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CONTRACTILE REPRIMING IN SNAKE TWITCH MUSCLE FIBRES

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SUMMARY

1. Contractile repriming has been studied in voltage-clamped snake twitch muscle fibres. Maintained depolarization causes a contractile response which inactivates after a few seconds. Repolarization of the fibre can restore its ability to contract to a subsequent depolarization. This restoration, or repriming, depends on the magnitude and the duration of the repolarization. At -100 mV the minimal period of repolarization which restores contractile response is 0·38 sec. The time for recovery to half maximal tension is about 0·68 sec, and restoration is complete at about 4 sec.

2. Repolarization to smaller levels of membrane potential results in a slower rate of repriming. For example, at -60 mV the mean minimal time for repriming was 2·89 sec, and nearly 17 sec of repolarization was required for full restoration of contractile response.

3. The rate of repriming was not influenced by lowering the external sodium concentration.

4. Repriming could be produced by repetitive, brief pulses of repolarization.

5. The restoration of contractile response and of outward inactivating current showed similar time courses.

INTRODUCTION

It has long been known that skeletal twitch muscle fibres give transient contractions on depolarization produced by raising external K^+. Hodgkin & Horowicz (1960) examined contractures in frog twitch muscle fibres produced by exposure to high concentrations of external K^+. Repolarization produced by lowering $[K^+]_o$ restored the capacity of the contractile

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system or its activating mechanism to respond to subsequent depolarization. Hodgkin & Horowicz designated this process as repriming. While the steady-state relationship between $[K^+]_o$ or membrane potential, and repriming of the contractile system has been studied thoroughly in single fibre preparations (Hodgkin & Horowicz, 1960; Lüttgau, 1963; Frankenhaeuser & Lännergren, 1967), most of the information on the time course of recovery of contractile responses following repolarization has been obtained from studies on small whole muscles (Curtis, 1964; Milligan & Edwards, 1965). The latter studies have the disadvantage that the time taken for diffusion prevents a direct measurement of the time course of repriming. In all of the above investigations, depolarization of frog or *Xenopus laevis* twitch muscle fibres was produced by exposure to high $[K^+]_o$. In the present study, short twitch muscle fibres of the snake were used in voltage-clamp experiments (see previous paper, Heistracher & Hunt, 1969*a*). This permitted rapid changes in membrane potential and the investigation of the relation between contractile repriming and membrane processes such as delayed rectification. The measurements of the time course of repriming as a function of membrane potential are presented in detail. Preliminary accounts of part of the results have appeared (Heistracher & Hunt, 1967).

METHODS

All experiments were carried out at room temperature, 20° C, using short (1–1·5 mm long) scale muscles from garter snakes. The details of the methods employed were the same as those described in a previous paper (Heistracher & Hunt, 1969*a*). In order to eliminate impulse activity and twitch responses, tetrodotoxin 10^{-7} w/v was usually added to the bathing solution. Instead of this, NaCl was replaced by choline chloride in some cases.

RESULTS

Restoration of contractile response following repolarization (repriming). Muscles whose active sodium permeability changes were blocked by tetrodotoxin (TTX) developed contractures following depolarization. With maintained depolarization, tension remained at a maximum for only a few seconds and then decayed. An example may be seen in Fig. 1. The muscle fibre was clamped at -100 mV except for periods of depolarization to 0 mV which were of sufficient duration to produce complete contractile inactivation. Repolarization, if of sufficient duration, restored the ability of the fibre to contract on a subsequent depolarization. If the fibre was polarized to -100 mV for less than 200 msec, the subsequent depolarization produced no detectable contractile response. If the period of repolarization was slightly longer, the depolarization which followed led to a small tension response which was much shorter in duration than

the control contracture. As the period of repolarization was further prolonged, the amplitude and duration of the contracture increased. It was usual for the amplitude of the contracture to recover completely before the contracture attained its controlled duration.

It may be noted from the current record in Fig. 1 that depolarizations which were preceded by long periods of polarization at -100 mV were associated with initially large outward currents which inactivated with time. It may be seen that there is a rough correlation between the size of the contractile response and the amount of inactivating current. Following the briefest period of polarization in Fig. 1 there was neither inactivating outward current nor any contractile response.

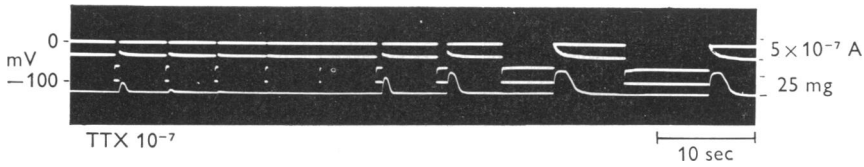

Fig. 1. Restoration of contractile response by repolarization of varying duration. At the left of the record, upper trace, is potential; middle trace, current; lower trace, tension. The fibre was clamped at 0 mV except for periods of polarization to -100 mV. Tetrodotoxin, TTX.

