Some Factors Affecting the Time Course of the Recovery of Contracture Ability Following a Potassium Contracture in Frog Striated Muscle

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ABSTRACT The recovery rate of contracture ability after a K contracture was shown to be initially dependent upon the rate of repolarization and later to be dependent upon a process which was sensitive to concentration and temperature changes in a manner consistent with chemical binding. It was shown qualitatively that repolarization did not depend on the presence of external calcium and the second process was studied by allowing the muscle to repolarize for 2 minutes in calcium-free solution following a K contracture. Recovery after this procedure was speeded by decreasing either the concentration of potassium in the contracture solution or its temperature and was slowed by either decreasing the calcium concentration of the recovery solution or its temperature or by increasing the duration of the exposure to potassium.

INTRODUCTION

Hodgkin and Horowicz (1960 *b*) found a delay in the recovery of the ability of a single muscle fiber to produce a spike or a contracture after it had been treated with a high concentration of potassium. They suggested that slow repolarization was responsible for part of this delay, but did not investigate the phenomena further. Preliminary unpublished experiments by Edwards had shown that the rate of recovery was more rapid if the calcium content of the recovery solution was increased or if the potassium concentration of the contracture solution was decreased. This suggested that some type of binding might be occurring during the recovery period and recoveries under various concentration conditions were studied to confirm the initial findings. The effect of temperature was also studied since it might be expected to have a marked effect on recovery rates as well. It was found that the recovery was

initially controlled by the rate of repolarization of the muscle membrane so the muscles were allowed to repolarize in Ca-free solutions in an attempt to separate this part of the recovery process more completely from that having some of the characteristics of chemical binding.

METHODS

In general, the methods and materials were the same as those described previously (Milligan, 1965). The sodium in all solutions was replaced with 90 per cent neutralized tris (Sigma brand of purified tris (hydroxymethyl)aminomethane). The general experimental procedure was as follows and deviations from it are mentioned in the appropriate places in the results. All contractures were produced by exposing the toe muscles of the frog to either 116 mM or 50 mM potassium for the designated times. These solutions contained no calcium. When no repolarization was allowed before recovery rates were measured, the muscle was placed directly into a recovery solution containing calcium and a normal potassium concentration of 2.5 mM. If repolarization was allowed the muscle was first put into a calcium-free solution containing normal potassium for 2 minutes and was then placed in a recovery solution containing calcium. Repolarization was done at 18°C. At specific times after the muscle had been placed in the recovery solution containing calcium it was again exposed to high potassium. The muscle was then allowed to recover for at least 10 minutes under appropriate control conditions for the experiment before the procedure was repeated using a different recovery time. The area under each experimental contracture was normalized by dividing it by the area of the previous control contracture. Thus each experimental contracture had its own control contracture. Control contractures usually decreased in magnitude during the experiment. The greatest decrease occurred in the first two or three controls, and the recoveries associated with these were duplicated at the end of the experiment. If they differed from the values obtained at the beginning, these later values were used. The normalized values of area, designated as Φ, were then averaged and plotted on the graphs as a function of recovery time.

RESULTS

Effects of Repolarization upon Recovery

According to Hodgkin and Horowicz (1960 *b*) the threshold concentration for potassium contractures is about 20 mM which corresponds to a resting potential of about 55 mv. It might be expected that a muscle would not be able to produce a second contracture following an exposure to a potassium solution until the fibers had repolarized to at least this level. Therefore, recovery rates for muscles allowed to repolarize for 2 minutes in a calcium-free solution before calcium was added were compared to the rates for recovery without repolarization. The average values for a group of six muscles are shown in Fig. 1. In the absence of repolarization, recovery did not begin until after about 15 seconds, but it was rapid after this time. This finding is similar to

that of Curtis (1964). Following repolarization in calcium-free tris solution, there was slight recovery at zero time and recovery continued from this time on after restoration of calcium. In both cases, the muscles had recovered to about the same extent after 1 minute, indicating that the recovery rate was dependent upon the rate of repolarization only during the first minute.

