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POTASSIUM CONTRACTURES IN SINGLE MUSCLE FIBRES

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It has been known for a long time that muscles can be made to contract by raising the potassium concentration in the external medium. In skeletal muscles these contractions are difficult to study, because potassium ions take some time to diffuse through the interspaces and the contractions which they set up are usually transient. The difficulties of interpretation become less acute if single fibres are employed; in that case, as was first shown by Kuffler (1946), a rise in external potassium concentration causes a rapid depolarization and a prompt contraction. The quantitative effects of sudden changes of potassium concentration on membrane potential have been described previously (Hodgkin & Horowicz, 1959, 1960); the present article deals with the effect of similar changes on the tension developed by single muscle fibres. For relevant work on whole muscles the excellent review of Sandow (1955) should be consulted.

METHODS

All the experiments were carried out with single fibres from the semitendinosus muscle of *Rana temporaria*. The apparatus and method of recording have been described in previous articles (Hodgkin & Horowicz, 1959, 1960). The only additional item of equipment was a device for changing solutions rapidly. In the method described previously, solutions were allowed to run into the cell from reservoirs at a height of about 50 cm above the cell; the flow obtained with this method was 1–3 ml./sec and the mean velocity in the channel containing the fibre was 10–30 cm/sec. In some of the present experiments (Fibres *D*, *E*, *F* in Table 1 and Figs. 1 and 2) the reservoirs were replaced by large syringes (internal diameter 2·1 cm) whose plungers were driven by pistons operated by compressed air between stops spaced 0·7 cm apart. This forced 2 ml. of solution through the cell in about 0·3 sec. Tests with dyes showed that the solution in the cell (volume about 0·3 ml.) was fully changed in one flush. The rate of flow (about 60 cm/sec) produced by the piston-operated syringes was too high for the experiments in which the membrane potential was recorded and most of the results described here were obtained with the original method.

Solutions were made up on the same lines as those described previously (Hodgkin & Horowicz, 1959, Table 1). When using relatively short exposures to high $[K]_o$, $[Cl]_o$ was generally kept at its normal concentration of 120 mM. For longer exposures solutions of constant [K] [Cl] product were employed, in order to avoid loading the fibre with Cl.

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RESULTS

The relation between potassium concentration and peak tension

The muscle fibres used here were of the quick, phasic type and did not give maintained contractions when depolarized with high potassium concentrations. The way in which the muscle relaxes in high $[K]_o$ will be described later; to begin with we shall consider the effect of relatively short exposures in which the contraction was cut short by a return to low $[K]_o$ and not by a spontaneous relaxation. Figure 1 shows the effect of different potassium concentrations on the tension generated by the

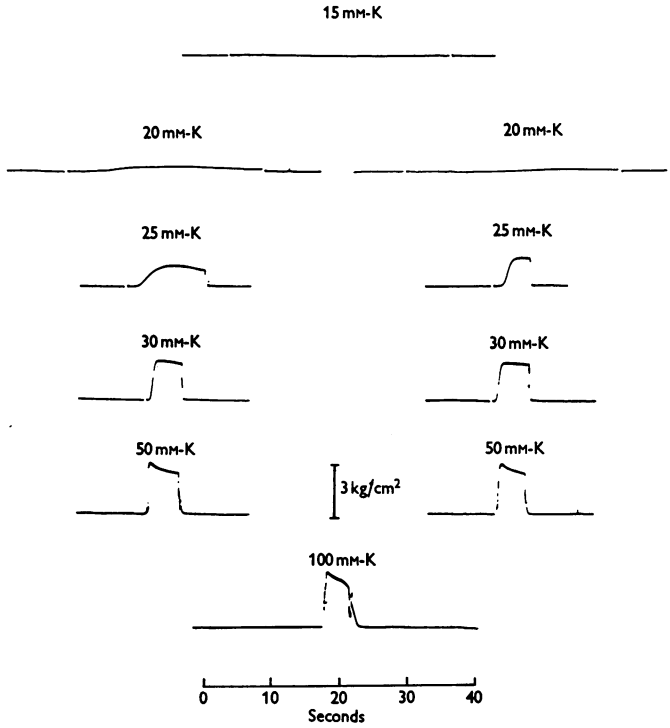

Fig. 1. Tension resulting from brief applications of potassium concentrations, varying between 15 and 100 mM-K, to fibre in choline-Ringer's fluid. Fibre *E*, diameter 88 μ, temperature 16·5° C; Na-free solutions containing [K + choline] = 120 mM, 121 mM-Cl 1·8 mM-Ca, 1·5 mM phosphate buffer. The potassium concentration at the beginning and end of each record was 2·5 mM. The breaks in the record, which were caused by the sudden flow of fluid through the cell, mark the times at which high $[K]_o$ was applied or removed. The fibre was allowed to rest for 7–10 min between contractures. The order of the records was 20–50 mM (left-hand column), 100–15 mM (bottom, right-hand and top). The fibre was stretched to 1·28 times slack length, giving a measured sarcomere distance of 2·7 μ.

fibre; solutions were made up with choline instead of sodium, so there were no action potentials or twitches when $[K]_o$ was increased. Contractions began at 20 mM-K, reached half their maximum at 25 mM-K and were nearly maximal at 50 mM-K. In this fibre the maximum tension at 100 mM-K was 3·0 kg/cm² and was 11 % greater than that produced by a 125/sec tetanus in Na-Ringer's fluid. Table 1 shows that similar values were obtained in other experiments.

