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THE POTASSIUM CHLORIDE CONTRACTURE OF THE HEART AND ITS MODIFICATION BY CALCIUM

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Evidence is accumulating (Kuffler, 1946; Katz, 1950; Sten-Knudsen, 1954; A. F. Huxley & R. E. Taylor, personal communication) that the first step in the process which initiates muscular contraction is the depolarization of the excitable membrane of the muscle fibre. Any agent which alters contraction independently of depolarization may therefore be presumed to influence the contractile mechanism directly or, alternatively, to affect a link in the process between depolarization and contraction. Calcium ions apparently act in this way since they are known to exert a striking effect on the contraction of the heart, particularly the 'hypodynamic' heart, without affecting the amplitude of the action potential. Furthermore, there is evidence (Krogh, Lindberg & Schmidt-Nielsen, 1944; Niedergerke, 1956) that calcium may act on a link between the depolarization of the membrane and the contractile events. In order to obtain further information on the action of calcium ions it seemed of interest to study their effect on contraction when the excitable membrane has been depolarized in a controlled manner. This has been done by investigating the effect of calcium on contractures of heart strips caused by the application of depolarizing potassium chloride solutions.

METHODS

Material and recording technique were similar to those of the preceding paper (Niedergerke, 1956). Strips of the heart ventricle of *Rana temporaria* and *R. esculenta* were used. The tension developed during propagated contractions or during a KCl contracture was measured with a transducer valve (RCA 5734). If KCl solutions are introduced into the chamber (Fig. 1), immersing only part of the strip, a potential difference will develop due to current flow between the upper and lower parts of the strip. This was measured by means of an AgCl–Ringer-agar electrode dipping into the bath, and the upper hook, a chlorided silver wire, connecting the strip to the transducer. In order to keep the electrode potential at the upper hook constant, the chlorided silver wire was insulated with shellac except in the small region attached to the strip. A piece of cotton-wool soaked in Ringer's solution covered the hook and strip at this point, and this prevented concentration changes when the upper part of the strip was exposed to air.

Control experiments with cotton wicks showed that drifts due to instabilities of electrode junctions and d.c. amplifier could be kept between 0 and 1 mV during 15 min, the longest time of continuous measurement. The symmetrical arrangement of the chain AgCl–Ringer's solution–bath solution–Ringer-agar–AgCl would avoid any p.d. due to changes in junction potentials arising from alteration of the bath solution, if the strip were electrically a simple fluid resistance. This was tested by using cotton wicks instead of a heart strip. Replacement of Ringer's solution by 100 mM-KCl solution then caused only a small and slowly developing potential (<1 mV), which probably arises from some slight asymmetry of the liquid junctions. A somewhat greater artifact is to be expected since the heart tissue has cable properties. Thus, the p.d. of the upper fluid junction (Ringer's solution–bath solution) will be shunted by the internal resistance of the cells.

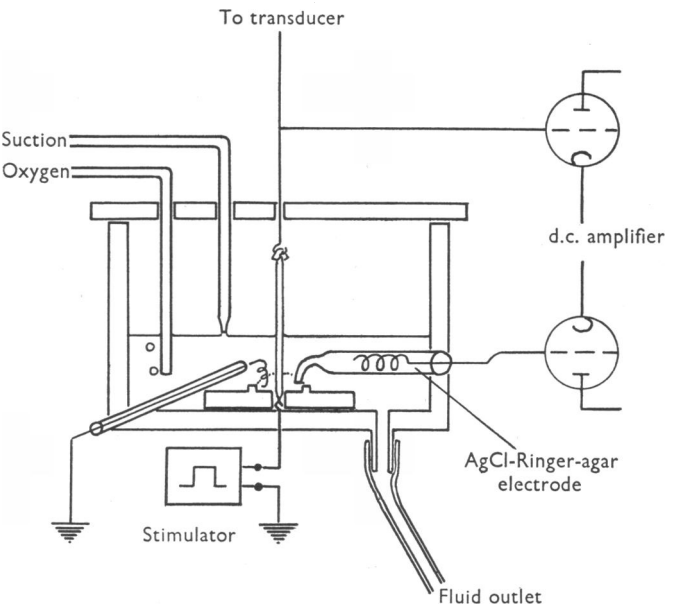


Fig. 1. Diagram of apparatus. Heart strip fixed to two chlorided silver hooks. Potential recorded between upper hook (which is also attached to the tension recorder [RCA 5734]) and the AgCl–Ringer-agar electrode, which dips into the bath. Electrical stimulation between lower hook and earthed electrode. Suction tube and fluid level of chamber are in position for potential recording. When the chamber is emptied a drop of fluid remains at the lower end of the strip; this maintains electrical connexion between strip and electrodes.

This will, in effect, cause a slight diminution of the observable p.d. due to KCl depolarizations (a reduction of less than 3 mV). In order to ensure that the potential was recorded at approximately the same region when the bath fluid was changed the fluid was kept at a constant level by means of a suction tube; alternatively, fluid changes were made with measured quantities of solutions.

The chamber was emptied through the outlet at the bottom, and the whole fluid was removed except for a drop which remained at the lower end of the strip and provided a connexion between the electrodes and the strip. Emptying and filling of the chamber took place in 2–4 sec, a time short in comparison with the events recorded during contracture.

