Equivalent Circuit of Frog Atrial Tissue as Determined by Voltage Clamp-Unclamp Experiments

MERRILL TARR and JOHN TRANK

From the Department of Physiology, University of Kansas Medical Center, Kansas City, Kansas 66103

ABSTRACT The equivalent circuit that has been used in the analysis of nerve voltage-clamp data is that of the membrane capacity in parallel with the membrane resistance. Voltage-clamp experiments on frog atrial tissue indicate that this circuit will not suffice for this cardiac tissue. The change in membrane current associated with a step change in membrane potential does not show a rapid spike of capacitive current as would be expected for the simple parallel resistance–capacitance network. Rather, there is a step change in current followed by an exponential decay in current with a time constant of about 1 msec. This relatively slow capacitive charging current suggests that there is a resistance in series with the membrane capacity. A possible equivalent circuit is that of a series resistance external to the parallel resistance–capacitance network of the cell membranes. Another possible circuit assumes that the series resistance is an integral part of the cell membrane. The data presented in this paper demonstrate that the equivalent circuit of a bundle of frog atrial muscle is that of an external resistance in series with the cell membranes.

INTRODUCTION

The equivalent circuit that has been used in the analysis of nerve voltage-clamp data is that of the membrane capacity in parallel with the membrane resistance. Recently, voltage-clamp experiments on cardiac tissue indicate that this simple equivalent circuit is not adequate for some cardiac tissue. Beeler and Reuter (1970) found in dog ventricular trabeculae that the membrane current associated with a small step change in potential did not show a rapid spike of capacitive current. Rather, there was a step change in current followed by an exponential decay in current having a single time constant of about 1 msec. Fozzard (1966) found in similar experiments on Purkinje fibers that there was an initial surge of current followed by a much slower exponential decay. We have obtained results on frog atrial tissue similar to those of Beeler and Reuter (1970).

The relatively slow capacitive charging current suggests that there is a resistance in series with the membrane capacity. Two possible equivalent circuits have been proposed by Beeler and Reuter (1970). One equivalent circuit assumes that the series resistance is external to the parallel resistance–capacitance network of the cell membrane. The other circuit assumes that the resistance in series with the membrane capacity is an integral part of the cell membrane. Beeler and Reuter (1970) concluded that the external series resistance circuit was applicable to dog ventricular trabeculae. The data presented in this paper demonstrate that the external resistance equivalent circuit is also applicable to a bundle of frog atrial muscle.

METHODS

Preparations   The preparations were bundles of frog (*Rana catesbeiana*) atrial muscle 150–400 μ in diameter and approximately 1 cm in length.

Sucrose-Gap Chamber   Fig. 1 shows schematically the sucrose-gap chamber. A bundle of atrial muscle was drawn through the holes (800 μ diameter) in the two Lucite partitions (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) (striped areas) which divided the chamber into three compartments. A short segment (<100 μ) in the middle of the preparation (test node) was isolated from the ends of the fiber by two streams of isosmotic sucrose solution filling the extracellular space. The center pool (test pool) was perfused with normal Ringer solution. The right pool was perfused with isosmotic KCl and the left pool was perfused with normal Ringer solution. All solutions were maintained at 14°–18°C. The solutions were removed from the chamber by vacuum lines attached to needles in the center and two side pools. The relative velocity of flow between the sucrose solutions and the solution in the center pool determined the width of the node: the flows were adjusted by needle valves. Under microscopic observation ($\times$ 25) the liquid junctions of sucrose and test solutions were adjusted to define a node width of less than 100 μ.

