Biphasic Potassium Contractures in Frog Muscle Fibers

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ABSTRACT Potassium-induced contractures were studied in single fibers from the semitendinosus muscle of *Rana pipiens*. Contractures elicited by solutions containing 60–117 mM potassium and 120 mM chloride were biphasic, consisting of a rapid initial contraction with a duration at 23°C of less than 1 sec followed by a slow response with a duration of many seconds. At 13°C, the initial response was greatly prolonged so that the two responses virtually fused into a single smooth contracture. Membrane potential in high potassium, high chloride solutions underwent a transient peak depolarization, probably as a result of time-dependent changes in membrane conductance during depolarization. It is proposed that this complex time course of depolarization gives rise to the biphasic contracture response.

Hodgkin and Horowicz (1960 *b*) have reported that the frog skeletal muscle fiber responded to potassium depolarization with a contracture whose magnitude varied continuously over a range of extracellular potassium concentrations of 20 to 50 mM. The rate of tension development showed a similar dependence on potassium concentration, and, in all cases, tension rose smoothly to a plateau value which was sustained for a number of seconds.

In the experiments described in the present report, an increase in extracellular potassium resulted, under certain conditions, in a biphasic contracture; an initially rapid development of tension was followed within less than 1 sec by partial relaxation and by a much slower secondary development of tension to a plateau level. Since it seemed possible that the initial transient component of this biphasic response reflected an "all-or-none" activation step, the phenomenon was studied in some detail. A preliminary account of these experiments has been presented to the Biophysical Society (Costantin, 1971).

METHODS

Single fibers dissected from the semitendinosus muscle of *Rana pipiens* were mounted in the experimental chamber at 1.25 to 1.33 times slack length. The stretched length of the fibers ranged from 1.6 to 2.8 cm.

Experimental Chamber

The experimental chamber was similar to that employed by Hodgkin and Horowicz (1959) and consisted of a channel 3 mm wide by 3 mm high into which solutions of different composition could be introduced. One tendon of the fiber was tied to a fixed stainless steel pin centrally located within the channel 7 mm from the site of fluid inflow; a stainless steel loop tied to the opposite tendon was attached to a 3 cm long extension of the anode pin of an RCA 5734 transducer. The transducer was mounted on a micromanipulator so that the muscle length could be adjusted to the desired value. The roof of the channel consisted of short lengths of 1 mm thick Lucite panels which covered the entire channel except for a gap of approximately 800 μ through which the anode extension of the transducer projected into the channel. The fiber was freely suspended in the channel in all experiments except those in which the membrane potential was to be recorded. In the latter experiments, a 1.75 mm high semicircular pedestal, which had been lightly coated with vaseline, was positioned in the channel about 1.5 cm from the inflow site, and the fiber rested on the surface of this pedestal over about 0.5–1.0 mm of its length. A 200 μ gap was left in the roof of the channel to permit insertion of a microelectrode into the portion of the fiber immobilized by contact with the pedestal. A pair of platinum wires imbedded in the floor of the channel were employed for electrical stimulation of the fiber. Tetani were elicited by a 250 msec train of 0.3 msec pulses delivered every 10 msec.

Electrical Recordings

The bath was connected to earth by a chlorided silver wire, and the transmembrane potential was recorded between two microelectrodes filled with 3 M KCl. The electrodes were coupled to unity gain voltage followers, and both the membrane potential and the signal from the RCA 5734 were recorded on a Brush direct writing oscillograph (Mark 220). Microelectrodes were chosen for low tip potentials and resistances between 5 and 10 megohms. Solution changes with both microelectrodes in the extracellular medium produced potential changes of less than 2 mv.

Fibers were visualized with a stereomicroscope at a magnification of 40 during impalement. A right-angle prism positioned alongside the experimental channel permitted a lateral view of the fiber and microelectrode and greatly facilitated the impalement procedure.

