MEMBRANE IONIC CURRENT OF THE PROPAGATED ACTION POTENTIAL
A NEW METHOD*

Fumitake INOUE

Department of Pharmacology, University of Alberta, Edmonton, Alberta 7, Canada

Summary Since HODGKIN et al. (1949) first recorded membrane ionic currents by the technique of voltage clamping the membrane of squid giant axons, it has been possible to predict precisely the ionic currents produced by an action potential. However, the ionic current produced during the propagating action potential can be obtained only after intricate experiments and laborious calculations. Employing the method used in the present report, the relationship between ionic current and membrane potential of nerve and muscle fibers can easily be obtained. According to the cable equation, the ionic current is described as the difference between the first and second derivatives of the action potential. It is also calculated that the ionic current should be zero at the foot of the action potential. Applying this condition to the cable equation, the ionic current-membrane voltage relation can then be obtained from the electronically differentiated transmembrane action potential. The fact that this method can be applied to muscle fibers whose membrane structure is complicated due to the tubular system is also discussed.

Some time ago JENERICK (1963, 1964), after painstaking measurements and calculations, obtained ionic currents in the membrane of active muscle fibers from the phase plane display of differentiated transmembranal action potential. COLE et al. (1955; cf. COLE, 1968) have also computed from the Hodgkin-Huxley model for the giant squid axon the relation between membrane potential and current during the propagation of an impulse. In the present study an attempt was made to obtain ionic current during the propagation of the action potential with a simple method of electronically differentiating the action potential.

ANALYTICAL METHOD

According to HODGKIN and HUXLEY (1952), the total membrane current ($I_m$)

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during the action potential of non-myelinated nerve fiber in a large volume conductor is expressed by the following equation:

\[
I_m = a/2R_i \theta^2 \cdot d^2V/dt^2 = C_m dV/dt + V/R_m
\]

\[
(1)
\]

\[
(1')
\]

where

- \( a \): the radius of the fiber (cm)
- \( R_i \): the specific resistance of the axoplasm in the fiber (\( \Omega \text{cm} \))
- \( R_m \): the membrane resistance per unit area (\( \Omega \text{cm}^2 \))
- \( C_m \): the membrane capacity per unit area (\( \mu \text{F/cm}^2 \))
- \( \theta \): the conducting velocity of the action potential (cm/sec)
- \( I_i \): the ionic current density across the fiber membrane per unit area (A/cm$^2$)

In the equation, membrane current is described as the second derivative of the action potential and is also expressed as the sum of the capacity current and the ionic current. Therefore, a plot of the ionic current against the membrane voltage during propagation of the action potential can be obtained if the first and second derivatives of the action potential are recorded.

The calibration of the first derivative of the action potential was obtained electronically, and it was possible to estimate its current density from the specific membrane capacity of the muscle fiber. On the other hand, it is impossible to calibrate the second derivative electronically. Furthermore, there is a large cumulative error in the calculation of the membrane current \( (I_m) \), since there are several coefficients \( (a, \theta, \text{and } R_i) \) involved in the calculation, and their values, estimated from the available data, vary widely. Therefore, the following calculations were made to estimate the ionic current.

The action current at the foot of the propagating action potential:

The solution of Eq. (1) is given by

\[
V = A \exp (\mu t)
\]

where \( A \) is a constant of integration and \( \mu \) is given by

\[
\frac{k + \sqrt{k(k + 4/\tau_m)}}{2}
\]

where \( k \) stands for \( 2R_i \theta^2C_m/a \) and \( \tau_m \) for the time constant of the fiber membrane (cf. Cole and Curtis, 1939; Tasaki and Hagiwara, 1957). In most cases \( k \gg 4/\tau_m \) (1: Ca 0.01-0.04 in frog sartorius) so that \( \mu \approx k \) and the time constant of the exponential rise of the action potential is

\[
V = A \exp (kt)
\]

The first derivative of the action potential is \( dV/dt = kA \exp (kt) \), hence \( dV/dt = kV \) and \( (dV/dt)/V = k \). Therefore, the initial part of the phase plane
Fig. 1. A: First and second derivatives of the propagating transaction potential-membrane potential relationships. The black disk on the right is zero potential; the one on the left shows the resting membrane potential indicated by R. Ordinate shows the rate of rise of the action potential as well as current intensity for the first derivative of the action potential. Action potential starts from R, moving clockwise as indicated by the arrows. Photo was retouched.