Voltage-Clamp Circuit   The remainder of Fig. 1 shows an equivalent circuit for the test node and the electronic apparatus necessary for control of the potential of the test node. The potential of the right pool (KCl) with respect to the center pool was measured with a high input impedance amplifier ($+V_i$) connected to an Ag-AgCl electrode in the right pool. The center pool was grounded through an Ag-AgCl electrode connected directly to the summing point (a virtual ground) of the I_m operational amplifier used to measure the membrane current. A finite resistance (combination of solution and electrode resistance) existed between the outside of the node membrane and the summing point of the I_m amplifier. The potential drop across the resistance (R_x) was measured by a high input impedance inverting amplifier ($-V_o$) connected to an Ag-AgCl electrode placed in close proximity to the tissue in the test node. In the voltage-clamp configuration (switch on the output of the control amplifier closed), the test node was placed in the feedback loop of the control amplifier which supplied through an Ag-AgCl electrode in the left pool the current required to make the potential of the test node ($V_i - V_o$) equal the magnitude of the command signal (C.S.). Starting from a holding potential at the resting mem-

brane potential, the potential of the node was displaced in rectangular steps (2 sec duration) and the associated membrane currents were measured with the I_m amplifier. Although it is not shown in Fig. 1, an electronic switching system was combined with this network in order to permit release of the voltage clamp at any time during the rectangular step change in node potential. A short-duration rectangular
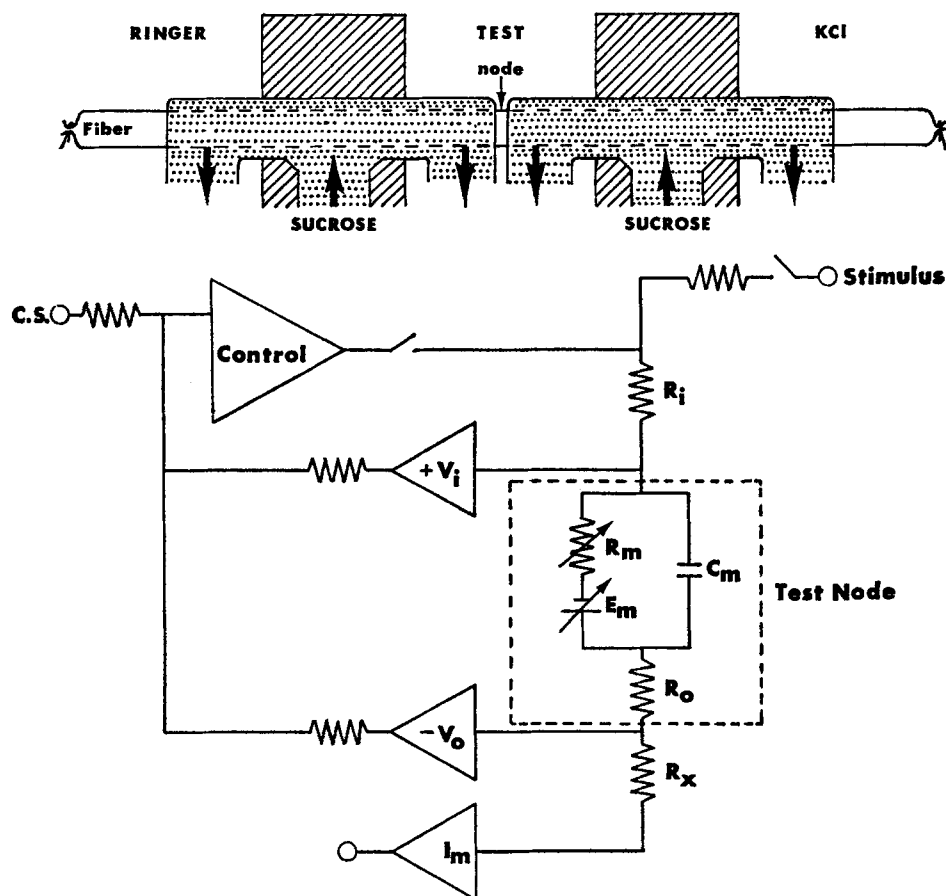

FIGURE 1. Schematic representation of the sucrose-gap chamber (upper half) and the equivalent circuit of the test node with associated voltage-clamp circuit (lower half).

pulse (500 μsec) was used to measure the external resistance (R_o) in series with the membrane capacity.