Solutions. The Ringer's solution contained NaCl 110·5 mM, KCl 2·5 mM, $CaCl_2$ 1·5 mM, $NaHCO_3$ 2 mM. $CaCl_2$ and KCl concentrations were changed by replacing osmotically equivalent amounts of NaCl. Only in the experiment of Fig. 6 (record *e*), where the $CaCl_2$ concentration exceeded the replaceable quantity of NaCl, an extra amount of solid $CaCl_2$ was added.

Experimental procedure. Consistent *potential* changes were obtained only when the part of the strip exposed to air (i.e. not treated with the KCl solutions) was greater than about 5 mm. This arrangement, however, was not satisfactory for recording the contracture *tension*, since the part of the strip in air acted as a long 'series elastic' component. In most experiments, therefore, tension and potential were recorded consecutively; the tension during immersion of the whole strip in KCl solutions, and potentials during partial immersion to a constant level about 10 mm from the upper end. Periods of KCl application lasting 2–3 min were separated by intervals of 30 min during which the strip was kept in oxygenated Ringer's solution and stimulated at a constant frequency. Under such conditions the electrical and mechanical responses to KCl solutions were well reproducible, particularly at medium Ca concentrations. This justifies the procedure of separately recording tension and potential changes.

RESULTS

General properties of the KCl *contracture*

Before studying the action of Ca on KCl contracture it was important to investigate what other factors influenced the size and time course of the contracture. Such factors were found to be the O_2 concentration of the KCl solution and the frequency at which the strip is stimulated before the onset of contracture. As in the previous paper (Niedergerke, 1956) experiments were started an hour after dissecting the strip, a period which allowed for the 'healing over' of cut fibre ends.

Effect of O_2 *concentration.* Fig. 2 shows the effect on the contracture of varying the O_2 concentration. During a preliminary 30 min period the strip was immersed in O_2-saturated Ringer's solution containing 1·5 mM-Ca, and stimulated at a frequency of about 9/min. The last minute of this 'conditioning' period is shown at the beginning of the records. Following the 9th propagated response, stimulation was interrupted and after a time slightly longer than the pulse interval of previous stimulation, a 100 mM-KCl solution, containing 1·5 mM-Ca, was applied. This KCl solution was O_2-saturated in records *a* and *c*, and N_2-saturated in record *b*. After a burst of twitch-like contractions, initiated by the KCl solution, the tension rises to a flat maximum and then declines again 'spontaneously', and as a rule much more slowly. It is clear that this decline of tension in the KCl is faster with N_2-saturated solution than with an O_2-saturated solution. A comparison of 2*a* with *c* shows the reproducibility of the KCl contracture which was better than that of the propagated 'twitch' responses.

Potentials were recorded after each tension measurement using the same solutions. Since there was no significant difference in the three potential traces, only one of them is shown in Fig. 2*d*. Corresponding to the tension records, the beginning of the action of KCl is marked by a short burst of diphasic action potentials only partially visible, and this is followed by a gradually increasing depolarization (bath electrode becoming negative with respect to the upper hook electrode). Withdrawal of the KCl solution causes a 'step', and refilling the chamber with Ringer's solution a sudden 'dip' in the

record. Repolarization in Ringer's solution is much slower than depolarization; recording was therefore stopped for 10 min, after which time repolarization seemed to be complete. The 'dip' (*t*) in the potential record is an artifact due to refilling the chamber temporarily above the previous level of the KCl solution. The 'step' (*h*), and the inequality of rates of depolarization and repolarization are of greater significance and will be further discussed below (p. 589).

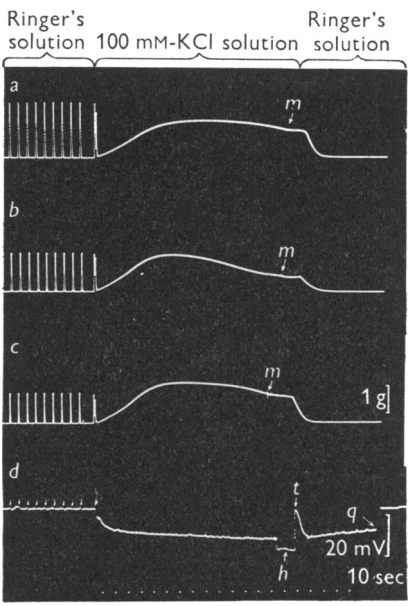


Fig. 2. Effect of oxygen lack on contracture. Tension, *a*, *b*, *c*; potential, *d*. Records *a* and *c*, contracture with 100 mM-KCl solution saturated with O_2; record *b*, contracture with an N_2-saturated 100 mM-KCl solution. *m*, mechanical artifacts, marking withdrawal of KCl solution. Record *d*, potential record taken 30 min after record *c* with O_2-saturated 10 mM-KCl solution. Only the 'feet' of the diphasic action potentials, in the conditioning period and during the initial burst of the contracture period, are visible at the slow film speed. *h*, 'step', due to withdrawal of KCl solution (see Fig. 5); *t*, 'dip' due to refilling chamber with Ringer above the previous recording level on the strip. At *q*, recording stopped for 10 min.