The close correlation between peak tension and the amplitude of inactivating outward current, as well as their common dependence on the duration of the preceding period of polarization, are further illustrated in Fig. 2. This provides a graphical representation of the results from an experiment similar to that shown in Fig. 1. The muscle was again bathed in tetrodotoxin solution, clamped at -99 mV, and depolarized to 0 mV. In the lower portion of Fig. 2, the peak tension developed during individual depolarizations is plotted as a function of the duration of the preceding period of polarization. In the upper portion of the Figure, the outward current at the beginning and end of each depolarization step is plotted. It may be seen that depolarization following periods of less than 0·3 sec at -99 mV failed to produce contraction and also elicited no inactivating current. When the period of polarization was prolonged to 0·3–0·4 sec, the subsequent depolarization steps led to contractures of small amplitude and were also associated with a small amount of inactivating current. Further prolongation of the polarization period caused a sharp increase in peak tension as well as in inactivating current during step depolarizations. Although there is a correlation between the amount of tension developed and the amount of inactivating current, recovery of maximal tension was usually attained with shorter periods of polarization than that required to produce maximal inactivating current.

Results obtained from eighteen other fibres clamped at −100 mV and tested by step depolarizations to 0 mV gave results very similar to those shown in Figs. 1 and 2. It was found consistently that the minimal duration of polarization for restoration of the contractile response coincided closely with that required to produce minimal detectable inactivating current. In these fibres the mean minimal time for repriming was 0·38 sec and the mean time necessary for recovery to half maximal tension, 0·68 sec.

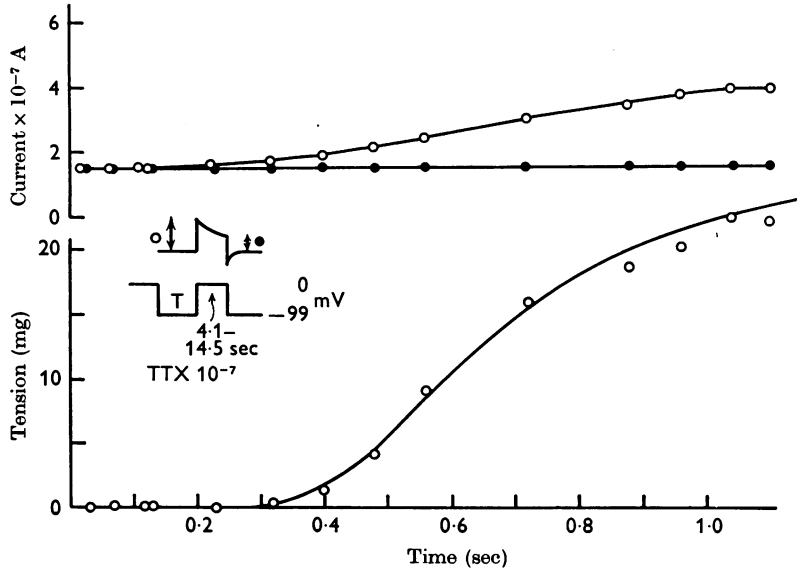

Fig. 2. Restoration of contractile response as a function of the duration of polarization. In the lower portion, the tension developed to a depolarizing step is plotted as a function of duration of polarization. The upper graph shows the current developed by the depolarizing pulse. Open circles represent peak current; filled circles, current at the termination of the pulse.

Dependence of the rate of repriming on membrane potential. The rate of repriming was markedly influenced by the membrane potential at which the fibre was held between depolarizations. An example is shown in the experiment illustrated by Figs. 3 and 4. In the upper portion of Fig. 3, a fibre was clamped at −97 mV, while in the lower record, at −58 mV. In both cases, depolarization steps were to 0 mV. The upper record was obtained a few minutes before the lower one. A comparison shows that the rate of repriming was much more rapid when the fibre was polarized to −97 mV. When the fibre was held at this potential for about 3·7 sec, a subsequent test depolarization produced a contracture with an amplitude similar to that observed after very long periods of repriming. However,

when the fibre was clamped at −58 mV for 5 sec, the restoration of the contractile response was still very incomplete.