The relatively rapid time course of the initial part of the recovery after repolarization is allowed suggests that it may be limited by diffusion of Ca into the extracellular space. The slower rate after 1 minute, however, suggests that something else may be rate-limiting at this time. In all recoveries of contracture ability discussed below the muscle was allowed to repolarize for

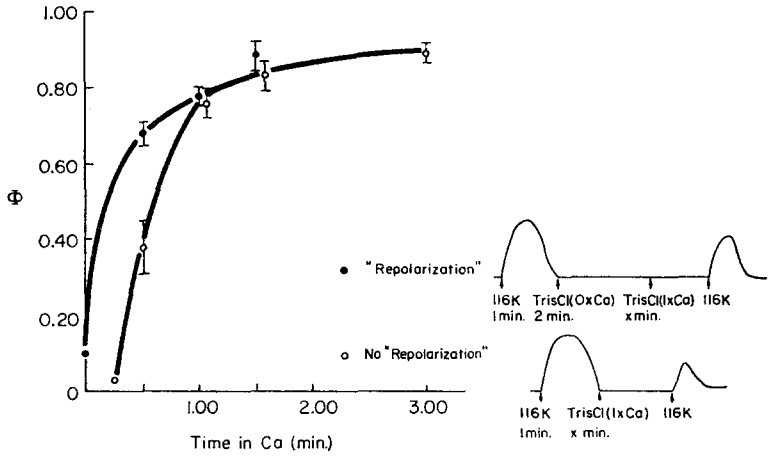

FIGURE 1. Time course of recovery of the ability to produce a second contracture afte a 116 mM potassium contracture without repolarization and with repolarization allowed in Ca-free solution. Φ is the ratio of the area of the second contracture to the area obtained immediately before the test procedure. Six muscles were used and the bar is the standard error of the mean.

2 minutes in a Ca-free solution before the recovery rate was determined in a solution containing calcium.

Repolarization Rates after Potassium Treatment

Because Ca-free solutions cause partial depolarization of muscle and preliminary experiments had shown that recovery of the ability to produce a second contracture after a potassium exposure was faster in solutions containing elevated calcium, experiments were done to determine whether the rate of repolarization after exposure to potassium was influenced by the Ca ion concentration of the recovery solution. Membrane potentials were sampled by a technique previously described (Milligan, 1965).

Sampling was done in the same 6 mm segment of muscle before and im-

mediately after exposure to a 116 mM potassium solution for 2 minutes. The solutions in which the muscle was allowed to repolarize contained various concentrations of Ca as indicated in Fig. 2. Generally repolarizations in three different solutions could be measured in a single muscle since the muscles were about 20 mm long. In some cases repolarizations in different solutions were measured in the same segment. All control values were obtained after the muscle had been soaked for at least 10 minutes in a tris Cl solution containing 1.8 mM Ca.

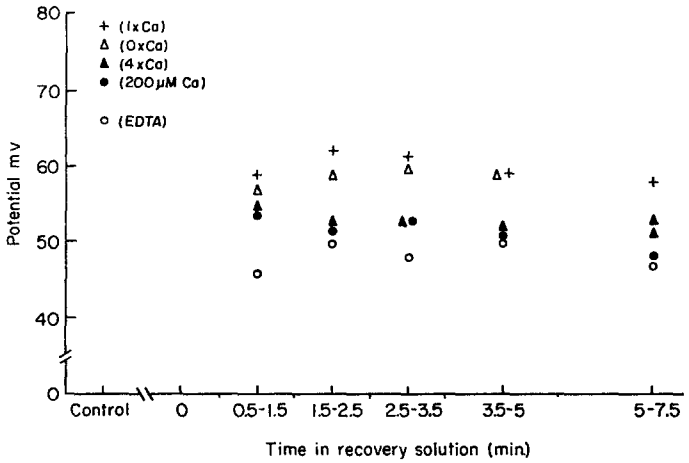

FIGURE 2. Time course of repolarization in solutions containing different amounts of calcium after a 2 minute depolarization and contracture in 116 mM potassium. Each point is the average of acceptable potentials measured within the respective time intervals. Five to twelve muscles were used for each solution and from thirty-two to two hundred and forty-eight separate fibers were measured for each point. The recoveries in up to three different solutions were measured in the same muscle. 1 × Ca is equal to 1.8 mM. The EDTA concentration was 1 mM.

