Effect of Membrane Polarization on Contractile Threshold and Time Course of Prolonged Contractile Responses in Skeletal Muscle Fibers

CARLO CAPUTO, PURA BOLAÑOS, and GILDA F. GONZALEZ

From the Laboratorio de Biofísica del Músculo, Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, Caracas 1010-A, Venezuela

ABSTRACT Short muscle fibers (<1.5 mm) from the m. lumbricalis IV digiti of *Rana pipiens* were voltage-clamped at −100 mV with a two-microelectrode technique, in normal Ringer's solution containing 10^{-6} g/ml tetrodotoxin. The activation curve relating peak tension to membrane potential could be shifted toward more negative or less negative potential values by hyperpolarizing or depolarizing the fiber membrane to −130, −120, or −70 mV, respectively, which indicates that contractile threshold depends on the fiber membrane potential. Long (>5 s) depolarizing (90 mV) pulses induce prolonged contractile responses showing a plateau and a rapid relaxation phase similar to K contractures. Conditioning hyperpolarizations prolong the time course of these responses, while conditioning depolarizations shorten it. The shortening of the response time course, which results in a decrease of the area under the response, is dependent on the amplitude and duration of the conditioning depolarization. Depending on the magnitude and duration, a conditioning depolarization may also reduce peak tension. When the area under the response is reduced by 50%, the level of membrane potential also affects the repriming rate. During repriming, peak tension is restored before the contracture area. Thus, when peak tension is reprimed to 80%, the area is reprimed by 50% of its normal value. Repriming has a marked temperature dependency with a Q_{10} higher than 4. These results are compatible with the idea that an inactivation process, voltage and time dependent, regulates the release of calcium from the sarcoplasmic reticulum during these responses.

INTRODUCTION

Steady or relatively prolonged (>1 s) depolarization of skeletal muscle fibers induces a contractile refractory state termed "inactivation," which is characterized by diminished or abolished tension output (Hodgkin and Horowicz, 1960; Frankenhaeuser and Lannergren, 1967). Repolarization of the fiber membrane

Address reprint requests to Dr. Carlo Caputo, Laboratorio de Biofisica del Musculo, Centro de Biofisica y Bioquimica, Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracas 1010-A, Venezuela.

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is necessary for the repriming of the fiber contractile capacity. Both inactivation and repriming are dependent not only on time but also on the level of the fiber membrane potential (Hodgkin and Horowicz, 1960), and may be affected by several other factors. Thus, a lowered external calcium concentration and some local anesthetics appear to increase the inactivation process and interfere with repriming (Frankenhaeuser and Lannergren, 1967; Caputo, 1972, 1976; Heistracher and Hunt, 1969*a–c*), while caffeine appears to have the opposite effect (Lüttgau and Oetliker, 1968; Caputo et al., 1981).

There are some reasons to consider that the area under the contractile response—that is, the tension-time integral—may represent an approximate measure of the amount of calcium released from the sarcoplasmic reticulum (SR) and available for binding to troponin (Caputo et al., 1984). Preliminary experiments have demonstrated that the time course of prolonged contractile responses may be affected by the membrane polarization level (Caputo, 1981). Furthermore, during repriming, peak tension is re-established before the time course of the reprimed responses recovers its normal value (Caputo and Fernández de Bolaños, 1979). These results suggest that the time course of these responses is a sensitive parameter for the study of the inactivation and repriming processes. In this work, we present a detailed description of the effects of membrane hyperpolarization and moderate depolarization (down to −50 mV) upon the contractile threshold, and the time course of prolonged contractile responses during inactivation and repriming.

The results are compatible with the idea that contractile activation, inactivation, and repriming processes are under tight control of the membrane potential, possibly through the intramembrane charge process (Schneider and Chandler, 1973).

METHODS

The experiments described in this work were carried out with bundles of 5–20 fibers dissected from the m. lumbricalis IV digiti of the hind limb of *Rana pipiens* and occasionally of *Leptodactylus insularis*. These fibers have a short length (~1.5 mm) and allow the use of a two-microelectrode voltage-clamp technique for the study of relatively slow processes (Heistracher and Hunt, 1969*a*; Bezanilla et al., 1971; Caputo et al., 1984). All fibers were voltage-clamped at a holding potential of −100 mV. Fiber tension was recorded with a series 400 Cambridge Technology (Cambridge, MA) force transducer and displayed on a model 220 Gould (Cleveland, OH) pen recorder. The experiments were carried out in normal Ringer's solution whose composition was the following (in mM): 115 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 10 mM Tris buffer, pH 7.4. Tetrodotoxin was added at a concentration of 10^{-6} g/ml. In some experiments, sulfate was substituted for chloride; in others, tetraethylammonium was used to abolish the delayed rectifier, without obvious effects on the contractile responses. The experiments were carried out at temperatures ranging from 21 to 10°C as stated in each case.

RESULTS

Contractile Threshold

Fig. 1 shows two sets of records obtained with the same fiber, whose membrane potential was held at either −100 (records on the left) or −130 mV (records on

the right). In these records, the upper trace shows the imposed membrane potential, while the lower trace shows the contractile response. It can be observed that depolarizations to −50 and −40 mV cause responses whose amplitude depends on the holding potential value, since when this is −130 mV, the responses are significantly greater, which indicates a potential-dependent shift in the