TABLE 1. Maximum tension developed in tetani and contractures

Fibre reference	Fibre diameter (μ)	Sarcomere length (μ)	Temperature (°C)	Tetanus tension per cm² 50/sec	Tetanus tension per cm² 125/sec	Contracture tension per cm²	Contracture tension / Tetanus tension (125/sec)
				← kg/cm² →		→	
A	82	—	20	3·9	3·9	4·4	1·11
				3·7	4·0	4·4	
B	71	—	20	2·7	2·8	3·4	1·13
						3·3	
						3·3	
						3·3	
						3·4	
						3·3	
						3·3	
				2·9	3·1	3·3	
D	98	2·8	18	3·1	3·2	3·6	1·07
				3·3	3·5	—	
E	88	2·7	17	2·3	2·6	3·0	1·11
				2·6	2·8	—	
F	73	2·7	19	2·8	3·2	3·5	1·08
				3·3	3·3	—	
J	80	(2·5)	20	3·6	3·8	4·1	1·08
						4·1	
						4·1	

Contractures were induced with 100 mM-K, 120 mM-Cl in *D*, *E*, *F* and with 190 mM-K, 2–4 mM-Cl in *A*, *B*, *J*.

The sarcomere distance was measured after the experiment by stretching the fibre to the experimental length in a cell in it which could be observed with a water-immersion objective. The sarcomere length in *J* is based on the fact that this fibre was stretched to 1·20 times slack length, whereas *D*, *E* and *F* were stretched to 1·35, 1·28 and 1·28, respectively. Diameters are means of several measurements including major and minor diameters; errors in cross-section might be ± 20 % but this does not affect the final column.

The relation between tension and log $[K]_o$ is given by the circles in Fig. 2. In the lower part of the curve the tension increased extremely steeply with potassium concentration, the tension at 25 mM being about 5 times that at 20 mM.

The records in Fig. 1 show that the tension rose with a long delay along an S-shaped curve when $[K]_o$ was raised to 20 or 25 mM, but dropped abruptly when 2·5 mM-K was restored. With 100 mM-K the situation was reversed, for the tension rose rapidly but did not drop at once when 2·5 mM-K was restored. These effects are probably explained by the

combined action of a delay in the response of the membrane potential to a change in $[K]_o$ and a very steep relation between membrane potential and tension (p. 393).

There was a slight difference between the contracture thresholds in choline- and in Na-Ringer's fluid. Tension log $[K]_o$ plots indicated that

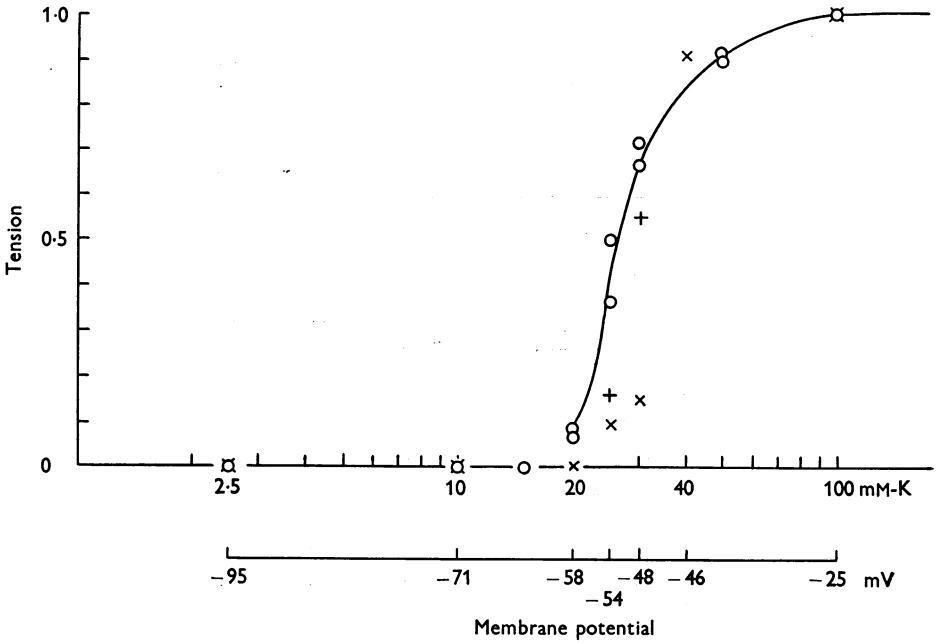

Fig. 2. Relation between peak tension and potassium concentration or membrane potential. ○, Fibre *E*, tension only. +, Fibre *G*, tension only. ×, Fibre *G*, tension and membrane potential. The numbers attached to the lower scale are the internal potentials measured at the same time as the tension. The scale for the potassium concentration is logarithmic and for potential is approximately linear, the difference in scale corresponding to 45 mV for a tenfold change. Choline-Ringer's fluid with K replacing choline. Fibre *E*, diameter 88 μ, maximum tension 3·0 kg/cm², temperature 16·5° C. Fibre *G*, diameter 75 μ, maximum tension 4·0 kg/cm², temperature 18° C.

in the presence of Na^+ the curve was shifted to the right by a factor of about 1·2. For example, 25 mM-K choline-Ringer gave roughly the same tension as 30 mM-K Na-Ringer. The effect is in the same direction, but much smaller, than that observed in heart muscle by Lüttgau & Niedergerke (1958).

The relation between membrane potential and tension

Figure 3 illustrates some simultaneous recordings of tension and membrane potential. Contractions began at a potential of about −54 mV

(25 mM-K) and were nearly maximal at −46 mV (40 mM-K). The relation between tension and membrane potential is shown by the crosses and the lower scale in Fig. 2. Figure 4 shows that a small increase in depolarization produced measurable tension and that this was suppressed by a small decrease in depolarization.

