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# THE RELATION OF MEMBRANE CHANGES TO CONTRACTION IN TWITCH MUSCLE FIBRES

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SUMMARY

1. Contractile responses in short twitch-type snake muscle fibres have been studied. These fibres are sufficiently short to allow fairly uniform changes in membrane potential along their length when current is passed through an intracellular micropipette. Active sodium permeability changes were blocked with tetrodotoxin (TTX), procaine, or by using solutions low in sodium. Current and voltage micropipettes were used to voltage-clamp these fibres. Depolarization steps to about $-40$ mV evoked contractile responses, maximal tension being developed between $-10$ and 0 mV. The relation between contraction and membrane potential was sigmoid.

2. Depolarization beyond a critical threshold produced an increment of outward current which inactivated with time. The threshold for this delayed rectification was normally similar to the threshold for contractile activation. Fibres exposed to high potassium showed a reversal of this inactivating current to slightly super-threshold depolarizing pulses. At membrane potentials near 0 mV, no inactivating current was noted, while stronger depolarizing pulses produced an inactivating current in the normal direction. Fibres in high potassium show the same threshold for initiation of contraction as in normal solution.

3. Thiocyanate, nitrate, and caffeine shifted the relation between membrane potential and contraction toward higher levels of membrane potential. The threshold for inactivating rectifying current failed to shift to a corresponding extent, although some shift in rectification which did not inactivate was evident.

4. When depolarization was maintained, contractile tension was maximal for several seconds, then gradually disappeared. The rate of this contractile inactivation depended upon the level of depolarization.

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## INTRODUCTION

In vertebrate twitch muscle fibres, contraction normally follows propagation of an action potential along the length of the fibre. The mechanisms whereby the muscle action potential brings about contractile activation are still incompletely understood, although it seems clear from the studies of Huxley & Taylor (1958) that potential changes from the surface of the fibre spread to the interior by means of the transverse tubular system. The relation between membrane potential and contraction has been investigated by Hodgkin & Horowicz (1960*a*), Lüttgau (1963), and others, using isolated twitch muscle fibres. By varying the external potassium concentration, the membrane potential could be changed uniformly along the length of the muscle fibre while tension was recorded. These studies have provided important information on the relation between membrane potential and tension development, on the inactivation of contraction which occurs during maintained depolarization, and on the repriming of the inactivated system which follows repolarization.

Frog muscle fibres, in which impulse activity is blocked, show changes in resistance of two sorts when their membrane potentials are varied: normal (delayed) and anomalous rectification. Depolarization normally results in a fall of membrane resistance (Jenerick, 1959; Katz & Lou, 1947; Narahashi, Deguchi, Urakawa & Ohkubo, 1960; Nakajima, Iwasaki & Obata, 1962; Adrian, Chandler & Hodgkin, 1966). This rectification in the 'normal' direction appears when depolarization exceeds a threshold value; it occurs after a delay and inactivates with time. Adrian *et al.* (1966) have found that the conductance change has a K:Na selectivity of 30:1. Anomalous rectification was first noted in fibres exposed to high $[K^+]_o$. In this circumstance the voltage–current relation shows rectification in the reverse or anomalous direction; that is, membrane resistance increases on depolarization (Katz & Lou, 1947; Adrian & Freygang, 1962; Nakajima *et al.* 1962). In fibres bathed in normal Ringer solution the threshold for delayed rectification and for impulse initiation are similar so that little evidence of delayed rectification is found unless impulse activity is blocked.

In fibres bathed in sodium-deficient solutions, or when active sodium permeability changes are blocked by tetrodotoxin, the thresholds for contraction and for the earliest appearance of delayed rectification are similar (Costantin, 1967, 1968; Kao & Stanfield, 1967; Lorkovic & Edwards, 1968). Further, the thresholds for contraction and for 'delayed' rectification have been found to shift to similar extents with changes in $[Ca^{2+}]_o$ (Costantin, 1967, 1968) and under the influence of various anions (Kao & Stanfield, 1967).

In the present study the relations between membrane potential,

membrane current, and contraction have been studied in short twitch muscle fibres of snake. These fibres are sufficiently short to allow fairly uniform changes in membrane potential along their length when current is passed via an intracellular micropipette (see also Orkand, 1962). This has permitted a more detailed study of the relation between membrane changes and contractile activation, inactivation and repriming. The results suggest that there is not a simple causal relationship between delayed rectification and contractile activation. A preliminary report has appeared (Hunt & Heistracher, 1967).

## METHODS

In the ventro-lateral skin of certain snakes there are short muscles composed of 3–12 parallel fibres between adjacent scales. The fibres are usually 1·0–1·5 mm in length. In the present study garter snakes (*Thamnophis*) were generally used. The scale muscles were dissected out by removal of the skin underlying them and leaving pieces of scale attached at both ends. The muscle was mounted in a flow chamber, one scale being fixed, the other being attached to a strain gauge.

The chamber was made of Plexiglas, the cross-sectional area in the vicinity of the muscle being about 9 $mm^2$. The muscle was illuminated from below, using a dark field condenser of long working distance. Fluid inflow could be selected from three inputs which entered the chamber just upstream from the preparation. After passing a baffle, fluid was removed from the chamber by suction. A porous Ag-AgCl electrode (Al-Chem Co.) was connected with the chamber via a 3 M-KCl bridge downstream from the preparation. The KCl in the bridge was periodically restored from a screw-driven syringe. Experiments were done at room temperature (20° C).

Micropipettes were made of Pyrex, filled with 3 M-KCl, and had resistances of 5–15 MΩ. They were broken under water about 3 mm from their tips and were then mounted on a thin (about 30 $\mu$) strip of chlorided silver tapered at the tip and fixed in place with Vaseline. The other end of the silver strip was connected to a pin jack which plugged into the unity gain amplifier. The strip was highly compliant in one direction, parallel to the long axis of the muscle fibre, allowing movement of the electrode tip during contraction and minimizing damage. Current electrodes were similar and usually 5–10 MΩ. A unity gain, high input impedance amplifier, employing field effect transistors (Fein, 1964), was used for recording, its output being fed to a Tektronix 565 oscilloscope. The circuit is shown in Fig. 1. The bath was connected to the input of an operational amplifier (Q 25 AH Philbrick, with 1 MΩ feed-back resistor). The input was kept at ground potential by feed-back, and the output of the amplifier indicated the current passed through the micropipette (1 V/$\mu$A).

In the voltage clamp mode, the current electrode was connected to the output of a high gain (25,000 ×), inverting operational amplifier (Data Devices D-12 modified for high output voltage swing, maximum +45 V). Through three resistors, several potentials could be brought to the summing point of the input. One was the output of the unity gain recording amplifier, another was a potential to determine the base line clamped potential, and the third was usually a step potential to produce a depolarization of variable amplitude and duration.

Tension was recorded by means of a semiconductor strain gauge, the output being connected to a high gain (3 A 3) DC amplifier of the Tektronix 565 oscilloscope. Maximal useable gain was 3 mg/cm of deflexion.