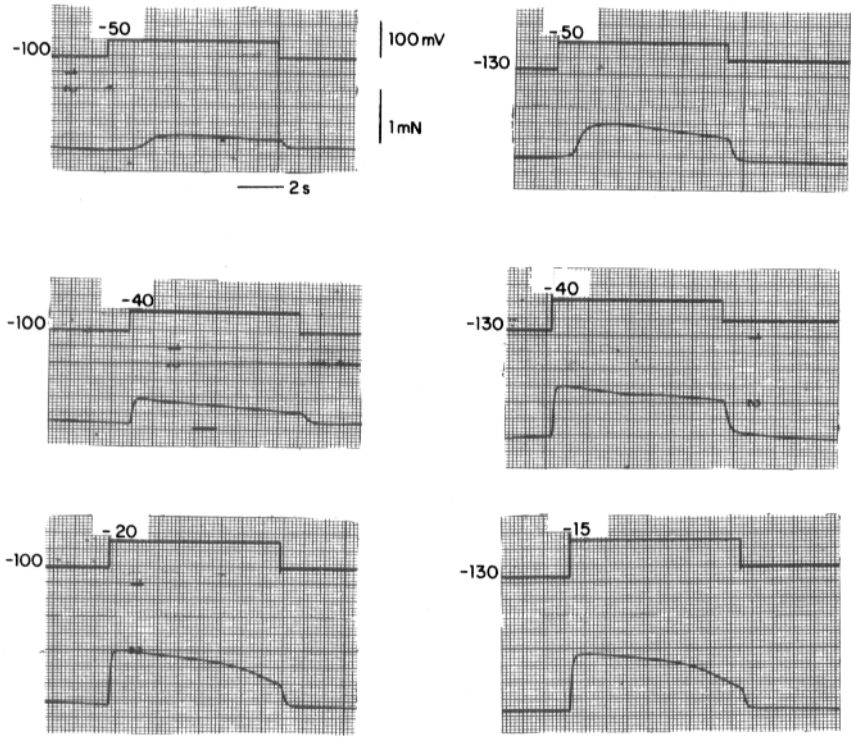

FIGURE 1. Contractile responses obtained with one fiber depolarized to different values of membrane potential from holding potential values of −100 or −130 mV. In each record, the upper trace shows the membrane potential, while the lower one shows the tension. Temperature, 13°C.

contractile threshold. With larger depolarizing pulses (to −20 mV), maximal tension was obtained independently of the value of the holding potential. An interesting effect results from the tension record obtained with a depolarization to −50 mV from a holding potential of −100 mV. It can be observed that tension development starts with a considerable delay after the onset of the depolarizing pulse. This lag is a consequence of the strength-duration relationship that determines contractile activation near threshold (Adrian et al., 1969; Costantin, 1974). In view of this behavior, the additional experiments to determine the effect of the holding membrane potential on the contractile threshold and the tension-voltage relationship were carried out using pulses of shorter duration. For the case of the experiment of Fig. 1, performed at 13°C, it is interesting to

notice that after the pulse was cut short and the fiber was repolarized to −100 mV, a fast relaxation occurred at a higher rate than the spontaneous relaxation, which occurred while the fiber was held depolarized. Fig. 2 shows plots of the tension-voltage relationship obtained with different fibers whose holding potential was either −100, −110 (filled symbols), or −130 mV (open symbols). In the figure, the tension is plotted as a fraction of the maximal tension value, which, as demonstrated in Fig. 1, does not change when the value of the holding membrane potential is more negative than −100 mV. The abscissa shows the membrane potential during the depolarizing pulses, whose durations varied

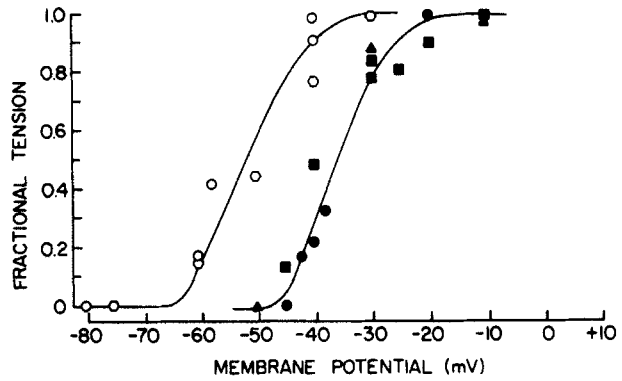

FIGURE 2. Effect of conditioning membrane potential (−130 mV: open symbols; −110 or −100 mV: filled symbols) on the relationship between peak tension of contractile responses and the membrane potential during the voltage-clamp depolarizing pulses. The tension is expressed as a fraction of the maximum fiber tension. The different symbols correspond to different fibers; thus, the results represented by the circles were obtained with the same fiber at 20°C. The other results were obtained at 15°C. The pulse duration was ≤1 s.

between 100 and 1,000 ms. The figure shows that when the membrane potential is hyperpolarized to −130 mV, the tension-voltage relationship is shifted toward more negative potentials by ~20 mV.

Additional experiments were carried out at less negative holding membrane potential values (−70 and −60 mV). A shift of the contractile threshold toward less negative potential was observed. For the range of membrane potential values between −130 and −70 mV, the principal effect on the tension-voltage relationship was a shift of the contractile threshold, without appreciable changes in the steepness of the curve describing the relationship. At less negative holding potentials, however, the peak tension started to decrease considerably, and the tension-voltage relationship was appreciably altered (C. Caputo and P. Bolaños, unpublished observations). Further experiments were carried out to measure the changes in the contractile threshold with greater precision, by applying pulses whose amplitude varied in a narrow range near the threshold. The results are shown in Fig. 3, which confirms the findings presented above and stresses the great variability existing between different fibers. In this figure, the contractile threshold, which was determined by measuring the membrane potential at which

the smallest tension developed could be detected, is plotted against the holding membrane potential value. The results obtained with each individual fiber are represented by the same symbol. Other experiments, in which the contractile threshold was determined visually by movement detection under the microscope, yielded similar results.

Contractile Response Time Course

Besides affecting contractile threshold and hence the tension-voltage relationship, the value of the holding membrane potential, in the range between −140 and

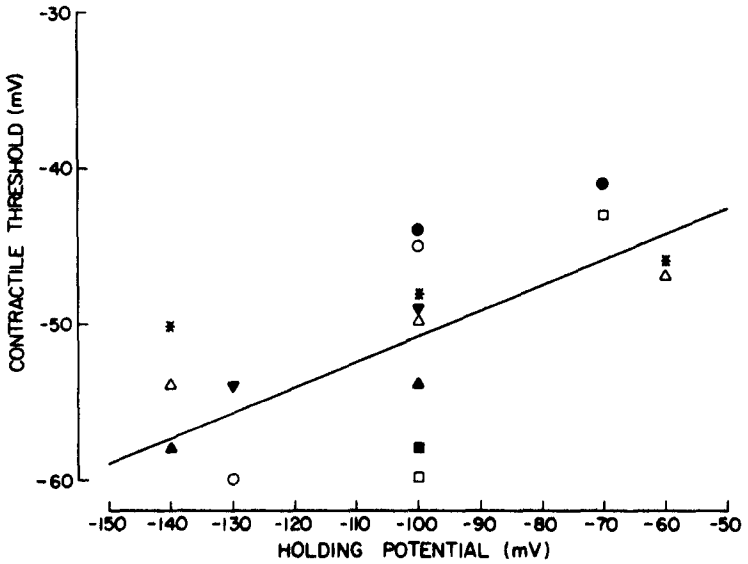

FIGURE 3. The relationship between the value of the contractile threshold and the holding membrane potential. The contractile threshold was determined by the membrane potential value at which the smallest tension could be recorded. The different symbols correspond to eight different fibers. The pulse duration for threshold determination was 400 or 500 ms. Temperature, 20°C.