Effect of the frequency of previous stimulation. If a heart strip, rendered 'hypodynamic' by a sufficiently low Ca concentration of the Ringer's solution, is stimulated at different frequencies, the tension response increases with the frequency (Niedergerke, 1956). This is one aspect of the 'staircase' which has been interpreted by saying that each contraction gives rise to some facilitating process which summates and allows successive contractions to develop a higher tension. Fig. 3 shows that the tension rise during a KCl contracture also depends on the frequency of the preceding stimulation. The strip was immersed during the 'conditioning' period in 1·5 mM-Ca Ringer's solution and

contractures were produced by oxygenated 100 mM-KCl solution which also contained 1·5 mM-Ca. In Fig. 3*a* and *c*, the frequency of conditioning stimulation in Ringer's solution was about 20/min, while in Fig. 3*b* it was less than 1/min. The rise of the KCl contracture is slower and its maximal tension smaller after the period of low-frequency stimulation in *b*, than after the higher frequency in *a* and *c*. This suggests that the same process which underlies the ordinary 'staircase' (illustrated by the different amplitudes of propagated responses during the initial bursts in *a*, *b*, *c*) facilitates also the KCl

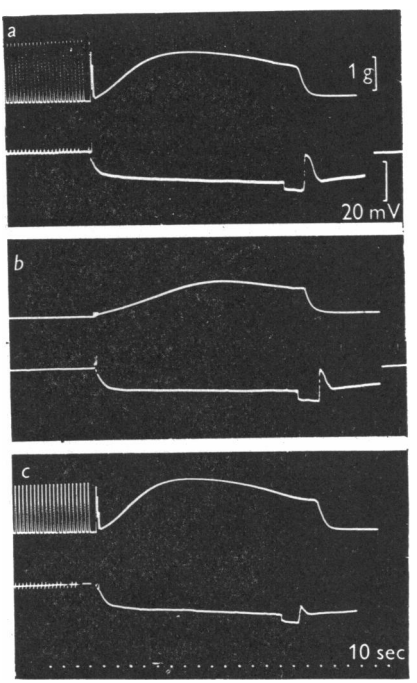


Fig. 3. 'Staircase' phenomenon of KCl contracture. Upper traces in each record, tension; lower traces, potential. Records taken in alphabetical order: *a* and *c* obtained after a conditioning stimulus frequency of 20/min, *b* after a conditioning frequency of 0·7/min. Contracture induced by 100 mM-KCl (1·5 mM-Ca) solution throughout.

contracture. In the potential records the depolarization after a low frequency of stimulation is also somewhat smaller than after a higher frequency. This effect is not very marked in Fig. 3 but it was consistently observed in three other experiments. The difference in the two conditions was 2–4 mV, i.e. about 10% of the maximal depolarization.

At this point the analogy with the staircase apparently fails, since it has repeatedly been stated that action potentials of the heart do not become larger as contractions increase during the staircase. In order to ascertain that this was true for the preparations used in the present experiment, a staircase was examined, with simultaneous recording of tension and monophasic action

potentials, on two of the strips from the above experiment. As found previously (Niedergerke, 1956), the amplitude of the monophasic action potential, if it changed at all during the staircase, became somewhat smaller while the tension increased.

Relation between KCl *concentration and strength of contracture.* Fig. 4 shows the changes in the height of the contracture, as the KCl concentration is increased (cf. also Fischer, 1924). A Ca concentration of 2·5 mM was used throughout and the different KCl concentrations were 100 mM (Fig. 4*a*, *d*), 50 mM (Fig. 4*b*) and 25 mM (Fig. 4*c*). Both the depolarization and the tension increase when the KCl concentration is raised, but the relations between the

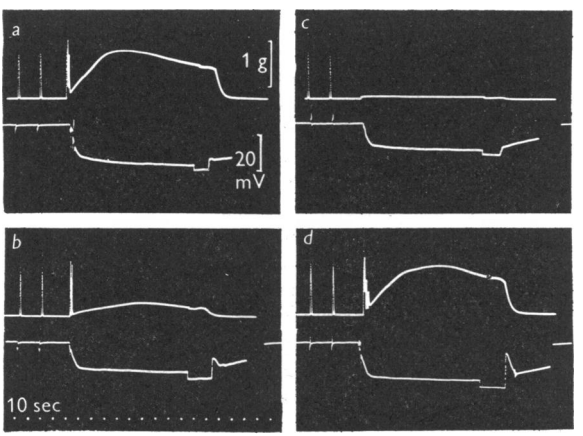


Fig. 4. Dependence of contracture on KCl concentration. Upper trace, tension; lower trace, potential in each record. Records taken in alphabetical order; *a* and *d*, 100 mM-KCl solution applied to the strip; *b*, 50 mM-KCl solution; *c*, 25 mM-KCl solution. Ringer's and KCl solutions contained 2·5 mM-Ca throughout.

two types of response and the KCl concentration are different. The depolarization shows a larger increase between normal (2 mM) and 25 mM-KCl, than between 25 and 50 mM, or between 50 and 100 mM-KCl. This would be expected from the known logarithmic relation between external KCl concentration and membrane potential in other cells (e.g. Hodgkin, 1951). By contrast, there is hardly any tension at 25 mM-KCl, and the main tension increment occurs between 50 and 100 mM-KCl.

Analysis of some features of the potential records

The extra potential of the 'step'. If the KCl solution is drained from the strip, an extra potential difference, the 'step', appears. This can be interpreted by assuming that the strip is less completely depolarized at the junction between the KCl bath and the normal, Ringer-treated, part than at a lower point, some distance from the junction. As a consequence, during the drainage the additional depolarization of the lower part would appear. This situation is illustrated schematically in Fig. 5*a* on a simplified electrical model of a single muscle fibre.