Figures 4 and 5 compare graphically the rates of repriming at −59 and at −99 mV. The plots are taken from experiments on two different fibres. The minimal time required for repriming in the fibre polarized to −59 mV was nearly 2 sec, whereas the fibre polarized to −99 mV showed a small

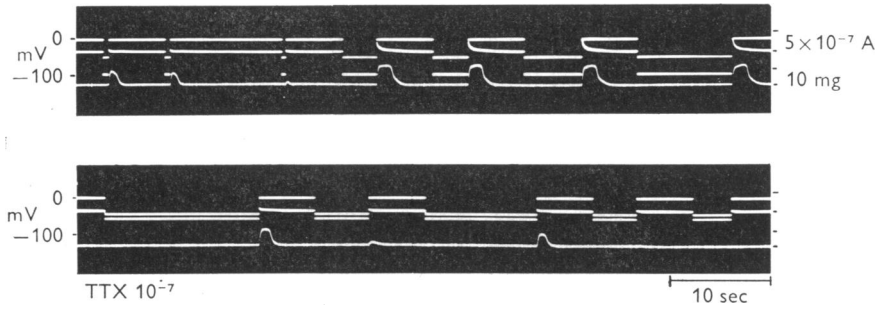

Fig. 3. Effect of membrane potential on rate of repriming. In both the upper and lower records the fibre was clamped at 0 mV except for periods of polarization. In the upper record, the fibre was polarized to −97 mV; in the lower record, to −58 mV.

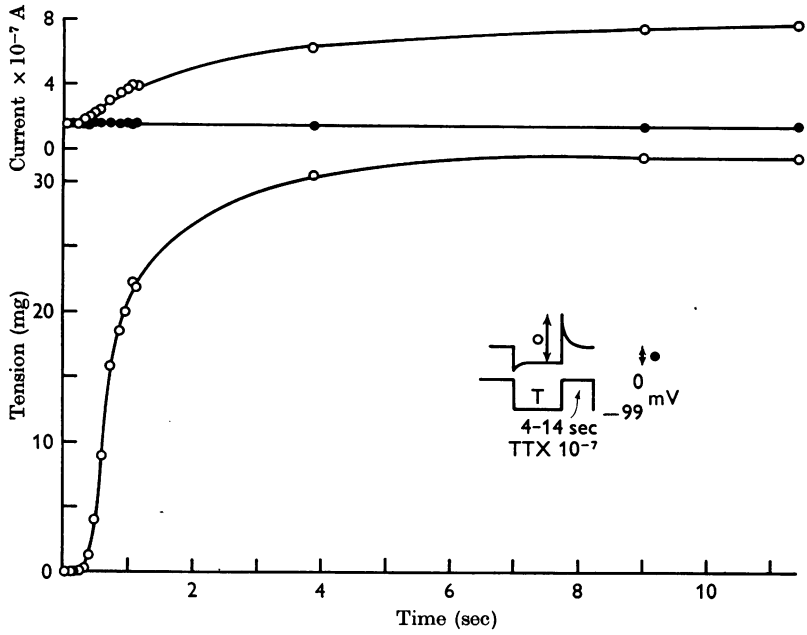

Fig. 4. Rate of repriming at −99 mV. Lower graph shows the contractile response when the fibre was depolarized following varying durations of polarization to −99 mV. Upper graph shows peak current (open circle) and final current to depolarizing pulses.

contractile response after only 0·3 sec of polarization. Also, the rate of repriming was much more rapid at −99 mV. It may also be noted that the inactivating current recovered much more rapidly following polarization at the higher potential. When repriming was studied in a single fibre at different potential levels, similar results were obtained.

In order to determine more accurately the relation between rate of repriming and membrane potential, a larger series of experiments was performed in which fibres were polarized to either −100, −80, or −60 mV.

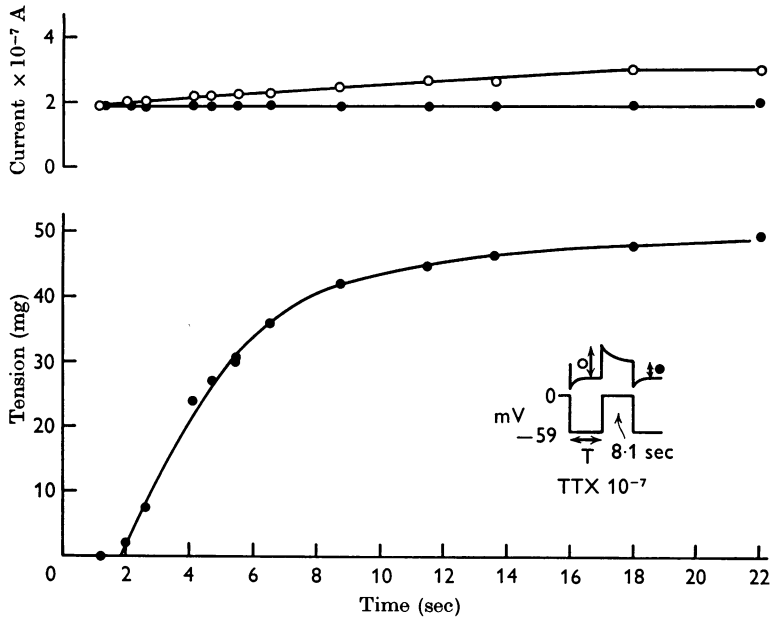

Fig. 5. Rate of repriming at −59 mV. Similar to the experiment shown in Fig. 4 except that the fibre was repolarized to −59 mV for varying periods of time.

