The Suppression of the Late After-Potential in Rubidium-Containing Frog Muscle Fibers

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ABSTRACT The late after-potential that follows trains of impulses in frog muscle fibers is virtually absent when most of the intracellular potassium is replaced by rubidium and the muscle is immersed in rubidium-containing Ringer's fluid. Its amplitude is also reduced in freshly dissected, potassium-containing muscle fibers that are immersed directly in Rb-Ringer's fluid. These findings are discussed in terms of the model for muscle membrane of Adrian and Freygang (1962 *a*, *b*) and in relation to the report of Adrian (1964) that Rb-containing muscle fibers do not exhibit the variations in potassium permeability as a function of membrane potential that are found in fibers with normal intracellular potassium concentration immersed in Ringer's fluid.

Adrian (1964) found that the replacement of intracellular potassium by rubidium in frog striated muscle fibers removes the increase in membrane conductance that is normally found with increasing hyperpolarization (anomalous rectification). The voltage-dependent changes in membrane conductance that were described by Katz (1948, 1949) were investigated further by Adrian and Freygang (1962 *a*, *b*) who were able to relate the conductance changes of fibers in Ringer's fluid to changes in potassium conductance. These changes were not present after most of the intracellular potassium was replaced by rubidium and the muscles were immersed in a Ringer's fluid that contained rubidium instead of potassium. The Rb-loaded muscles in Rb-Ringer's fluid behaved as if their permeability to cations was low when the membrane potential was about −90 mv; the membrane was permeable

almost exclusively to chloride ions. In short, one can describe the condition as a loss of anomalous rectification in the Rb-loaded muscles in Rb-Ringer's fluid. Except for a slight decrease in the rate of fall of the action potential, the Rb-loaded muscles in Rb-Ringer's fluid had the same action potentials as those of potassium-containing muscles in Ringer's fluid. Therefore, the mechanism for the outward flow of cations that recharge the membrane capacity during the falling limb of an action potential must have been intact. This mechanism, which has been called delayed rectification, presumably allows the outward movement of rubidium rather than of potassium in Adrian's experiment.

The late after-potential that follows a train of impulses can be calculated from the equations that describe the model of Adrian and Freygang (Freygang, Goldstein, and Hellam, 1964). The assumption is made that potassium accumulates in a region of the fiber that corresponds to the intermediary space of the model during the train of impulses. In the model the walls of the intermediary space have the property of anomalous rectification. Freygang, Goldstein, Hellam, and Peachey (1964) have recently provided evidence that the intermediary space of the model is the transverse tubular or T-system.

Arguing by analogy, in Rb-Ringer's fluid one would expect development of an accumulation in the intermediary space of rubidium rather than of potassium as an effect of a train of impulses. A late after-potential would not be expected to follow, however, because anomalous rectification is absent in these fibers and therefore the route has been removed by which the accumulation of rubidium would exert a depolarizing effect. That is, the permeability of the walls of the intermediary space to rubidium is too low to permit depolarization of the membrane by accumulated rubidium. This reasoning leads to the prediction that the late after-potential should be small in the experiments that are described here. These experiments, thus, provide one means of testing the assumption that potassium is the species of ion that accumulates in the intermediary space during a train of impulses.

A preliminary account of part of this work has appeared (Hellam, Freygang, and Peachey, 1964).

METHODS

The intracellular potassium of sartorius muscles of *Rana pipiens* was replaced by rubidium by soaking the muscles for 5 days at 9°C in a solution of the following composition: Rb_2SO_4 = 50 mM, Na_2SO_4 = 12.5 mM, $CaSO_4$ = 9 mM, Na_2HPO_4 = 2.15 mM, NaH_2PO_4 = 0.85 mM, and glucose = 5 mM as has been described by Adrian (1963). The solution was stirred and bubbled with oxygen. After the muscles had been transferred to a Rb-Ringer's fluid the experiments were performed while the resting potentials remained greater than −70 mv. Resting potentials fell below this level within 30 to 45 minutes after the muscles were transferred to the Rb-Ringer's fluid. The composition of this solution was as follows: NaCl = 115 mM, RbCl =