Bathing fluids of the following composition (in millimolar) were usually used: (A) NACl 145, KCl 4·3, $CaCl_2$ 3·5, $MgCl_2$ 1·7, $NaHCO_3$ 15, and glucose 9·8; or (B) NaCl 158, KCl 2·15, $CaCl_2$ 3·5, $MgCl_2$ 1·7, $Na_2HPO_4$ 2·15, $NaH_2PO_4$ 0·85, and glucose 9·8. Solution A was equilibrated with 95 % $O_2$, 5 % $CO_2$. Variation in $[Ca^{2+}]_o$ was made without alteration in other

electrolytes. When $[Na^+]_o$ was reduced, choline was used as a substitute. Usually tetrodotoxin (Sankyo, Tokyo, Japan), in a concentration of $1 \times 10^{-7}$ (w/v) was used to block impulse activity. In order to determine whether solutions containing foreign anions produced any change in tip potential of the recording micropipette, the latter was usually withdrawn after completion of a series of measurements and the solution changed from one containing $Cl^-$ to foreign anion and back to $Cl^-$.

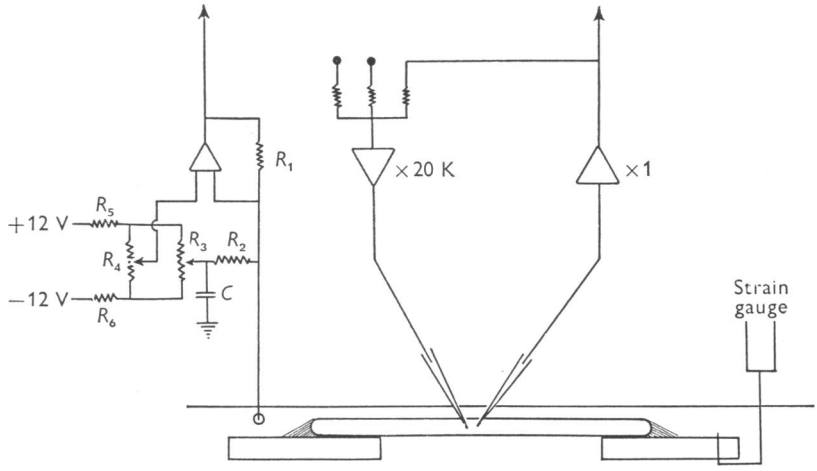


Fig. 1. Schematic view of experimental set-up. $C = 0{\cdot}01\ \mu F$; $R_1 = 1\ M\Omega$; $R_2 = 22\ M\Omega$; $R_3 = 1\ k\Omega$; $R_4 = 1\ k\Omega$; $R_5 = 10\ k\Omega$; $R_6 = 10\ k\Omega$.

## RESULTS

*Distribution of potential change along the fibre length.* When current is passed through an intracellular micropipette at one point along a long muscle fibre well away from its ends, the resultant potential change ordinarily decays to 1/e in one length constant. More generally, including the case where the total length of a fibre is small compared to its length constant ($\lambda$), and one electrode (either that used for applying current or for recording voltage) is placed at the centre of the fibre, the following relation (Wiedmann, 1952) holds:

$$V = V_0 \frac{\cosh[(L-X)/\lambda]}{\cosh[L/\lambda)}, \qquad (1)$$

where

$V_0$ = potential at $X = 0$,
$X$ = distance between recording and current electrodes,
$V$ = potential at $X$,
$L$ = fibre length from centre to end,
$\lambda = \sqrt{(r_m/r_i)}$,
$r_m$ = membrane resistance $X$ unit length,
$r_i$ = internal resistance/unit length.

The electrical characteristics of long (*ca.* 10 mm) twitch muscle fibres in the costocutaneous muscle of the snake are shown in Table 1. Except for their length, these fibres appeared similar to the short scale muscle fibres. It may be seen that the length constant ($\lambda$) as measured from near the centre of the fibre, was approximately 2 mm in these fibres which averaged about 60 $\mu$ in diameter. These measurements were made by impaling the fibre far from its end with a current electrode and examining the potential changes at various distances in response to a hyperpolarizing current pulse.

TABLE 1. Input resistance and length constant ($\lambda$) in costocutaneous fibres ($[K^+]_o$ = 2·15 mM)

| Fibre diam. ($\mu$m) | Input res. MΩ | $\lambda$ (mm) |
|---|---|---|
| 60 | 0·545 | 1·70 |
| 50 | 0·38 | 1·94 |
| 50 | 0·62 | 2·12 |
| 50 | 0·87 | 2·00 |
| 70 | 0·60 | 1·77 |
| 50 | 0·76 | 2·31 |
| 60 | 0·70 | 2·32 |
| 35 (?) | 0·575 | 2·21 |

In the present experiments, the maximum fall in input resistance during delayed rectification was to about 10 % of its resting value. In long muscle fibres, where $L$ is much larger than $\lambda$, this change in input resistance would cause a tenfold decrease in $\lambda$ since

$$\frac{V_0}{I_0} = \frac{1}{2}\sqrt{(r_i r_m)} = \frac{1}{2} r_i \lambda.$$

However, in the short muscle fibres used in this study, where $L$ is short compared to $\lambda$, the following relation holds (Falk & Fatt, 1964)

$$\frac{V_0}{I_0} = \frac{1}{2}\sqrt{(r_i r_m)} \coth \frac{L}{\lambda}.$$

The input resistance is therefore proportional to $\lambda \times \coth L/\lambda$. In this circumstance, if the input resistance decreased tenfold, there would be only a 3·5-fold decrease in $\lambda$ (when $\lambda$ = 2·4 and $L$ = 0·6 mm).

Figure 2 shows the expected distribution of potential change if current is passed through a micropipette in the centre of a 1·2 mm fibre, when $\lambda$ has a resting value of 2·4 mm or when it is decreased to 0·68 mm, a value expected during the fall in input resistance to about 10 % of its resting value. Also shown is the spatial distribution expected if $\lambda$ = 1·2, a value predicted if $V_0/I_0$ falls to 1/4. These represent predicted distributions of potential change when the membrane is displaced from its resting potential level.

Experiments were carried out on scale muscle fibres in which current and recording electrodes were placed in the centre of a fibre and a second recording electrode impaled the fibre at one end. An example is shown in Fig. 3 where the centrally located current and voltage electrodes were used in the voltage clamp mode. Impulse activity in this fibre was blocked. A small step depolarization produced a voltage change at the end of the fibre which rose more slowly, but reached nearly the same level as the potential change in the centre of the fibre. Larger depolarization steps, which produced a delayed rectification, were associated with a slightly greater

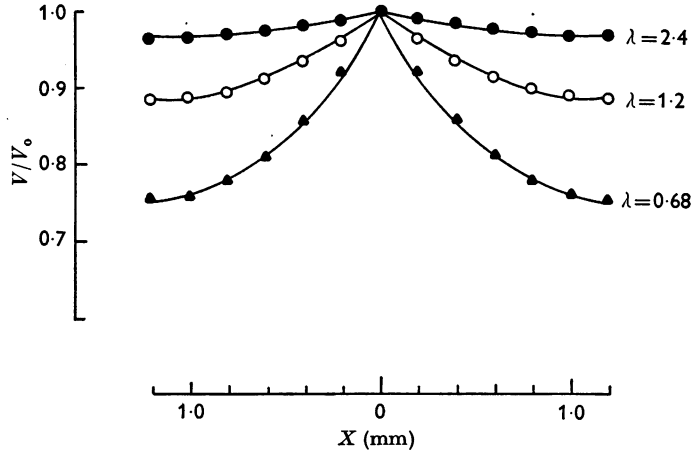


Fig. 2. Calculated distribution of potential change along the length of a 1·2 mm muscle fibre when current is passed through an intracellular pipette in the centre of the fibre.

difference in potential change between centre and end of the fibre as may be seen in Fig. 3. The above considerations indicate that the non-uniformity in the distribution of potential change along the length of the fibre is relatively small under the conditions of the present experiments. Under the least favourable conditions, the potential change at the end of the fibre may differ from that in the centre by about 25 %. Usually the difference would be expected to be much smaller.