Solutions The Ringer solution had the following composition (in millimoles per liter): NaCl, 111; KCl, 5.4; $CaCl_2$, 1.8; tris (hydroxymethyl) amino methane, 10. The pH of this solution was adjusted to 7.0 at 25°C by adding 12.4 N HCl. Isosmotic KCl was 121 mM; isosmotic sucrose was 193 mM.

Nomenclature Membrane potentials (V_m) and clamp potentials (V) are measured with respect to the zero extracellular potential. A change in clamp potential

from the holding potential (V_H) is designated as ΔV: depolarization a positive ΔV, hyperpolarization a negative ΔV. The membrane current (I) is measured with respect to zero membrane current. Changes in membrane current from the holding current are ΔI, I_o, I_s, I_{co} (see text).

RESULTS

Fig. 2 shows the change in membrane current resulting from a 20 mv step depolarization of the test node. The current rises rapidly to a maximum value I_o and then decays exponentially with a time constant of 1 msec to a steady-state value of I_s; the capacitive current continues to flow even though the clamp potential has attained a steady value.

The relatively slow capacitive charging current suggests there is a resistance in series with the membrane capacity. Two possible equivalent circuits similar
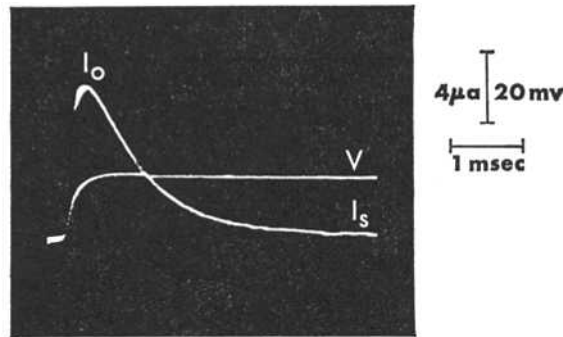

FIGURE 2. The membrane current associated with a step change in V of $+20$ mv under voltage-clamp conditions. Current and voltage traces are superimposed before the step change in V.

to those proposed by Beeler and Reuter (1970) are shown in Fig. 3. Circuit No. 1 assumes that there is an external resistance in series with the parallel resistance-capacitance network of the cell membrane. Circuit No. 2 assumes that the membrane capacity is in series with a resistance directly attributable to part of the cell structure. In both circuits, the change in membrane current (ΔI) associated with a step change in potential would decay exponentially from some maximum value (I_o) to some steady-state value (I_s) according to the following equation (see Appendix for derivation)

$$\Delta I = I_s + (I_o - I_s) \exp(-t/\tau).$$

In circuit No. 1, the potential (V) under voltage-clamp control is the sum of the membrane potential (V_m) and the potential drop (IR_o) due to the membrane current (I) flowing through the series resistance (R_o); i.e., V equals $V_m + IR_o$. The difference between V_m and V (i.e., $V_m - V$) can be measured

by suddenly releasing the voltage clamp during the flow of membrane current. The potential measured immediately after release of the clamp is the V_m just before release of the clamp. Thus, a step change of potential equal to $(V_m - V)$ should occur upon release of the voltage clamp. The magnitude and direction of the step change in potential should obey the relation $-IR_o$, where I is the membrane current flowing just before unclamp.