Solution Changes

Different solutions were introduced into the experimental channel by a multiple tap, and fluid was removed from the other end of the channel by a suction device. Contractures were elicited at intervals of 10 min or longer, and exposure to solutions with elevated $[K]_o[Cl]_o$ product was limited to 10 sec or less to avoid significant changes in the intracellular chloride ion concentration (Hodgkin and Horowicz, 1959). The flow of fluid into the channel was controlled in three different ways. In most experiments, solutions were permitted to flow freely from a reservoir by gravity. The flow rate with this arrangement was about 2 ml/sec. When microelectrode impalements were to be made, the solution was delivered by a constant flow infusion

pump (Harvard Apparatus Co., Millis, Mass., Model 975) at a rate of 0.77 ml/sec. When very rapid solution changes were desired, 3 ml of solution were delivered to the experimental channel from a syringe whose plunger was driven between two stops by a stretched spring. After 1 ml of solution had been delivered, the excursion of the plunger was damped by an opposing spring. With this arrangement the first milliliter of solution was delivered in 140 msec, the second milliliter in the next 240 msec, and the third milliliter was delivered over the subsequent 1–2 sec. Tests with dye solutions indicated that solution changes in the channel were more than 99% complete following delivery of the first milliliter of solution.

Solutions

The standard solutions employed are shown in Table I. Choline chloride was obtained from Eastman Organic Chemicals, and was recrystallized from absolute

TABLE I

COMPOSITION OF SOLUTIONS

Concentrations in mmoles per liter solution

(*a*) Solutions containing *x*K, 120 Cl

Solution	K^+	Cl^-	Na^+	Choline$^+$	Ca^{++}	Mg^{++}	$(PO_4)^*$	(EGTA)*
A	2.5	121	117.5	—	1.8	—	1.5	—
B	2.5	121	2.5	115	1.8	—	1.5	—
C	117.5	121	2.5	—	1.8	—	1.5	—
D	2.5	120	117	—	—	5.4	3.0	2.5
E	109.5	120	10	—	—	5.4	3.0	2.5
F	—	121	120	—	1.8	—	1.5	—

(*b*) Sulfate and propionate stock solutions

Solution	K^+	Cl^-	Na^+	Ca^{++}	$(PO_4)^*$	Propionate$^-$	SO_4^-	Sucrose
G	—	—	82.5	8	1.5	—	48	113
H	80	—	2.5	8	1.5	—	48	113
I	2.5	3.6	117.5	1.8	1.5	117.5	—	—
J	117.5	3.6	2.5	1.8	1.5	117.5	—	—

* PO_4 and EGTA (ethyleneglycol-*bis*-(β-aminoethylether)*N*,*N*′-tetraacetic acid) were added as stock solutions of the sodium salt at pH 7.2. The final solution pH ranged from 6.9 to 7.1.

ethanol. Propionate stock solutions were made by titration of propionic acid to pH 7 with NaOH or KOH.

Two general types of bathing solutions were employed, those in which the chloride concentration was equal to that of normal Ringer (solution A), and those in which the chloride concentration was lowered as the potassium concentration was raised. The former solutions are characterized in the text as *x* K, 120 Cl and were made with appropriate combinations of solutions A and C, B and C, or D and E. The latter solutions are characterized as *x* K, *y* Cl and were of two kinds: (*a*) those in which sulfate was substituted for chloride by appropriate combinations of solutions F, G and H, and (*b*) those in which propionate was substituted for chloride by

appropriate combinations of solutions F, I, and J. Unless otherwise indicated, all solutions which bathed the fiber contained tetrodotoxin (TTX) in concentrations of 25–100 γ/100 ml. A propagated twitch was not obtained in TTX-containing solutions.

Temperature

Most experiments were performed at room temperature which was usually 22°–23°C but ranged as high as 26°C. The temperature variation in the course of any given experiment was less than 1°C. In those experiments in which the effect of temperature was examined, a continuous flow of solution from a temperature-controlled reservoir was maintained in the experimental channel. Temperature was monitored with a thermistor probe (Yellow Springs Instrument Co., Yellow Springs, Ohio, Model 520) placed in the channel. The steady flow of solution was adjusted manually to the minimum level which would maintain the channel fluid within 1°C of that of the reservoir. The temperature of the channel fluid could be controlled within ±0.5°C with this arrangement. The temperature was held constant for about 3 min before each test contracture was elicited.

