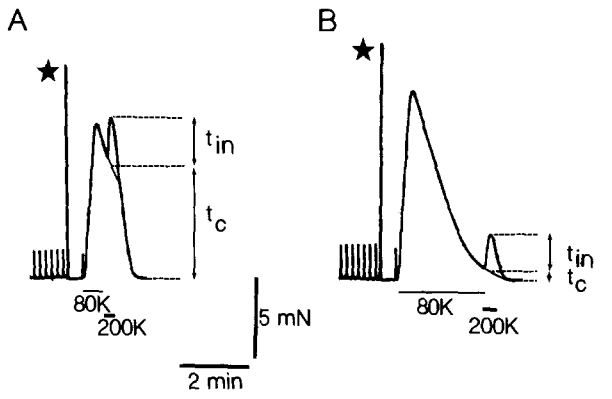

Fig. 6. Measurement of IN(x) during the decay of K^+ contractures. Twitches and a tetanus (★) were recorded immediately before conditioning contractures in 80 mM K^+. The decay of the conditioning contracture is interrupted by a test 200 mM K^+ contracture after depolarizations for 55 sec in *A* or for 150 sec in *B*. The broken lines and vertical arrows indicate test tension (t_{in}), extrapolated conditioning contracture tension (t_c) and baseline tension. Exposure to 80 mM K^+ and 200 mM K^+ are shown by the unbroken lines below the records. Calibration bars: horizontal, 2 min; vertical, 5 mN.

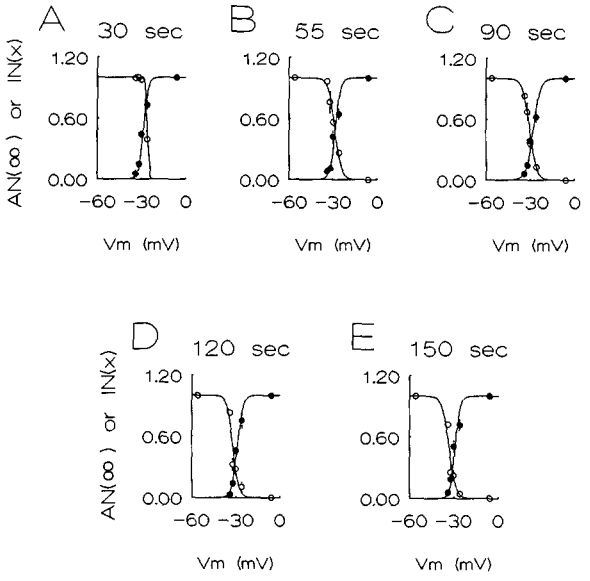

Fig. 7. Overlap of AN(∞) and IN(x) obtained from least-squares fits of Eqs. (1) and (2) to K^+ contracture data obtained with conditioning depolarizations lasting 30 sec (*A*), 55 sec (*B*), 90 sec (*C*), 120 sec (*D*) and 150 sec (*E*). Filled circles: average amplitude of conditioning K^+ contractures. Open circles: average amplitude of test 200 mM K^+ contractures. Vertical bars: ±1 SEM where this exceeds the symbol. *A*: V_{an} = −28.8 mV, k_{an} = 1.5 mV, V_{in} = −26.2 mV and k_{in} = 0.5 mV. *B*: V_{an} = −28.4 mV, k_{an} = 1.7 mV, V_{in} = −28.8 mV and k_{in} = 2.2 mV. *C*: V_{an} = −28.0 mV, k_{an} = 2.3 mV, V_{in} = −30.4 mV and k_{in} = 2.3 mV. *D*: V_{an} = −28.9 mV, k_{an} = 1.8 mV, V_{in} = −32.0 mV and k_{in} = 1.9 mV. *E*: V_{an} = −29.0 mV, k_{an} = 1.6 mV, V_{in} = −33.2 mV and k_{in} = 2.2 mV.

could be calculated from the inactivation variables derived from experimental data and an activation variable with a V_{an} that was shifted by +3 to +4 mV to more positive membrane potentials.

Measurements of Inactivation during the Decay of K^+ Contractures

The results thus far raised the question of whether tension at any time during the decay of K^+ contractures could be predicted by the measured activation and inactivation variables. To address this question, we obtained inactivation curves during the decay of condition K^+ contractures (Fig. 6). Conditioning K^+ contracture tension (t_c) at the time of the peak of the test 200 mM K^+ contracture was measured from the extrapolated decay of the conditioning contracture (broken line, Fig. 6). The magnitude of the test 200 mM K^+ contracture (t_{in}) was the peak tension in 200 mM K^+ minus t_c.