*Contractile activation.* All the fibres examined in the scale muscle were of the twitch type, that is, they normally showed propagated action potentials and all-or-none contractile responses to above-threshold stimuli. In the experiments to be reported, impulse activity was blocked by substituting choline for sodium, or by adding tetrodotoxin or procaine HCl to the bathing solution. Depolarization of the fibre above a threshold level then produced contractures which were graded by the magnitude and duration of the depolarization steps. Such contractures are analogous to

the responses obtained in frog twitch muscle fibres in which the spike mechanism was blocked and the fibres depolarized by raising external $[K^+]$ (Hodgkin & Horowicz, 1960*a*).

A typical response of a fibre in a bathing solution containing tetrodotoxin is shown in Fig. 4. Current and voltage electrodes were introduced into the centre of the fibre and the membrane potential was held by a voltage clamp at $-100$ mV (resting potentials were between 70 and 90 mV).

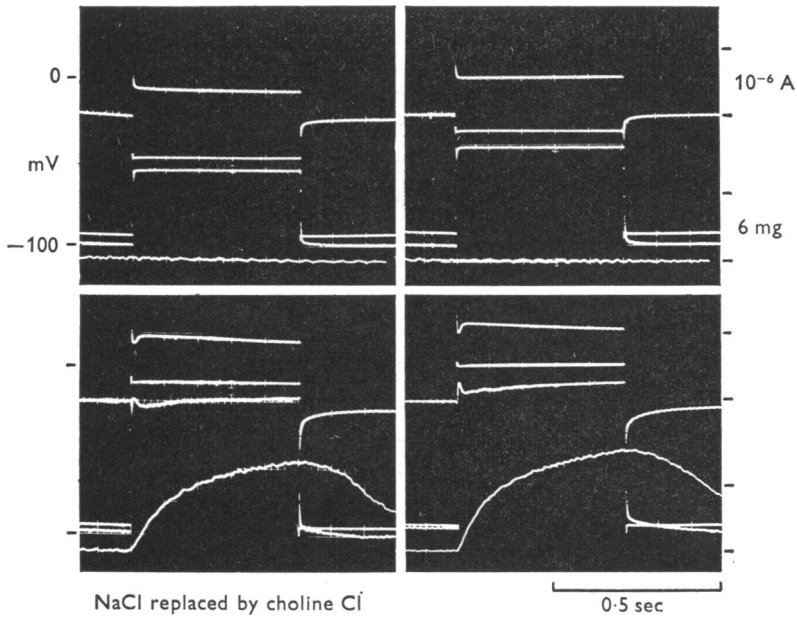


Fig. 3. Potential, current, and tension in a voltage-clamped fibre. Current and recording electrodes were introduced into the centre of the fibre and an additional electrode at one end. The centre recording electrode was connected to a high gain amplifier in a voltage-clamp mode. Upper trace, current; second trace, voltage at the centre of the fibre; third trace, voltage at the end of the fibre; fourth trace, tension. The voltage scale applied to the centre recording electrode. The potential from the end recording micropipette is at the same gain and the same resting potential. The beam was deflected downward so that it could be seen more readily. Note delayed onset of rectification.

Graded depolarizations of constant duration were then applied. Step depolarizations produced no tension until the membrane potential reached $-44$ mV. Maximal tension usually occurred at levels of membrane potential between $-10$ and 0 mV. In the fibre shown in Fig. 4, the contraction was maximal at 0 mV. Further depolarization (not shown) caused no further increase in the contractile response. The latency of the onset of contraction to such depolarization steps was approximately 5–10 msec.

The tension recording allowed a moderate amount of shortening due to compliance in the rather long lever system. These latency values would undoubtedly be shorter under more rigidly isometric conditions.

The relation between tension developed and membrane potential was sigmoid as shown in Fig. 5. This is similar to the relation found in frog

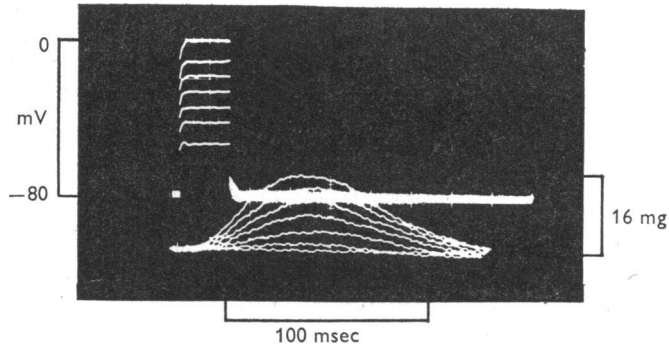


Fig. 4. Contractile responses to graded depolarization steps. Upper trace, potential; lower trace, tension.

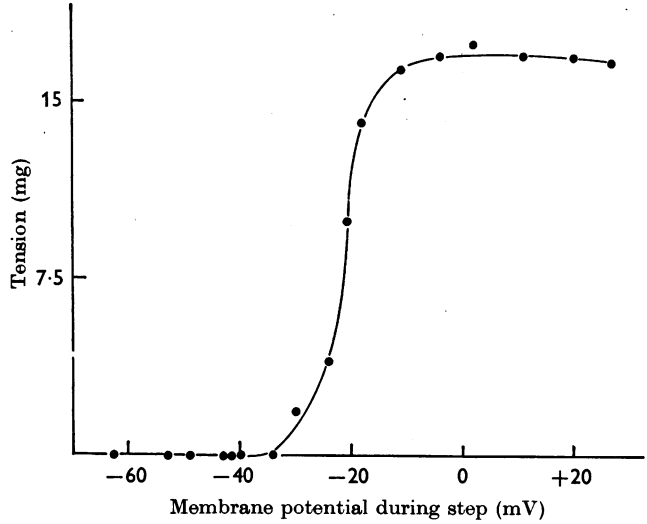


Fig. 5. Relation between tension and membrane potential during depolarization steps.

twitch muscle fibres by Hodgkin & Horowicz (1960*a*), and also by Lüttgau, (1963). Similar results were found by Frankenhaeuser & Lännergren (1967) in *Xenopus* twitch muscle fibres. However, the threshold for contractile response was at a somewhat more depolarized level, about −40 mV (range −45 to −25 mV), than in frog twitch fibres. This is probably due, in part, to the higher concentrations of external $Ca^{2+}$ used here. We generally used 3·5 mM-$Ca^{2+}$, about double the concentration used by Hodgkin & Horowicz (1960*a*).

Lowering the calcium concentration shifted the curve relating tension to membrane potential to the left, but it appears that at identical calcium concentrations there might be some threshold difference between snake and frog twitch muscle fibres. Our observations are too limited to be certain about this point.