RESULTS

The observation which forms the basis of this report is illustrated in Fig. 1. The records in the left-hand column show contractures of a single fiber in

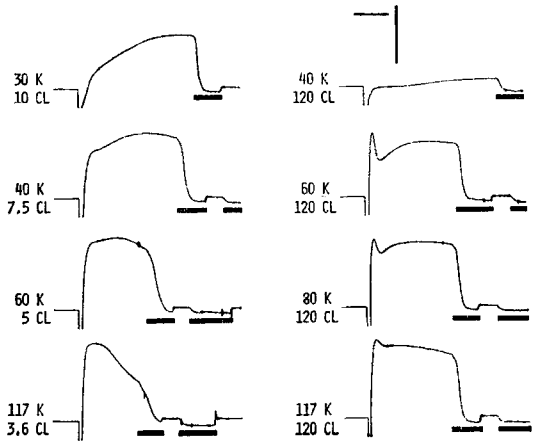

FIGURE 1. Contracture force resulting from elevation of extracellular potassium. The sudden downward deflection in each record marks the introduction of high potassium solution by a spring-driven syringe; the composition of the high potassium solution is indicated at the left of each force record (see text for details). The horizontal lines indicate the times during which normal Ringer flowed through the experimental channel. Contractures were elicited in the following sequence: 30 K, 10 Cl; 40 K, 120 Cl; 40 K, 7.5 Cl; 60 K, 120 Cl; 60 K, 5 Cl; 80 K, 120 Cl; 117 K, 3.6 Cl; 117 K, 120 Cl. Initial and final tetanic tension were identical and equal to 2.5 kg/cm^2. Vertical bar = 2 kg/cm^2. Horizontal bar = 2 sec. Temperature = 22°C.

response to a series of high potassium, low chloride solutions. In this experiment chloride was replaced by propionate, but identical results were obtained in sulfate solutions. The product $[K]_o[Cl]_o$ was constant and equal to normal Ringer solution, except in the trial with 117 mM potassium, where the chloride concentration was 3.6 mM. These records are similar to the results of Hodgkin and Horowicz (1960 *b*). The peak contracture tension increased with increasing extracellular potassium, and, over the lower range of potassium concentrations, a marked increase in the rate of tension development was seen with small increases in the extracellular potassium. The frog semitendinosus muscle gives a phasic contracture in response to a sustained depolarization, and the duration of the contracture decreases with increasing depolarization, that is, with increasing potassium concentrations. In the record labeled 117 K, 3.6 Cl, the onset of spontaneous relaxation can be seen before the reintroduction of normal Ringer solution.

In the records in the right-hand column of Fig. 1, the potassium concentration was elevated at a constant chloride concentration of 120 mM. At the lower potassium concentrations, the developed tension at a given potassium concentration was less in 120 Cl than in low chloride solutions. This was a consistent finding, and presumably arose because the depolarization in response to a given potassium concentration was less in the presence of a high extracellular chloride concentration (Hodgkin and Horowicz, 1959). At higher potassium concentrations, the contracture was clearly biphasic. Although in most fibers the time course of the two components of the contracture showed considerable overlap, some characteristics of the individual components were evident in the force records. The initial component, for example, was clearly transient, in that the rapid development of force was followed by the onset of relaxation within less than 1 sec. The secondary component developed much more slowly, and force was usually well-maintained throughout the few seconds of exposure to high potassium. The duration of the initial response was generally somewhat prolonged at higher potassium concentrations, and the rate of tension development in the secondary response increased. The result was that the two responses usually fused into a more or less smooth single contracture at 117 K, 120 Cl, the highest potassium concentration employed. This sequence of changes in the contracture records is well-illustrated by the fiber in Fig. 1.

A biphasic contracture was consistently observed with exposure to sufficiently high K, 120 Cl solutions. In 16 fibers tested with 60 K, 120 Cl, 13 gave a biphasic response to 60 K, and the remainder gave only the slow secondary response. All gave a biphasic response when tested with higher K, 120 Cl solutions.