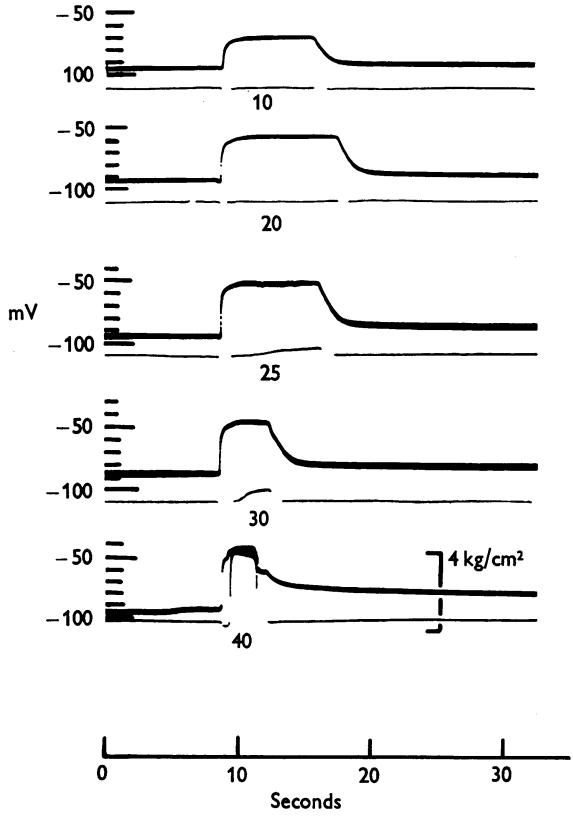

Fig. 3. Simultaneous recording of internal potential (upper trace) and tension (lower trace) showing effect of brief application of potassium concentrations varying between 10 and 40 mM to fibre in choline-Ringer's fluid containing 2·5 mM-K. The record in 30 mM-K was taken immediately after that in 25 mM-K and this may have reduced the size of the mechanical response; earlier measurements, in which the fibre was allowed to rest between contractions, gave 3·4 times more tension in 30 mM-K than in 25 mM-K. Fibre *G*, diameter 75 μ, temperature 18° C. Na-free solutions with [K]+[choline] = 120 mM, 121 mM-Cl, 1·8 mM-Ca, 1·5 mM-phosphate buffer. The tension record has been slightly retouched.

The sharpness of the contractile threshold

If the latency and slow rise of tension depend solely on the time taken by the membrane potential to pass through a critical region, a comparison of tension and potential during the rising phase of a contracture should

give the relation between the two variables; for short contractures the same relation ought to hold for the sudden relaxation associated with a reduction of $[K]_o$. These measurements could not be carried out in a direct manner because the onset of contraction was associated with irregularities in the recorded potential. Figure 5 illustrates the procedure adopted. Curve *A* gives the membrane potential for the sequence 10 → 20 → 10 mM-K, and *B* the potential for 10 → 30 → 10 mM-K; the latter resulted in a substantial rise in tension which is shown in curve *b*. Curve *B** was drawn on the assumption that the irregularities in *B* were errors and that the internal potential rose smoothly towards the steady value determined by the potassium concentration. Since the microelectrode was jerked out of the fibre at the end of the period in 30 mM-K,

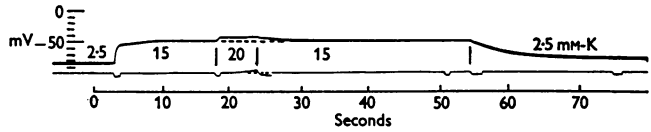

Fig. 4. Effect of sudden changes in potassium concentration on internal potential (upper trace) and tension (lower trace). Fibre *H*, diameter 119 μ, temperature 18° C, sulphate solutions containing 8 mM-$CaSO_4$ and [Na+K] = 83 mM (see Hodgkin & Horowicz, 1959, Table 1, solutions *D* and *E*). At 51 and 75 sec the solution already in the cell was flushed through for a second time. The tension developed in 20 mM-K was 4 % of the maximum contraction tension (3·2 kg/cm²). The record was taken several minutes after impalement and the resting potential in 2·5 mM-K had declined 7 mV during this period; 2 hr earlier the resting potential was 95 mV. The changes in tension and potential produced by replacing 15 with 20 mM-K are best seen by looking along the record.

the repolarization phase of *B** had to be inferred from the repolarization observed in record *A*. If the tension in the initial part of curve *b* is plotted against membrane potential, it is found that each millivolt increase in depolarization is associated with an elevenfold rise in tension. This is illustrated by the circles near the tension record, which were calculated on the assumption that tension $\propto \exp(V/0{\cdot}417)$. The same equation gave an approximate fit to the phase of relaxation at the end of the period in high $[K]_o$. Similar results were obtained in two other experiments, but the value of the exponential constant was somewhat higher, the mean in the three experiments being 0·7 mV. This figure is subject to large errors, but it is clear that the tension generated in the region of the mechanical threshold is acutely sensitive to small changes in membrane potential.

Absolute value of the contractile threshold

In five experiments with fibres equilibrated in choline-Ringer's fluid, the critical potential for development of measurable tension was found to average −54 mV (range from −48 to −58 mV). Similar measurements

were not made with ordinary Ringer's fluid because the fibres often gave a twitch when $[K]_o$ was increased; this would have damaged the membrane if the electrode had been inserted. Since about 20 % more K was required to give a contracture in Na than in choline solutions the critical potential in the presence of Na may be taken as about -50 mV. Critical potentials of from -40 to -45 mV were obtained in fibres which had been equilibrated in sulphate solutions containing 10 mM-K and 70 mM-Na; these fibres had resting potentials of -65 mV instead of the usual -95 mV.

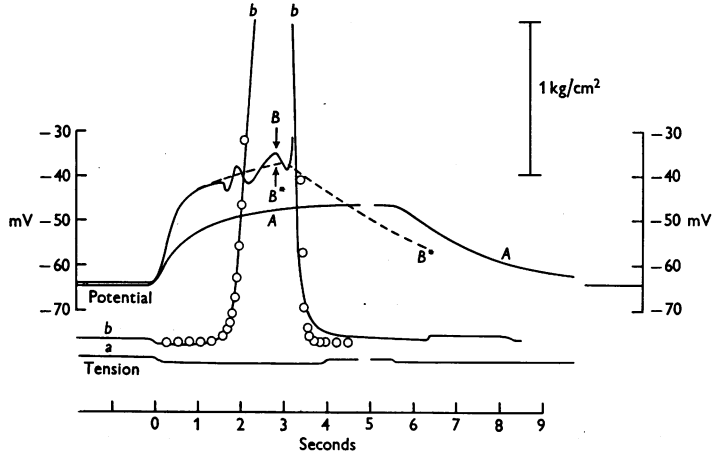

Fig. 5. Simultaneous records of membrane potential (A, B) and tension (a, b). The changes in solution were

Aa: 10 → 20 → 10 mM-K (0-Cl)
Bb: 10 → 30 → 10 mM-K (0-Cl).