KCl solution, introduced from the right, with the fluid level at $x=0$, establishes a membrane e.m.f., E_2, along the fibre, from $x=0$ to $x=l$, while the left side of the fibre, suspended in air and

surrounded by a film of Ringer's solution has a larger membrane e.m.f., E_1. The p.d. recorded in this position is then

$$V_1 = -r_0 \int_{-b}^{0} i\,dx.$$

If the fluid level is shifted from $x=0$ to $x=a$ the recorded potential is

$$V_2 = -r_0 \int_{-b}^{0} i\,dx - r_0 \int_{0}^{a} i\,dx.$$

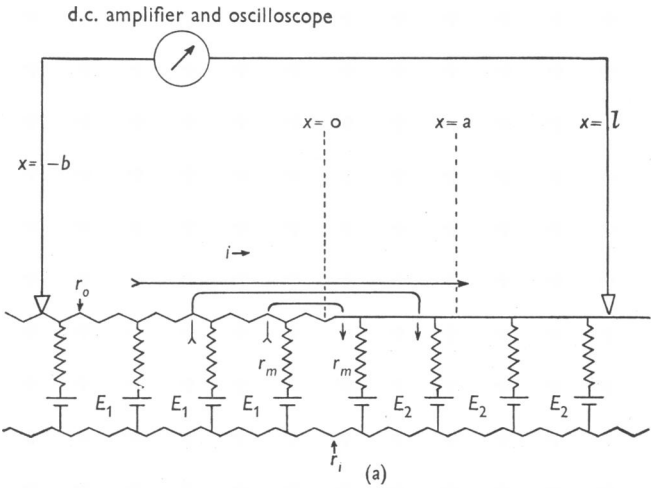
It is easy to see that V_2 is markedly greater than V_1, unless at the junction the membrane resistance r_m for $x>0$ is very small compared with the membrane resistance for $x<0$.

The experiment of Fig. 5*b* was carried out to test this interpretation. Two potential curves were obtained from a strip bathed in 100 mM-KCl solution. Record 1 was obtained with the usual procedure, KCl solution was applied to a given level and withdrawn 20 sec before Ringer's solution was introduced, showing the familiar 'step'. For record 2, KCl solution was introduced to a leve 2 mm higher than the previous recording level and after 20 sec lowered to the previous level. From this time on, recording position and experimental procedure for both records were the same. The extra potential in curve 2 is practically equal to the step in curve 1, and occurs during the early lowering of the KCl solution from $x=0$ to $x=a$; there is little extra potential when the KCl solution is finally drained from the chamber.

This feature is not specific for heart muscle strips but has also been found, giving an effect of the same magnitude, on small bundles of the semitendinosus muscle.

The difference in rate of depolarization and repolarization. The depolarization in KCl develops much faster than the repolarization in Ringer's solution. Four factors might be responsible for this difference. (i) Relaxation after a KCl contracture starts as soon as Ringer's solution is applied and a region previously contracted will be pulled, from the Ringer bath into air, by the elastic force of the stretched remainder. Diffusion of residual KCl from this air-exposed region will obviously be slower than from other parts which remain bathed in Ringer's solution. Since the potential is recorded from the neighbourhood of this region, repolarization will therefore be slowed. This artifact, however, would be very small if—as in the experiment illustrated in Fig. 5*c*—Ca-free solutions which minimize contracture are employed. This is further shown by the procedure of Fig. 5*c*: two records are superimposed; during repolarization of one the potential was recorded continuously, while in the other case there were two intermissions during which the whole strip was immersed in Ringer's solution. The time course of depolarization in the two curves is nearly the same, showing that the artifact discussed above is hardly noticeable. (ii) A more important factor is the logarithmic relation between KCl concentration and membrane potential. The rise and fall of the recorded potential could therefore not be expected to represent simply the time course of diffusion of KCl in the extracellular space. The logarithmic relation will tend to make the development of depolarization fast compared with the rise of K concentration in the extracellular space, and conversely will cause repolarization to be apparently slower than the fall of the extracellular K concentration. (iii) If a part of the strip is depolarized, a component of electrotonic spread from this region will occur in a direction perpendicular to the axis of the strip because of the syncytial connexions of the heart tissue. This will cause the deeper parts of the tissue to become depolarized electrotonically even *before* the KCl concentration in these regions attains a high value. By the same mechanism the superficial layers will be kept depolarized after the return of the Ringer's solution until the KCl has been soaked out from the centre. This electrotonic spread will contribute to the difference between the rates of depolarization and repolarization if, as seems likely, the membrane resistance is reduced by K-rich solutions.

