

THE CONTROL OF MEMBRANE IONIC CURRENTS BY  
THE MEMBRANE POTENTIAL OF MUSCLE\*

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ABSTRACT

Comparisons between electrotonic potentials and certain predicted curves allow the identification of the membrane potential at which the sodium and potassium currents are switched on in frog sartorius. The activation potentials (the membrane potentials at which the ionic currents are great enough to be resolved by the method) are functions of the resting potential and time but not of ionic concentration. In the normal fiber, the activation potential for sodium lies nearer the resting potential and depolarizations set off sodium currents and action potentials. Below a resting potential of 55 to 60 mv. sodium activation is lost and conduction is impossible. A tenfold increase of calcium concentration lowers (moves further from the resting potential) the sodium activation potential by 20 to 25 mv. whereas the potassium activation potential is lowered by only 15 mv. Certain consequences of this are seen in the behavior of the muscle cell when it is stimulated with long duration shock.

The action potential appears to be generated by various voltage- and time-dependent changes of membrane conductance. The initiating step, sodium activation (an increase of the membrane's sodium conductance), begins when the membrane potential is reduced by electrical shock or advancing action potential to some characteristic value below the resting level. As the stimulus further depolarizes, activation increases until the restoring forces are overcome and the potential is driven toward the peak of the spike by the large, initial sodium current. There is, until this point is reached, a range of potential in which activation is present but not great enough to be regenerative. In this region are found local responses; these are the subject of this investigation.

The striated muscle fiber has not yet yielded to voltage clamp techniques. Certain other procedures can be employed which allow the identification of sodium and potassium activation. Indications of the rates and magnitudes of

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these processes are also obtained. We have not directly measured the ionic currents of the muscle membrane. The method gives the potential and time when these are switched on or changed from their resting values. The term "activation" will be used for the onset and magnitude of these currents.

#### *Methods*

Two microcapillary electrodes (10 megohm resistance) separated by 50 to 100  $\mu$  were placed into a single fiber of *Rana pipiens* sartorius muscle lying in a suitable bathing fluid. Rectangular current pulses of 100 millisecond duration and ranging in strength from threshold and below were delivered through one electrode. A series resistance of 120 megohms and periodic tests insured that these were constant current pulses. The recording electrode led to an oscilloscope *via* a neutralized input capacity preamplifier (Bioelectric Instruments, type D). The low frequency components of the desired signal did not require a wide pass band, but a 1 kilocycle band width was chosen nevertheless. The oscilloscope trace was photographed and its image was enlarged and traced onto coordinate paper for analysis. Laboratory temperatures were maintained between 24 and 26°C. The Ringer's fluid was varied between the following limits: NaCl 111 mM to 0 mM, KCl 0.5 mM to 62.5 mM, CaCl<sub>2</sub> 1.8 mM or 18 mM. Phosphate buffers were added at 1 mM and pH 7.1-7.3. Recrystallized choline chloride was used to replace NaCl when necessary.

#### RESULTS

*Rationale.*—The similarity of the resting membrane to a distributed, parallel resistance, capacitance line is assumed. On this model depends the interpretation of all our present measurements of these membrane electrical properties. Hodgkin and Rushton (1946) have furnished equations (error functions) relating the polarization voltage of such a membrane to a function of time, polarizing current, membrane conductance, and several other variables. In our recording situation these latter can be regarded as constant. Since time and current are known, only the membrane conductance and a scaling constant are to be determined. The first few electrotonic potentials of a series are elicited with very weak shocks to avoid activation. Comparisons between these always show that voltage is directly proportional to current at any time after "make" as the equation demands. Therefore, in this interval the membrane conductance has not been altered by the cathodal polarization. Larger, subthreshold shocks generally cause local responses (accelerating depolarizations or humps on the electrotonic potential). Parameters for the error function are chosen from the first few records where the membrane remained passive. Multiplying these by the ratio of the currents, we obtain voltages which the membrane should reach if it continues to behave linearly. The local response curves are compared point by point to these calculated curves. Early portions always fit. Only after a characteristic potential is reached do the actual records depart from the predicted curves. This is best illustrated by plotting the discrepancy,  $\Delta V$ , between

the two curves as a function of membrane voltage,  $V$ . (See Figs. 1 and 3.) As long as the membrane properties remain in the resting state, the electrotonic potential corresponds to the expected error function and  $\Delta V$  is zero. After the activation potential is reached, the membrane voltage swings above or below and  $\Delta V$  becomes positive or negative. These voltage deflections are in a direction to be caused by sodium or potassium currents, respectively.