In A 20 mM-K was applied for 20 sec and the gap in the record represents 14·5 sec. In B 30 mM-K was applied for 3 sec. The micro-electrode was jerked out of the fibre when relaxation started. Curve B^*, which is based on B and A, gives the assumed time course of the membrane potential. The circles against the tension curve b were calculated from B^* by the equation

$$T = 0{\cdot}284 \exp \frac{V+40}{0{\cdot}417},$$

where T is tension per unit area in kg/cm² and V is the internal potential in mV. Fibre K, diameter 117 μ, temperature 20° C, sulphate solutions as in the experiment of Fig. 3. Another record showed that the tension in 30 mM-K reached a maximum value of 3·2 kg/cm² and that with a maintained exposure to 30 mM-K the duration of the contracture was about 20 sec.

The time course of the contraction during a maintained depolarization

As is to be expected in a phasic muscle, the contraction was not maintained when the fibre was depolarized for periods longer than 5–30 sec. Figure 6 shows simultaneous recordings of membrane potential and tension

during a contraction produced by high $[K]_o$. The record starts with the fibre in a solution containing 190 mM-K and 3·6 mM-Cl; the membrane potential was close to zero. The fibre contracted when first put into high $[K]_o$ but had relaxed completely before the beginning of the record. On applying Ringer's fluid (2·5 mM-K, 120 mM-Cl) the fibre repolarized to about −90 mV. After 43 sec in Ringer's fluid, 190-K 3·6-Cl was again applied; the membrane potential fell rapidly to zero and the fibre developed a peak tension of 4·3 kg/cm². The contraction was not maintained and after a few seconds the fibre was fully relaxed, in spite of the fact that the membrane potential remained close to zero.

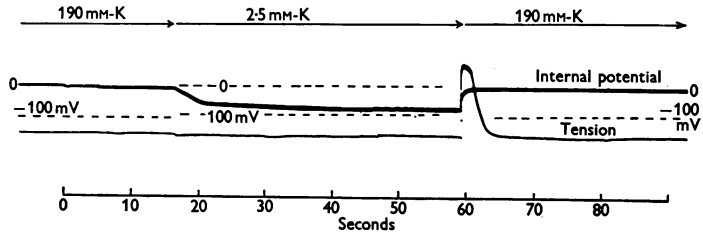

Fig. 6. Simultaneous records of membrane potential and tension for following changes in solution

190 mM-K 3·6 mM-Cl → 2·5 mM-K 120 mM-Cl → 190 mM-K 3·6 mM-Cl.

The peak tension developed was 4·3 kg/cm². In this experiment the internal potential was recorded against an agar-Ringer electrode in the external solution. The dotted lines allow for the junction potential. (In the other experiments illustrated the effect of the junction potential was small or was eliminated by recording with a 3 M-KCl electrode in the external solution.) The fibre was impaled at zero time. On reapplying Ringer's fluid after the end of the record the fibre repolarized to −75 mV, showing that the electrode had not been dislodged by the contraction. Fibre *C*, diameter 62 μ, temperature 21° C.

A characteristic feature of the contractures recorded from single muscle fibres is the rather sudden transition from a plateau, in which the tension fell slowly, to a rapid exponential phase of relaxation (Figs. 6, 7, 9 and 10). Occasionally the tension was constant during the plateau but a gradual decline was more usual. A decline in tension comparable to that in the plateau of the contracture was also seen if the fibre was stimulated at 50 or 125/sec for more than a second. This gradual decline took place before the irregular fall in tension resulting from failure of action potentials had begun.

Another interesting point about the contractures produced by high $[K]_o$ is that the duration of the plateau and the time constant of the phase of relaxation both shortened progressively as the potassium concentration was increased from 50 to 190 mM (Fig. 7).

A tentative explanation of the plateau and of the shortening of the contracture with increasing $[K]_o$ can be given in the following way. Suppose that depolarization releases an activator which is destroyed in a first-order reaction with a rate constant of about 30 sec^{-1}. A very brief depolarization of an action potential might then give a twitch lasting 30–100 msec, whereas a maintained depolarization would lead to a contraction lasting until the precursor of the activator was nearly exhausted.