−60 mV, affects the time course and, to a lesser extent in this potential range, the peak tension of contractile responses to prolonged voltage-clamp depolarizations. Fig. 4 shows that the time course of voltage-clamp contractures can be markedly prolonged by conditioning hyperpolarization and shortened by conditioning depolarization. The results shown in this figure were obtained with two different fibers. The upper panel, *A*, shows that for the case of one fiber, hyperpolarizing the membrane to −130 mV for 46 s appreciably prolongs the time course of a response to a test depolarization to −10 mV (response on the left), as compared with the response obtained starting from a holding potential of 100 mV (response on the right). The two lower panels, *B* and *C*, obtained with another fiber, show that prolonging the duration of a conditioning depolarization is increasingly effective in shortening the contracture time course. For the case of panel *B*, a 20-s conditioning depolarization to 45 mV causes a 12%

decrease in the contracture area, without affecting its peak tension. Panel *C* shows that prolonging the duration of the conditioning depolarization to 40 s causes a 33% decrease in the contracture area and only a 12% decrease in its peak tension.

The changes demonstrated in Fig. 4 occur mainly through a modification of the plateau phase, although the rate of the spontaneous relaxation phase is also modified. The results indicate that the tension-time integral, i.e., the area under the contracture, under conditions such as those of Fig. 4, can be a more sensitive

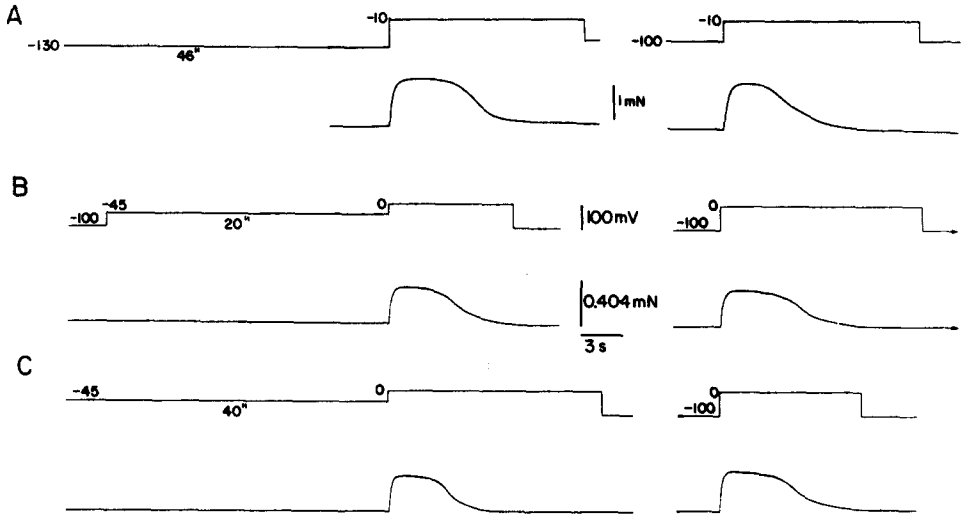

FIGURE 4. Effect of membrane polarization on the time course of contractile responses to prolonged depolarizations to −10 or 0 mV in two fibers. The upper records show the effect of conditioning hyperpolarization to −130 mV for 46 s of the response of one fiber at 13°C. The lower records show the effect of conditioning depolarization in another fiber at 10°C. In this case, the fiber was depolarized to −45 mV for either 20 (records in the middle) or 40 s (records on the bottom). The records on the right show the responses to the same test pulse from a holding potential of −100 mV.

parameter than peak tension for studying the voltage dependence of the calcium release process. (Further justification for this statement will be given later.)

Fig. 5 summarizes the results obtained with three different fibers, which show the increased effectiveness of prolonging the conditioning pulse duration in affecting the response time course. In this graph, the fractional value of the area of the response obtained with the conditioning prepulse, with respect to that obtained from a holding potential of −100 mV, is plotted against the prepulse duration. In the graph, the results obtained with each fiber are represented by the same symbol. The results, obtained with subthreshold conditioning depolarizations, clearly indicate that the time integral of the responses continuously decreases with increasing prepulse duration.

Fig. 6 summarizes the results obtained with several different fibers (each identified by a different symbol), which were either hyperpolarized or depolarized to different values of membrane potential, shown on the abscissae, for a fixed period of 46 s before applying the test depolarization. The ordinates show the fractional value of the response area. Confirming the findings presented in the previous figures, the results indicate that conditioning hyperpolarization

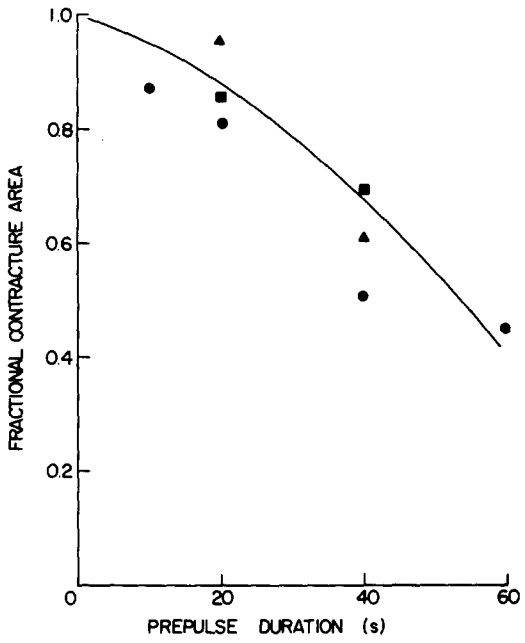

FIGURE 5. Effect of the duration of conditioning depolarization on the area under the contractile response to a prolonged test depolarization to 0 mV. The area of the contractile response obtained with the conditioning depolarization is expressed as a fraction of the value obtained in its absence. Each symbol represents the results obtained with a different fiber. In every case, the conditioning depolarization was subthreshold but near the contractile threshold (to about −50 or −45 mV). Temperature, 12°C.

increases, and conditioning depolarization decreases, the tension-time integral of voltage-clamp contractures. In Fig. 6, the continuous line was fitted by linear regression analysis using all the experimental points. The broken line represents the linear regression curve calculated without using the points obtained at membrane potentials less negative than −60 mV. Although the fitting is less precise, it allows the possibility, shown by the dotted line, that near the contractile threshold, the relationship between contractile inactivation, expressed as a reduction of the response time course, and membrane potential becomes more steep. Experiments in progress support this statement (C. Caputo and P. Bolaños, unpublished results).