Figure 6 shows an experiment in which the duration of a depolarization step was varied. The fibre was clamped initially at $-100$ mV and depolarized to 0 mV for varying periods of time. The record shows three traces.

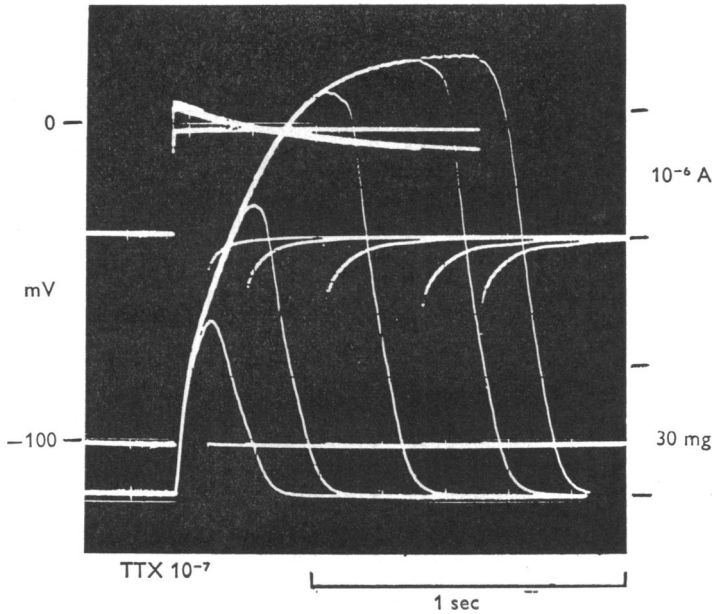


Fig. 6. Responses to depolarizing pulses of increasing duration. Upper trace, current; centre trace, potential; lower trace, tension. The fibre was in tetrodotoxin $1 \times 10^{-7}$ w/v (TTX).

From above downwards these are current, potential, and tension. As the depolarization step was lengthened, peak tension increased until it reached a maximal value. Maximal tension was approximately 105 mg. The maximal tensions in these fibres generally ranged from 1 to 2 kg/cm$^2$. This is somewhat smaller than the values reported by Hodgkin & Horowicz (1960*a*), but in the same range as some values found for *Xenopus* twitch muscle fibres (Frankenhaeuser & Lännergren, 1967). Under more rigidly isometric conditions, we would expect larger values for these snake muscle fibres.

Several additional features may be seen in Fig. 6. The current which accompanies the depolarization step inactivates with time, reaching a steady value after about one second. Other records will show that this

current had a delayed onset. One may also note that there was a transient current after the return of the membrane potential from its depolarized level to its base line clamped value; this off-current may be seen to increase with increasing duration of the depolarization step. It may also be seen in Fig. 6 that the peak tension for the shortest duration depolarization step occurs slightly later than the cessation of the step; with longer depolarizations, peak tension occurred at approximately the same time. The rate of relaxation is similar for all but the shortest duration pulse. These findings show that contractures produced in response to a depolarization, which is supermaximal when of sufficient duration, may be graded by the duration of the depolarization step.

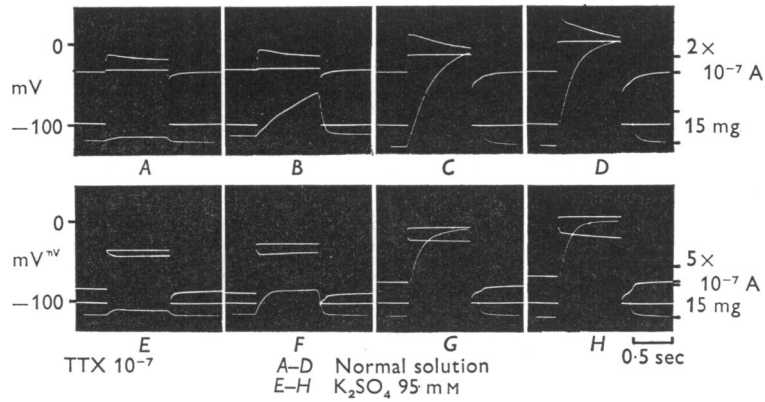


Fig. 7. Current and tension changes in a muscle fibre in normal or high potassium solution. In *A* to *D* the fibre was in a normal bathing solution; in *E* to *H* 95 mM-$K_2SO_4$ replaced NaCl. At the left of each record, the upper trace is current; middle trace, potential; lower trace, tension.

*Delayed rectification and the effects of high potassium.* Weak depolarization steps were associated with an outward flow of current which, apart from capacitative transients, remained nearly constant during the step. When depolarization exceeded a critical threshold, an increment of outward current appeared which inactivated approximately exponentially with time. At 20° C the time constant of this inactivation was about 1 sec. It may be seen in Fig. 7*A–D* that the rate at which this component of outward current reached its peak value increased as the size of the depolarizing step was increased. Although no attempt was made to determine systematically the delay in onset of this current, such delay was clearly observed with slightly superthreshold depolarizing pulses. This inactivating outward current appears similar to delayed rectification in frog muscle (Adrian *et al.* 1966). At the end of a long depolarizing pulse, when the current had reached a steady value, the current–voltage relationship

showed a slope indicating that there remained a residual rectification in the normal direction (see later figures). In some fibres the voltage–current relationship at membrane potentials higher than the threshold for delayed rectification showed some degree of anomalous rectification; however, the amount of anomalous rectification in these fibres is apparently less than in frog twitch fibres. In the latter, after inactivation of delayed rectification the current–voltage relation shows a conspicuous rectification in the anomalous direction (Nakajima *et al.* 1962), whereas in snake muscle fibres, the residual rectification is in the normal direction. When depolarization steps exceeded the threshold for delayed rectification, the slope of the relation between peak current and potential changed abruptly. The slope then remained quite constant as the size of the depolarization step was further increased. This suggests that the conductance change associated with delayed rectification becomes maximal near threshold and remains rather constant as depolarization increases. However, the rate at which the delayed rectification reached its peak increased markedly as depolarization was increased above threshold.

As was noted above, the threshold for delayed rectification in snake muscle normally coincides with the threshold for contraction, as is the case in frog muscle (Costantin, 1967, 1968; Kao & Stanfield, 1967; Lorković & Edwards, 1968). This may again be noted in Fig. 7*A–D*. In this fibre the threshold for contraction was at $-40$ mV.

Since the delayed rectification channel involves a conductance with a high selectivity to $K^+$, it was of interest to examine the relations between current, potential, and contraction in a fibre bathed in a solution containing high K. Figure 7*E–H* shows the results of replacing NaCl in the external solution by 95 mM-$K_2SO_4$. The resting potential in this solution was near 0 mV. The fibre was clamped at $-100$ mV and then subjected to depolarization steps of graded amplitude but constant duration. The threshold for initiation of contractile response was the same as when the fibre was in normal solution. This was a consistent finding in other experiments. The threshold for the appearance of a current which inactivates with time was also unchanged by switching from a normal solution to 95 mM-$K_2SO_4$. With levels of depolarization slightly above threshold, it can be seen that the inactivating fraction of the current is inward rather than outward as in normal solution. With larger depolarization steps it may be seen that the inactivating current continued to be inward, but became progressively smaller as the step approached 0 mV. When the fibre interior was made positive during the step, the inactivating current reversed and became outward. In spite of the fact that the inactivating portion of the current associated with delayed rectification was reversed in direction, no apparent change in contractile activation resulted. These results suggest that con-

tractile activation cannot depend simply on an inactivating outward current through the delayed rectification channel (see Discussion). The findings show that delayed rectification is present in snake twitch muscle fibres exposed to 95 mM-$K_2SO_4$ and clamped at $-100$ mV (cf. Nakajima *et al.* 1962, Fig. 2) and that the delayed inactivating current is carried mainly by $K^+$.