2.5 mM, $CaCl_2$ = 1.8 mM, Na_2HPO_4 = 2.15 mM, NaH_2PO_4 = 0.85 mM. The experiments were done at room temperature, which was 22°C. Control experiments were done in which the muscles were soaked for 5 days under identical conditions except that 50 mM K_2SO_4 replaced the Rb_2SO_4 of the soaking solution. In these muscles, records of spikes and late after-potentials were then obtained when the muscle was returned to Ringer's fluid.

In another series of experiments, freshly dissected sartorius muscles were placed directly in Rb-Ringer's fluid. The effects on the late after-potential of the replacement

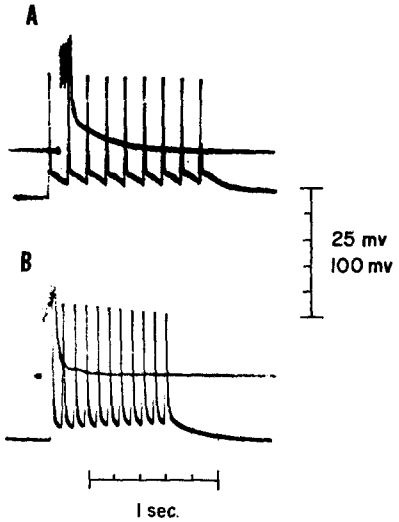

FIGURE 1. Suppression of the late after-potential in a Rb-loaded muscle fiber. Gain 4 times greater in upper than in lower trace in A and B. Time scale refers to upper trace in both parts. Sweep speed is not equal in lower traces of A and B; both, however, show trains of impulses with 10 milliseconds separating each impulse. The muscle in the experiment of part A was immersed in K_2SO_4 solution for 5 days before the experiment which was done with the muscle in Ringer's fluid. The resting potential was −79 mv. Part B was recorded from a muscle that had been immersed for 5 days in the Rb_2SO_4 solution and transferred to Rb-Ringer's fluid. The resting potential was −74 mv.

of external potassium by rubidium without prior replacement of intracellular potassium by rubidium were then examined over a period of several hours in this solution.

The other methods that were employed in these experiments were the same as those described previously (Freygang, Goldstein, and Hellam, 1964), as was the method of calculation of the theoretical late after-potential that would be expected under the conditions of these experiments. Electron microscopy of Rb-containing fibers was done in a manner similar to that previously described for K-containing fibers (Freygang, Goldstein, Hellam, and Peachey, 1964).

RESULTS

The upper trace in Fig. 1A illustrates the late after-potential that follows a train of 9 impulses in a muscle fiber immersed in Ringer's fluid. The muscle

had been soaked for 5 days in a 50 mM K_2SO_4 solution that was otherwise identical to the Rb_2SO_4 solution. The action potentials are shown in the lower trace which is of faster sweep speed and lower gain than the upper trace. Fig. 1B is an experiment similar to the one illustrated in Fig. 1A except that rubidium replaced potassium in the soaking solution and in the Ringer's fluid. The late after-potential which followed the early after-potentil in the rubidium-containing muscles was virtually absent, being less than 2 mv. In K-containing fibers the peak amplitude was 13.9 ± 0.7 mv. Results similar to those in Fig. 1B were obtained from 11 fibers in 5 muscles after potassium was replaced by rubidium. The resting potentials in these fibers ranged from −70 to −88 mv; the mean was −77 mv.

Seven muscles in which intracellular potassium had been replaced by rubidium were analyzed by flame photometry by Dr. R. H. Adrian. If one assumes an extracellular volume of 12.5 per cent and uses the formula for muscles that have been soaked for long times (Desmedt, 1953; and Boyle, Conway, Kane, and O'Reilly, 1941),

$$C_{fw} = \frac{C_m - 0.125\, C_o}{0.645},$$

the mean intracellular cation concentrations were as follows: Rb = 108, K = 36, Na = 31, all expressed as mM/kg fiber water. The rather high value for sodium probably results from the presence of some damaged fibers.