Biphasic Contractures in Choline Solutions In the experiments of Hodgkin and Horowicz (1960 *b*), biphasic contractures were not seen, and the general

form of the contracture response was apparently independent of the chloride concentration. Tetrodotoxin was not available to Hodgkin and Horowicz, and in their experiments, choline chloride was substituted for sodium chloride to prevent action potentials. In the present study, five fibers were examined in choline solutions both with and without tetrodotoxin. The fiber was placed in solution B for periods up to 30 min, and contractures were elicited with high K, 120 Cl solutions made by combining appropriate amounts of solutions B and C. Biphasic contractures similar to those of Fig. 1 were seen with these high K, 120 Cl solutions, so that it seems unlikely that the use of choline in the experiments of Hodgkin and Horowicz can account for the difference between their findings and the results of the present study.

Comparison between Contracture Tension and Tetanic Tension It was important to establish that the biphasic contracture response was not a reflection of fiber damage. Accordingly, in 14 fibers, a 250 msec isometric tetanus was recorded in normal Ringer solution (solution A), a series of contractures, including a contracture in response to 117 K, 120 Cl, was then obtained in TTX-containing solutions, and a second isometric tetanus was recorded following return to TTX-free Ringer solution. Tetani elicited 5–10 min following return to TTX-free solution were generally reduced in size, but recovery of tetanic force did occur in about 20 min. In these 14 fibers, the peak tetanic force recorded after the series of contractures ranged from 85 to 115% of the initial tetanus (average = 101%). The initial and final tetani were averaged and compared to the two components of the contracture response to 117 K, 120 Cl. The initial component averaged 87 ± 3% of tetanic tension (range: 63 to 102%), and the secondary component averaged 75 ± 5% of tetanic tension (range: 31 to 98%).

The ability to produce full tetanic force upon return to normal Ringer solution persisted after prolonged periods in TTX-containing solutions and after multiple contractures. The fiber in Fig. 2, for example, remained continuously in solutions containing 25 γ/100 ml of TTX for 2 hr, and underwent 16 contractures over a 4 hr period. Tetanic tension at the end of this period was 112% of the initial response. Fibers which were damaged in the course of the experiment were easily recognized, since they produced progressively weaker contractures, and, upon return to TTX-free Ringer solution, were either electrically inexcitable or gave weak and unsustained tetani. Fibers which exhibited these signs of deterioration were not studied further.

The maximum and minimum diameters of the fiber mounted in the experimental chamber were measured with a dissecting microscope at a magnification of 80, and the fiber cross-sectional area was calculated on the assumption that the fiber was an ellipse with major and minor axes equal to one-half the observed maximum and minimum diameters. (For a discussion of the uncertainties involved in this determination, see Blinks, 1965.) Tetanic tension in

these 14 fibers was 3.6 ± 0.3 kg/cm², a value comparable to that obtained by Hodgkin and Horowicz (1960 *b*).

In two additional fibers, the membrane potential was recorded in normal Ringer solution with TTX after a biphasic contracture had been elicited with 80 K, 120 Cl. The membrane potential was −88 mv in one fiber and −94 mv in the second.

Biphasic Contractures at Constant $[K]_o [Cl]_o$ *Product* The finding of a biphasic contracture with high potassium, 120 Cl raised the possibility that two responses might also be involved in the contracture elicited by solutions with constant $[K]_o [Cl]_o$ product. A careful inspection of the records in the left-hand column of Fig. 1 indicates that this might be the case. In the contractures with 40 K, 7.5 Cl and 60 K, 5 Cl, a definite inflection is present in the force records at about the time at which the initial component is relaxing in the high chloride solutions. Such an inflection was frequently seen in the contractures elicited by solutions with constant $[K]_o [Cl]_o$ product, but no record was obtained in which a distinct fall and secondary rise in contracture force could be detected.

Effect of Temperature on the Biphasic Contracture The effect of lowered temperature on the biphasic response to high K, 120 Cl solutions was examined in two fibers; the most striking effect was a prolongation of the initial rapid component of the contracture. The experimental records from one of these fibers are shown in Fig. 2. Contractures over a range of potassium concentrations were elicited at 21°C and at 13°C. It is clear that the duration of the initial response was much prolonged at the lower temperature (compare the two records during exposure to 60 K, 120 Cl). With higher K concentrations at 13°C, a single smooth contracture was obtained, presumably because the prolongation of the initial response and the more rapid development of the secondary response which can be seen with higher K concentrations at 21°C (see also Fig. 1) resulted in a fusion of the two responses at 13°C.