*Effect of thiocyanate, nitrate, and caffeine.* Caffeine, as well as certain foreign anions, is known to shift the relation between membrane potential

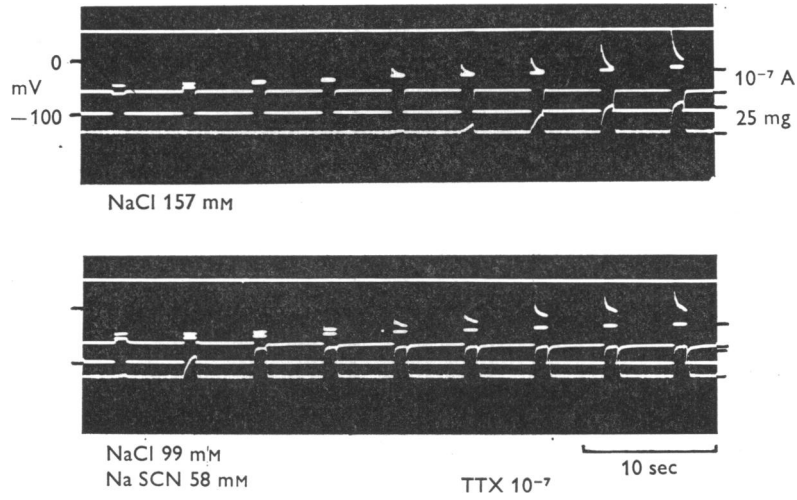


Fig. 8. Responses to graded depolarization steps with a fibre in normal solution or one in which 58 mM-$Cl^-$ was replaced by $SCN^-$. Lowermost trace, tension; next trace, potential; next trace, current.

and contraction in the direction of higher levels of membrane potential (Etzensperger & Gasciolli, 1963; Sandow, Taylor, Isaacson & Seguin, 1964; Lüttgau & Oetliker, 1968; Hodgkin & Horowicz, 1960*a*). Kao & Stanfield (1967) found on replacing $Cl^-$ by foreign anions that the threshold for rectification, measured after current had reached a steady value, shifted to a similar extent as did the threshold for contraction.

We have investigated the actions of thiocyanate and nitrate on snake twitch muscle fibres. The replacement of 58 mM-$Cl^-$ by $SCN^-$ caused a marked shift in the threshold for contraction, as may be seen in Fig. 8. The upper record shows the responses of a fibre in normal $Cl^-$ to graded depolarizing steps of constant duration from a base line clamped potential of $-100$ mV. With incrementing steps, the first appearance of inactivating outward current was associated with a small contracture. With further increases in the amplitude of the depolarizing step, tension increased, as did the amount of outward current which inactivated. The lower record

shows the responses to a roughly comparable series of depolarizing steps after $Cl^-$ had been replaced in part by $SCN^-$. The threshold for contraction was shifted markedly, but there appeared to be little, if any, change in the potential level at which the inactivating current first became evident. A plot of the relations between membrane potential, current, and tension is shown in Fig. 9. The peak current near the beginning of the depolarizing step and the final current near the end of the step are both shown. The difference between these two curves represents the fraction of outward current which inactivated. With the fibre in normal $Cl^-$, the threshold for contraction was approximately $-35$ mV. The threshold for the appearance of inactivating current was at approximately the same level.

On replacing part of the chloride by thiocyanate, the threshold for contraction shifted to about $-55$ mV, and the curve relating tension to membrane potential became much steeper. The level at which inactivating current appeared was only slightly changed by $SCN^-$; however, the voltage–current relationship in the steady state showed an inflection at approximately the level of the contraction threshold. Thus, in thiocyanate the fibre showed some rectification coinciding with the mechanical threshold level. These findings are in keeping with those of Kao & Stanfield (1967); however, the results do show that the threshold for inactivating current does not shift to the same extent as does the contractile threshold. Similar results were found after replacing $Cl^-$ by $NO_3^-$. There was a shift of contractile threshold to a higher level of membrane potential without any significant change in the threshold for inactivating current. It is of incidental interest that the fibre shown in Fig. 9 exhibited a slight degree of anomalous rectification at levels of membrane potential greater (more negative) than those producing normal rectification.

Caffeine is known to shift the relation between tension and membrane potential towards higher values of the latter (Etzensperger & Gasciolli, 1963; Sandow *et al.* 1964; Lüttgau & Oetliker, 1968). We have examined the relation of this effect to the currents which accompany depolarizing pulses. A plot of such an experiment is shown in Fig. 10. Caffeine ($1 \times 10^{-3}$ w/v) was added to the bathing solution. The threshold for contraction shifted by about 20 mV, but there was no change in the level at which inactivating outward current appeared. While it is possible that a small amount of steady-state current rectification might have been missed in this experiment, if present, it would be very small. This experiment provides another example of a shift in contractile threshold without a corresponding shift in the threshold for inactivating rectifying current.

*Effect of procaine.* While the effects of procaine will be considered in detail in a subsequent paper (Heistracher & Hunt, 1969*b*), one aspect of its action has particular relevance here. It provides an example of

another kind of dissociation between inactivating outward current and contractile response. Procaine can produce a marked reduction in the inactivating current associated with depolarizing pulses without significant change in the amount of tension produced. This is illustrated in Fig. 11 which is a plot of successive tension responses to depolarization steps from −99 mV to −1 mV. The amplitude of the current at its peak and at the end of the depolarization step is also shown. With the fibre bathed in a