Contractile Repriming

It is known that after complete inactivation, the steady state repriming process is dependent on the fiber membrane potential (Hodgkin and Horowicz, 1960; Caputo et al., 1981). During repriming after potassium contractures, muscle fibers recover their capacity to develop maximal tension before recovering their normal contracture time course (Caputo and Fernández de Bolaños, 1979).

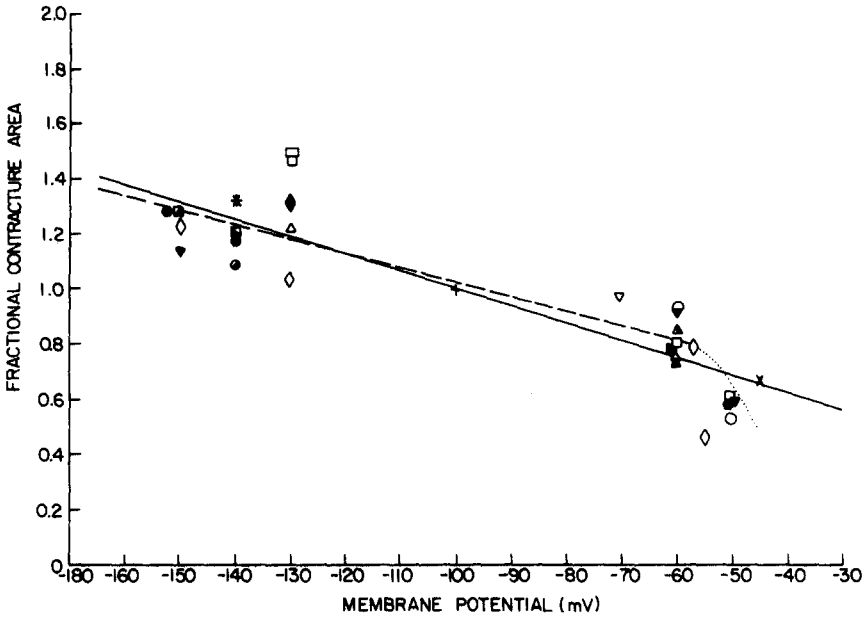

FIGURE 6. Effect of holding membrane potential (−150 to −50 mV) on the area under the contractile responses to maximal (0 mV) and prolonged test depolarizing pulses. The response area is expressed in the same way as in Fig. 5. In the graph, each symbol represents the results obtained with the same fiber. The continuous line represents the linear regression fit given by $y = 0.006x + 0.377$, where y represents the fractional area and x is the absolute membrane potential. This regression, calculated taking into account all the experimental points, gives a determination coefficient $r^2 = 0.800$. The broken line represents the regression fit calculated without the experimental points obtained at potential values less negative than −60 mV. This regression line is described by $y = 0.005x + 0.50$ with a value of $r^2 = 0.742$. The dotted curve was drawn by eye to fit the experimental points between −60 and −50 mV. The experimental points were obtained with membrane changes of 46 s duration. The temperature was 15°C, in all cases.

However, in this case, the repriming process time course is affected by the relative slowness of the solution changes and by the time it takes the fiber membrane to repolarize when the external potassium concentration is lowered (Caputo, 1972). The voltage-clamp technique provides a better approach for the study of contractile repriming (Heistracher and Hunt, 1969*b*, *c*; Caputo et al., 1981). The following experiments were carried out to study the effect of

membrane potential on the kinetics of repriming of both peak tension and the time course of contractures after the fibers had been brought into a refractory state following a test contracture by a procedure similar to that shown in Fig. 7. The upper records of Fig. 7, obtained with one fiber at 21°C, show that after a test contracture induced by depolarizing the membrane to 0 mV from a holding potential of −100 mV, brief repolarizations of 0.5 s to −70 (record on the left), −100 (record in the middle), or −150 mV (record on the right) are increasingly effective in repriming the fiber contractile capacity. The lower records, obtained at 15°C, show a similar run, carried out with a different fiber, using a longer repriming period (1 s). The results indicate that at both temperatures, repriming

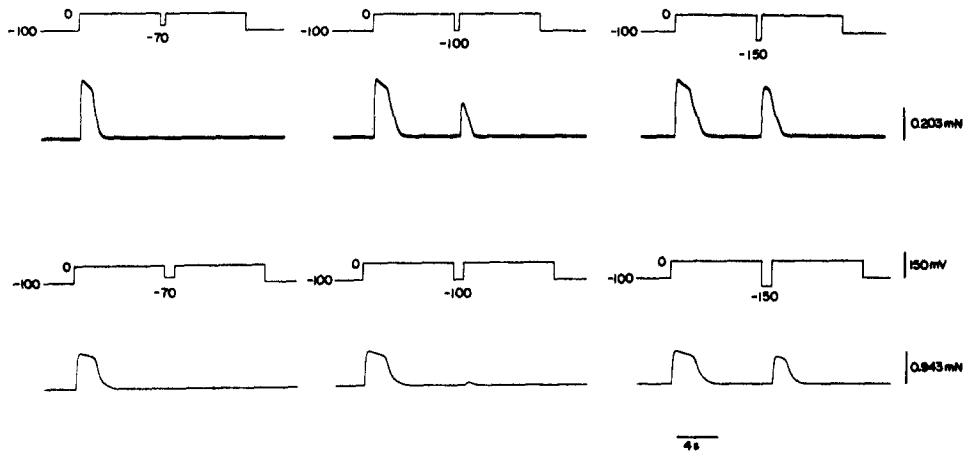

FIGURE 7. The effect of membrane potential on contractile repriming. The upper records were obtained with one fiber (P40A010284) at 21°C. The repriming period at −70, −100, or −150 mV was 0.5 s. The lower records were obtained with another fiber (P25240184) at 15°C. In this case, the repriming interval was 1 s.

at −150 mV is more pronounced than repriming at −100 mV. Furthermore, they indicate that tension is reprimed at a faster rate than the response area.