This hypothesis was tested by unclamping during the inward sodium current resulting from a step depolarization of the test node. The results of such an experiment are shown in Fig. 4. Fig. 4 A shows the inward sodium current resulting from a 60 mv step depolarization. Fig. 4 B shows the result of unclamping the test node at the peak of the inward current (see arrow). Associated with the unclamp was a step change in potential of $+12$ mv followed by
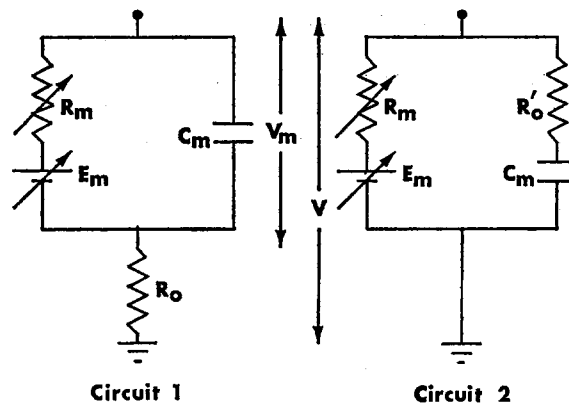

Circuit 1   Circuit 2

FIGURE 3. Two possible equivalent circuits which could explain the relatively slow capacitive charging current.

an action potential; only upstroke phase and peak of the action potential are shown in Fig. 4 B. A $+10$ mv, 500 μsec pulse was used to measure I_o (see spike of outward current in Fig. 4 A and B preceding the 60 mv depolarization). In circuit No. 1, I_o is related to R_o by the relationship $R_o = \Delta V/I_o$, where ΔV equals $+10$ mv in this case. In Fig. 4 B, I_o equals 2.2 μa and, therefore, R_o equals 4.6 kΩ. The $+12$ mv potential step upon unclamping agrees well with the product of R_o and the inward current $(-2.7 \mu a)$ flowing just before unclamp (i.e., $-IR_o = +12.4$ mv).

The relationship between $(V_m - V)$ and $-IR_o$ obtained from unclamping at various times during the inward current from three different fibers is shown in Fig. 5. The data show a good agreement between $(V_m - V)$ and $-IR_o$ as would be expected if the equivalent circuit of atrial tissue were that of circuit No. 1.

Table I gives the results of experiments in which the equivalent circuit
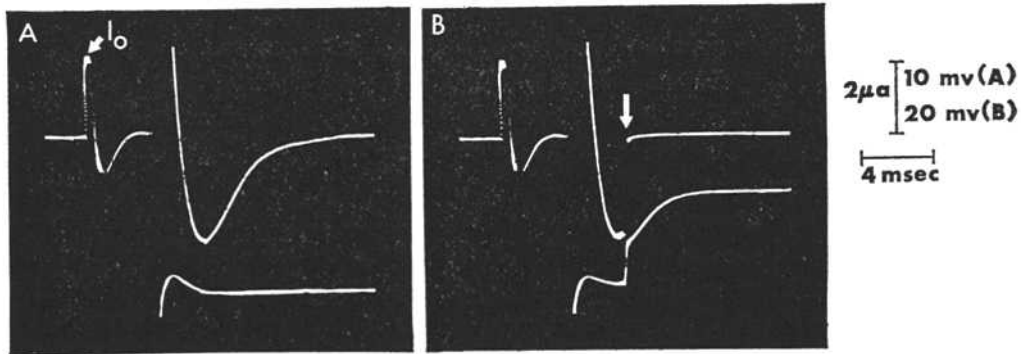
FIGURE 4.   Change in membrane current (upper trace) associated with a step depolarization of 60 mv (lower trace) from a holding potential of −80 mv. Inward current is shown as a downward deflection from the holding current. The 20 mv voltage calibration line at the right of Fig. 4 B applies to Fig. 4 B; the 10 mv calibration applies to Fig. 4 A. Due to the high voltage gain, only the upper part of the step potential (+60 mv) was recorded. The spike of outward current (I_o) preceding each +60 mv pulse resulted from a 500 μsec pulse of +10 mv used to determine R_o.
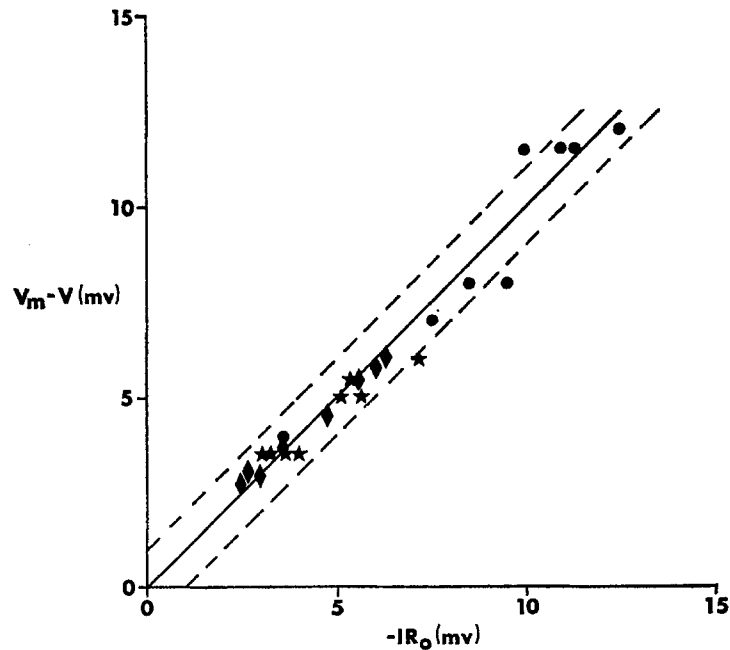