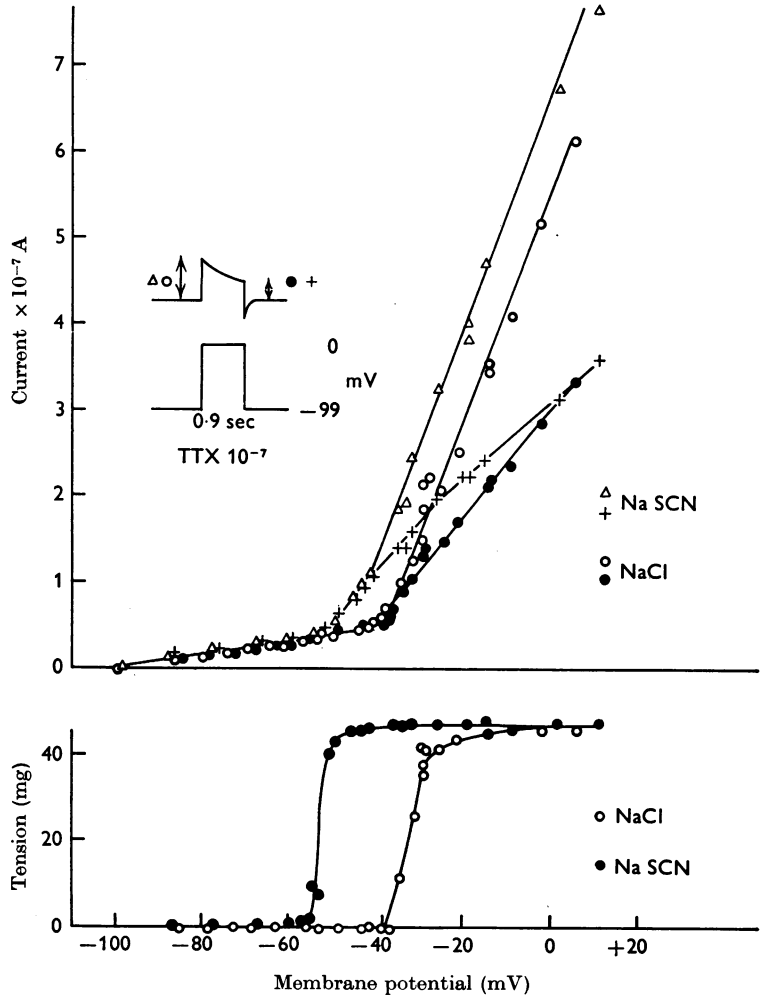


Fig. 9. Plot of the relation between tension and membrane potential and between current and membrane potential in normal solution and in one containing 58 mM-NaSCN. The open triangles and open circles are measurements of peak current near the beginning of a depolarizing step. The filled triangles and filled circles are the current values at the end of the step.

solution containing tetrodotoxin, $1 \times 10^{-7}$ w/v, each step produced a contracture reaching a maximal tension of about 13 mg and an outward current a large fraction of which inactivated by the end of the 800 msec depolarizing step. On switching to a bathing solution containing procaine

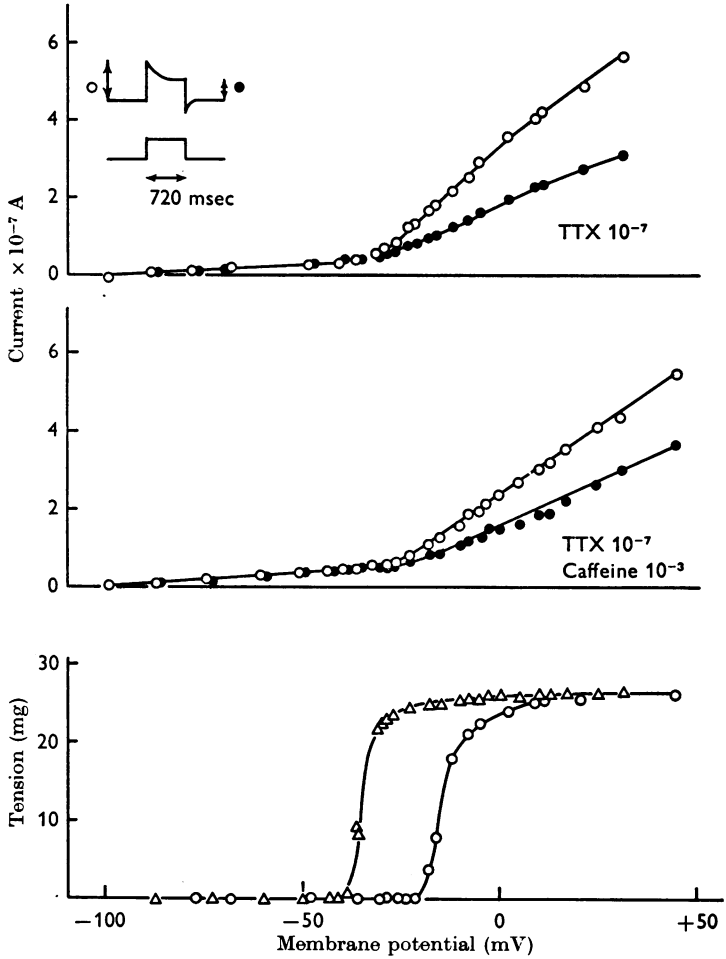


Fig. 10. The relation between tension and membrane potential and between current and membrane potential in a fibre bathed in normal solution containing tetrodotoxin ($1 \times 10^{-7}$ w/v) and one containing tetrodotoxin, $1 \times 10^{-7}$ w/v and caffeine, $1 \times 10^{-3}$ w/v. Open circles represent peak current; filled circles, current at the end of a depolarizing pulse. Tension: open circles, normal solution; triangles, caffeine $10^{-3}$.

HCl $5 \times 10^{-3}$ w/v, in place of tetrodotoxin, the tension and the current at the end of the pulse remained essentially the same, but the amount of inactivating current was markedly reduced. This effect occurred within a few seconds and reached a maximum within 30 sec. On changing the

solution back to one containing tetrodotoxin, the effect reversed but with much slower time course. From this result alone, one could not conclude that inactivating current and contractile activation are unrelated, for the depolarization step is supermaximal and a small amount of inactivating current persists in the presence of procaine. The argument could be advanced that the residual inactivating current suffices to saturate the contractile activating mechanism. However, the very marked reduction in

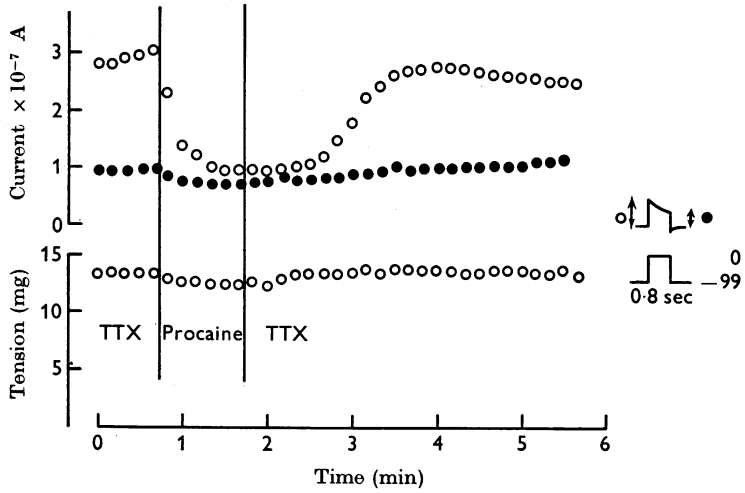


Fig. 11. Current and tension in a fibre subject to repeated depolarizing steps in the presence of tetrodotoxin or procaine. Peak current, indicated by open circles; current at the end of depolarizing steps, by filled circles. For details, see text.

inactivating current without change in contractile response lends weight to the evidence presented above in indicating that the inactivating current and contractile activation are not causally related.

*Off-current.* On return to a base line clamped potential, after a step depolarization, there was a brief phase of current flow inward relative to the base line current which followed the capacitative transient. Examples may be seen in Fig. 7 *A–D*. The magnitude of this 'off-current' varied with the size of the depolarization step, increasing in amplitude with increase in the size of the step. The magnitude and time course of this off-current varied considerably from one fibre to another. Its total duration was usually about 0·1 sec. In most fibres its decay was roughly exponential.