In order to estimate the extent to which each of the latter two factors contribute to the form of the records, the same experiment as in Fig. 5*c* was carried out on bundles of the semitendinosus muscle which do not possess syncytial connexions. This provides a fair comparison if the bundles are of the same width as the heart strips. It was found that a difference in rise and fall of the potentials still existed, but it was much less pronounced than with the heart strips. This suggests



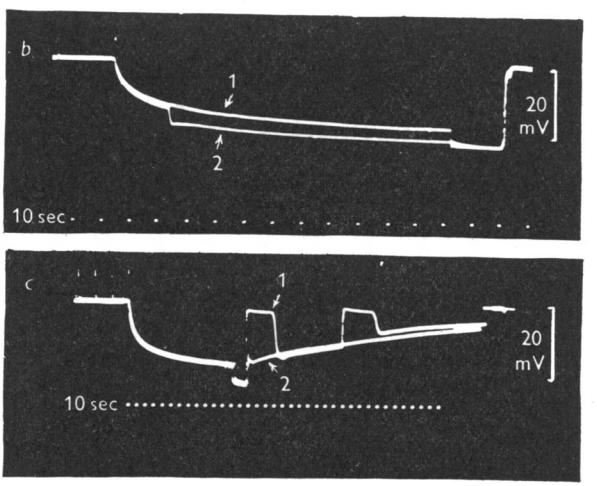


Fig. 5. Some features of the potential records. (*a*) recording conditions on a single muscle fibre. x is the distance along the fibre; r_i, the resistance per unit length of the fibre inside, is assumed to be constant for all x; r_0 is the resistance per unit length of the external fluid, r_m the membrane resistance × unit length. Electrodes for leading off potentials at $x = -b$ and $x = l$. (The distances $x = 0$ to $x = -b$, and $x = 0$ or $x = a$ to $x = l$ are assumed to be $\gg \lambda$, the space constant of the fibre on either side of $x = 0$.) Left side of fibre from $-b$ to 0 surrounded by a film of Ringer's solution and suspended in air with membrane e.m.f. E_1. Right side of fibre soaked in KCl bath solution from $x = 0$ to $x = l$ with e.m.f. E_2. (*b*) the significance of the potential 'step'. Two superimposed potential records. Record 2 was taken by raising initially the KCl solution to a level 2 mm higher than in record 1. After 20 sec the fluid level of the KCl solution was lowered to the same level as that used in record 1. Note that the potential drop in record 2, at this moment, has approximately the same size as the 'step' in record 1 which was obtained when the bath was drained. (*c*) potential curve free from mechanical artifact. Two superimposed records of depolarizations by Ca-free 100 mM-KCl solution, taken from the same point on the strip. Record 2 recorded continuously, record 1 with two interruptions in the phase of repolarization during which the entire length of the strip was short-circuited by Ringer's solution. The diameter of strip used in record *b* and *c* was exceptionally large, 1·4 mm: this is the reason for the comparatively low rate of depolarization.

that the syncytial structure of the heart is an important factor in determining the time course of the recorded potentials. (iv) It is conceivable that metabolic activity is directly involved in establishing the normal membrane potential after treatment with K-rich solutions. This is suggested by Csapo & Wilkie's (1956) recent experiments on the frog's sartorius, showing very slow recovery from depolarization at a low temperature.

Modifications of potassium chloride contracture due to calcium ions

The experiments of this section were carried out with O_2-saturated solutions, and with a constant rate of preliminary stimulation, thus controlling two otherwise disturbing factors.

The effect of different calcium concentrations on the contracture. Ca enhances the tension developed in propagated contractions of the heart in a striking manner (cf. Niedergerke, 1956). That this holds true also for the tension developed in a KCl contracture is shown in Fig. 6. Contractures were elicited by applying solutions containing 100 mM-KCl but varying concentrations of Ca. Each new Ca concentration was introduced with the Ringer's solution 30 min before onset of contracture so as to 'equilibrate' the strip with the required Ca level.

As shown in Fig. 6, the peak tension of the contracture rises with the Ca concentration up to a concentration of about 4 mM-$CaCl_2$. In two other experiments a maximum tension was obtained at a Ca concentration between 5 and 10 mM. With higher concentrations there is little further change in the amplitude of contracture but its rate of rise continues to increase and the rate of relaxation continues to decline.

The potential records at the various Ca concentrations did not show significant differences. One of them has been reproduced in Fig. 6*f* to provide a comparison of the time courses of contracture and depolarization. The general result is that Ca appears to enhance contractile strength without altering the depolarization produced by a given KCl concentration. Small changes of KCl depolarizations due to added Ca may however exist. There is evidence that in muscle (Fleckenstein, Wagner & Goggel, 1950) and crab nerve (Guttmann, 1940) Ca reduces depolarization produced by a given KCl concentration. Although this would be an effect in the 'wrong' direction, it seemed worth while examining more closely whether any changes in depolarization could be held responsible for the facilitating action of Ca on the contracture. The experiment illustrated in Fig. 7 excludes this possibility. In records 1 and 3, contractures were elicited by 50 mM-KCl in the presence of 10 mM-Ca. In record 2, 100 mM-KCl was used to produce a contracture in a Ca-free solution. It can be seen that, when the Ca concentration was high, the *smaller* depolarization was associated with a large contracture (records 1 and 3), while the *larger* depolarization in the Ca-deficient solution had little effect. Hence the action of Ca cannot be due to a direct effect on depolarization.