FIGURE 5.   Relationship of the step change in potential ($V_m - V$) associated with unclamping the frog atrial bundle during the inward sodium current (I) to the value of $-IR_o$ (abscissa). The solid line is a line of equivalence and the dotted lines are ± 1 mv from the equivalence line. The different symbols represent data obtained from different fibers.

parameters of frog atrial fibers were determined using circuit No. 1 as the equivalent circuit. A pulse of $+10$ mv was used in each experiment. The equations used for calculation of R_o, R_m, and C_m are given in the Appendix (Equations 10–12).

TABLE I

FROG ATRIAL TISSUE EQUIVALENT CIRCUIT PARAMETERS
CALCULATED FOR CIRCUIT NO. 1

τ	R_o	R_m	C_m
msec	*kΩ*	*kΩ*	*μF*
0.87	8.7	57.9	0.12
0.62	7.1	92.9	0.09
0.67	11.8	238.2	0.06
0.51	3.4	139.5	0.15
0.57	2.6	97.4	0.22
2.13	5.7	44.3	0.42
1.28	11.8	54.8	0.13
1.44	15.4	24.6	0.15
0.98	11.1	55.5	0.11
0.75	4.5	62.1	0.18
0.71	3.1	96.9	0.23
(0.96)	(7.7)	(87.6)	(0.17)

Average values in parentheses.

DISCUSSION

The data presented in this paper demonstrate that the step change in potential $(V_m - V)$ upon unclamping equals $-IR_o$ as would be expected if the equivalent circuit for frog atrial tissue were that of a resistance (R_o) in series with the parallel resistance–capacitance network of the cell membranes.

A step change in potential equal to $-IR_mR_o'/(R_m + R_o')$ would occur if circuit No. 2 were the equivalent circuit (see Appendix, Equation 25). To exclude circuit No. 2 as a possibility, it is necessary to exclude the possibility that $R_mR_o'/(R_m + R_o')$ equals R_o. The values for R_o given in Table I can be converted to R_o' by the following equation: $R_o' = R_o(R_m + R_o)/R_m$, where R_o and R_m are the values given in Table I. (This equation can be derived by substitution of Equations 10 and 11 into Equation 17—see Appendix.) For the average values given in Table I, $(R_m + R_o)/R_m$ is approximately 1.1, and R_o' is approximately 1.1 R_o. For the example shown in Fig. 4 B, R_o equals 4.6 kΩ. Therefore, a value of 50.6 kΩ for R_m would be required for $R_mR_o'/R_m + R_o'$ to equal R_o. If this were the value for R_m, then it would be impossible from the unclamp data to distinguish between circuits No. 1 and No. 2.