Effect of a Conditioning Depolarization on the Biphasic Contracture In an attempt to characterize the biphasic contracture further, the effect of a conditioning depolarization on the contracture response was examined in three preparations. Fibers were bathed in 20 K, 15 Cl (made from solutions F, G, and H) for 2 min before exposure to 80 K, 120 Cl or 117 K, 120 Cl solutions. No contracture force was recorded in these solutions. Upon exposure to the higher K, 120 Cl solutions, however, the initial component of the contracture appeared to be abolished by pretreatment with 20 K, 15 Cl (Fig. 3, records 2, 4, and 7). Two of these fibers were also exposed to 10 K, 30 Cl before contractures were elicited, and the initial component was abolished in one fiber and persisted in the other (see Fig. 3, record 6).

The four control responses in the left-hand column of Fig. 3 illustrate the variability of the contracture response of a single fiber to the same test solu-

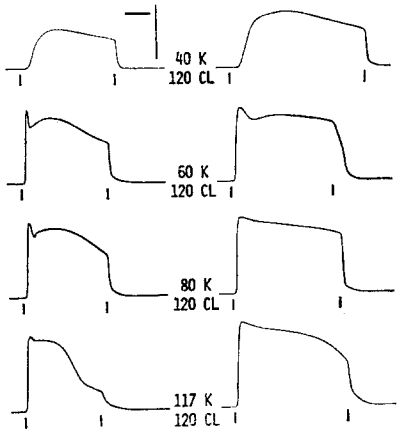

FIGURE 2. Effect of temperature on time course of potassium contracture. All solutions were introduced by gravity flow, and the flow artifacts have been edited out of these *tracings* of the original force records. High K solutions were introduced at the first vertical line in each record, and normal Ringer was reintroduced at the second vertical line. Left-hand column, contractures at 21°C. Right-hand column, contractures at 13°C. The composition of the high K, 120 Cl solution for the two contractures in each row is given in the center column. Contractures were elicited in the following order: 40 K; 60 K; 80 K; 117 K, all at 21°C; then 60 K; 40 K; 80 K; 117 K, all at 13°C. A contracture with 60 K, 120 Cl at 13°C was obtained before this sequence, and a contracture with 60 K, 120 Cl at 21°C was obtained after this sequence. Each was quite similar to the corresponding contracture shown in the figure. Initial tetanic tension = 2.9 kg/cm². Final tetanic tension = 3.2 kg/cm². Vertical bar = 2 kg/cm². Horizontal bar = 2 sec.

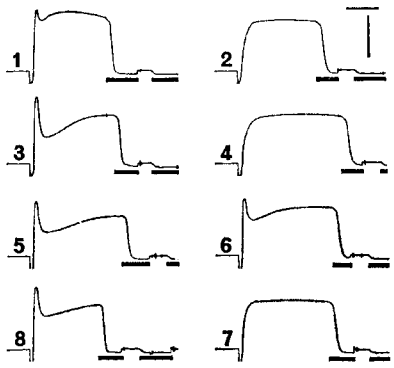

FIGURE 3. Effect of a conditioning depolarization on potassium contracture. The sudden downward deflection in each record marks the introduction of an 80 K, 120 Cl solution by a spring-driven syringe. The horizontal lines indicate the times during which normal Ringer flowed through the experimental channel. The number at the left of each record indicates the order in which the contractures were elicited. The solution bathing the fiber before the contracture contained 2.5 K, 120 Cl in records 1, 3, 5, and 8; it contained 20 K, 15 Cl in records 2, 4, and 7, and 10 K, 30 Cl in record 6. Initial tetanic tension = 3.5 kg/cm². Final tetanic tension = 3.6 kg/cm². Vertical bar = 2 kg/cm². Horizontal bar = 2 sec. Temperature = 23°C.

tion, in this case 80 K, 120 Cl. Each record shows a biphasic contracture, but the rate of force development of the secondary component is quite variable. Despite this variability, it appears from Fig. 3 that pretreatment with either 10 K, 30 Cl or 20 K, 15 Cl accelerated the rate of force development of the secondary component. This effect was also seen in the other two fibers in this series.