Another way of expressing the result of the experiment in Fig. 7 is to say

that Ca alters the relation between depolarization and contractile tension. In the experiment illustrated in Fig. 8 the relation between depolarization and plateau tension of a contracture has been measured on a strip at two Ca concentrations. Depolarizations were varied by applying solutions containing KCl in different concentrations and with either 2 mM-Ca or 10 mM-Ca. With 2 mM-Ca the relation between plateau tension and KCl concentration has the features already known from Fig. 4. Little tension was developed with

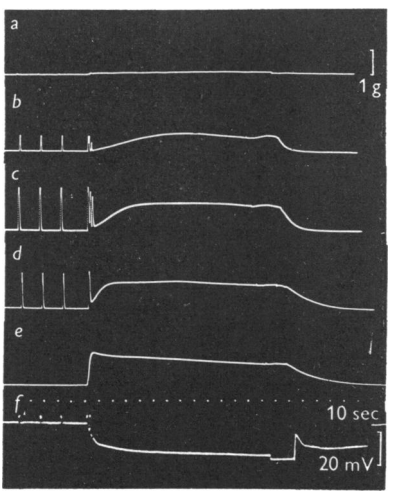


Fig. 6

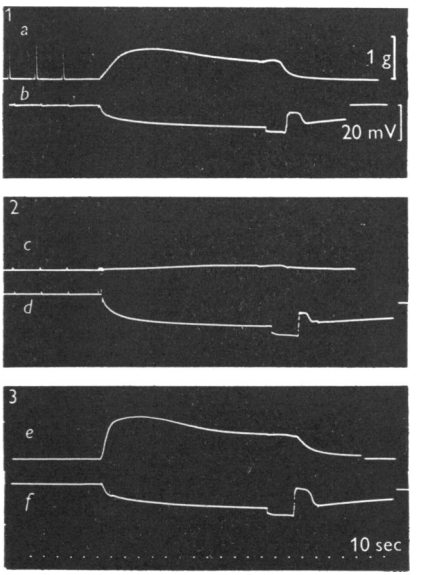


Fig. 7

Fig. 6. Dependence of contracture on Ca concentration. Contractures with 100 mM-KCl solutions, but varying Ca concentrations. Tension records: *a*, Ca-free; *b*, 1·6 mM-Ca; *c*, 4 mM-Ca; *d*, 10 mM-Ca; *e*, 25 mM-Ca. These concentrations were present during contracture period as well as the conditioning period except in *a*, where strip was bathed in Ca-free Ringer's solution only during the last 10 min of the conditioning period. Potential record *f* taken in 10 mM-Ca, 100 mM-KCl solution.

Fig. 7. Ca does not enhance tension by enhancing depolarization. Upper traces, tensions; lower traces, depolarizations in each record. Records 1 and 3 with 10 mM-Ca 50 mM-KCl solution, and record 2 with Ca-free 100 mM-KCl solution. The Ca concentrations were present during contracture periods as well as the conditioning periods, except in *c* and *d* where strip was bathed in Ca-free Ringer's solution only during the last 10 min of the preparatory period.

25 mM-KCl, the main increment in tension being obtained between 50 and 100 mM-KCl. High Ca alters this relation so that the smallest detectable contracture occurs at a lower KCl concentration, and the main tension increments are observed up to about 50 mM-KCl.

These results suggest a simple explanation for the results in Fig. 6, viz. that Ca influences the strength of a contracture as well as the rates of contraction

and relaxation. Suppose that contracture tension is determined solely by the instantaneous amplitude of the depolarization, and that the effect of Ca is merely to alter the quantitative relation between the two values. Fig. 9 illustrates this hypothesis further: the 'curves' of Fig. 8 were converted into tension–depolarization curves by assuming (*a*) that the depolarization with 10 mM-KCl amounts to 20% of the depolarization with 100 mM-KCl (see Weidmann, 1956, p. 41), and (*b*) that the relation between depolarization and KCl concentration is logarithmic in the range of 10–100 mM-KCl. The reconstructed curves of tension development resemble the experimental results closely (cf. Fig. 6).

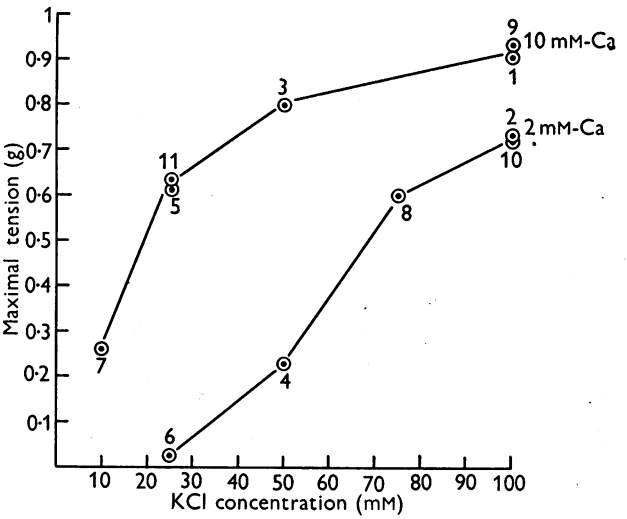


Fig. 8. Relation between tension and KCl concentration at two different Ca concentrations. Maximal contracture tension of a strip plotted against varying KCl concentrations. Strip remained equilibrated with 2 mM or 10 mM-Ca during 30 min conditioning period and the contracture time. Frequency of constant electrical stimulation during conditioning period—4/min. The order in which the measurements were made is indicated by the numbers.

Effect of calcium applied or withdrawn during a potassium chloride contracture. The effect of Ca on the strength of propagated contractions of a heart strip occurs rather quickly, and it has been suggested that the action takes place close to the cell surface (Niedergerke, 1956). It was of interest to investigate whether such a rapid change can also be observed during a KCl contracture. In the experiments illustrated in Fig. 10*a* and *b*, contractures were produced with solutions whose Ca concentration differed from those of the Ringer's solution with which the strips had previously been equilibrated.