We regard the point of departure from the  $\Delta V = 0$  axis as the activation potential because only a change in the electrical properties of the membrane can cause such an alteration of potential. The finite time course of the activation process will tend to shift the point of departure as the current and  $dV/dt$  increase. Weak currents will drive the membrane voltage slowly and tend to minimize the effect of time; *i.e.*, membrane voltage is better able to meet the demands set by the activation process. As current is increased, the electrotonic potential is developed more rapidly. Any delay or slowness in the effects of activation (which will drive the membrane voltage above or below the expected values) after the proper activation potential is reached will allow the membrane to further follow the error function before  $\Delta V$  becomes positive or negative. Such delays will shift the plots along the  $V$  axis and will roughly indicate the rate constant of the activation process.

It is assumed that in the absence of the  $\text{Na}^+$  ion, activation is the result of an increased  $\text{K}^+$  conductance.

*Sodium Activation.*—In Ringer's fluids with a normal  $\text{Na}^+$  concentration (111 mM), the electrotonic potentials fit the error functions until a depolarization of more than 10 mv. is produced. Above this the potential is gradually deflected upwards in a depolarizing direction. The excess depolarization ( $\Delta V$  in Fig. 1) is almost directly proportional to the actual depolarization  $V$ . Fig. 1 shows a series of electrotonic potentials obtained from one fiber. The stimulus current was raised with the indicated steps and the  $\Delta V, V$  plot shows how activation begins at a fixed level of 67 mv. (voltage remaining across membrane). The positive  $\Delta V$  curves seem to be a measure of the sodium current. When the  $\text{Na}^+$  concentration is lowered, the magnitude of  $\Delta V$  is smaller at any given  $V$  although the initial point of inflection from the  $V$  axis is unchanged. This fits the assumption that activation is a membrane property; voltage-dependent, not concentration-dependent. If  $\text{Na}^+$  is completely replaced by choline, positive  $\Delta V$  are never seen.

If the activation process develops at some finite rate, the  $\Delta V, V$  curves should be shifted. We have not been able to estimate the time constant for the sodium activation. Plots from almost all fibers lie on top of each other, as in Fig. 1, indicating the method is too slow to resolve the process. There is no doubt that the potassium activation is slower in development and initiation (see below).

The activation potential is not a fixed value but varies with the resting potential (when altered with  $\text{K}^+$  concentration). (See Fig. 2.) Sodium activation

is found only in fibers with resting potentials above 60 mv. The filled circles along the abscissa below this potential indicate the resting potentials of fibers showing no sodium activation (positive  $\Delta V$ ). Since the potassium activation potential is very near the sodium activation potential in the 60 mv. region,

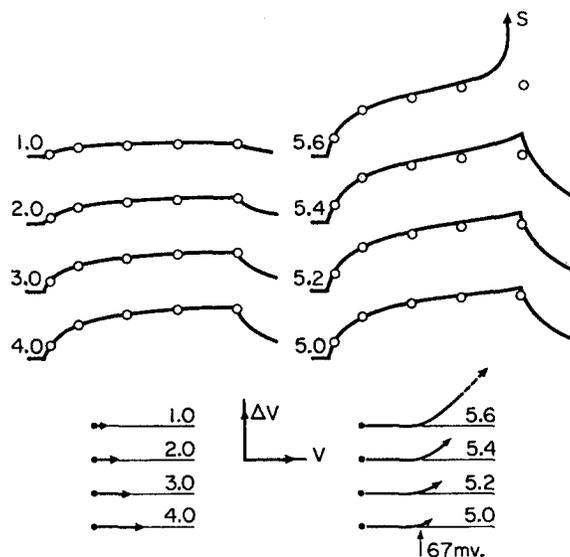


FIG. 1. Electrotonic potentials (solid lines in upper portion) obtained from one fiber, resting potential 98 mv., in response to 100 millisecond constant current pulses. The relative current strengths are given by the numbers next to each record. The open circles are the potentials the membrane should have reached if it remained passive. These values are determined by extrapolation from the first four records which agree among themselves very well. Plotted below against the membrane voltage  $V$  are the differences,  $\Delta V$ , between the actual potential and the extrapolated potential. These curves begin at the resting potential, indicated by the dot at the left, and proceed toward the right. These positive  $\Delta V$  are probably caused by sodium current and occur only when the fiber is depolarized below the activation potential of this fiber which is taken as 67 mv. The length of the  $\Delta V$  and  $V$  axes in the center corresponds to 30 mv. A spike occurred at S.

conduction is marginal. Were it not for a shorter time constant for the sodium current, conduction would be quite haphazard. Depolarizations could trigger off potassium (repolarizing) or sodium (depolarizing) currents. Any further reduction of the resting potential causes a loss of the sodium activation and conduction becomes impossible.