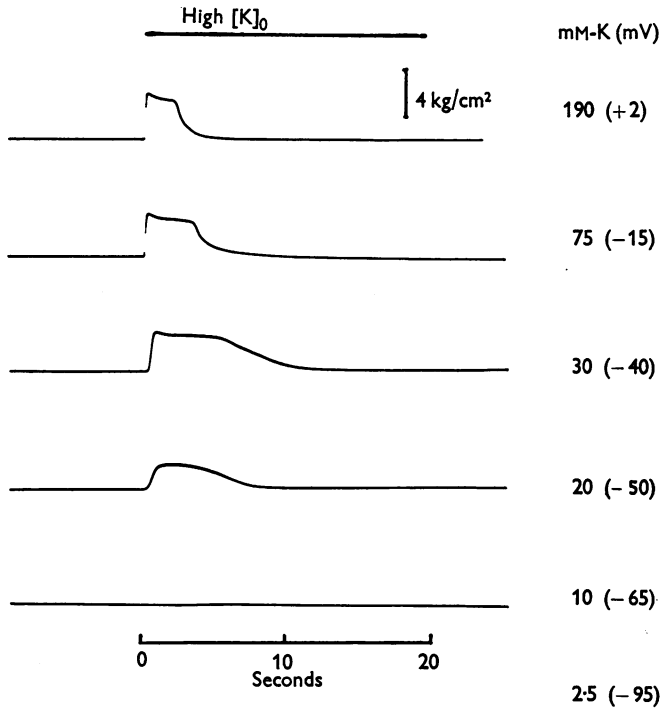

Fig. 7. Tension resulting from sudden applications of high $[K]_0$ and low $[Cl]_0$. The chloride concentration was $(300/[K]_0)$ mM, except with $[K]_0 = 190$ mM where there was 2·5 mM-Cl; solutions were of the constant K, Cl product type (see Hodgkin & Horowicz, 1959, Table 1, *F* and *G*). Potentials were taken from Fig. 4 of that paper. The high-K low-Cl solutions contained some Na, but twitches were prevented by keeping the fibre in choline-Ringer's fluid (2·5 mM-K, 120 mM-Cl) before switching on the test solution. Fibre *I*, diameter 106 μ, temperature 21° C.

Since the depolarization produced by potassium concentrations greater than 50 mM is greater than the mean depolarization in a train of action potentials at a frequency of 100/sec, one would expect high potassium concentrations to raise the concentration of activator above the level required to saturate the contractile mechanism. On this basis the duration of the plateau is the time for the concentration of activator to fall from

a supramaximal to a maximal value, and the duration of the relaxation phase is the time for the concentration to fall from a maximal to a threshold value (Fig. 8). High potassium concentrations might shorten the contracture by promoting the rate of liberation of the activator from its precursor and by reducing the rate at which the precursor is resynthesized. If something like this is correct it should be possible to shorten the plateau, or to eliminate it altogether, by reducing the amount of precursor.

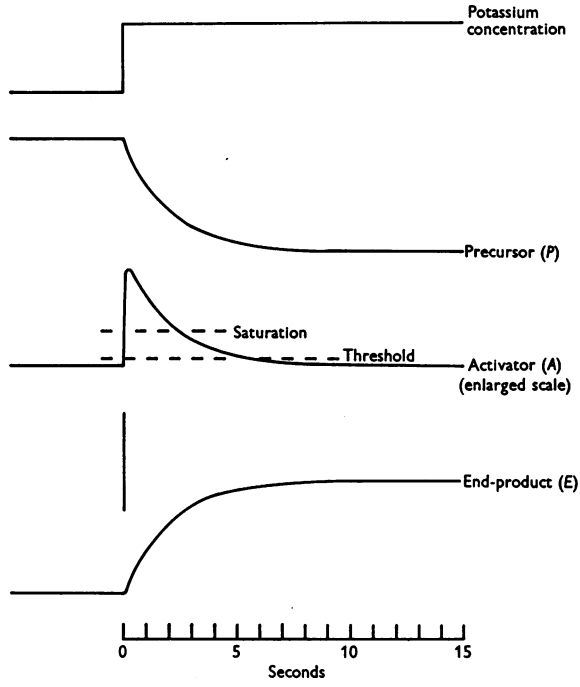

Fig. 8. Diagram showing rise and fall of hypothetical 'activator' during a potassium contracture. The curves are drawn as

$$\text{Precursor } (P) = e^{-\alpha t};$$

$$\text{Activator } (A) = \frac{\alpha}{\beta-\alpha}(e^{-\alpha t}-e^{-\beta t});$$

$$\text{End product } (E) = 1-(P+A),$$

where $\beta = 30\ \text{sec}^{-1}$; $\alpha = 1\ \text{sec}^{-1}$. Scale for (A) 29 times that for (P) or (E). The equations were obtained by neglecting back reactions in the system: $P \xrightarrow{\alpha} A \xrightarrow{\beta} E$.

Figure 9 illustrates an experiment suggested by this hypothesis. Curve $A1$ shows the tension produced by applying 100 mM-K to a fibre which had been equilibrated in Ringer's fluid (2·5 mM-K), whereas $A2$ shows the effect of applying 100 mM-K after the fibre had been recovering for a relatively short time (38 sec) in Ringer's fluid. It will be seen that the second contraction is smaller, that it had no plateau and that it could be

superposed on the falling phase of the first contraction. These findings agree well with the idea that relaxation depends on the exhaustion of a store of activator, but could no doubt be explained in other ways, for example, by assuming a constant level of activator and the slow development of an inhibitory process. Similar results were obtained in a series of experiments with a higher potassium concentration (190 mM), provided the first exposure to high $[K]_o$ was short (Fig. 9*B*). However, if the first exposure was long, the relaxation after the second contraction was more

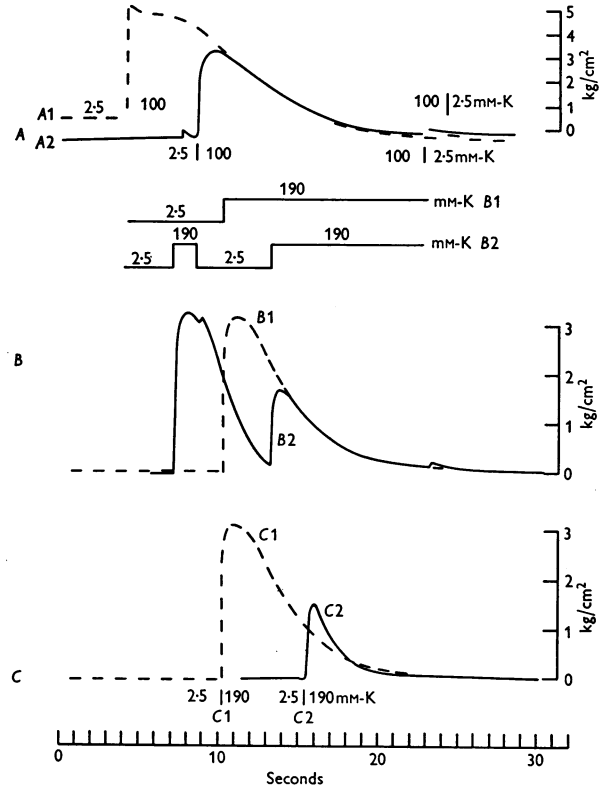

Fig. 9. Comparison of contractures produced by applying high [K] to resting fibres and to those recovering from previous exposure to high [K].