The average amplitudes the test 200 mM K^+ contractures elicited at different times during the conditioning K^+ contractures are given in Fig. 7, as well as the amplitude of the conditioning K^+ contractures. The inactivation curve obtained at 30 sec was very steep, possibly because most test 200 mM K^+ contractures elicited after 30 sec at potentals between −40 and −25 mV (i.e., between 30 to 80 mM K^+) either reached, or were slightly greater, than T_{max} and may have been limited by saturation of the contractile proteins rather than calcium release. Since T_i was uncertain in these cases, it was given a value of 1.00. The overlap between the activation and inactivation variables was greatest at 30 sec (Fig. 7*A*) and decreased progressively to 150 sec (Fig. 7*E*).

Unlike the bell-shaped relationship between pedestal tension and membrane potential, tension during the decay phase of the contractures (from 30 to 120 sec) increased progressively with depolarization (Fig. 8*A* to *E*). Tensions measured at 30 and 55 sec were similar to tensions predicted from the activation and inactivation parameters (unbroken lines Fig. 8*A* and *B*). The predicted tension became increasingly larger than the measured tension at longer times (Fig. 8*C* to *F*). The broken lines in Fig. 8*C* to *E* show tension predicted using IN(x) from experimental data and a hypothetical AN(∞) in which V_a was shifted to fit the data. The necessary shift in V_{an} was minimal at 30 and 55 sec and reached maximal values between 55 and 180 sec.

Discussion

Tension during the decay of K^+ contractures was measured and compared with tension predicted using an Independence model for the activation and

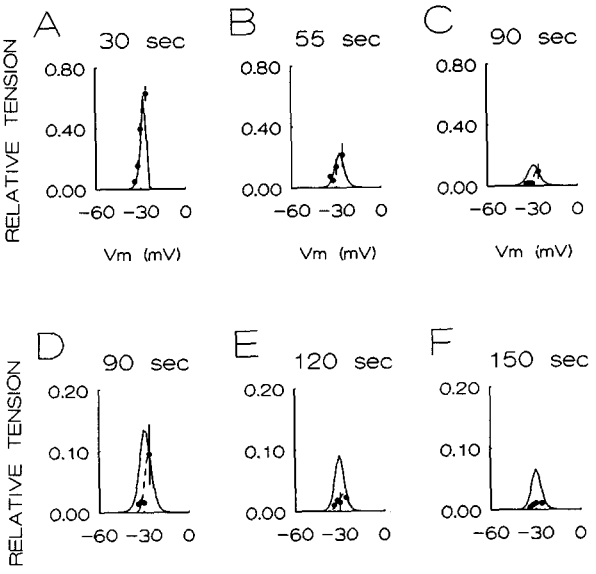

Fig. 8. Average measured (filled circles) and predicted (unbroken lines) tensions with depolarizations of 30 sec (*A*), 55 sec (*B*), 90 sec (*C* and *D*), 120 sec (*E*) and 150 sec (*F*). Vertical bars: ±1 SEM where this exceeds the symbols. Tension was predicted from the AN(∞) and IN(x) curves in Fig. 7. Broken lines: tension predicted from the IN(x) curve in Fig. 7 and a hypothetical AN(∞) obtained by altering V_{an} and k_{an} until the predicted and measured tensions were similar. Final values for the hypothetical AN(∞) were: $V_{an} = -27.2$ mV and $k_{an} = 1.2$ mV in *C* and *D*; $V_{an} = -26.5$ mV and $k_{an} = 1.5$ mV in *E*; $V_{an} = -24.8$ mV and $k_{an} = 1.85$ mV in *F*.The vertical scale is changed between *C* and *D* to better display the smaller tensions at longer times. The 90 sec graph is shown on both scales (*C* and *D*).

inactivation processes in EC coupling (*see* Introduction). The model predicted that (i) tension would decay to a pedestal level during maintained depolarization and (ii) the pedestal tension would increase when K^+ contractures were recorded in the presence of perchlorate ions. Both predictions were confirmed in a qualitative sense. However, closer inspection of the data and tensions predicted by the model showed that, although the predicted tension was exactly the same as the measured tension immediately after the peak of the K^+ contracture (i.e., after 30 to 55 sec in the high K^+ solution), the calculated tension became increasingly larger than the measured tension during the decay and plateau. The results suggest that the Independence model for EC coupling may not be an appropriate description of the activation and inactivation processes that control K^+ contracture tension.