In Fig. 10*a*, trace 2, the Ca concentration was 5 mM before, and zero during the contracture period. In traces 1 and 3, the Ca concentration remained constant, at 5 mM, throughout. The effect of Ca removal is indicated by the

difference between traces 2 and 1 or 3; the difference is not very striking in this case, but it is significant, for the controls are nearly identical.

In Fig. 10*b*, traces 1 and 2, Ca-free Ringer's solution was applied during the last 7 min of the pre-contracture period. Contracture was produced in trace 1 with Ca-free 100 mM-KCl solution and in trace 2 with 5 mM-Ca, 100 mM-KCl solution. In trace 4, the strip remained equilibrated with 5 mM-Ca during conditioning and contracture period. The difference between traces 1 and 2 of Fig. 10*b* shows the effect of adding 5 mM-Ca. Trace 2 does not reach the same tension as trace 4, probably because the time of exposure to 5 mM-Ca is too

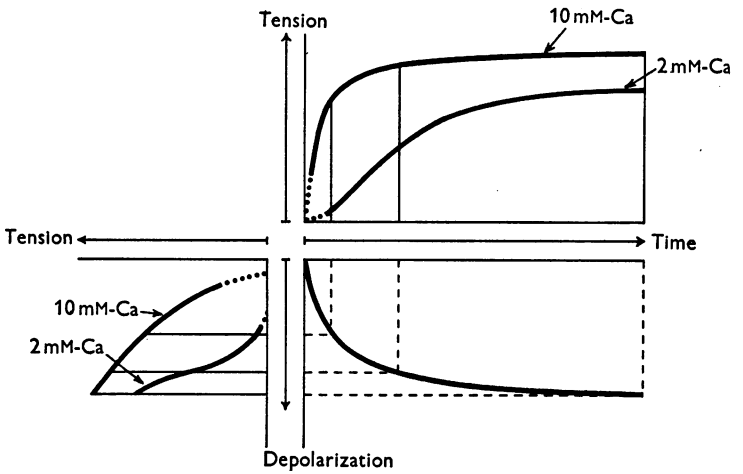


Fig. 9. Construction of two tension records from experimental data. *Lower part of diagram*, right, record of a depolarization by a 100 mM-KCl solution, redrawn from Fig. 7*b*; left, Fig. 8 transformed into a tension–depolarization diagram. *Upper part of diagram*, right, two constructed tension records of contractions due to 100 mM-KCl solution with either 2 or 10 mM-Ca. Projections from three points of the potential record indicate the procedure of the construction.

short. A rough estimate of the speed with which these changes occur is of interest. Contracture tension decreased by about 25% in 2·5 min during Ca removal and in the same period it increased to about 75% of the equilibrium value due to Ca addition. This time course of the Ca effect is of a similar order to that determined previously with propagated contractions (Niedergerke, 1956).

The striking effect which Ca can produce is further brought out in Fig. 11*b*. A strip soaked in Ca-free Ringer's solution for 10 min was treated with a Ca-free 100 mM-KCl solution. This caused depolarization accompanied by a barely noticeable tension development. 90 sec later 10 mM-Ca was added to the depolarized muscle, causing immediate strong contracture with little further change in the potential. The control experiment (Fig. 11*a*) was similar to that in Fig. 10*b* (2), high Ca (10 mM in 100 mM-KCl solution) being applied

directly after a preparatory 10 min period in Ca-free Ringer's solution. A comparison of the two tension records (11*a* and *b*) shows that the final plateau is almost the same in the two cases, whereas the rate of rise of the contracture in Fig. 11*b*, with the delayed Ca application, is faster.

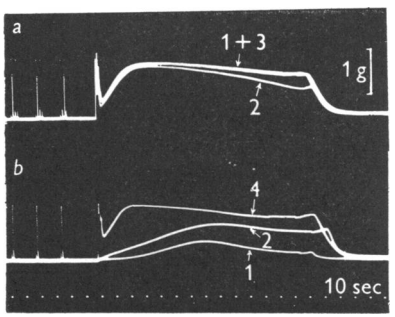


Fig. 10

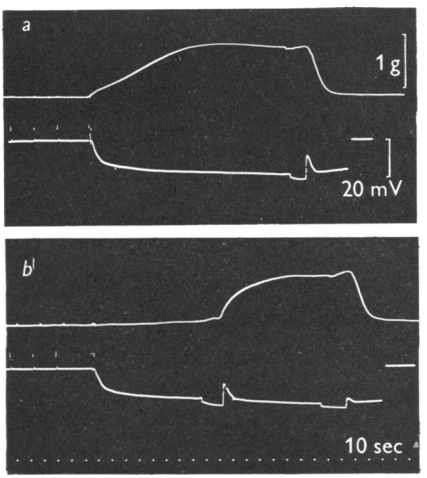


Fig. 11

Fig. 10. Tension changes due to Ca removal or addition during contracture period (diameter of strip 1 mm). Record *a*, Ca removal; traces 1 and 3, 5 mM-Ca Ringer's solution during conditioning period, 5 mM-Ca + 100 mM-KCl solution during contracture; trace 2, 5 mM-Ca Ringer's solution during conditioning period, Ca-free 100 mM-KCl solution during contracture. Record *b*, Ca addition; trace 1, Ca-free Ringer's solution during the last 7 min of the conditioning period and Ca-free 100 mM-KCl solution during contracture; trace 2, Ca-free Ringer's solution during the last 7 min of the conditioning period, 5 mM-Ca + 100 mM-KCl during contracture; trace 4, 5 mM-Ca Ringer's solution during conditioning period and 5 mM-Ca + 100 mM-KCl during contracture.