Test depolarizations were, in all cases, to 0 mV, and all experiments were carried out in a bathing solution containing tetrodotoxin 10^{-7} w/v. The results derived from thirty-five experiments on thirty-one muscle fibres are shown in Fig. 6. Only those fibres are included in which repriming curves were carried to a level of complete restoration of tension. In order to compare fibres which developed different maximal tensions, tension is expressed in per cent of the maximal tension produced by test depolarizations following complete repriming.

The uppermost graph in Fig. 6 shows the restoration of tension after repriming at −100 mV. The minimal time for repriming was between 0·2 and 0·6 sec, with a mean of 0·38 sec. As the duration of polarization was increased, peak tension increased rapidly, and then more slowly. The mean

time for restoration of half maximal tension was 0·68 sec; after approximately 2 sec, restoration was nearly 90 per cent complete; and when the period of polarization exceeded 5 sec, the contractile response was maximal. Further prolongation of the polarization period did not increase the maximal tension although the duration of contracture was sometimes

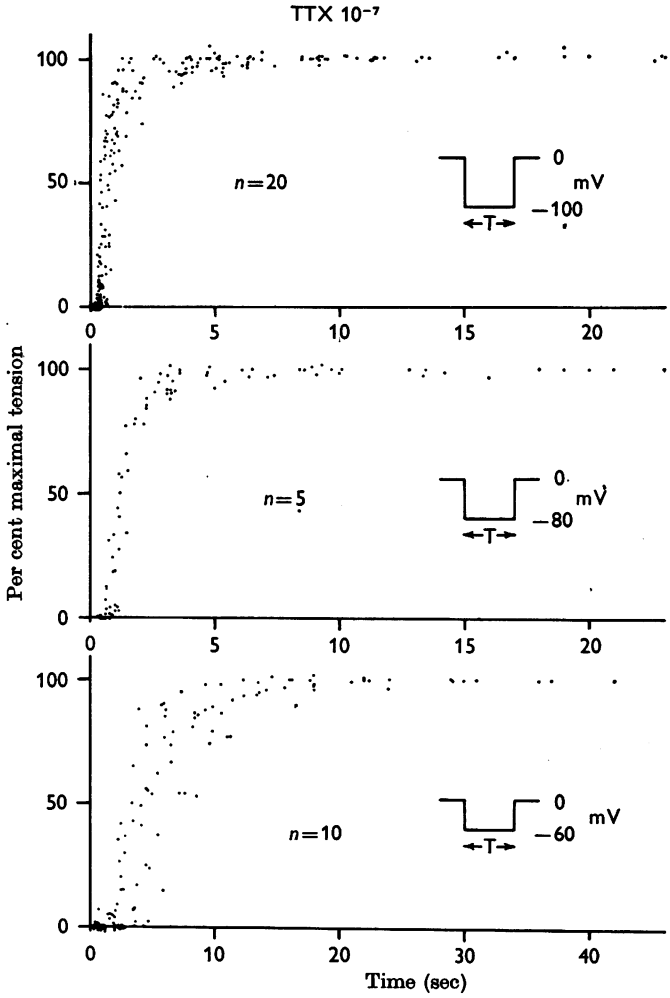

Fig. 6. Effect of membrane potential on rate of repriming. Composite plots of repriming rates from a number of fibres indicated by *n*. Contractile responses have been normalized. For details, see text.

lengthened (see above). When fibres were clamped at −80 mV, the rate of repriming was somewhat slower. Also, the minimal time required for repriming was slightly greater than at −100 mV. In the lowermost graph, the results of polarizing fibres to −60 mV are shown. Here the mean

minimal time for repriming was 2·89 sec, and it required approximately 17 sec of polarization for most of the fibres to reach 90–100 % restoration of their contractile responses. The results indicate clearly that the rate of repriming depends markedly upon the membrane potential to which the fibre is polarized. The relation between restoration of contractile response and membrane potential during repriming has previously been studied only after relatively long periods of polarization. Present results indicate that there are marked differences in the rate of repriming at levels of polarization which produce a uniform and complete degree of restoration after longer periods of time (Hodgkin & Horowicz, 1960).