The measured tensions at long times could be predicted if the activation curves were shifted to more positive membrane potentials, i.e., if the amount of activator decreased from its peak value as depolarization was maintained. Such a decrease in activator concentration would occur if the activator was removed to an inactive state, i.e., if the reaction was sequential.

Clearly, tension data provide summed information about each of the processes involved in the activation of tension and this could include the responses of the contractile proteins as well as the multiple steps in excitation-contraction coupling. The following discussion will show that it is not unreasonable to suggest that slow tension changes in mammalian muscle during prolonged depolarization reflect slow changes in calcium concentration that are controlled by the state of the voltage sensor.

As mentioned in the Introduction, the voltage dependence of tension in mammalian muscle is similar to that of asymmetric charge movement in specific fiber-types in many diverse situations (*see* Dulhunty, 1992, for review) and appears, perhaps fortuitously, to be little influenced by differences in the calcium sensitivity of the contractile proteins (Stephenson & Williams, 1981). The parallels between tension and charge movement suggest that calcium release and steady-state calcium concentrations are closely tied to the state of the voltage sensor in mammalian muscle. The decline in tension attributed to inactivation follows a decline in myoplasmic calcium concentration seen with calcium sensitive dyes (Brum, Rios & Stevani, 1988), and thus cannot be attributed to changes in the contractile proteins. That caffeine can generate maximum tension after fibers have been exposed to high potassium solutions for many hours (Axelsson & Thesleff, 1958) shows that (a) the calcium release channel is not inactivated, (b) calcium stores in the sarcoplasmic reticulum are not depleted and (c) the contractile proteins can still generate maximum tension. Studies of immobilization of asymmetric charge movement (Chandler, Rakowski & Schneider, 1976) show that the voltage sensor undergoes inactivation and is thus likely to be responsible for the decay of K^+ contracture tension.

Although we assume that tension largely reflects the state of the voltage sensor, we cannot exclude the possibility that K^+ contracture tension could be influenced by changes in the mechanism coupling the voltage sensor to the calcium release channel, the calcium release channel itself, or in the calcium sensitivity of troponin. An additional possibility is that changes in potassium permeability occur during long depolarizations, although microelectrode studies have shown that membrane potential remains constant during long exposures to high potassium if external chloride concentrations are low (Dulhunty, 1979). Although these factors and the requirement for a threshold calcium concentration for tension may influence K^+ contracture tension, they would

be expected to effect measurements of K^+ contracture activation, inactivation and pedestal tension in the same way and would not alter the conclusions of this paper.

The alternative sequential "state" model for the response of the voltage sensor was postulated by Luttgau et al. (1986) and by Dulhunty and Gage (1988). The model is similar to kinetic models that have been proposed for ion channels (Aldrich, Corey & Stevens, 1983) and is reasonably applied to the voltage sensor for EC coupling which is known to reside in a voltage-dependent, dihydropyridine-sensitive, calcium channel protein (Tanabe et al., 1990) and is homologous with voltage-dependent sodium channels. Thus, it is possible that the same conformational changes gate activation and inactivation of both ion channels and EC coupling. Preliminary results obtained with the sequential model for the voltage sensor suggest that tension during the decay and pedestal phases of K^+ contractures, in control solutions and in the presence of perchlorate ions, can be predicted by appropriate changes in the rate constants for the transitions between each of the states (Dulhunty, 1992) (P.W. Gage and A.F. Dulhunty, *unpublished observations*).

That pedestal tensions were not seen in all preparations was not surprising. The variability between the activation and inactivation data obtained in individual preparations allowed for the possibility that the parameters may not overlap in some fibers and, thus, a pedestal tension would not be expected.

In conclusion, the results show that the activation and inactivation processes that control the rise and decay of K^+ contracture tension are not independent. Since activation and inactivation are properties of the voltage sensor, the two processes in this molecule, as in other voltage-dependent molecules, are likely to be linked by interdependent conformational changes.

We are grateful to Professor P.W.Gage for stimulating discussion and for comments on the manuscript. We are also grateful to Suzanne Curtis for her help with the experiments.

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