Effect of Extracellular Calcium on the Biphasic Contracture The slow time course of the secondary rise in tension in high K, 120 Cl contractures raised the possibility that the secondary component was mediated by an influx of extracellular calcium. This possibility was directly tested in three fibers by the following experiment. After a biphasic contracture had been elicited by an 80 K, 120 Cl solution, the fiber was bathed for up to 9 min in calcium-free solution containing 5.4 mM Mg^{++} and 2.5 mM EGTA (solution D). A contracture was then elicited by an 80 K, 120 Cl solution made by appropriate mixing of solutions D and E. In all three fibers examined, the contracture was biphasic, and both initial and secondary components were similar to those elicited by the 80 K, 120 Cl solution containing 1.8 mM Ca^{++}. It would appear that extracellular calcium is not required in the development of biphasic contractures.

A Possible Mechanism for the Biphasic Contracture

The frog twitch muscle fiber possesses a well-organized transverse tubular, or T system, a network of tubules which extends through the fiber cross-section at the Z line and whose lumen is in direct continuity with the extracellular space (Peachey, 1965; Huxley, 1964). Depolarization of the T system is generally believed to be a step in the activation process (Huxley and Taylor, 1958; Adrian, Costantin, and Peachey, 1969).

The slow development of tension in the secondary component of the biphasic contracture suggested that this component might arise from direct depolarization of the T system by diffusion of potassium into the tubular lumen. Some support for this idea comes from the observation that pretreatment of the fiber with 10 K, 30 Cl or 20 K, 15 Cl increased the rate of force development of the secondary component, since under these conditions the potassium concentration throughout the T system would be somewhat elevated before exposure to high K, 120 Cl solutions. More convincing evidence would be an inverse relation between the rate of tension development in the slow component and the diameter of the muscle fiber. Unfortunately, the large overlap between the two components which was found in most fibers, coupled with the variability of the slow response itself (see Fig. 3), made it impossible to establish such a correlation in the present experiments.

If the slow component does result from a direct effect of high potassium in the T system, then the initial component must presumably result from an effect of high potassium on the surface membrane, an effect which is inacti-

vated in rather less than 1 sec. Nakajima, Iwasaki, and Obata (1962) have shown that a large depolarization of a frog twitch fiber results in an increase in the potassium conductance of the muscle membrane, or delayed rectification, and that this conductance increase is inactivated within less than 1 sec. In the present experiments, the application of high K, 120 Cl solution to the muscle fiber might be expected to produce sufficient depolarization to activate delayed rectification, and the surface membrane should depolarize to the potassium equilibrium potential. Electrotonic spread of this surface depolarization (Adrian, Costantin, and Peachey, 1969) should depolarize some or all of the T system and activate the contractile mechanism. With inactivation of delayed rectification, the relatively high chloride permeability of the surface membrane (Hutter and Noble, 1960; Eisenberg and Gage, 1969) should result in a partial repolarization of the surface membrane (and therefore of the T system), and some relaxation might occur. The contractile mechanism would then be reactivated as potassium diffused into the T system and directly depolarized the T tubule membranes. Such a mechanism implies that the potential change across the surface membrane in response to a high K, 120 Cl solution should show an early maximal depolarization, and this prediction was tested directly.

Membrane Potential Changes in High K Solutions Since it was not feasible to record the membrane potential of a vigorously contracting single muscle fiber, the time course of the membrane potential response to high potassium was followed in hypertonic solutions; such solutions have been shown to markedly inhibit muscle contraction (Hodgkin and Horowicz, 1957). Solutions A and C were made approximately twice isotonic by the addition of 350 mM of sucrose per liter; the addition of sucrose caused an 8% increase in solution volume so that the final ion concentrations were 8% less than the values in Table I. Three fibers were examined. After a contracture was elicited in response to 117 K, 120 Cl, the fiber was bathed in hypertonic solution A for 10 min, and the membrane potential was recorded. Then, with the microelectrode still impaling the fiber, the experimental chamber was perfused with hypertonic solution C (109 K, 112 Cl). In the three fibers tested, the resting potential ranged from −74 to −80 mv, and exposure to 109 K, 112 Cl resulted in a prompt depolarization to a membrane potential of −21 to −27 mv followed by a repolarization of 13–15 mv. In each case the time course of repolarization was similar to that seen in Fig. 4 (lower trace), that is, repolarization began only after 1 sec and was complete in about 2 sec, while the time course of the early contracture was considerably more rapid (Fig. 4, upper trace). Thus, although the general form of the depolarization is compatible with the suggestion that the initial component of the contracture relaxes because of repolarization of the surface membrane as delayed rectification is inactivated, the time courses of the force transient and of the potential transient appear to be somewhat different.