*Sodium-Free Solution.*—As previously mentioned, replacement of the  $\text{Na}^+$  ion by choline abolished all positive  $\Delta V$ . The electrotonic potentials which occur in these fluids all fit the error function until driven greater than 20 mv. At this point, the curves smoothly move back toward the original resting potential

(Fig. 3). This we assume is caused by an increase in the membrane's potassium conductance. The shape of the  $\Delta V, V$  plots is not greatly dependent upon the  $K^+$  concentration in distinction to the experiments with altered  $Na^+$  concentrations. There should be a change in membrane current ( $\Delta V$ ) as the driving force is altered by changes in external  $K^+$  concentration. Matters are complicated by the fact that external  $K^+$  affects the resting membrane conductance.

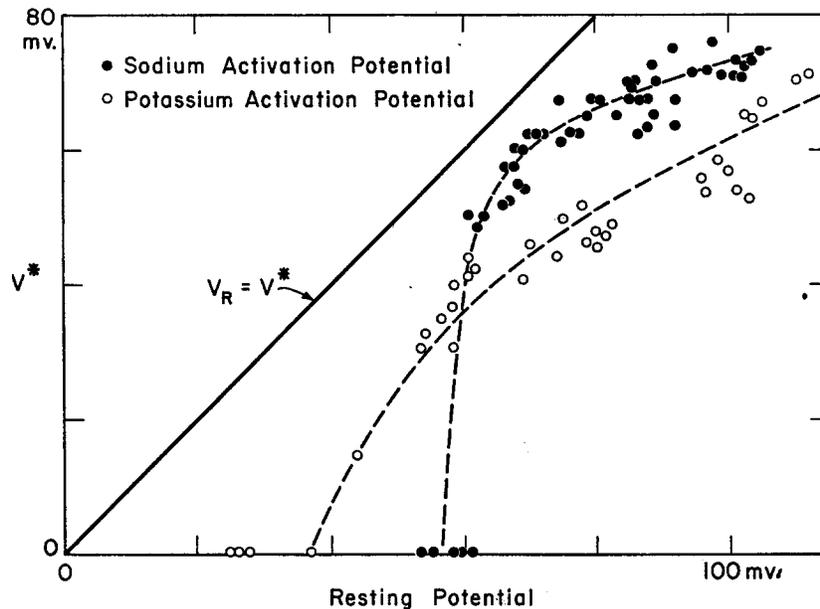


FIG. 2. The dependence of the activation potential for sodium and potassium on the resting membrane potential. Activation potentials are the voltages remaining across the membrane when the ionic currents are switched on. Points along the abscissa show resting potentials where activation was not found. For example, sodium activation is lost at 55 to 60 mv., potassium activation is lost near 40 mv.

Since we do not know how far the activation process alters the membrane from its resting state, we cannot predict what the  $\Delta V, V$  plots should do when external  $K^+$  is changed.

The displacement of each successive  $\Delta V$  curve in Fig. 3, as compared to Fig. 1, suggests a longer time constant for the  $K^+$  current.

The potassium activation potential is also a function of the resting potential (Fig. 2). In the normal range it is located well below the sodium activation level, conforming to our expectations and insuring that action potentials will be produced by cathodal shocks. Below the usual blocking level of 55 to 60 mv. the curves have crossed and conduction is impossible.

Potassium activation is lost below *ca.* 35 mv. The circles near 27 mv. in Fig.

2 represent data from thirteen fibers in a high KCl (62.5 mM) Ringer's fluid. In this experiment the membrane was driven to zero and beyond (inside positive) without departing more than 1 mv. from the predicted error function. Rectification was lost as a consequence.

*Other Observations.*—The activation process has been observed to fatigue. Muscles were placed in a Ringer's fluid made hypertonic with sucrose. While these muscles do not contract and pull off the electrodes, their electrical be-