But it can be demonstrated that an R_m of 50.6 kΩ at the peak of the inward current (i.e., just before unclamp) is not realistic. From the chord conductance

relationship, the equilibrium potential (E_m) for the ionic current (I_i) flowing just before unclamp is $V - I_iR_m$. In Fig. 4 B, the inward current flowing just before unclamp is -2.7 μa, and V is -20 mv (pulse of $+60$ mv from a -80 mv holding potential). Assuming this current to be entirely ionic, E_m would have to be $+116$ mv for R_m to be 50.6 kΩ. This would be a minimum value for E_m, since in addition to ionic current, capacitive current equal to $(\Delta V/R_c')$ exp $(-t/\tau)$ would also be flowing during the peak of the inward current. In Fig. 4 B, the inward current reaches a maximum value 3.2 msec after the step change in potential. Assuming a τ of 1.0 msec, the capacitive current would equal $+0.54$ μa $[(60$ mv/5.05 k$\Omega)$ exp $(-3.2)]$. The ionic current would then equal -3.24 μa (i.e., $I_i = I - I_c$) and E_m would become $+143$ mv. This inward current is carried by sodium ions (Rougier et al., 1968 and Tarr, 1971) which have an equilibrium potential of about $+40$ mv. Thus, an unrealistic value for E_{Na} would have to occur for the data to be consistent with circuit No. 2.

In addition to the above arguments excluding circuit No. 2, the following considerations are important. If circuit No. 2 were the equivalent circuit, then the inward sodium current should be the difference between the membrane current before and after removal of the inward current by application of tetrodotoxin or removal of Na^+. We have found that such a subtraction procedure gives an unrealistic current-voltage relationship for the sodium ionic current in that E_{Na} is greater than $+50$ mv. Recently, Haas et al. (1970) reported that the membrane potential as recorded by microelectrodes under voltage-clamp conditions showed a small hump (up to 5 mv) at the peak of a large inward current. Such a result is consistent with circuit No. 1, but it is not consistent with circuit No. 2.

Recently, Beeler and Reuter (1970) proposed a similar circuit for dog ventricular trabeculae as we propose for frog atrial tissue. In contrast, Fozzard (1966) proposed a more complicated circuit for Purkinje fibers. In his circuit, two capacities separate the myoplasm from the surface of the Purkinje strand: a capacity in parallel to the membrane resistance and one in series with a resistance. Each circuit would appear to be consistent with the structure of the respective tissue. A 100 μ diameter Purkinje fiber is composed of a relatively small number (10–15) of individual cells (Sommer and Johnson, 1968). A fraction (about 20%) of the individual cell membranes comprises the periphery of the Purkinje fiber and the remainder (about 80%) of the cell membranes are connected to the periphery through the narrow clefts ($\sim$300 A) between the tightly packed cells. The equivalent circuit proposed by Fozzard (1966) for Purkinje fibers is reasonable. In response to a step change in voltage, there should be two components of capacitive current: an initial rapid component related to charging the membrane capacitance at the periphery of the bundle, and a much slower component related to charging the remainder of the membrane capacitance through the cleft resistance. In contrast, a 100 μ diameter

bundle of frog atrial tissue contains approximately 1250 cells (4 μ diameter). Approximately 80 of the cells would comprise the periphery of the bundle. Thus, approximately 6% (80/1250) of the total cell membrane area would be in direct contact with the fluid bathing the bundle. The majority of the cell membranes would be connected to the periphery through relatively narrow (200–400 A) intercellular clefts (Staley and Benson, 1968). Thus, the capacitive current related to charging the capacitance of the peripheral cells would be negligible, and a one time-constant equivalent circuit would be a reasonable first-order approximation to explain the charging of the capacitance of the majority of the cell membranes through the distributed resistances of the intercellular clefts.