The effect of duration of the depolarization step on the subsequent off-current is illustrated in Fig. 12. The fibre was initially clamped at − 100 mV and then depolarized to 0 mV for varying durations. The onset of this depolarization step was associated with an outward current which reached a maximal value with very brief pulses. With longer pulses, it decayed

with a half-time of about 0·5–1·0 sec to a steady level, which was about 40 % of its maximum. Cessation of the depolarization step was followed by an off-current which became larger as the duration of the depolarization step was increased, reaching a maximum with depolarizations of about 1·0 sec in duration. Further increases in the duration of depolarization caused the off-current to become smaller until it finally reached a steady level. In Fig. 12, the relation between duration of the depolarization step and maximal tension developed is also shown.

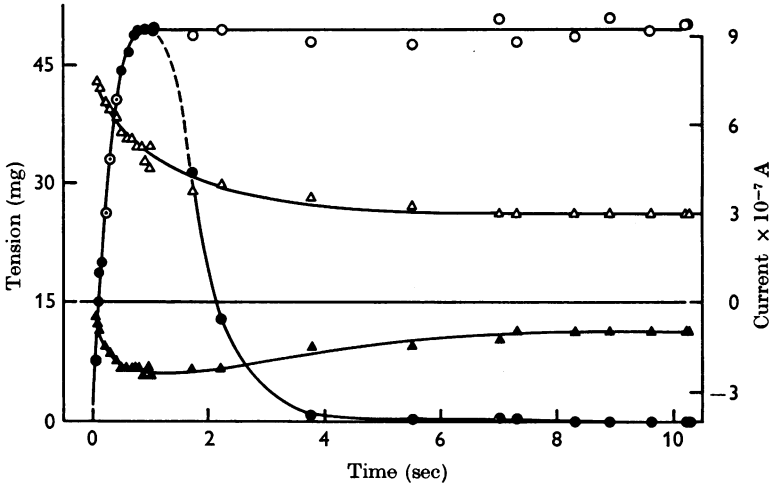


Fig. 12. The relation between tension development outward current near end of depolarizing step and off-current. Abscissa, duration of depolarizing pulse from −100 to 0 mV; circles indicate tension developed as a function of duration of depolarization; open circles indicate peak tension; filled circles indicate time course of tension response to a prolonged depolarization step; open triangles indicate current towards end of depolarizing pulse; filled triangles indicate 'off' current following cessation of depolarizing pulse. Ordinate for tension is on left; for currents, on right.

While the basis for the off-current is not well understood, some of the following factors seem important. It seems clear that the off-current does not represent a residuum of delayed rectification which inactivates with time. In the first place, off-current appeared following depolarization steps which were subthreshold for delayed rectification. Secondly, as may be seen in Fig. 12, depolarization pulses which are terminated shortly after delayed rectification reaches a maximum are not associated with a maximal off-current. In experiments on repriming, to be reported in a subsequent paper (Heistracher & Hunt, 1969*a*), it was found that depolarization steps following clamping of the fibre membrane at −100 mV for varying durations led to the same amplitude of off-current, although delayed

rectification varied from 0 to maximum. On the other hand, with very short depolarization pulses, the amplitude of off-current increased, markedly as depolarization exceeded the threshold for delayed rectification. It was shown that a recording electrode at the end of the fibre revealed a return of potential to the base line level which mirrored the time course of the off-current when the fibre was voltage clamped by current and voltage electrodes at its centre. In other words, on cessation of the depolarization step, the membrane potential at the end of the fibre returned to its base line value as the off-current diminished.

One possible explanation for the above findings is as follows: outward current associated with a depolarization step flows, in part, through the sarcoplasmic reticulum-transverse tubular system. This current may be carried principally by $K^+$. At rest, the tubular contents are similar to the external solution. Outward current could be associated with an accumulation of $K^+$ in the T-system. The accumulation of potassium ions in the T-system could result in a change in the $K^+$ equilibrium potential. On return to the base line potential level, the passage of current inward relative to the control level would be needed to hold the membrane at its previously clamped value. The change in tubular $K^+$ concentrations would depend upon several factors: (1) the amount of outward current and the fraction thereof passing through the tubular-sarcoplasmic reticulum membrane; and (2) the diffusion of $K^+$ from the tubules to the external fluid and the establishment of a steady-state concentration.

The decay of the off-current is much too rapid to be accounted for by diffusion from tubule to external fluid. The current accompanying repolarization may account for a relatively rapid decrease in tubular $K^+$ concentration and off-current would decrease in parallel. Another factor of possible importance might be a fall in resistance of the sarcoplasmic reticulum-tubular membrane as a consequence of the increase in $K^+$ concentration. Changes in concentration of $K^+$ within the transverse tubular-sarcoplasmic reticulum compartment might account for the slow return of the potential at the end of a fibre when the centre is repolarized at the end of a depolarization step. The return of the membrane potential to a more uniform level along the length of the fibre might parallel the return of tubular $K^+$ to its resting value. As noted above, there is very little direct evidence for this suggestion, although it is consistent with the findings.

*Contractile inactivation.* When depolarization was maintained, contractile tension remained at its maximal value for several seconds and then gradually declined to zero. This inactivation of the contractile response is similar to that which occurs in frog twitch muscle fibres exposed to a high concentration of external potassium (Kuffler, 1946; Hodgkin & Horowicz,

1960*a*). The rate of inactivation varied to some extent from one fibre to another. It was markedly prolonged by lowering temperature. The rate of inactivation depended upon the level of depolarization, as may be seen in Figs. 13 and 14.

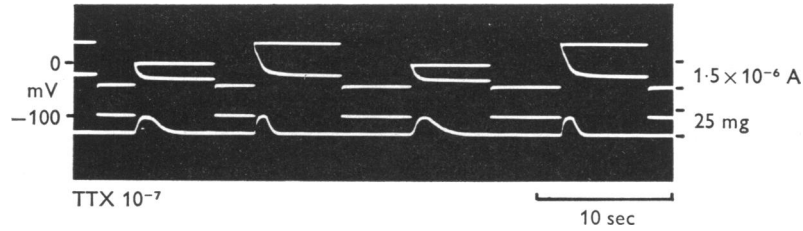


**Fig. 13.** Effect of depolarizing to 0 or +40 mV on contractile inactivation. At the extreme right, upper trace is current; middle trace, potential; lower trace, tension.

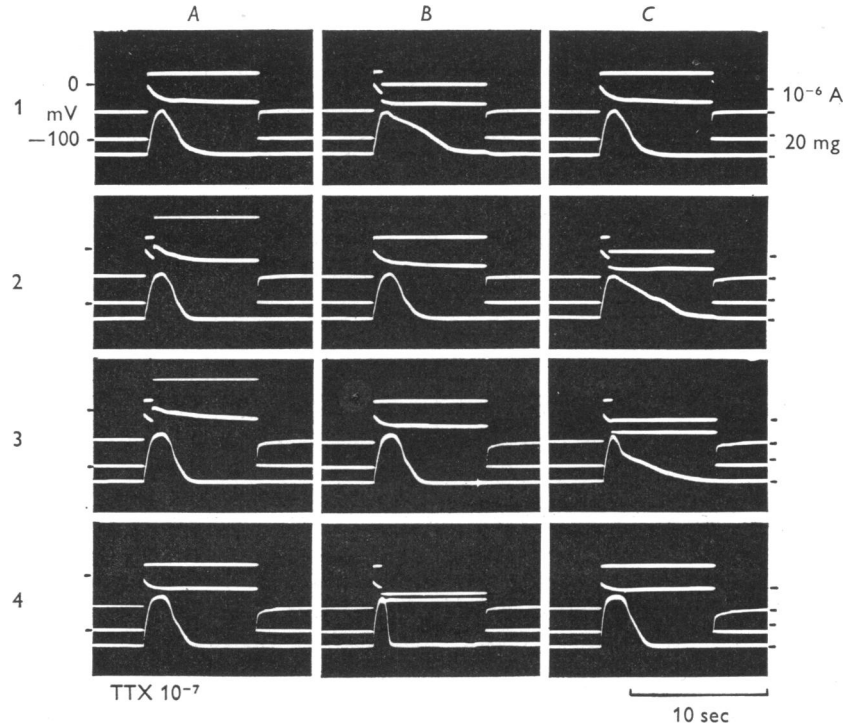


**Fig. 14.** Effect of membrane potential on rate of inactivation. At the beginning of each record, upper trace is current; middle trace, potential; lower trace, tension. Fibre was in tetrodotoxin $1 \times 10^{-7}$ w/v.