The decay of the late after-potential of the rubidium-loaded fibers was estimated from the equations that describe the model of Adrian and Freygang. It was assumed that P_K was fixed and equal to 0.22×10^{-6} cm/sec., which is about 1/10 of the usual value for P_K at a membrane potential of −92 mv, in order to correspond to the estimated rubidium permeability of muscle membrane. The calculated result is illustrated in Fig. 2. The calculated late after-potential is suppressed when this condition is imposed. When P_K was reduced by one order of magnitude but allowed to vary with membrane potential (as in Freygang, Goldstein, and Hellam, 1964, Table II, p. 941), the calculated curve was essentially the same as the curve shown for fixed P_K in Fig. 2. With normal P_K, the calculated late after-potential had a progressively smaller peak amplitude as resting potential was lowered from −94.5 to −66.8 mv but this reduction was not sufficient to coincide with the experimental results. With resting potential between −76 and −80.5 mv the peak amplitude of the calculated late after-potential was reduced threefold, to about 1 mv, by the second method of reducing P_K. These results are in accord with the experimental observation that the late after-potential was small and of similar time course for fibers with resting potentials between −70 and −88 mv.

When muscles with normal intracellular potassium concentration were placed in Rb-Ringer's fluid the effect on the late after-potential was qualitatively similar to that seen in the experiments just described. Immersion in Rb-Ringer's fluid for several hours was required before the late after-potential became strikingly suppressed and it was rarely entirely eliminated. Fig. 3 shows the peak amplitudes of late after-potentials in 41 fibers from 8 muscles plotted against duration of immersion in Rb-Ringer's fluid. Although there was a small decrease in amplitude after about 10 minutes in Rb-Ringer's fluid, the time required for the peak of the late after-potential to become smallest was about 7 hours. The mean peak amplitude for trains of 10 spikes at 10 msec. intervals for 8 control fibers in Ringer's fluid was 13.9 ± 0.7 mv (mean ± SE). At 3 hours in Rb-Ringer's fluid this value for 9 fibers was 3.4

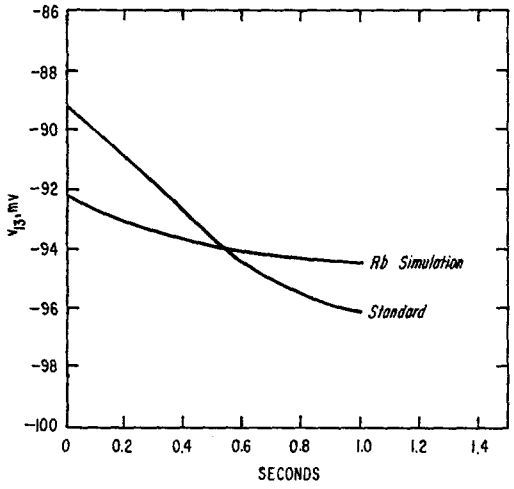

FIGURE 2. Simulation of the late after-potential in a Rb-loaded muscle fiber. The standard curve is the same as that previously described by Freygang, Goldstein, and Hellam (1964). The simulation of the late after-potential in a Rb-loaded fiber was calculated with the same parameters as the standard except that P_K was held constant at 0.22×10^{-6} cm/sec. $[K]_s$ = 10 mM at zero time in both curves. (See text.)

± 0.6 mv, and for the 14 fibers impaled between 6 and 8 hours in Rb-Ringer's fluid the mean peak amplitude was reduced further to 1.9 ± 0.4 mv. It is also apparent that fibers in Rb-Ringer's fluid often show larger late after-potentials following longer trains at higher frequency than those which follow the usual trains of 10 impulses (a characteristic of fibers in Ringer's fluid (Freygang, Goldstein, and Hellam, 1964)). The early recovery of the late after-potential when the muscles were returned to Ringer's fluid is also shown.