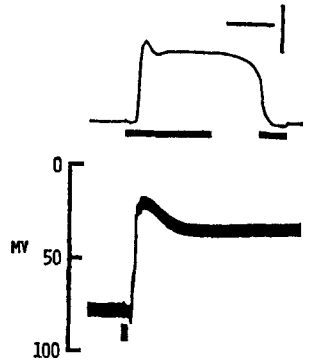

FIGURE 4. Upper trace, contracture elicited by 117 K, 120 Cl delivered at 0.77 ml/sec by infusion pump during first horizontal line. Normal Ringer reintroduced during second horizontal line. Tetanic tension = 3.1 kg/cm². Vertical bar = 2 kg/cm². Horizontal bar = 2 sec for both records. Lower trace, membrane potential of same fiber in hypertonic solution. 109 K, 112 Cl delivered at 0.77 ml/sec by infusion pump during entire record beginning at vertical mark. Temperature = 23°C.

Two additional fibers were examined in a 74 K, 0 Cl hypertonic solution (solution H with 350 mm of sucrose added per liter). The resting potential in both was −76 mv, and the membrane potential 1 sec after exposure to 74 K, 0 Cl was −32 mv in one fiber and −33 mv in the other. One fiber showed no repolarization, and the second repolarized by 3 mv with approximately the same time course as the fiber of Fig. 4. Thus, the large repolarization seen in Fig. 4 appears to require the presence of a high chloride concentration in the bathing medium.

DISCUSSION

The present experiments have shown that the potassium-induced contracture in single muscle fibers from *Rana pipiens* consists of two components, an initial transient contraction whose duration at room temperature is rather less than 1 sec, and a more slowly developing contraction which can be maintained for many seconds. The transient component was elicited by potassium concentrations somewhat higher than the threshold for the slow component, and it appeared to be abolished by prior exposure to 20 K, 15 Cl, a medium in which the membrane potential should be about −47 mv (Hodgkin and Horowicz, 1959). The duration of the initial component was markedly prolonged at low temperatures. These findings raised the possibility that the initial component was the result of a regenerative and self-limited release of activator at a critical level of depolarization, a process analogous to the increase in sodium conductance during the action potential. Such a step in excitation-contraction coupling has been suggested previously (Lüttgau, 1963; Costantin and Podolsky, 1967).

It seems more likely, however, that the time course of contracture is bi-

phasic because the time course of depolarization within the T system is biphasic following a sudden increase in the extracellular potassium concentration. As discussed above (see Results), a transient peak in depolarization is to be expected following the application of high K, 120 Cl solutions because of the rapid inactivation of delayed rectification, and such a peak in depolarization was observed. In high K solutions with constant $[K]_o [Cl]_o$ product, the decreasing potassium conductance of the surface membrane might also lead to a transient repolarization in the T system. Since equilibration of the tubular lumen with the bathing medium is relatively slow (Hodgkin and Horowicz, 1960 *a*), the equilibrium potential of the T tubules will be rather near the resting potential for a brief period following a sudden increase in the potassium concentration of the bathing medium. As the conductance of the surface membrane falls with inactivation of delayed rectification, the T system should repolarize to a membrane potential somewhere between the equilibrium potential of the surface membrane and that of the tubular membrane. In the absence of a large surface repolarization such as was seen with 109 K, 112 Cl, the magnitude of repolarization within the T system might be rather small, and the separation of the contracture response into an early and a late component should be less distinct. This prediction agrees well with the experimentally observed difference between the contractures elicited by high K, 120 Cl and those elicited by solutions with a constant $[K]_o [Cl]_o$ product (see Fig. 1).