For short repriming periods, repolarization to −70 mV appears to be ineffective. Figs. 8 and 9 summarize the results obtained in several experiments of this type. Fig. 8 shows the time course of repriming of peak tension and contracture area at 21°C at repriming potentials of −100 and −150 mV. Fig. 9 shows the results of similar experiments carried out at 15°C. In this case, results obtained with a repriming potential of −70 mV have also been included. In the graphs, each point represents the mean value of several determinations with their standard errors. From Figs. 8 and 9 it is clear that the repriming rates for both peak tension and response are dependent on the membrane potential, occurring faster when the repriming potential is more negative. It is interesting to notice that even under the best conditions, complete repriming of both tension and area was not achieved, probably because of insufficient repriming time. In agreement with the work of Heistracher and Hunt (1969*b*), it appears that under voltage-clamp conditions, repriming occurs faster than when potassium contrac-

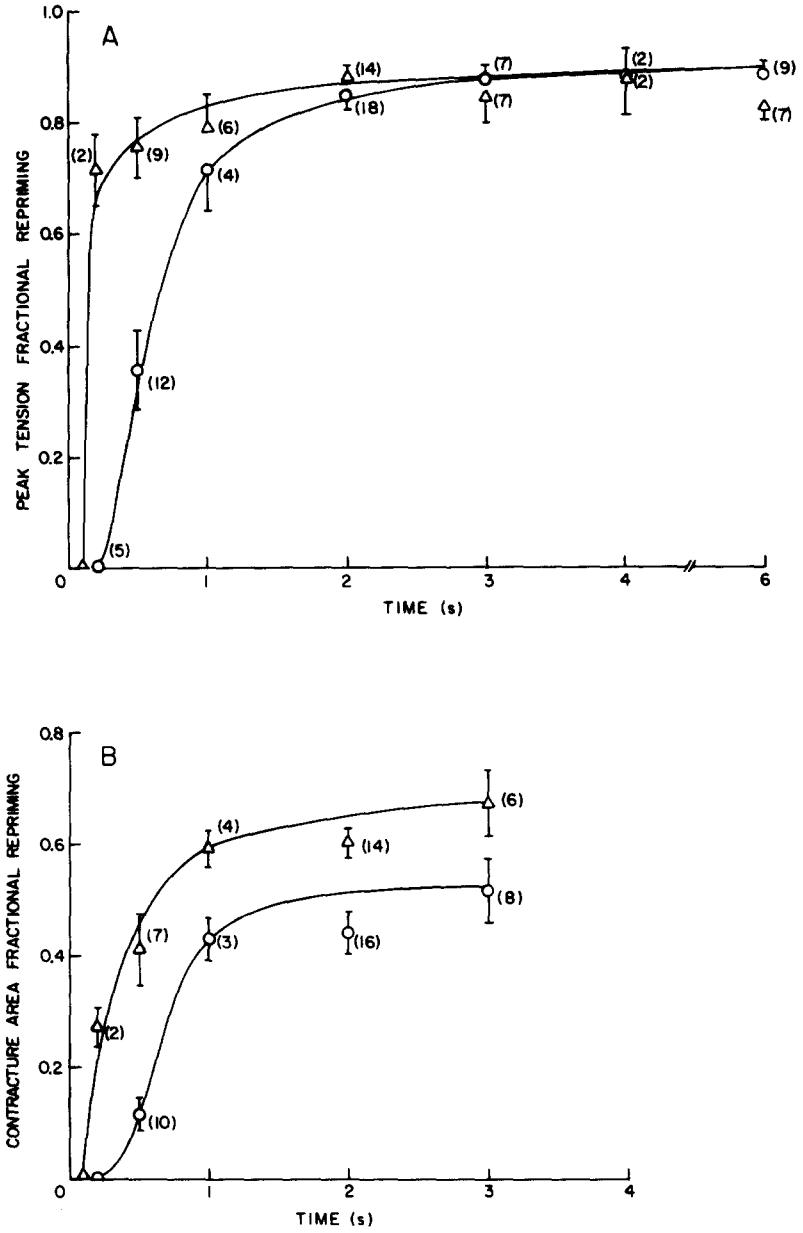

FIGURE 8. The effect of membrane potential on the time course of contractile repriming at 21 °C. The upper graph shows the repriming time course of contracture peak tension, at 100 (○) or −150 (△) mV for several fibers. The lower graph shows the repriming of the contracture area for the same experiments as in the upper graph. Both peak tension and contracture area are expressed as fractional values of the first response. Each point represents the mean ± SEM. The number of measurements in each case is shown beside each point.

tures are employed, with half-repriming times for peak tension of 0.6 and 0.2 s at −100 and −150 mV, respectively, at 21°C. Comparison of Figs. 8 and 9 demonstrates the marked temperature dependence of the repriming process.