B: Current-voltage relation during the propagated action potential. Subtraction of the first and second derivatives of the action potential in A is shown as the ionic current across the membrane. Abscissa is the membrane voltage ($V_m$), and $R$ is the resting potential of the muscle fiber. Ordinate is the current intensity ($I_i$). Negative sign represents net inward flux of the current, presumably carried by sodium ion. Action current starts at $R$, travels counterclockwise along arrows, and returns to $R$.

The record of the first derivative of the action potential versus the membrane potential is a straight line with the slope of $k$ (Fig. 1).

Equation (1) becomes \( \frac{d^2V}{dt^2} = k\left(\frac{dV}{dt} + \frac{I_i}{C_m}\right) \).

When multiplied by \(1/(dV/dt)\), it becomes

\[ \frac{d(dV/dt)}{dt} = k\left(\frac{dV}{dt} + \frac{I_i}{C_m}\right)/(dV/dt). \]

The left side of the equation represents the slope in the phase plane record (\(dV/dt: V\)) (Fig. 1) and is substituted for $m$. Then:

\[ m\frac{dV}{dt} = k\left(\frac{dV}{dt} + \frac{I_i}{C_m}\right) \]

\[ I_i = C_m(m/k - 1)dV/dt \]

This relationship was also obtained by Jenerick (1964), using another approach. This equation predicts that, because $m = k$, the ionic current should be zero at the foot of the propagating action potential. Therefore, the gain of the channel recording the second derivative of the action potential was chosen such that the initial trajectory of both derivatives would be the same. Using this gain setting, the difference between the derivatives should depict the ionic current.
RESULTS

A typical record of the propagated transmembrane action potential *versus* its $dV/dt$ and $d^2V/dt^2$ is shown in Fig. 1A. It was obtained from a frog sartorius muscle fiber instead of a nerve fiber, because of the difficulty of acquiring the latter. Current calibration on the vertical axis was calculated on the assumed value of 2.5 µF/cm² for the specific surface membrane capacity. The same current calibration was amplified to ionic current. The net ionic current across the membrane during the action potential, which was obtained by the subtraction of the phase plane records in Fig. 1A, is shown in Fig. 1B. An identical record, shown in Fig. 2, was obtained by two operational amplifiers and a difference amplifier. Inward flux of ionic current is shown as negative. Once the ionic current begins to flow, it rises steeply and reaches a maximum near the peak of the action potential. Inward and outward currents may be carried by the sodium and potassium ions, respectively, as suggested by voltage clamp experiments. Thereafter, ionic current dwindles due to a decrease of sodium conductance and a rapid increase of potassium conductance, as in the case of the squid giant axon. A short time after passing the peak of the action potential, the direction of the net ionic current reverses from an inward to an outward direction around zero potential level and the outward current fades away at the beginning of the negative afterpotential. The net ionic current during the negative afterpotential at this stage is equal throughout the entire fiber length, and therefore there is no longitudinal current flow.

Fig. 2. Phase plane record as in Fig. 1B, simultaneously with a nondifferentiated action potential. However, in this case the differentiation of the action potential was achieved by employing operational amplifiers, and the ionic current, *i.e.*, the difference between the first and the second derivatives, was obtained by using a difference amplifier. The Z axis was obtained by using intensity modulation of the oscilloscope trace.

Cole’s computed diagram (1968) is qualitatively similar to Figs. 1 and 2. These phase plane records of the ionic current are slightly different from those obtained by voltage clamp methods. There is no outward current at the beginning of the propagating action potential, and this agrees with the theoretically derived characteristic.

DISCUSSION

There is some doubt about application of this method to the muscle fibers.
Since the study of Falk and Fatt (1964), the existence of the two time constants of the membrane has received strong support in studies of skeletal and heart muscles. They found that tubular capacitance which consists mostly of membrane capacitance, was in series with resistance.