In no case did the average potential return to the control value during the repolarization and in a small series of seven muscles, values obtained 20 minutes or more after repolarization was allowed, were the same as those obtained immediately after repolarization was allowed. This effect may be due to some type of irreversible change produced by exposure to 116 mM K immediately after numerous punctures of the muscle membranes during the control measurements. For this reason the results can only be considered to be qualitative. It is, however, apparent that under all conditions the muscle has repolarized as much as it is going to within 1 minute of the removal of high concentrations of potassium and when the differences in control values are taken into consideration this repolarization is independent of the external concentration of calcium.

Characteristics of Recovery After Potassium Treatment

Since the time course of recovery after a potassium contracture was slower than would be expected if only physical factors were involved, its dependence on factors affecting chemical reactions such as concentration and temperature was studied. In each case a standard recovery using normal potassium and calcium concentrations and normal temperatures was done so that valid comparisons could be made. However, only qualitative comparison between groups can be made because the large amount of variation in the frogs used allowed selection of only small uniform groups.

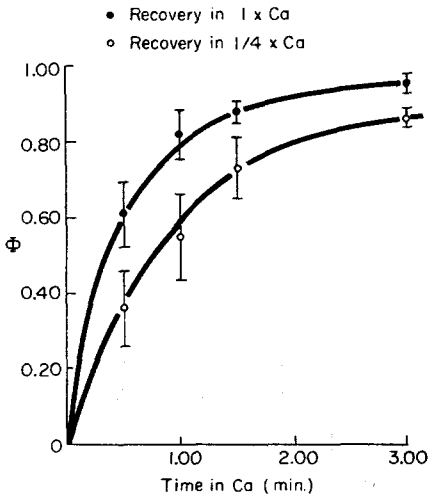

FIGURE 3. Time course of recovery in either 450 μM Ca or 1.8 mM Ca after 2 minutes in 116 mM K followed by 2 minute repolarization in Ca-free solution. Three muscles were used and the bar is the standard error of the mean.

CONCENTRATION EFFECTS The recovery rate was slowed if the calcium ion concentration of the recovery solution was reduced from 1.8 mM to 450 μM (Fig. 3). This effect of lowered concentration of calcium in the recovery solution was much greater after a contracture followed by repolarization in calcium-free solution than it was after a 10 or 15 minute washout in calcium-free solution without a prior exposure to potassium (Milligan, 1965). The more linear relation between Φ and external calcium concentration in the range below 450 μM which adequately explained the concentration effect on recovery after washout cannot be used to explain the greater magnitude of this effect under the circumstances described here.

If, on the other hand, the concentration of potassium in the contracture solution was decreased, the recovery rate was faster (Fig. 4). This faster recovery after 50 mM K exposure occurred even though the area under the contracture curve in this case was as large as twice that produced by 116 mM K.

TEMPERATURE The recovery was slowed considerably if it occurred in a cold (8°C) solution of tris Cl (Fig. 5). This was most evident for times longer than 1 minute, by which time the calcium concentration in the extracellular space had theoretically equilibrated with that in the solution. Under the conditions of these experiments, it is likely that repolarization of the mem-

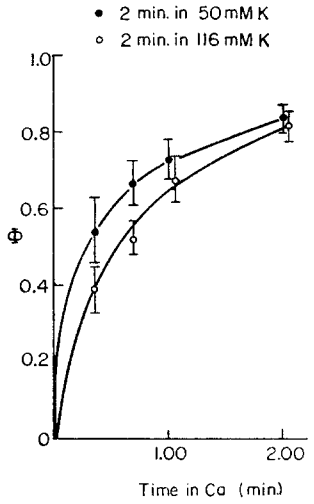

FIGURE 4. Time course of recovery in 1.8 mM Ca solution after 2 minutes in either 50 mM K or 116 mM K followed by 2 minutes repolarization in Ca-free solution. Each point is the average of four muscles and the bar is the standard error of the mean.