Fig. 11. Effect of Ca on a depolarized strip, diam. 1·1 mm. In both records, upper traces, tensions; lower traces, depolarizations. The records started after soaking the strip in Ca-free Ringer's solution for 10 min. *a*, application of 10 mM Ca 100 mM-KCl solution; *b*, application, initially, of a Ca-free 100 mM-KCl solution; 90 sec later, 10 mM-Ca was added to this solution, causing an immediate rise of tension.

DISCUSSION

It is very probable (see, for example, Kuffler, 1946; Katz, 1950; Fleckenstein *et al.* 1950) that KCl-rich solutions cause contracture by depolarizing the surface membrane of muscle cells rather than by changing the intracellular electrolyte content. Two findings support this view: (1) The relative rates of contraction and relaxation following application and withdrawal of KCl solutions are of the same order as the rates of depolarization and repolarization. There is therefore hardly time for K ions to diffuse across the cell membrane to exert a direct action on the contractile proteins. (2) Contractures in K_2SO_4

solutions have the same appearance as those in KCl solutions. Since muscle cells take up K and swell in KCl, but not in K_2SO_4 solutions (Overton, 1904) such intracellular changes are not likely to be of importance in the development of contractures.

The 'spontaneous' decline of the contracture which was not associated with a decline in potential may be of complex origin. Two factors which possibly contribute are: (1) an exhaustion of some energy store, usually maintained by oxidative metabolism—this is suggested by the difference observed when oxygen- or nitrogen-saturated solutions were used (Fig. 2); (2) a loss of Ca from the cell may occur during contracture when the bath contains less Ca than required for maximal tension development (this is suggested by the experiment in Fig. 10*a*).

The action of calcium. Ca enhances the contracture associated with a given depolarization. In particular, Ca was found to reduce the 'threshold' depolarization necessary to produce noticeable tension. Furthermore, it alters the relation between depolarization and tension in such a way that tension increments become relatively much greater in the lower range of depolarization. This effect helps to explain why high Ca increases the rate of development and reduces the rate of relaxation of the contracture (cf. Fig. 9).

It is still uncertain on which part of the cell Ca produces its action. That an easily exchangeable fraction of the cellular Ca is involved was inferred from a study of the time course of Ca acting on propagated contractions (Niedergerke, 1956), and is supported by the present experiments. Thus, Ca produced striking effects in the relatively short time of 1–2 min when it was added at the beginning or even during a KCl-depolarization (Figs. 10, 11). It may be that in these experiments the effect of calcium was accelerated by increased rate of entry through the depolarized cell membrane (cf. Flückiger & Keynes, 1955). The effect of Ca removal during contracture suggests that the contractile process does not require a very firm binding of the Ca by which it has been activated.

It is a matter of conjecture why the effect of Ca on skeletal muscles is not present, or is so much less pronounced (Denton, 1948). Factors which might explain this difference are the following. (*a*) Spontaneous decline of the contracture is much more rapid in most skeletal muscles than in the heart. This may obscure a facilitating action of Ca. (*b*) Skeletal muscles seem to lose Ca less easily than the heart muscle (compare for example, Taubmann, 1934, with Lieb & Loewi, 1918) and are therefore perhaps always fully activated. (*c*) Ca increases the membrane potential of some skeletal muscle fibres (Jenerick & Gerard, 1953; Gossweiler, Kipfer, Poretti & Rummel, 1954), but not of the heart (Weidmann, 1955). In addition, it has been reported that it diminishes depolarization by a given KCl concentration on skeletal muscle (Fleckenstein *et al.* 1950). These membrane effects might cause the threshold depolarization

of contracture to rise and thus cancel any facilitating effect on the contractile process.

Staircase phenomenon. The dependence of the KCl contracture on the rate of previous stimulation (Fig. 3) was found to be similar to the staircase of propagated contractions. In contrast to the staircase, however, the greater contracture response was accompanied by a greater amount of depolarization. The possible extent to which the altered depolarization contributes to this change in the contracture has not been assessed.

SUMMARY

1. Tension and surface depolarization of strips of frog's ventricle were recorded during potassium contractures. This was used as a method to study the influence of calcium on the contractile response when the excitable membrane has been depolarized to a given extent.

2. High calcium increases the amplitude and rate of rise of the contracture, and slows its relaxation. The effect on the tension reaches a maximum at a medium calcium concentration (about 5 mM), whereas the speed of contracture and relaxation continues to change with high calcium concentrations.

3. The effects of calcium on the potassium contracture are not associated with significant changes in the time course or amount of the depolarization.

4. When calcium is added to, or withdrawn from, a depolarized muscle, rapid changes occur in the strength of the contracture. Thus, a depolarized muscle can be caused to contract strongly and rapidly by transferring it from a low to a high calcium concentration.

5. The effects of calcium can be summarized by saying that calcium alters the relation between depolarization and tension: it enhances tension at all levels of depolarizations, but particularly strongly at small depolarizations.

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