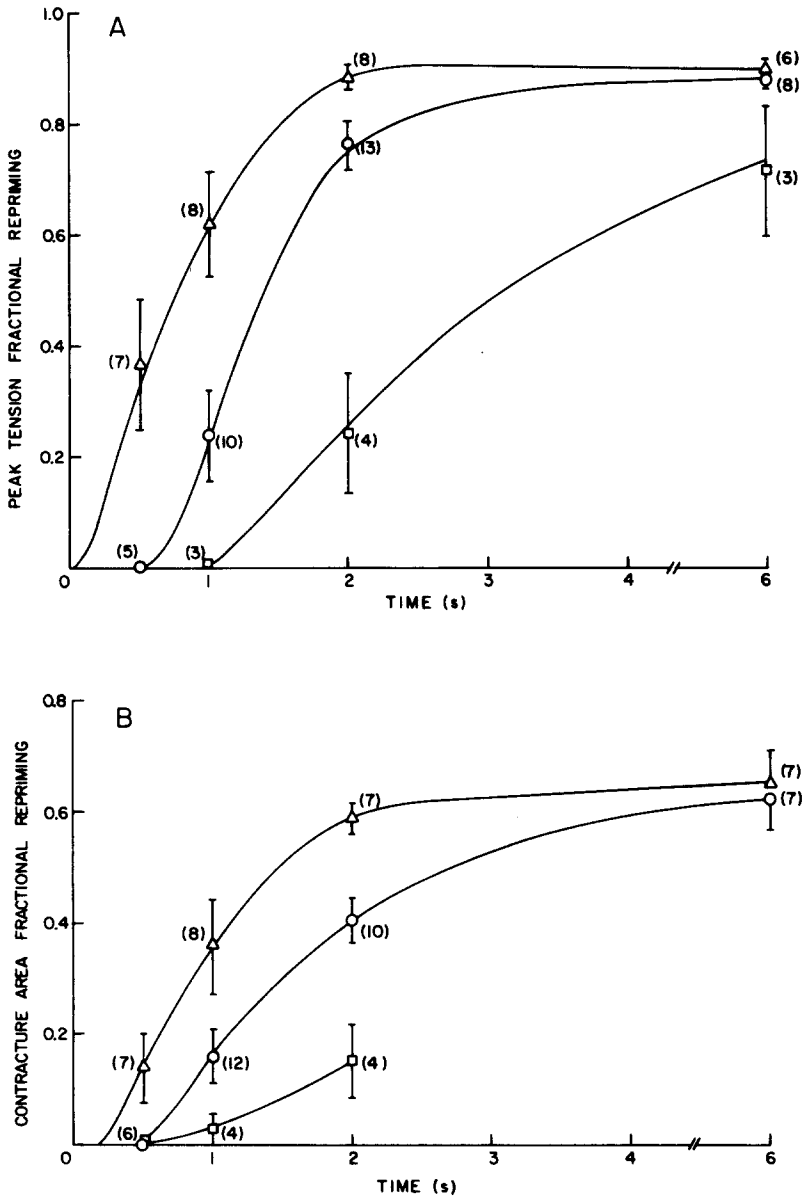

FIGURE 9. Effect of membrane potential on contractile repriming at 15°C. The graph and the symbols have the same meaning as in Fig. 8. These results were obtained with a different group of fibers. □, −70 mV; ○, −100 mV; △, −150 mV.

Using the initial repriming rates from the curves of the two figures, a Q_{10} value of >4 can be calculated for the repriming process.

The results of the experiments of contractile inactivation and contractile repriming allow us to obtain information about the relationship existing between two parameters of the contractile responses considered in this work: peak tension and the tension-time integral. Fig. 10 shows a plot of the fractional peak tension

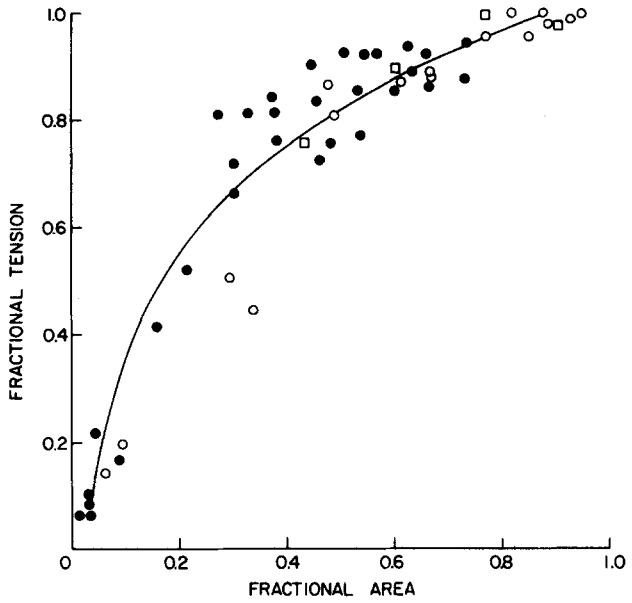

FIGURE 10. Relationship between peak contracture tension and contracture area under different conditions. The open symbols were obtained with inactivation experiments, while the filled symbols were obtained during repriming experiments. The squares represent the few results obtained with fibers dissected from *Leptodactylus insularis*. The circles were obtained with *Rana pipiens*. Each point represents a response from a given fiber in which both peak tension and the area under the tension curve were measured. The continuous line was drawn to fit the expression $Tfr = 1.02 + 0.29 \ln Afr$ obtained by logarithmic regression analysis of the experimental points, which gave a value of $r^2 = 0.95$. The results were obtained at temperatures ranging from 21 to 13°C.

against the fractional area of several responses obtained in the two types of experiments. In the graph, each symbol represents an individual response. The open symbols represent responses obtained during inactivation experiments, while the filled symbols represent the results obtained during repriming experiments. No correction has been made for the failure to achieve complete reprimming in the latter experiments. The continuous line has been drawn to fit the experimental data (50 points) by a logarithmic regression curve calculated by the expression:

$$Tfr = 1.02 + 0.29 \ln Afr,$$

where *Tfr* and *Afr* represent the fractional tension and area, respectively, and the coefficients 1.02 and 0.29 were obtained by logarithmic regression analysis of the experimental data. The determination coefficient, r^2, was 0.92. The figure shows that the same relationship describes the results obtained during inactivation or during repriming. Taking the data as shown in the figure, it appears that during inactivation, a decrease of the response area of 50% is necessary before tension declines below 80% of its maximal value. During repriming, on the other hand, tension is recovered to 80% of its final value before the response recovers 50% of its final area value.

DISCUSSION

Since the work of Hodgkin and Horowicz (1960), it has been recognized that prolonged depolarization of muscle fiber membranes causes the onset of a process that leads to contractile refractoriness. This process, also termed inactivation, is characterized by a diminution or abolishment of tension output in response to further depolarization. Strictly speaking, the refractory state, as described originally by Hodgkin (1960), referred to a state reached after the spontaneous relaxation following a potassium contracture. Similarly, the term "inactivation," employed by Frankenhaeuser and Lannergren (1967), described the decreased tension output caused by a conditioning depolarization, normally suprathreshold. In the present work, "inactivation" does not imply previous contractile activation by the conditioning depolarizations, since these were subthreshold, except in the repriming experiments. (Recent experiments have shown the possibility of complete inactivation without activation; C. Caputo and P. Bolaños, unpublished observations). Recovery or repriming from the refractory state occurs only if the membrane is repolarized beyond a critical membrane potential value for a determined period. Both inactivation and repriming are most probably related to the mechanism that provides the required voltage sensitivity to contractile activation.