In the experiment shown in Fig. 13, a fibre was clamped at −100 mV and then depolarized to either 0 mV or +40 mV for approximately 6 sec. The contracture was appreciably longer when the fibre was depolarized to 0 mV than when the potential was changed to +40 mV. However

maximal contractile tension was nearly the same in both cases. It may be noted that the rate of tension development was somewhat faster when the potential was stepped to +40 mV rather than to 0 mV. Figure 14 illustrates another experiment in which the relation between level of membrane potential and contractile inactivation was investigated. In all figures the fibre was clamped at a base line level of −100 mV and initially stepped to +15 mV. The effect of maintaining this level of membrane potential is shown in Fig. 14, records *A*1, *A*4, *B*2, *B*3, *C*1 and *C*4. The contractile response rises to a maximum and, after several seconds, declines rapidly to 0. In Fig. 14, *B*1, after the membrane was held for about 0·6 sec at +15 mV, a second step brought the potential to 0 mV. It may be seen that the inactivation then proceeded much less rapidly, and some tension still remained at the end of the 8 sec period of depolarization. Figure 14, *C*2 shows a similar slowing of the rate of inactivation when the second step brought the membrane potential to approximately −10 mV. In Fig. 14, *C*3 the second step brought the membrane potential to about −20 mV. In this case, the rate of inactivation was decreased, but the amount of tension maintained during the depolarization pulse was considerably less. This is because the second step brought the membrane potential to a level below maximum for contractile response. Record *B*4 shows the effect of further reducing the level of depolarization during the second step. In this case, the membrane potential was shifted from +15 to −40 mV. This shift was associated with a prompt relaxation. Clearly the second step brought the membrane potential to a level subthreshold for contraction. Records *A*2 and *A*3 show the results of a second step from +15 to +50 mV. This produced no conspicuous change in the rate of inactivation as compared to those records in which the membrane was held at +15 mV. In other experiments, it was possible to show an increase in the rate of inactivation when the membrane was subject to a double step experiment, the first step being to 0 mV, the second to a more positive value; however, such an effect was evident at only very critical intervals.

The above experiments suggest that the rate of contractile inactivation is membrane potential dependent. Inactivation appears to be accelerated by increasing the level of depolarization beyond that required to cause maximal contraction.

## DISCUSSION

The similarity in threshold for mechanical response and for delayed rectification has been noted previously (Costantin, 1967, 1968; Kao & Stanfield, 1967; Lorkovic & Edwards, 1968). Kao & Stanfield (1967) found that the shift in threshold for contraction was quite similar to the shift in threshold for the appearance of rectification when the muscle was exposed

to certain foreign anions. The presence of rectification was determined from the slope of voltage-steady current curve. In the present experiments, it has been found that thiocyanate or nitrate causes a shift in mechanical threshold, but that this is not accompanied by an equal change in the threshold for that portion of the rectifying current which appears with delay and which inactivates with time. There is, however, a comparable shift in the appearance of rectification as judged by a change in slope in the voltage-steady current curve. When fibres were exposed to caffeine, mechanical threshold shifted without apparent change in the threshold for inactivating rectification or for rectification as determined in the steady current condition. Our findings suggest that delayed, inactivating rectification and contractile activation may, under some circumstances, be dissociated. Furthermore, the amplitude of the rectifying current which inactivates, may be strikingly reduced by procaine without a reduction in the tension produced by supermaximal depolarizing pulses. By bathing a muscle fibre in a solution containing 95 mM-$K_2SO_4$, the threshold for contractile activation is unchanged. With slightly supermaximal depolarization pulses, the inactivating current is reversed in direction. These findings suggest that outward current through the inactivating rectifying channel is not responsible for contractile activation.

Kuffler (1946) showed that frog twitch muscle fibres exposed to a high concentration of external $K^+$ gave contractures which lasted for some seconds and then relaxed. Hodgkin & Horowicz (1960*a*) found that depolarization produced by exposure of frog twitch muscle fibres to 190 mM of external $K^+$ produced a contracture which remained at its maximum for about two seconds and then rapidly declined. They also noted that lower concentrations of external $K^+$ were associated with a less rapid rise in tension, but with a greater prolongation of the contractile response. The present studies confirm these observations and show further that the rate of contractile inactivation is membrane potential dependent, and not simply due to the rate of contractile activation. Double-step experiments have shown that once the fibre is activated at its maximal rate, subsequent shifts in membrane potential can alter the rate at which the fibre inactivates (cf. Foulks & Perry, 1966). The rate of inactivation in snake twitch muscle fibres at 20° C appears to be slightly faster than that in frog twitch muscle fibres. Preliminary experiments indicate that the rate of contractile inactivation becomes markedly slowed when the temperature is reduced to about 5° C.

The present results may be compared with the study of Orkand (1962) on the relation between membrane potential and contraction in single short muscle fibres of crayfish. He also found that the rate of increase in tension depended upon the amplitude of the depolarization step and that

the relation between membrane potential and tension development was the same in high and in low $K^+$ solutions.

Several mechanisms have been suggested for the activation of contraction by the release of calcium from the sarcoplasmic reticulum. This release might result from outward current through the sarcoplasmic reticulum-transverse tubular system. Hodgkin & Horowicz (1960*a*) suggested that $Ca^{2+}$ might be bound with a carrier yielding a complex with a net negative charge. Current flow from sarcoplasm to reticulum would then cause movement of this complex into the sarcoplasm where $Ca^{2+}$ would be released. The present results do not negate this possibility. However, they do exclude the rectifying channel which inactivates with time as the source of the required outward current.

Alternatively, the release of $Ca^{2+}$ might be due to a potential-dependent increase in $Ca^{2+}$ conductance of the sarcoplasmic reticulum membrane. If the resistance of the latter were high compared to that of the sarcoplasmic reticulum-transverse tubular junction, outward current through tubular system might trigger a release of $Ca^{2+}$ by depolarization of the sarcoplasmic reticulum membrane. The increase in $Ca^{2+}$ conductance might inactivate with time and contractile inactivation would then accompany the active uptake of $Ca^{2+}$ by sarcoplasmic reticulum (Ebashi & Lipmann, 1962; Hasselbach & Makinose, 1961). Repolarization might restore the ability of the membrane to undergo the increase in $Ca^{2+}$ conductance. The present experiments do not decide between these two alternatives, although the latter appears more likely (see also Ebashi, 1965).

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