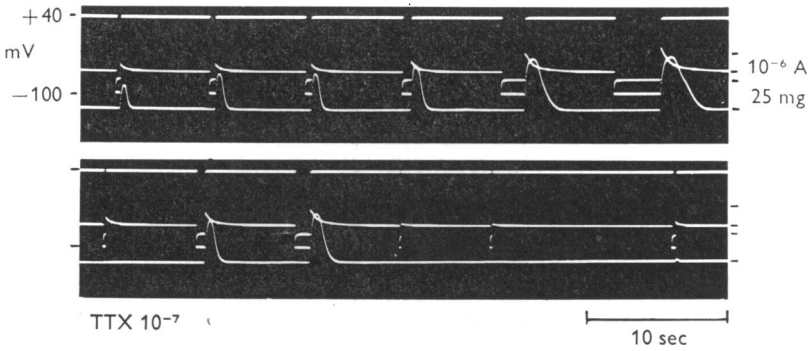

Fig. 7. Effect of depolarizing to +40 mV on the rate of repriming at −100 mV. At the left of the figure, upper trace, potential; middle trace, current; lower trace, tension. See text.

Further evidence for the dependence of the rate of repriming on membrane potential will be presented in the paper which follows (Heistracher & Hunt, 1969*b*), in which it will be shown that the rate of repriming is markedly slowed by procaine.

Repriming at −100 mV in fibres depolarized to either 0 or +40 mV. In order to investigate whether depolarization to +40 mV rather than to 0 mV exerts any influence on subsequent repriming at −100 mV, two sets of experiments were carried out. In one group, fibres were clamped at −100 mV for varying periods and then depolarized to +40 mV for constant periods. Following this, a similar series was obtained with depolarizations to 0 mV. In the other type experiment, each depolarization to 0 mV alternated with one to +40 mV throughout the experiment in which the duration of polarization was varied. Figure 7 shows a typical experiment in which the fibre was clamped at −100 mV for varying periods and depolarized to +40 mV. The results are essentially similar to those described above in which fibres were depolarized from −100 mV to 0 (see Fig. 1). After a minimal period of repriming, restoration of contractile

amplitude and duration increased rapidly as polarization was prolonged. Together with this increase in contractile response, there was an increase in the outward current which inactivated. Figure 8 shows a plot of the restoration of tension as a function of duration of polarization in a fibre which was depolarized either to -1 or $+39$ mV. There appears to be no significant difference in the rate of repriming.

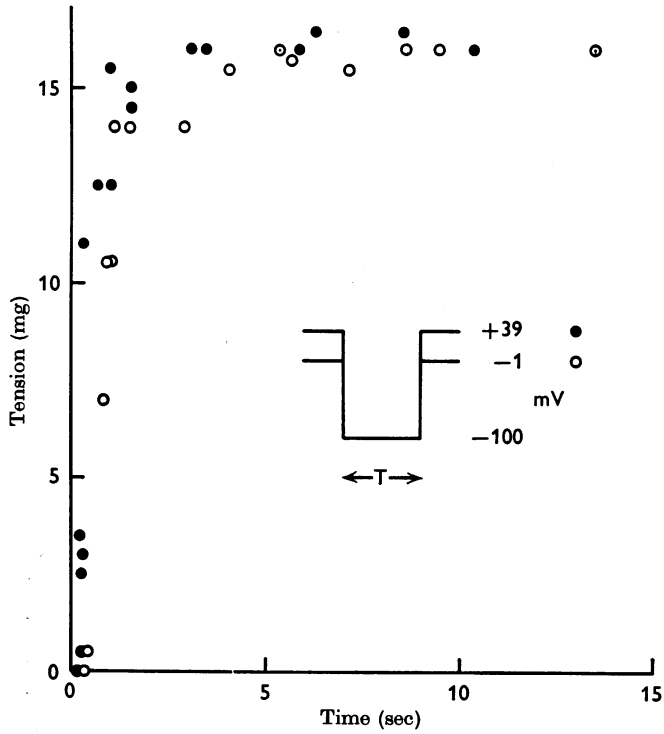

Fig. 8. Comparison of the rate of repriming at -100 mV when a fibre is depolarized to -1 or $+39$ mV. Ordinate, tension developed. Abscissa, duration of polarization. Note the lack of significant difference in the repriming rates.

Repriming in solutions containing low $[Na^+]_o$ *and normal* $[Ca^{2+}]_o$. In several fibres the rate of repriming was studied after the bathing solution was switched to one containing choline Cl rather than NaCl. All these fibres were clamped at -100 mV and depolarized to 0 mV. The repriming curve from six fibres is shown in Fig. 9. Peak tensions are expressed as percentage of maximal peak tension obtained when contractile restoration was complete. Comparison of Fig. 9 with the uppermost graph of Fig. 6 indicates that the rate of repriming was not appreciably changed by replacing Na in the bathing solution by choline.