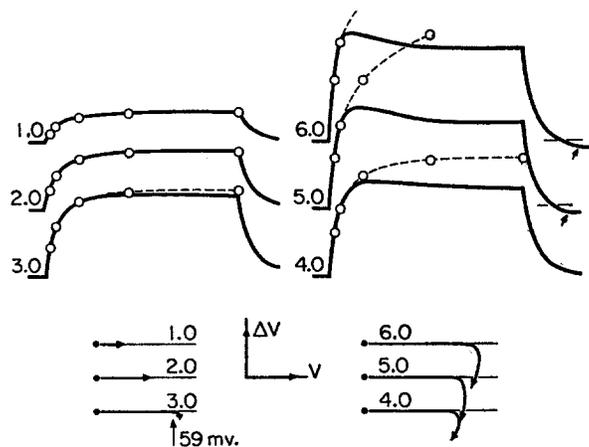


FIG. 3. Electrotonic potentials from a fiber bathed in a sodium-free Ringer's fluid, resting potential 100 mv. All electrotonic potentials are at the expected values (open circles) or below, in contrast to Fig. 1. Activation occurs after a larger depolarization and shows a time delay (indicated by the displacement of the lower plots). The undershoot, indicated by the arrows in the late records, is consistent with an increased potassium conductance. Fibers with high resting potentials like this one are below the potassium concentration potential.

havior is slightly modified. When tetanized with equal intensity 100 millisecond shocks delivered at 1 to 5 per second, the spike potential is lowered and becomes a slow hump or local response. This may occur after five or ten such shocks. In depolarized fibers in which sodium activation is suppressed, the potassium activation can be similarly fatigued, leaving only a large error function electrotonic potential. (The decay of the electrotonic potential (negative  $\Delta V$ ) seen in Fig. 3 is the fatigable portion.)

Several exploratory experiments with calcium concentration increased tenfold indicated that despite a mild hyperpolarization of the resting potential, the potassium activation was lowered (moved further from the resting potential) by about 15 mv. We cannot say yet whether the  $K^+$  curve of Fig. 2 is shifted bodily downward or displaced along the axis. It also seems that the difference

between the sodium and potassium curves is decreased; *i.e.*, sodium activation is lowered by 20 to 25 mv. and its time constant by our method is increased. These observations are strikingly similar to the behavior of the squid giant axon currents under similar conditions of increased calcium (Frankenhaeuser and Hodgkin, 1957). In particular, the shift of the sodium and potassium activation

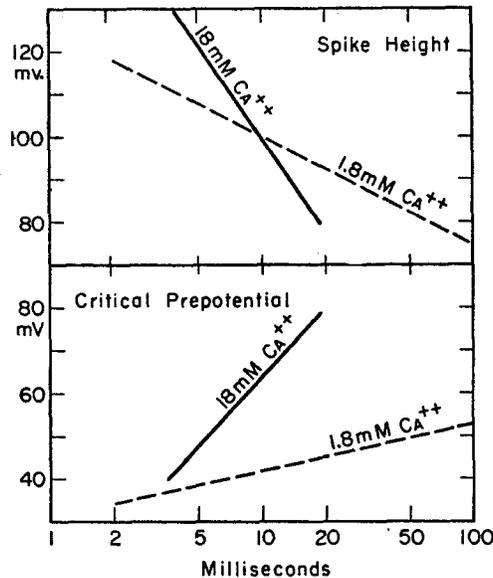


FIG. 4. When strong, long duration shocks were delivered to the impaled fiber multiple firing was elicited. From two to six spikes can occur during the application of the stimulus. The spike height and critical prepotential (estimated as the potential at which the trace begins a rapid, upward deflection into the leading edge of the spike) were measured as function of time after make. The lines in the figure indicate the average slope and position of these data taken from fifty fibers in normal fluids (1.8 mM Ca<sup>++</sup>) and sixteen fibers in a high calcium fluid (18 mM Ca<sup>++</sup>).

potentials with calcium in muscle is virtually identical with the shifts reported by them.

Setting these two opposing processes closer in potential and rate leads to the following predictions which are consistent with experimental observations. (a) If potassium current can be switched on at a rate approaching that of sodium, action potentials should occur only at early times during the application of a long d.c. shock. The voltage must be moving rapidly in time to allow the sodium activation to stay ahead of the potassium activation which tends to restore matters. (b) Thresholds should be raised because the sodium activation potential is set further from the resting potential. (c) The normal separation of the sodium

and potassium activation potentials accounts nicely for the weak powers of accommodation in muscle. In the presence of high concentrations of calcium, when the activation potentials are closer together, accommodation is increased.

These latter observations are set forth in Fig. 4. Spikes were elicited with 100 millisecond shocks. The spike height and critical prepotential were measured as a function of time after make. Only those records were analyzed in which two or more responses occurred during the stimulus interval. This was done in order to pit the sodium and potassium currents against each other. Any restoring effects left after the first spike of a train would have to be overcome by what we suspect is an impaired or diminished sodium activation in high calcium fluids. As the figure indicates, spike height is more rapidly reduced and critical prepotential is more rapidly increased. While there is a complete absence of spike activity after 20 milliseconds in high calcium solutions, normal fluids allow spikes as late as 100 milliseconds (perhaps longer) after make.

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