At this point, it is important to stress the difference between the results obtained with brief (<400 ms) or prolonged (>1 s) conditioning depolarizations, with regard to several phenomena related to contractile activation. Thus, while prolonged depolarization leads to inactivation and refractoriness (Hodgkin and Horowicz, 1960; Frankenhauser and Lannergren, 1967), short depolarizing conditioning pulses induce potentiation of contractile responses to short test pulses (Bezanilla et al., 1971; Caputo et al., 1984), increased arsenazo III calcium signals (Miledi et al., 1981), and a decrease in the contractile threshold toward more negative membrane potentials (Horowicz and Schneider, 1981; Dulhunty, 1982).

The effect of the membrane polarization level on the time course of voltage-clamp contractures appears to be dependent on both the duration and amplitude of the conditioning polarization, which is in agreement with the idea that the time course of these responses is determined by the time course of an inactivation process that is voltage and time dependent (Caputo, 1972; Caputo and Fernández de Bolaños, 1979), and which can be considered to control calcium release from the SR. The relationship between the time course of these responses and the process of calcium release is possibly affected by the presence, in the myoplasm,

of different calcium buffering systems (SR calcium pumping sites, parvalbumins, etc.). Nevertheless, there is evidence indicating that calcium release occurs throughout the duration of these responses (Caputo, 1972), which provides some justification for considering the time course of these responses as reflecting approximately the process of calcium release. The results presented here demonstrate that the first sign of the onset of the inactivation process, induced by a conditioning membrane depolarization, is a marked shortening of the time course of the prolonged responses (contractures) obtained under voltage-clamp conditions. During repriming, on the other hand, peak tension is re-established before the responses recover their normal time course. There appears to be a definite relationship between the peak tension and the time course (expressed as the tension-time integral) of these responses which is the same, regardless of whether inactivation or repriming processes are occurring.

Membrane hyperpolarization causes an increase in the response time course, which could be explained assuming that even at a holding membrane potential of −100 mV the fibers are moderately inactivated, and that hyperpolarization removes such a slow inactivation.

Besides depending on time and voltage, the onset and time course of contractile inactivation also appear to be affected by other factors, such as the concentration of extracellular calcium (Frankenhauser and Lannergren, 1967; Caputo, 1981) or the presence of substances like caffeine (Lüttgau and Oetliker, 1968; Caputo et al., 1981), local anesthetics (Caputo, 1972, 1976; Heistracher and Hunt, 1969*b*, *c*; Almers and Best, 1976), and lanthanum (Anderson and Edman, 1974; Dörrscheidt-Käfer, 1981). Besides the above-mentioned effects, these compounds also have a marked effect on the contractile threshold (see references quoted above). Interestingly, the contractile threshold is also affected by the membrane polarization level, as has been shown here. Thus, membrane hyperpolarization causes a shift toward more negative potentials, while membrane depolarization has the opposite effect.

An interesting question is whether there exists a causal relationship between the value of contractile threshold and the inactivation state of the fibers. Such a relationship could be inferred considering that some of these compounds produce the same effect as membrane polarization on the contractile threshold, contracture time course, and repriming capacity of the fiber. For instance, caffeine produces the same effects as membrane hyperpolarization, while tetracaine mimics membrane depolarization (see the quoted references). However, further work seems necessary to confirm this point, since lanthanum acts differently, causing a shift of the contractile threshold toward less negative potentials, while prolonging considerably the contracture time course (Anderson and Edman, 1974; Dörrscheidt-Käfer, 1981).

The effects of conditioning membrane depolarizations described in this work are paralleled by similar changes that affect another phenomenon, the intramembrane charge movement, which supposedly provides the required voltage sensitivity to the mechanism responsible for calcium release from the SR during contractile activation (Schneider and Chandler, 1973; Schneider, 1981). In fact, not only inactivation but also repriming of the charge movement follows depolarization and repolarization of the fiber membrane (Chandler et al., 1976).

Steady depolarization causes a decrease of the charge moved with the leading edge of the depolarizing pulse (ON charge), and when this signal is reduced to ~40% of its normal value, contractile tension is completely abolished (Horowicz and Schneider, 1981). Thus, the present results are consistent with the idea that nonlinear charge movement (Schneider and Chandler, 1973; Schneider, 1981) or a component of this signal is involved in the regulation of contractile activation (Huang, 1981; Hui, 1982; Vergara and Caputo, 1983). To explain our results on the basis of this hypothesis, it seems necessary to assume that the progressive reduction of the ON charge movement causes the onset or speeding of the inactivation process, which controls calcium release and whose first contractile manifestation is the shortening of the contractile response time course, followed by tension reduction, and finally by its complete abolishment. This could mean that the onset and time course of contractile inactivation is determined by the amount of the ON charge moved.

The alternative possibility that the inactivation phenomena described in this work might be related to voltage-dependent calcium currents, which have been demonstrated in muscle fibers (Sánchez and Stefani, 1978), should also be considered. Although it is generally recognized that these calcium currents develop much too slowly to play any role in contractile activation during a twitch or to contribute to the rising phase of contractile responses in general, their magnitude could in principle be sufficient to support the maintenance of tension during prolonged contractile responses. The shortening of contracture time course observed in low-calcium solution would appear to be in agreement with such a hypothesis (Lüttgau, 1963; Caputo and Gimenez, 1967; Caputo, 1972). However, there is clear evidence indicating that this is not the case (González-Serratos et al., 1982). It has been shown (Caputo, 1981) that the normal contracture time course can be restored by replacing calcium with nickel. Nickel has been reported to reduce calcium currents and to be unable to flow through the calcium channels (Almers and Palade, 1981). Lanthanum, which is also a known inhibitor of, and is reportedly unable to flow through, calcium channels, causes a marked prolongation of the contracture time course (Anderson and Edman, 1974; see also González-Serratos et al., 1982). Although the fate of calcium entering the fibers during prolonged depolarization remains to be explained, these works indicate that calcium entry is not necessary for the maintenance of the contracture time course, and that this latter parameter is most probably determined by an inactivation process at the level of the coupling mechanism and possibly of the charge movement phenomenon. The role of calcium currents could be limited to the long-term replenishment of intracellular stores.