Repriming with single pulses or trains of pulses. The characteristics of the repriming process were further studied by examining the contractile responses following repetitive polarizing pulses. The fibres were clamped at 0 mV except during the polarizing pulses to −100 mV. All experiments in this group were carried out in muscle fibres in a bathing solution containing tetrodotoxin 10^{-7} w/v and 3·5 mM-$[Ca^{2+}]_o$.

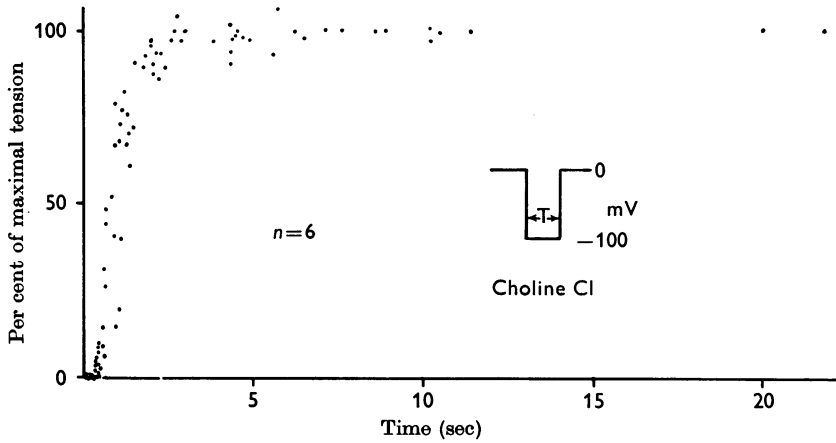

Fig. 9. Rate of repriming in a sodium-deficient solution. The rate of repriming at −100 mV in six fibres was examined in a solution in which all NaCl was replaced by choline Cl.

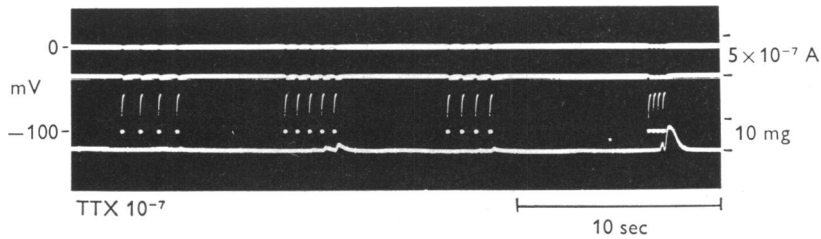

Fig. 10. Repriming to brief recurrent pulses of hyperpolarization. Frequency of the polarizing pulses was varied, but the duration of each pulse remained constant.

Figure 10 shows a record from a typical experiment. The fibre was clamped at 0 mV, and, at intervals of about 10 sec, four to five polarizing pulses were given. Each repolarization was to −100 mV for a duration of about 0·1 sec. The interval between pulses of a train was varied. In the first series of pulses the interval was about 1 sec. Following the first three pulses, no contractile response occurred; following the fourth, a very small tension appeared. In the second series of pulses, the interval was about 0·6 sec. Contractile responses may be seen to follow the third, fourth and fifth pulses. In the third series, the interval between pulses was 0·75 sec,

and the amount of contractile response is intermediate between the first and second series. The fourth series of pulses is at a higher frequency, the pulse interval being 0·3 sec. There is a small amount of tension developed after the third pulse, and a much larger contractile response after the completion of the train. The amount of repriming developed by pulsatile repolarization can be seen to depend upon the interval between pulses.

In Fig. 11 hyperpolarizing pulses of the same duration and amplitude as those in Fig. 10 were given at a constant interval of 0·3 sec. The number of pulses was varied from three to ten. At the end of the series, a long polarizing pulse was given which produced nearly maximal restoration of the contractile response. When three brief hyperpolarizing pulses were given

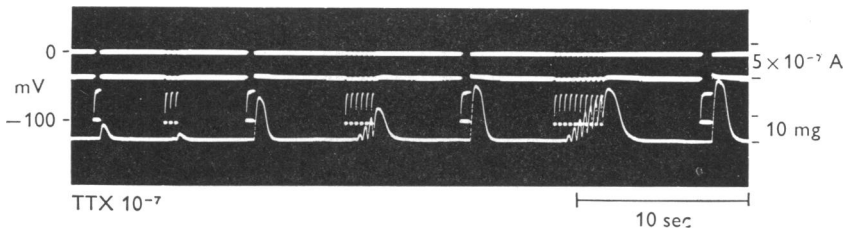

Fig. 11. Contractile repriming to brief hyperpolarizing pulses. The duration and frequency of pulses was constant, but the number of pulses varied. At the right is the response following a long repolarizing step.

a contractile response followed only the last. As the train length was increased, it may be seen that there was a cumulative restoration of tension, and following the 10-pulse train repriming was almost complete. This was not associated with a detectable amount of inactivating outward current, whereas the long duration repolarization which followed was.