The data in Table I give a mean value of membrane resistance of approximately 88 kΩ and a capacitance of 0.17 μF. Thus, the membrane time constant $(R_m C_m)$ would be about 15 msec. Tarr and Sperelakis (1964) reported values of 5–10 msec for frog ventricular fibers, and a value of about 20 msec has been reported for Purkinje fibers (Weidmann, 1952; Fozzard, 1966). Recently, Weidmann (1970) reported a value of 4.4 msec for ventricular fibers of sheep and calf hearts.

The data in Table I were taken from fibers having diameters of 160–240 μ. Assuming an average bundle diameter of 200 μ, a node length of 100 μ, and a bundle composed of a maximum density of 4 μ diameter cells (Staley and Benson, 1968), the test node would contain 2500 cells $[(100\,\mu)^2/(2\,\mu)^2]$ and have a membrane area of 3 $\times$ 10^{-2} cm (2500 $\times$ membrane area of a cell 4 μ in diameter and 100 μ in length). Thus, the specific membrane resistance would be approximately 2600 ohm-cm^2 and the specific membrane capacity would be approximately 5.7 μF/cm^2. These values are similar to those previously reported for other cardiac tissue. A specific membrane resistance of approximately 2000 ohm-cm^2 has been reported for Purkinje fibers (Weidmann, 1952), and a value of 9100 ohm-cm^2 has been reported for sheep and calf ventricular fibers (Weidmann, 1970). Weidmann (1952) reported a value of 12 μF/cm^2 for the specific capacitance of Purkinje fibers. Fozzard (1966) separated the total capacitance into that in parallel with the membrane resistance (2.4 μF/cm^2) and that in series with the cleft resistance (7 μF/cm^2). Recently, Weidmann (1970) reported a value of 0.8 μF/cm^2 for ventricular fibers.

APPENDIX

Equations for the Equivalent Circuits

Circuit No. 1

In this circuit (see Fig. 3) the potential (V) under voltage-clamp control is the sum of the membrane potential (V_m) and the voltage drop (IR_o) resulting from the mem-

brane current (I) flowing through the series resistance (R_o). In equation form,

$$V = V_m + IR_o.\tag{1}$$

For a step change of ΔV in clamp potential imposed on the circuit at $t = 0$, Equation 1 can be written as

$$\Delta V = \Delta V_m + \Delta IR_o,\tag{2}$$

where ΔV_m is the change in membrane potential from the original membrane potential and ΔI is the change in membrane current from the holding current. The following equation describes ΔV_m

$$\Delta V_m = (\alpha \Delta V)[1 - \exp(-t/\tau)],\tag{3}$$

where $\alpha = R_m/(R_m + R_o)$ and $\tau = R_m R_o C_m/(R_m + R_o)$. Equation 3 can be rewritten as

$$\Delta IR_o = \Delta V - \alpha \Delta V \exp(-t/\tau).\tag{4}$$

Evaluation of Equation 4 at $t = 0$ gives

$$\Delta I = I_o = \Delta V/R_o,\tag{5}$$

where I_o is the initial change in membrane current associated with the step change in V at $t = 0$. Substitution of Equation 5 into Equation 4 gives the following equation

$$\Delta I = I_o - \alpha I_o + \alpha I_o \exp(-t/\tau).\tag{6}$$

Evaluation of Equation 6 at $t = \infty$ gives

$$\Delta I = I_s = I_o - \alpha I_o,\tag{7}$$

where I_s is the steady-state change in membrane current. Substitution of Equation 7 into Equation 6 gives

$$\Delta I = I_s + \alpha I_o \exp(-t/\tau)\tag{8}$$

which can also be written as

$$\Delta I = I_s + (I_o - I_s) \exp(-t/\tau),\tag{9}$$

since $\alpha I_o = I_o - I_s$ (see Equation 7).