Another way in which the late after-potential might be removed is through a reduction in the size of the T-system since Freygang, Goldstein, Hellam, and Peachey (1964) found that the time course of the decay of the late after-potential seems to be proportional to the size of the T-system. To examine this possibility, 3 muscles were fixed for electron microscopy and examined

in the manner described previously. No reduction in the size of the T-system was found. The mean longitudinal dimension of transverse tubules in 145 measurements in Rb-loaded fibers of the 3 muscles was 310 ± 80 A (mean ± SE), as compared to 260 ± 70 A in 56 measurements in 3 control muscles in Ringer's fluid. The difference of the means, 50 A, is considered to be near the limits of sensitivity of the method for the correlation of time course of the late after-potential with size of the T-system. The absence of the late after-potential in these fibers cannot, therefore, be attributed to shortening

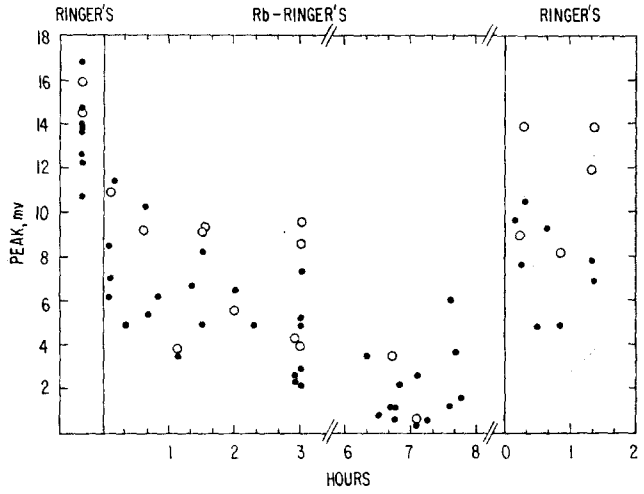

FIGURE 3. Change in the peak amplitude of late after-potential with time after immersion of muscle, without previous treatment, in Rb-Ringer's fluid and after being returned to Ringer's fluid. Points indicate peaks of late after-potentials that followed trains of 10 impulses at 10 millisecond intervals; open circles indicate peaks of late after-potentials that followed trains of 19 to 22 impulses at 5 millisecond intervals. Ringer's, 8 fibers; Rb-Ringer's, 41 fibers; returned to Ringer's, 10 fibers.

of the time course of the late after-potential to some imperceptibly small value by a decrease in size of the T-system.

DISCUSSION

As was pointed out in the Introduction, these experiments provide a relatively simple, indirect test of the hypothesis that potassium, or rubidium in Rb-containing fibers, accumulates in a part of the muscle fiber corresponding to the intermediary space of the Adrian-Freygang model during a train of impulses. The finding that the late after-potential was suppressed in Rb-containing fibers is in accord with this hypothesis; the accumulation of rubidium should not have exerted a depolarizing effect because of the relative

impermeability of the walls of the space to rubidium after the train of impulses had ended.

Adrian (1964) also showed that the potassium permeability of unexcited membrane was greatly reduced and did not display anomalous rectification in a K-containing, freshly dissected muscle which was immersed in Rb-Ringer's fluid. Therefore, and in accord with the interpretation above, in the experiments described here in which fresh muscle with normal intracellular potassium concentration was immersed in Rb-Ringer's fluid, the late after-potential was slightly suppressed initially (Fig. 3) because the membranes of the space were relatively impermeable to potassium. After 8 hours, however, the intracellular rubidium concentration may have become sufficient to allow an accumulation of both potassium and rubidium in the intermediary space during the train of impulses. This would then approach the conditions found in the Rb-containing fibers. As in Rb-containing fibers, the membrane potential was determined by the distribution of chloride across the membrane after the train of impulses had subsided.

The results are interpreted as suggesting that, in normal muscle, potassium probably accumulates in the intermediary space during a train of impulses when the train is followed by a late after-potential.

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