When a fibre is reprimed by pulsatile polarization, the periods between such pulses may be expected to produce some degree of inactivation. There appears to be some cumulative effect on repriming by a series of hyperpolarizing pulses. As has been seen above, a sufficiently long train of pulses can produce complete repriming. Figure 12 shows a graph of an experiment in which repriming following a single pulse or a train of pulses was compared. The membrane was held at 0 mV except for periods of polarization to − 99 mV. The restoration of tension as a function of duration of polarization for single pulses is shown by the open circles. The rate of recovery is quite similar to the examples shown earlier. The filled circles represent the rate of recovery following trains of pulses. The duration of each pulse was 0·39 sec. The frequency of the train was 2/sec. In the curve showing repriming for trains of pulses, time is measured as the sum of the durations of the polarizing pulses during the train. When the duration of the single pulse coincided with one pulse of the train, the reprim ing was,

of course, similar. The rate of restoration of tension was then more rapid to single pulses than to trains until the number of pulses increased to twenty or more, when the amount of repriming following the trains of pulses was nearly the same as after single pulses. This experiment indicates that the restoration of tension following a single pulse may be greater than that following a like duration of polarization in the form of a pulse train. This is undoubtedly due to some inactivation occurring in the intervals between the pulses of the train. However, as the pulse train becomes longer, the additive effect on repriming produces a complete restoration of contractile response in spite of the intermittent inactivation.

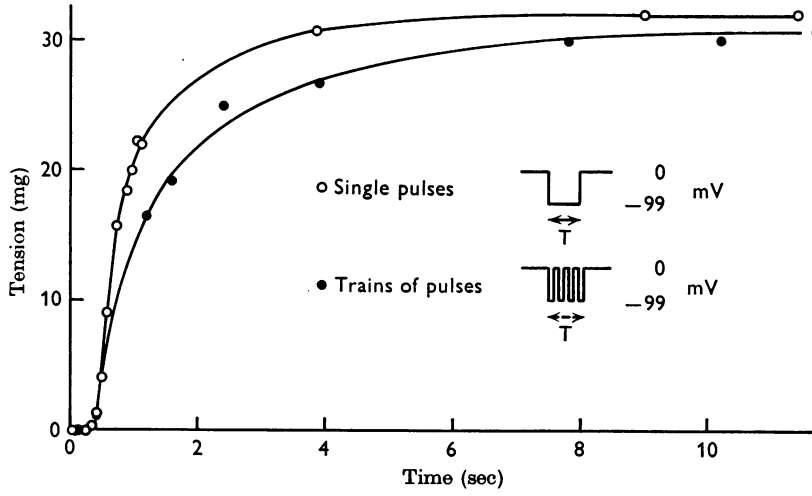

Fig. 12. Comparison of repriming to single pulses or trains of pulses.

By delivering a train of repolarizing pulses, the contractile response could be maintained at its maximal level. An example is shown in Fig. 13. The fibre was initially clamped at 0 mV then polarized to −100 mV for approximately 10 sec. A long depolarization step to 0 mV produced a maximal contracture, and the peak tension was maintained for about 1·5 sec. The tension then slowly returned to the resting level. After another period of polarization for 10 sec, the fibre was again depolarized to 0 mV. The second depolarization caused maximal tension to develop, and while this was at its peak, a train of brief repolarizations to −100 mV was initiated. Twenty-three pulses at a frequency of 4·5/sec were given, and the tension remained at its maximal level until about 1 sec after the last pulse of the train. It may also be noted that the inactivating outward current associated with the depolarization step had partially decayed when the train of hyperpolarizing pulses was begun. The first few hyperpolarizing pulses were followed by an increase in outward current, but following subsequent

pulses the current between pulses was at essentially the same level as during a comparable period in the previous depolarization in which no hyperpolarizing pulses were given. This experiment indicates that a fibre can maintain a tension slightly in excess of the maximal tetanic tension for a long period of time if repriming is maintained by a succession of brief periods of polarization.