A. Fibre diameter *ca.* 50 μ, temperature 20° C. *A* 1, effect on resting fibre of replacing 2·5-K 120-Cl with 100-K 120-Cl. *A* 2, same change in solution but applied when the fibre had been recovering in 2·5-K 120-Cl for 38 sec after removing 100-K in record *A* 1.

B. Fibre diameter 71 μ, temperature 20° C. The potassium concentrations are shown on the figure. The 2·5-K solution was Ringer's fluid (120-Cl) and the 190-K was an isotonic sulphate solution containing 3·6 mM-Cl.

C. Same fibre and solutions as *B*. *C* 1, application of 190-K 3·6-Cl to resting fibre. *C* 2, application of 190-K 3·6-Cl to fibre recovering from *C* 1 (115 sec in 190-K 3·6-Cl followed by 18·5 sec in 2·5-K 120-Cl).

rapid than after the first (Fig. 9*C*) and the two curves were no longer superposable. Another example of the difference between the effects of long and short exposures to high $[K]_o$ is mentioned on p. 400.

The 'steady' relation between membrane potential and the state of the contractile system

When single fibres are immersed in high $[K]_o$ they contract and then relax completely. On returning them to low $[K]_o$ they repolarize, and after a short time (usually 5–30 sec) they give a second contraction when reimmersed in high $[K]_o$. The return of the contractile system or its activating mechanism to a resting condition will be referred to as the priming or repriming of the system and the converse process which causes relaxation in high $[K]_o$ will be said to make the fibre mechanically refractory.

The question considered here is the steady relation between the potassium concentration (or membrane potential) and the extent to which the contractile system is refractory. The experimental procedure is illustrated by Fig. 10. The contractures on the left were obtained by applying 190 mM-K, 2·5 mM-Cl after the fibre had rested for 10 min in Ringer's fluid. Immediately after these contractions the fibre was immersed for 1 min in a test solution containing x mM-K and $(300/x)$ mM-Cl (see Hodgkin & Horowicz, 1959). After 1 min in the test solution 190 mM-K was applied for a second time. If the potassium concentration in the test solution was low the fibre contracted and the size of the second contracture was taken as an index of the amount of restoration. The records showed that there was no restoration in 40 mM-K, a little in 30 mM-K and practically complete recovery in 20, 10 or 2·5 mM-K. The reason for choosing an equilibration time of 1 min in the test solution was that recovery in 2·5 mM-K was very nearly complete in that period and that with a solution such as 30 mM-K the restoration reached a maximum at about 1 min and then underwent a slow decline. The decline may perhaps be a result of the very large rise in oxidative heat production which occurs when muscles are immersed in solutions containing 20–30 mM-K (Hill & Howarth, 1957).

Figure 11 gives the relation between the potassium concentration and the state of the contractile system. Probable values of the membrane potential are given by the lower abscissa. The filled circles are the height of the second contraction and the crosses the areas under the second contraction. The latter are considered to be a better index of the extent to which the contractile system has been restored. It will be seen that curve 3, which runs through the crosses, is a mirror image of the relation between membrane potential and initial tension (open circles, curve 1).

The initial-tension curve was obtained in the usual way by starting with the fibre in 2·5 mM-K, switching on x mM-K and measuring the height of the contraction in this solution.

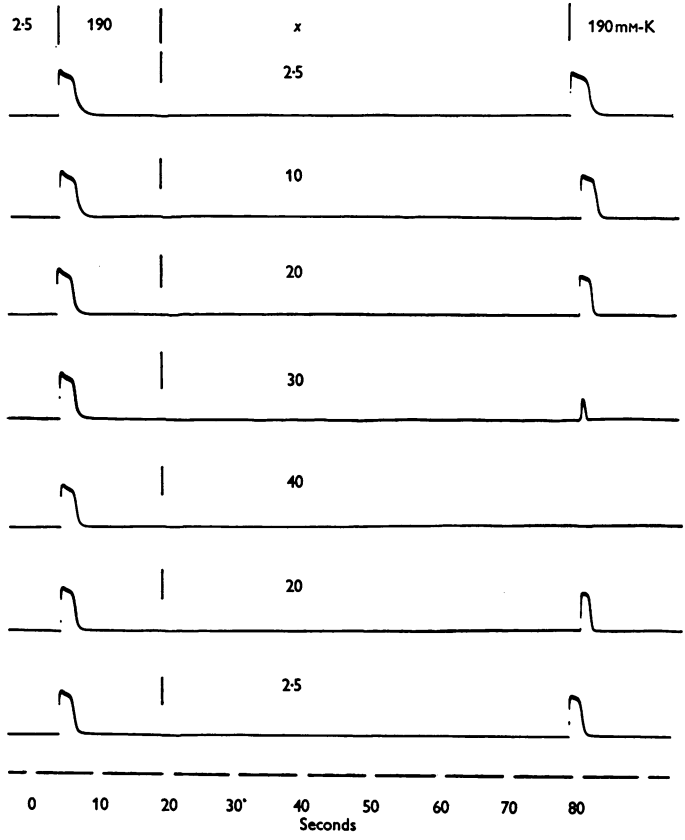

Fig. 10. Effect of allowing fibre to recover for 1 min in solution containing x mM-K and $(300/x)$ mM-Cl on tension resulting from second application of 190 mM-K. The sequence of solutions was: 2·5-K 120-Cl; 190-K 2·5-Cl; x-K $(300/x)$-Cl; 190-K 2·5-Cl. All records were at the same gain; the peak tension in the first contracture was 4·1 kg/cm². Fibre J, diameter 80 μ, temperature 20° C.