The following equations can be used to determine the equivalent circuit parameters

$$R_o = \Delta V/I_o\tag{10}$$
$$R_m = (\Delta V/I_s) - R_o\tag{11}$$
$$C_m = \tau/(1 - I_s/I_o)(R_o).\tag{12}$$

The time constant (τ) can be calculated by measuring the current at a given time (t) and using the equation

$$\tau = -t/\ln(I_t - I_s)/(I_o - I_s),\qquad(13)$$

where I_t is the current at time (t).

Circuit No. 2

Equation 9 can also be derived for this circuit. The change in membrane current (ΔI) associated with a step change in V at $t = 0$ is the sum of the change in ionic current (ΔI_i) and the change in capacitive current (ΔI_c). In equation form,

$$\Delta I = \Delta I_i + \Delta I_c.\qquad(14)$$

But ΔI_c equals $I_{co} \exp(-t/\tau)$ and Equation 14 becomes

$$\Delta I = \Delta I_i + I_{co} \exp(-t/\tau),\qquad(15)$$

where I_{co} is the value of ΔI_c at $t = 0$. I_{co} is also equal to $(I_o - I_s)$, where I_o and I_s are the initial and steady-state changes in membrane current, respectively. Since $\Delta I_i = I_s$, Equation 15 becomes Equation 9.

The following equations can be used to determine the equivalent circuit parameters

$$R_m = \Delta V/I_s\qquad(16)$$
$$R_o' = \Delta V/(I_o - I_s)\qquad(17)$$
$$C_m = \tau/R_o'.\qquad(18)$$

Equation 13 can be used to calculate τ.

Upon unclamping circuit No. 2, the potential (V_m) will be the open circuit potential given by

$$V_m = (g_m/\Sigma g)E_m + (g_o'/\Sigma g)E_c,\qquad(19)$$

where $g_m = 1/R_m$, $g_o' = 1/R_o'$, $\Sigma g = g_m + g_o'$, E_m is the equilibrium potential for the ionic current flowing just before unclamp, and E_c is the potential on the membrane capacitance just before unclamp. The step change in potential upon unclamping (i.e., $V_m - V$) is given by the following equation

$$V_m - V = (g_m/\Sigma g)E_m + (g_o'/\Sigma g)E_c - V.\qquad(20)$$

Equation 20 can be rewritten as the following equation

$$V_m - V = (g_m/\Sigma g)(E_m - V) + (g_o'/\Sigma g)E_c - V(1 - g_m/\Sigma g).\qquad(21)$$

Since $(1 - g_m/\Sigma g)$ equals $g_o'/\Sigma g$, Equation 21 can be rewritten as follows

$$V_m - V = (g_m/\Sigma g)(E_m - V) + (g_o'/\Sigma g)E_c - (g_o'/\Sigma g)V.\qquad(22)$$

The voltage on the membrane capacitance (E_c) equals $(V - I_cR_o')$, where I_c is the capacitive current flowing just before unclamp, and Equation 22 becomes

$$V_m - V = (g_m/\Sigma g)(E_m - V) - (g_o'/\Sigma g)I_cR_o'. \tag{23}$$

The following substitutions can be made in Equation 23: $(E_m - V) = -I_iR_m$, where I_i is the ionic current flowing just before unclamp; $g_m/\Sigma g = R_o'/(R_m + R_o')$; and $g_o'/\Sigma g = R_m/(R_m + R_o')$. The following equation is then derived

$$V_m - V = -(I_i + I_c)(R_mR_o'/R_m + R_o'). \tag{24}$$

The total membrane current (I) flowing just before unclamp is $I_i + I_c$, and Equation 24 becomes

$$V_m - V = -IR_o'R_m/(R_m + R_o'). \tag{25}$$

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