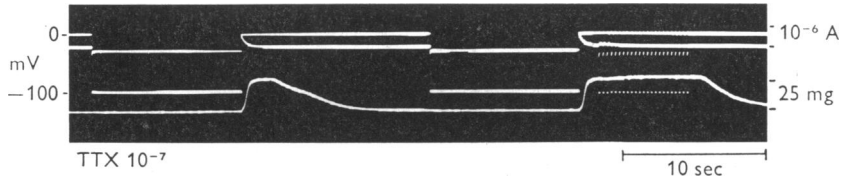

Fig. 13. Maintenance of maximal contractile tension by repeated brief hyperpolarizing pulses.

DISCUSSION

The present study confirms the finding that restoration of the contractile response after prolonged depolarization depends upon the level to which a twitch muscle fibre is polarized (Hodgkin & Horowicz, 1960; Lüttgau, 1963; Zachar & Zacharová, 1966; Frankenhaeuser & Lännergren, 1967; Lüttgau & Oetliker, 1968). The present results indicate the time course of this repriming. It is clear that the rates of repriming vary considerably at levels of membrane potential, which, in the steady state, produce complete repriming. Thus, in snake twitch muscle fibres bathed in solutions containing tetrodotoxin, restoration of the contractile response to depolarization is complete after polarization for less than a minute to levels slightly higher than the threshold for contractile activation. Yet, the rate at which repriming occurs varies markedly in the range -50 to -100 mV. Polarization to higher levels of membrane potential produced very little change in the rate of repriming.

Complete repriming can be maintained by repetitive brief pulses of hyperpolarization. In the normal twitch type muscle fibre, a maintained tetanic contraction usually occurs at a relatively low frequency (about 20–50/sec in mammalian muscle). At such frequencies the period of time during which the fibre is polarized must be in excess of the time needed for complete repriming, particularly since inactivation produced by depolarization is probably only partial. The need for repriming in twitch muscle fibres is met by the intermittent nature of their excitation.

At the present time, there is little direct evidence on the mechanism by by repriming takes place. If one assumes that contractile inactivation is associated with an inactivation of a calcium conductance in the sarcoplasmic reticulum membrane, repriming might be associated with a

reactivation of this conductance mechanism. The system might then be analogous to the inactivation and reactivation of the active sodium conductance in nerve or muscle membrane. In frog and *Xenopus laevis* muscle fibres, the recovery of electrical and mechanical response in Ringer solution, following a potassium contracture, occur at the same time (Hodgkin & Horowicz, 1960; Frankenhaeuser & Lännergren, 1967). In snake twitch muscle fibres there is a close correlation between the time course of repriming and of reactivation of the delayed potassium conductance. Both these findings make the assumption that repriming is associated with a reactivation of a calcium conductance in the sarcoplasmic reticulum membrane seem reasonable. On the other hand, there appears to be a minimal time necessary at any given membrane potential before there is any restoration of the contractile response. This might be due also to the need for release of a minimal quantity of calcium into the sarcoplasm before contractile activation occurred. The minimal time for repriming is about one half the time to half recovery of the contractile response (see also Heistracher & Hunt, 1969*b*). If the amount of repriming were related to the quantity of Ca^{2+} released by a test depolarization, and if, as suggested above, a minimal concentration of Ca^{2+} is needed for contraction, it might be reasonable to expect this relationship between minimal time for repriming and time to half recovery.

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REFERENCES

CURTIS, B. A. (1964). The recovery of contractile ability following a contracture in skeletal muscle. *J. gen. Physiol.* **47**, 953–964.

FRANKENHAEUSER, B. & LÄNNERGREN, J. (1967). The effect of calcium on the mechanical response of single twitch muscle fibres of *Xenopus laevis*. *Acta physiol. scand.* **69**, 242–254.

HEISTRACHER, P. & HUNT, C. C. (1967). Contractile inactivation and repriming in twitch muscle fibres. *J. gen. Physiol.* **50**, 2499.

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

HEISTRACHER, P. & HUNT, C. C. (1969*b*). The effect of procaine on snake twitch muscle fibres. *J. Physiol.* **201**, 627–638.

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

LÜTTGAU, H. C. (1963). The action of calcium ions on potassium contractures of single muscle fibres. *J. Physiol.* **168**, 679–697.

LÜTTGAU, H. C. & OETLIKER, H. (1968). The action of caffeine on the activation of the contractile mechanism in striated muscle fibres. *J. Physiol.* **194**, 51–74.

MILLIGAN, J. V. & EDWARDS, C. (1965). Some factors affecting the time course of the recovery of contracture ability following a potassium contracture in frog striated muscle. *J. gen. Physiol.* **48**, 975–983.

ZACHAR, J. & ZACHAROVÁ, D. (1966). Potassium contractures in single muscle fibres of the crayfish. *J. Physiol.* **186**, 596–618.