Recovery of twitches and repriming of the contractile system after a period in high $[K]_o$

Figure 12, record A, shows the effect of applying 190-K 3·6-Cl for 60 sec during a period when the fibre was being stimulated steadily at 0·78 shocks/sec. The twitches were abolished by the high-K solution and did not reappear until 11 sec after restoring Ringer's fluid. The recovery time varied with the duration of the exposure to high K; times as short as 6 sec were observed after exposures of 3 sec, but if 190 mM-K was applied

for 3–10 min the recovery time might increase to about 1 min. The recovery time also appeared to grow progressively longer as the fibre deteriorated. As one would expect, the recovery was quicker if concentrations lower than 190 mM-K were used to depolarize the fibre.

It was interesting to know if there was any relation between the recovery of twitch amplitude and the repriming of the contractile system. This could be done simply by reapplying 190 mM-K during the period when the fibre was recovering in 2·5 mM-K.

Record *B* in Fig. 12 shows that when 190 mM-K was reapplied at a time when the twitch had nearly recovered it gave full tension but a contracture which was shorter than normal. Record *C* shows that when 190 mM-K was reapplied before the twitches had appeared it gave no contracture and

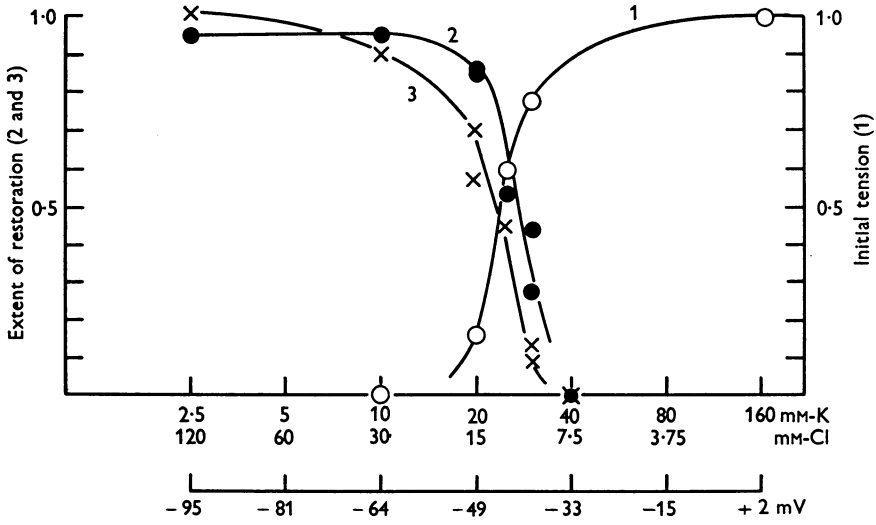

Fig. 11. Relation between potassium concentration and initial tension (curve 1) or degree of restoration (curves 2 and 3).

The abscissa gives the potassium or chloride concentrations of the test solution on a logarithmic scale. The lower scale, which is approximately linear, gives probable values of membrane potential, taken from Fig. 4 of Hodgkin & Horowicz (1959). A correction for activity coefficient has been made in plotting the right-hand point (190 mM-K 2·5 mM-Cl) on the potassium scale.

For curve 1 (○) the ordinate is the initial tension on increasing K from 2·5 to x mM, relative to the maximum tension of 4 kg/cm². (Except for the right-hand point [K] [Cl] = 300 (mM)² throughout.)

For curve 2 (●) the ordinate is the tension in 190 mM-K 2·5 mM-Cl after 1 min recovery in x mM-K $(300/x)$ mM-Cl.

For curve 3 (×) the ordinate is the area under the tension–time curve for the same contractures as in curve 2. The ordinates in curves 2 or 3 are given relative to the peak tension or area in the first contraction. Experimental details as in Fig. 10.

that when the twitch was small the contracture was small and brief. The conclusion is that the ability to give both twitches and contractures depends on the same underlying process.

One possible explanation of the simultaneous recovery of twitches and contractures is that the action potential recovers quickly and that both twitches and contractures are limited by the disappearance of the state of mechanical refractoriness. If this were so, there should be a large spike at a time when the fibre is still unable to contract. The expectation was not fulfilled, for all fibres tested were found to remain electrically inexcitable as long as they were mechanically quiescent. The first electrical

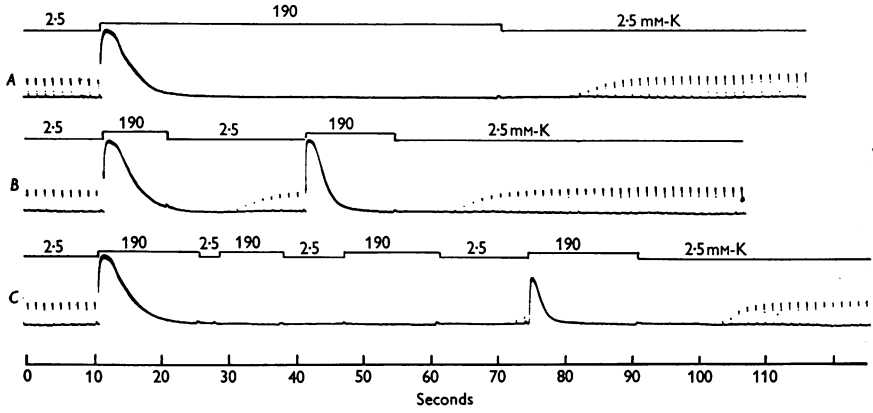

Fig. 12. Records of tension during twitches and contractures. The fibre was stimulated throughout at 0·78/sec with a shock which was well above threshold in Ringer's fluid. The external solution was either Ringer's fluid (2·5 mM-K, 120 mM-Cl) or 190 mM-K, 3·6 mM-Cl. All records at the same gain; the peak tension in the first contracture was 3·2 kg/cm². Fibre *B*, diameter 71 μ, temperature 20° C.

responses, which were greatly reduced in amplitude and probably decremental, appeared at the same time as the first twitches and both then grew in amplitude. The delay in recovery of the spike was partly due to the slow return of the resting potential (Hodgkin & Horowicz, 1960) but this was probably not the full explanation. After being kept 10 min in 190-K 3·6-Cl two fibres remained inexcitable for about 1 min in 2·5-K 120-Cl, although a resting potential of 70–80 mV was established in 20–30 sec. Apparently exposure to high $[K]_o$ has some slow effect which can persist for a minute or two and which impairs both the electrical and the mechanical response of the fibre. It is unfortunate that shortage of time and the difficulty of the experiments prevented us from making any proper investigation of this phenomenon.