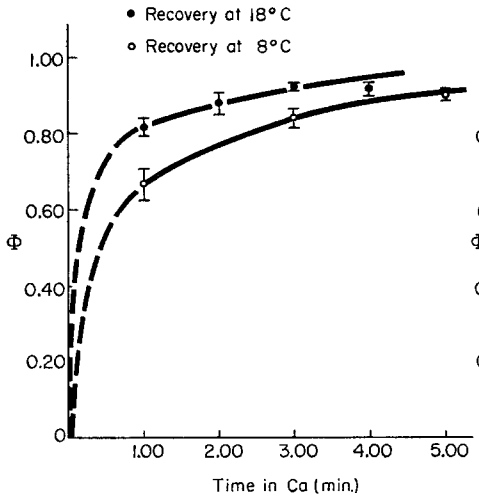

FIG. 5

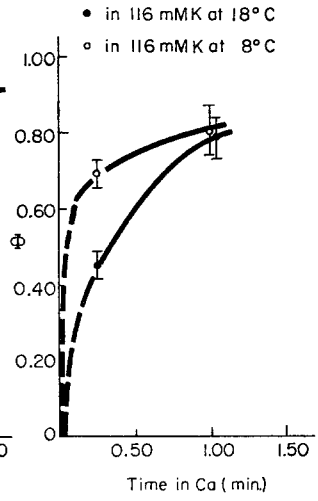

FIG. 6

FIGURE 5. Time course of recovery in 1.8 mM Ca at either 18°C or 8°C following a 2 minute exposure to 116 mM K and subsequent repolarization in Ca-free solution bath at 18°C. Eight muscles were used.

FIGURE 6. Time course of recovery in 1.8 mM Ca after 2 minutes in 116 mM K at either 18°C or 8°C followed by 2 minute repolarization in Ca-free solution. Four muscles were used.

brane potential was not impaired at low temperature (Grieve, 1960). If, however, the recovery rate was measured at 18°C, after the prolonged contractures produced by cold 116 mM potassium solution at 8°C, and compared to the recovery after contractures produced at 18°C with repolarization in both cases, the former was faster (Fig. 6). Similar results have been reported by Lorković (1961) for the sartorius and rectus muscles. The results suggest that during recovery there is a temperature-sensitive reaction involving calcium and that a temperature-sensitive reaction occurs during the potassium treatment as well, probably involving potassium.

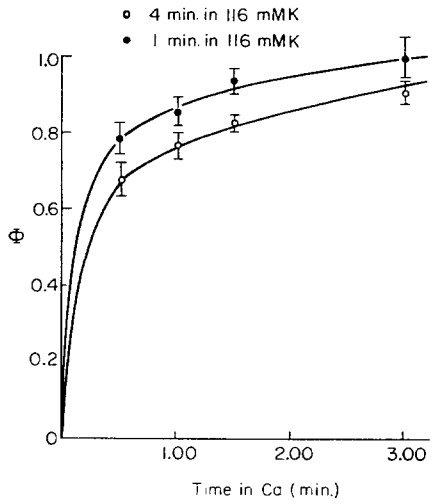

FIGURE 7. Time course of recovery in 1.8 mM Ca solution after either 1 minute or 4 minutes in 116 mM K followed by 2 minute repolarization in Ca-free solution. Six muscles were used.

EXPOSURE TIME Recovery rates were also slower if the exposure time to potassium was increased. The recovery was only about 90 per cent complete in 3 minutes after a 4 minute exposure to 116 mM K, but was 100 per cent complete at this time after a 1 minute exposure to 116 mM K (Fig. 7). This effect was not a linear function of exposure time, and while qualitative differences appeared to be present it was difficult to demonstrate differences between 2 and 4 minute exposure times to 116 mM K. If the muscle was exposed to 116 mM potassium solutions for periods longer than 6 minutes, it deteriorated rapidly and the various recovery times could not be obtained.