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REFERENCES

Adrian, R. H., W. R. Chandler, and A. L. Hodgkin. 1969. The kinetics of mechanical activation in frog muscle. *J. Physiol. (Lond.)*. 204:207–230.

Almers, W., and P. M. Best. 1976. Effects of tetracaine on displacement currents and contraction of frog skeletal muscle. *J. Physiol. (Lond.)*. 262:583–611.

Almers, W., and P. T. Palade. 1981. Slow calcium and potassium currents across frog muscle membrane: measurements with a vaseline-gap technique. *J. Physiol. (Lond.)*. 312:159–176.

Anderson, K. E., and K. A. P. Edman. 1974. Effects of lanthanum on potassium contractures of isolated twitch muscle fibres of the frog. *Acta Physiol. Scand.* 90:124–131.

Bezanilla, F., C. Caputo, and P. Horowicz. 1971. Voltage clamp activation of contraction in short striated muscle fibres of the frog. *Acta Cient. Venez.* 22:75–76.

Caputo, C. 1972. The time course of potassium contractures of single muscle fibres. *J. Physiol. (Lond.)*. 223:483–505.

Caputo, C. 1976. The effect of caffeine and tetracaine on the time course of potassium contractures of single muscle fibres. *J. Physiol. (Lond.)*. 255:191–208.

Caputo, C. 1981. Voltage activation of contraction and relaxation in frog skeletal muscle. *In* The Regulation of Muscle Contraction: Excitation-Contraction Coupling. A. D. Grinnell and M. A. B. Brazier, editors. Academic Press, Inc., New York. 81–95.

Caputo, C., F. Bezanilla, and P. Horowicz. 1984. Depolarization-contraction coupling in short frog muscle fibers. A voltage clamp study. *J. Gen. Physiol.* 84:133–154.

Caputo, C., and P. Fernández de Bolaños. 1979. Membrane potential, contractile activation and relaxation rates in voltage clamped short muscle fibres of the frog. *J. Physiol. (Lond.)*. 289:175–189.

Caputo, C., and M. Gimenez. 1967. Effects of external calcium deprivation on single muscle fibers. *J. Gen. Physiol.* 50:2177–2195.

Caputo, C., G. Gottschalk, and H.-Ch. Lüttgau. 1981. The control of contraction activation by the membrane potential. *Experientia.* 37:580–581.

Chandler, W. K., R. F. Rakowsky, and M. F. Schneider. 1976. Effects of glycerol treatment and maintained depolarization on charge movement in skeletal muscle. *J. Physiol. (Lond.)*. 254:284–316.

Costantin, L. L. 1974. Contractile activation in frog skeletal muscle. *J. Gen. Physiol.* 63:657–674.

Dörrscheidt-Käfer, M. 1976. The action of Ca^{2+}, Mg^{2+}, and H^{+} on the contraction threshold of frog skeletal muscle. Evidence for surface charges controlling electro-mechanical coupling. *Pflügers Arch. Eur. J. Physiol.* 363:33–41.

Dörrscheidt-Käfer, M. 1981. Comparison of the action of La^{3+} and Ca^{2+} on contraction threshold and other membrane parameters of frog skeletal muscle. *J. Membr. Biol.* 62:95–103.

Dulhunty, A. 1982. Effects of membrane potential on mechanical activation in skeletal muscle. *J. Gen. Physiol.* 79:233–251.

Frankenhaeuser, B., and J. Lannergren. 1967. The effect of calcium on the mechanical response of single twitch muscle fibres of *Xenopus laevis*. *Acta Physiol. Scand.* 69:242–254.

González-Serratos, H., R. Valle-Aguilera, D. A. Lathrop, and M. C. Garcia. 1982. Slow inward calcium currents have no obvious role in muscle excitation-contraction coupling. *Nature (Lond.)*. 248:292–294.

Heistracher, P., and C. C. Hunt. 1969*a*. The relation of membrane changes to contraction in twitch muscle fibres. *J. Physiol. (Lond.)*. 201:589–611.

Heistracher, P., and C. C. Hunt. 1969*b*. Contractile repriming in snake twitch muscle fibres. *J. Physiol. (Lond.)*. 201:613–626.

Heistracher, P., and C. C. Hunt. 1969*c*. The effect of procaine on snake twitch muscle fibres. *J. Physiol. (Lond.)*. 201:627–638.

Hodgkin, A. L., and P. Horowicz. 1960. Potassium contractures of single muscle fibres. *J. Physiol. (Lond.).* 153:386–403.

Horowicz, P., and M. F. Schneider. 1981. Membrane charge moved at contraction thresholds in skeletal muscle fibres. *J. Physiol. (Lond.).* 314:595–633.

Huang, C.-L. 1981. Pharmacological separation of charge movement component in frog skeletal muscle. *J. Physiol. (Lond.).* 324:375–387.

Hui, C. S. 1982. Pharmacological dissection of charge movement in frog skeletal muscle fibers. *Biophys. J.* 39:119–122.

Lüttgau, H.-Ch. 1963. The action of calcium ions on potassium contractures of single muscle fibres. *J. Physiol. (Lond.).* 168:679–697.

Lüttgau, H.-Ch., and H. Oetliker. 1968. The action of caffeine on the activation of the contractile mechanism in striated muscle fibres. *J. Physiol. (Lond.).* 194:51–74.

Miledi, R., S. Nakajima, I. Parker, and T. Takahashi. 1981. Effects of membrane polarization on sarcoplasmic calcium release in skeletal muscle. *Proc. R. Soc. Lond. B Biol. Sci.* 213:1–13.

Sánchez, J. A., and E. Stefani. 1978. Inward calcium current in twitch muscle fibres of the frog. *J. Physiol. (Lond.).* 283:177–209.

Schneider, M. F. 1981. Membrane charge movement and depolarization-contraction coupling. *Annu. Rev. Physiol.* 43:507–517.

Schneider, M. F., and W. K. Chandler. 1973. Voltage dependent charge movement in skeletal muscle: a possible step in excitation-contraction coupling. *Nature (Lond.).* 242:244–246.

Vergara, J., and C. Caputo. 1983. Effects of tetracaine on charge movements and calcium signals in frog skeletal muscle fibers. *Proc. Natl. Acad. Sci. USA.* 80:1477–1481.