DISCUSSION

The size and rapid onset of the maximal potassium contractures in single muscle fibres strongly supports the conclusion of Kuffler (1946), Sten-Knudsen (1954) and Sandow (1955) that the event which normally induces contraction is a change of membrane potential rather than a longitudinal current. With the present method there might be small longitudinal currents during the fraction of a second required to change solutions, but at longer times any longitudinal currents should be exceedingly small compared with those in the propagated impulse. Nevertheless, the tension generated with high $[K]_o$ in an Na-free solution can remain for several seconds at about the same level as that in a maximal tetanus. Since the fibre relaxes when $[K]_o$ is reduced and since a maximal contraction can be repeated many times, it cannot be argued that any irreversible effect is involved.

The observation that the maximum contracture tension is about 10% greater than the maximal tetanic tension is new but not altogether surprising. At 19° C the mean depolarization associated with a 125/sec train of spikes is 40–50 mV as against the steady depolarization of 60–100 mV produced by 100–190 mM-K (unpublished records and Hodgkin & Horowicz, 1959). A quantitative comparison cannot be made until more is known about mechanical activation, but it does not seem unreasonable that the steady depolarization should be more effective, in spite of the higher peak reached in the train of spikes.

There seems to be general agreement that in normal muscle fibres the strength of current, or the degree of depolarization, required to activate the mechanical system is not much greater than the threshold for the propagation of excitation (Ramsey & Street, 1938; Kuffler, 1946; Taylor, 1953). Since the action potential normally arises at a potential of -45 to -60 mV (Fatt & Katz, 1951; Jenerick & Gerard, 1953) our value of about -50 mV for the contractile threshold is not unreasonable. Although it was difficult to obtain quantitative results, our experiments show that there is an extremely steep relation between tension and membrane potential in the region of the mechanical threshold. A sharp threshold might be expected if depolarization started an autocatalytic or regenerative reaction. However, there was evidence against any regenerative process, for contractures were never 'all-or-nothing' and the tension varied continuously and reversibly with membrane potential. A possible explanation of the steep relation is that there is no appreciable tension until one reaction initiated by depolarization overtakes another which has the opposite effect. (Something of this kind seems needed to explain latency relaxation.) Alternatively it might be supposed that the fibre does not

develop measurable tension until many sites have been activated. An experiment which ought to be done is to see whether any motion, detectable with a high-power microscope, occurs at potassium concentrations which are just below the mechanical threshold.

In the second part of the paper it is shown that contractures produced by potassium concentrations greater than 50 mM consist of an initial plateau and a second phase in which the tension falls in an approximately exponential manner to its resting value. The durations of the plateau and the time constant of the relaxation shorten progressively as the depolarization is increased. It is suggested that relaxation might be caused by the exhaustion of an activator, or by the accumulation of something produced by the activator, and that the brevity of the contractures in high $[K]_o$ may be due to release of activator at a rate which is wastefully high.

The experiments show that a fibre which has relaxed in high $[K]_o$ can be restored to a condition in which it is once more able to contract by reducing the potassium concentration to less than 30 mM. Restoration of the contractile system usually occurs in under a minute and in the steady state the degree of restoration is related to membrane potential by an S-shaped curve which is roughly the mirror image of the curve relating initial tension to membrane potential. A tentative hypothesis is that reduction of the normal potential difference across the membrane liberates an activator which is used up in generating tension, whereas an increase in membrane potential prevents the activator being destroyed and allows its concentration in a store to be built up to a high level.

Our experiments give no information about the localization of the hypothetical activator, but from the work of Huxley & Taylor (1958) it is conceivable that the electrical effect of the normal resting potential might be to concentrate a negatively charged particle in some part of the endoplasmic reticulum (Porter & Palade, 1957; Huxley, 1959). On this basis depolarization would start a contraction by allowing an activating particle —possibly a negatively charged Ca complex—to move into the main part of the muscle, and the duration of the contraction might depend on the time taken to exhaust the activator stored in the reticulum.

SUMMARY

1. A sudden increase of the external potassium concentration, $[K]_o$, from 2·5 to 100 mM-K caused a single muscle fibre to develop a tension of 2–4 kg/cm^2 in a fraction of a second.

2. The maximum contracture tension was about 1·1 times the maximum tetanus tension.

3. Development of tension started at 20–30 mM-K and was related to log $[K]_o$ or membrane potential by a steep S-shaped curve.

4. The membrane potential at which contractions started was usually about -50 mV.

5. The relation between membrane potential and tension was reversible and, with fibres in Na-free solutions, there was no evidence of a regenerative process.

6. When $[K]_o$ was maintained at a high level the tension declined to its resting value along a characteristic curve consisting of an initial plateau and a subsequent phase of rapid relaxation.

7. The duration of the plateau and the time constant of relaxation shortened progressively as the potassium concentration was increased.

8. After a fibre had relaxed in high $[K]_o$ it could be restored to a condition in which it was once more able to contract by reducing $[K]_o$ for 10–30 sec below 20–30 mM. The degree of restoration was related to log $[K]_o$ (or membrane potential) by an S-shaped curve with a half-value at 20–25 mM-K (or -50 to -45 mV).

9. When Ringer's fluid was used to restore a fibre which had relaxed in high $[K]_o$, the action potential, twitches and the ability to give contractures all returned at about the same time.

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