The rate of repolarization after varying times of exposure to potassium was measured with multiple sampling using a micropipette in one muscle. Repolarization occurred in tris Cl containing 1.8 mM Ca. The results are shown in Fig. 8. The order in which the various potassium treatments were given is shown beside the control averages. The failure of the values to return to control levels is thought to be due to reasons already discussed. When the differences in control values are taken into account, it can be seen that the time courses of repolarization were similar and did not change much after the first

minute, except perhaps in the case of the 6 minute exposure to potassium, where it was reasonably uniform after the second minute. It seems unlikely that the difference between recovery rates after short and longer exposures to potassium can be attributed to membrane repolarization since all muscles were in calcium-free solutions for 2 minutes after the potassium exposure which would allow complete repolarization.

DISCUSSION

Complete recovery of contracture ability in frog muscle after a previous potassium contracture involves at least two processes. During the first minute

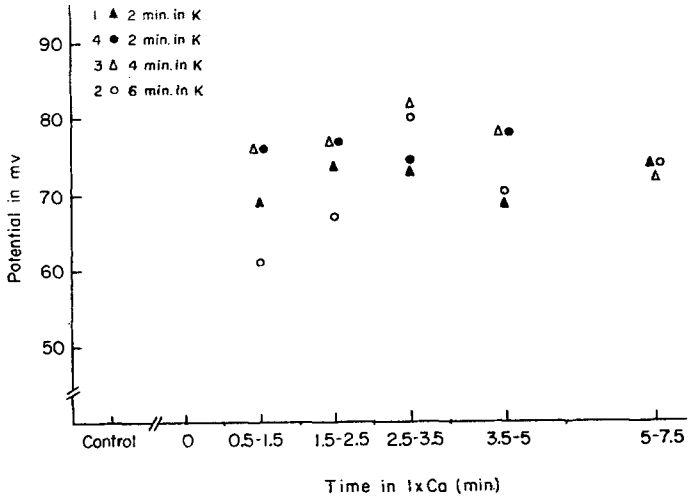

FIGURE 8. Time course of repolarization in normal Ca in one muscle after exposure to 116 mM K for varying lengths of time. Each point is the average of acceptable potentials, in eight to thirty-nine individual fibers, measured in that respective time interval indicated. Each repolarization was done in a separate segment of muscle.

of recovery, repolarization controls the rate, and after this time a process which has characteristics of chemical binding is the controlling factor. These two processes appear to be independent of each other as far as calcium is concerned since repolarization occurs readily in its absence.

The 15 second delay before recovery starts in the absence of repolarization must correspond to the time period when none of the muscle fibers has repolarized to a potential above threshold. Curtis (1964) has calculated that this time is approximately that required to reduce the average extracellular concentration of potassium ions to 5 mM and assumes that the muscle is essentially repolarized at this time. However, Hodgkin and Horowicz (1960 *a*, *b*) have shown that repolarization is delayed several seconds in a single fiber where the external concentration can be changed almost instantaneously and a

longer delay would be expected in the toe muscle due to longer diffusion times. It is apparent that the muscle can give another response to high potassium before it has completely repolarized.

When repolarization is allowed in calcium-free solutions, the recovery during the first 30 to 40 seconds after the restoration of calcium is likely to be limited by the diffusion of calcium ions back into the extracellular space since a potassium contracture cannot be produced in their absence (Frank, 1960). Therefore at short time intervals it is difficult to separate the recovery process associated with the calcium-free treatment from that associated with exposure to potassium ions. The portion of recovery after about 40 seconds is, however, certainly due to the potassium exposure since recovery from a 15 minute exposure to calcium-free solution is almost complete at this time (Milligan, 1965).

These findings strongly suggest that bound calcium is removed from "fast" muscle during a potassium contracture. This removal may be responsible for the spontaneous relaxation which occurs in these contractures, but more quantitative evidence must be obtained to substantiate this speculation.

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