The major difficulty with the suggestion that a biphasic depolarization produces the biphasic contracture response is the difference in the time course of the two responses (see Fig. 4). It should be emphasized, however, that contractures were recorded in isotonic media and the records of membrane potential were made in hypertonic media. There is some suggestion in recent voltage-clamp experiments on frog fibers by Adrian, Chandler, and Hodgkin (1970) that inactivation of delayed rectification is slowed in hypertonic solution. They reported that at 20°C, the time constant for inactivation was 0.4–1.2 sec at a membrane potential of +10 mv in hypertonic Ringer solution and 0.16 sec at +2 mv in isotonic sulfate Ringer solution.

Comparison With Previous Studies

The present experiments were undertaken in an attempt to duplicate the experimental method of Hodgkin and Horowicz (1960 *b*) for use in another investigation, so that the finding of a biphasic contracture in contrast to the single smooth contracture which they described was somewhat surprising. The only obvious difference between these two studies is that Hodgkin and Horowicz employed *Rana temporaria* while the present experiments employed *Rana pipiens*. Although it is possible that there is a qualitative difference in excitation-contraction coupling between these two species, the basic requirements for a biphasic depolarization of the T system with the sudden applica-

tion of a high K, 120 Cl solution are certainly present in *Rana temporaria*, that is, the surface membrane has an appreciable chloride permeability (Hutter and Noble, 1960), delayed rectification is transient during a maintained depolarization (Adrian, Chandler, and Hodgkin, 1970), and the T system equilibrates relatively slowly with the bathing medium (Hodgkin and Horowicz, 1960 *a*). A more likely explanation is suggested by the experiment illustrated in Fig. 2, in which prolongation of the initial transient response at 13°C virtually obscured the biphasic character of the contracture. Although the experiments of Hodgkin and Horowicz were performed at temperatures not very different from those of the present study (16.5°–21°C vs. 21°–26°C), relaxation of an initial transient response might simply be inherently slower in *Rana temporaria*. The finding that the peak contracture tension averaged 88% of tetanic tension with 117 K, 120 Cl in the present study and 109% of tetanic tension with 100 K, 120 Cl in the experiments of Hodgkin and Horowicz is compatible with this idea, since it suggests that some relaxation occurs sufficiently rapidly in *Rana pipiens*, but not in *Rana temporaria*, to prevent the development of maximal fiber tension during a potassium contracture.

The Relation between Graded Contractures and Graded Activation of the Contractile Mechanism

The apparently graded responsiveness of the contractile mechanism to elevated potassium concentrations has been taken as evidence for a graded responsiveness of the activation process to depolarization (Hodgkin and Horowicz, 1960 *b*). In the light of the biphasic contractures found in the present study, however, the possibility must be considered that graded contractures result from the complex time course of surface and tubular membrane depolarization following a sudden elevation of the extracellular potassium concentration. Immediately following elevation of the extracellular potassium, T tubule depolarization presumably occurs by electrotonic spread of depolarization from the surface membrane. Since it has been shown that under certain conditions, a significant gradient of depolarization can exist between the surface membrane and the most axially located T tubules (Adrian, Costantin, and Peachey, 1969), it is possible that the entire fiber cross-section is not activated during the initial transient component of the potassium contracture. Direct depolarization of the T system by diffusion of potassium into the T tubule lumen is a relatively slow process and is presumably responsible for the secondary component of the contracture observed in the present study. If the step in excitation-contraction coupling which results in the release of activator involves an inactivation step analogous to the inactivation of sodium conductance in the action potential mechanism, this relatively slow direct depolarization of the T tubule by diffusion of potassium could conceivably produce less than complete activation. The

finding in the present study that the secondary component of the contracture response to 117 K, 120 Cl is about 86% of the initial component is compatible with this suggestion. It should be emphasized that the results of the present experiments do not provide evidence for a regenerative as opposed to a graded release of activator in excitation-contraction coupling. They do, however, suggest that the ability of the single muscle fiber to produce graded contractures cannot be taken as evidence to exclude either model of excitation-contraction coupling. Recently Adrian, Chandler, and Hodgkin (1969) have presented evidence in support of a regenerative step in activator release.

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