The general cable equation, the original form of Eq. (1) is

\[
d^2V/dx^2 = (r_o + r_i)i_m
\]

which is valid for any membrane property as long as \( r_o \) and \( r_i \) remain linear, where \( x, i_m, r_o \) and \( r_i \) represent distance, membrane current, external and axoplasmic resist resistance per cm respectively, and \( r_o \) is assumed to be zero in a large volume conductor. Then, \( i_m = YV \), where \( Y \) is the admittance of the membrane per cm, and the admittance of the muscle fiber having the tubular system becomes

\[
Y = \frac{1}{r_m} + \frac{1}{r_s} \cdot \frac{1}{1 + 1/(\omega c s)^2} + j \left[ \frac{\omega c_s}{(\omega c s)^2 + 1} \right]
\]

where
- \( r_m \): surface membrane resistance per unit length \([R_m \ (\text{per unit area}) = 2,000 \ \Omega \text{cm}^2]\)
- \( \omega \): angular velocity of the action potential \([\text{Ca} 900 \ Hz \text{ in frog sartorius}]\)
- \( c_s \): membrane capacity per unit length \([C_s \ (\text{not} \ c_s) = \text{Ca} 5 \ \mu F/cm^2] \ T\text{-system}\)
- \( r_s \): membrane resistance per unit length in series with \( c_s [R_s \ (\text{per unit area}) = \text{Ca} 400 \ \Omega \text{cm}^2]\)
- \( c_m \): surface membrane capacity per unit [length \([C_m \ (\text{not} \ c_m) = 2.5 \ \mu F/cm^2]\)

Using the values given above, Eq. (2) is simplified in the following manner:

\[
d^2V/dt^2 = 2R_s \theta^2 \alpha (1/R_m + 1/R_s + j\omega c_m) V
\]

Strictly speaking, Eqs. (1) and (3) may not be applied during the active state of an excitable fiber, because the cable theory, at present, deliberately avoids the non-linear properties of the membrane (Taylor, 1963). Nevertheless, \((1/R_m + R_s)V\) in Eq. (3) may be substituted by \(I\), in the active state, because \( R_m \) is assumed to be very small in the active state of the membrane. When \( j\omega \) is substituted by the operator \((d/dt)\), Eq. (3) may be transformed into Eq. (1)'.

According to Falk and Fatt (1964), the potential drawn by \( C_s \) reaches 25 mV at the peak of the action potential, and its current intensity is 0.24 mA/cm² \((\text{peak voltage of the action potential } - 25 \text{ mV})/R_s\). Hence, the maximal membrane current in Figs. 1 and 2 may be one quarter larger than that obtained. Freygang et al. (1967) found that the foot of the action potential of the muscle fiber is still exponential, even though the membrane has two time constants due to its tubular system. They showed that the combined time constant of the foot of the action potential \(1/\mu \) is 15% slower than that calculated with a single surface membrane time constant. Therefore, the cable equation of muscle fiber at the foot of the action potential should be described by the following equation from Eq. (3):
The space constant for DC current is described by
\[ \lambda^2 = \frac{r_m}{r_i} = \frac{aR_m}{2R_i}. \]
Therefore the equation above is:
\[ \mu^2 - \frac{R_m + R_e}{R_i} \frac{\theta^2}{\lambda^2} = k\mu. \]
This means the first derivative is the same as the second derivative of the action potential at the foot and there is no net membrane current at the foot. Therefore, the analysis done here is still valid with approximate values for the muscle fiber whose membrane has two time constants.

This method cannot be applied to the Loligo axon, in which \( 4/\tau_m \) is significant compared with \( k \). In Carcinus and cockroach axons the ratio \( k: 4/\tau_m \) is 1: \( Ca \) 0.1 and thus perhaps suitable for this method. However, Sepia and lobster axons are most suitable, since in these the ratio is only a few percent and the equivalent circuits of their membranes are simple.

In conclusion, by this simple procedure the ionic current of the propagating action potential can be obtained from most excitable fibers.

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