

Voltage Clamp Simulations for Multifiber Bundles in a Double Sucrose Gap: Radial vs. Longitudinal Resistance Effects

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Abstract. Voltage clamp responses of a single excitable fiber were simulated using a core conductor model including a high external resistance (R_s) in series to the fiber membrane to allow for intercellular clefts in a multifiber preparation. In terms of specific resistance, R_s was between 68 and 264 Ωcm^2 . Internal resistivity (R_i) was taken to be zero or 200 Ωcm . The aim of the study was to quantify the expected antagonistic effects of external and internal resistances on Na current measurements. With $R_i = 0$, the external resistance was found to cause a strong depression of fast inward current compared to an ideal space clamp at command potentials between -30 and 30 mV. With $R_i = 200$ Ωcm , the depression of inward current was partially removed. The effects of R_s and R_i on membrane current measurement were illustrated by cable analysis assuming a quasi-steady state of the fiber at peak time of inward current.

Key words: Sucrose gap — Voltage clamp — Na current — Series resistance — Computer simulation

Introduction

In multicellular preparations, a quantitative analysis of transmembrane ionic currents by the voltage clamp method is limited by axial and radial voltage gradients due to intracellular and extracellular resistances. The distorting effect of an internal (longitudinal) resistance on membrane current measurement has been studied in a previous paper (Solchenbach et al. 1986) using the model of a single, thin fiber in a double sucrose gap arrangement where the external resistance was set to zero level. In the present paper a single fiber from the interior of a bundle is considered, connected to the bundle surface through narrow intercellular clefts. The restricted extracellular pathway is represented by a lumped resistor in series to the cell membrane of the test compartment. Again, emphasis is put on the distortion of fast initial inward current.

Model and Methods

The double sucrose gap arrangement employed in the present simulations has been described in a previous paper (Solchenbach et al. 1986; Fig. 2 and eqs. (1–7)). The preparation is a cylindrical fiber of a radius $a = 3 \times 10^{-4}$ cm; the test node length is $d = 0.02$ cm, the sucrose gap length $b = 0.14$ cm. The fiber membrane is characterized by a capacity of $2 \mu\text{F}/\text{cm}^2$, a time and voltage independent potassium conductance, $g_K = 0.5 \text{ mS}/\text{cm}^2$, and a sodium conductance, g_{Na} , of the Hodgkin and Huxley (H-H; 1952) type, with a maximum conductance, $\bar{g}_{Na}$, of 10, 50, or 120 mS/cm². The internal resistivity of the fiber is taken to be zero or $200 \Omega\text{cm}$. The sucrose gap leakage resistance R_{sh} is $20 \times R_{ax}$ with $R_i = 200 \Omega\text{cm}$ and has the same absolute value with $R_i = 0$. The external series resistance is chosen to match the cleft resistance of a fiber bundle of about $60 \mu\text{m}$ diameter (cf. Haas and Brommundt 1980). As was shown in the latter paper (where the bundle was treated as a radial continuum) the external resistance is dependent on both the cleft width and the resistivity of the external medium as well as on membrane conductance. With increasing conductance, the radial current flow becomes more and more restricted to the outer layers of the bundle so that the effective external resistance will decrease. With $\bar{g}_{Na} = 10 \text{ mS}/\text{cm}^2$ the resistance in series to the fully activated membrane was about $300 \Omega\text{cm}^2$ while it was only $\approx 60 \Omega\text{cm}^2$ with $\bar{g}_{Na} = 120 \text{ mS}/\text{cm}^2$. In the present study the lumped resistor R_s is assumed to be 7, 3, or $1.8 \text{ M}\Omega$ for $\bar{g}_{Na} = 10, 50$, or $120 \text{ mS}/\text{cm}^2$. With a nodal membrane area $A = 2\pi ad = 3.77 \times 10^{-5} \text{ cm}^2$, the respective values of specific external resistance are 264, 113, or $68 \Omega\text{cm}^2$. Since a series resistance of an order as above greatly increases the stability of the feedback system, the specification of the gain factor of the control amplifier is not critical and there is no need to introduce a phase lead as was done in the simulations with $R_s = 0$. G is thus taken to be 1000, with $\tau = 1 \text{ ms}$, while $\tau_1 = \tau_2 = 0$. With these values an adequate feedback control of the voltage monitored across the right sucrose gap is obtained. The voltage under control is the sum of the voltage drop across R_s plus the transmembrane potential at the right end multiplied by the isolation factor of the gap: $E = IR_s + V_d(1 - q)$.

The methods used for numerical integration of the differential equations were described previously (Solchenbach et al. 1986).

Results

Fig. 1 illustrates the effect of an external series resistance, R_s , on membrane current, voltage, and conductance changes associated with a depolarizing step command. Simulations with $\bar{g}_{Na} = 10 \text{ mS}/\text{cm}^2$ and $R_s = 7 \text{ M}\Omega$ were performed setting the internal resistivity, R_i , to zero level (left column) or to $200 \Omega\text{cm}$ (middle column) and are compared with results obtained with $R_s = 0$; $R_i = 200 \Omega\text{cm}$ (right column) and the ideal clamp response of a free membrane patch. With $R_i = 0$, membrane current and voltage at any time are independent of the position along the test node. The typical effect of a series resistance (left column) is to shift the membrane potential from the command level in the depolarizing (hyperpolarizing) direction during transmembrane inward (outward) current flow (cf. Kootsey and Johnson 1972; Ramón et al. 1975). This effect is clearly seen in Fig. 1 A and C. In the beginning of a step clamp the series resistance causes a marked delay in membrane potential change (C). While the output voltage of the control amplifier jumps to a steady level within a few microseconds (not shown), the membrane potential rises in an almost exponen-

tial manner with a time constant which approximately equals the product of membrane capacitance and series resistance, i.e. ≈ 0.5 ms in this case. The slow development of membrane depolarization is reflected by a delay in the Na activation process. Time to the peak of Na conductance, or Na current, is about 1.4 ms against 0.8 ms in the ideal H-H kinetics (*B* and *D*). After activation of the Na system, the membrane potential overshoots the command level (-20 mV) by about 10 mV. This means a reduction of the driving force, $(V_m - E_{Na})$, for inward current by 16 % compared to an ideal clamp and a similar reduction of the current itself, the peak values being 94 and 115 $\mu\text{A}/\text{cm}^2$, respectively. Since the membrane current has an appreciable component of capacitive current, the peak of Na current precedes the peak of membrane current by about 0.5 ms. The magnitude of peak Na conductance (*D*) is slightly decreased by the series resistance. In terms of the m , h kinetics, the positive effect of the voltage overshoot on the activation variable m is overbalanced by the negative effect of the time delay on the inactivation variable h . The apparent speed of inactivation, as assessed from the decay of the Na current wave, is almost the same in the model and in the control (*B*).

When the series resistance is combined with internal resistivity (middle column), the voltage deviations due to R_s are modified by cable complications. Membrane potential, current, and conductance changes spread from the I-end to the V-end and vary in shape and size. The typical overshoot of membrane potential after activation of the Na system is clearly seen at all positions along the test node (Fig. 1 *G*). The overshoot is largest (≈ 15 mV) at the right end of the fiber. Since the voltage monitored across the right sucrose gap follows the command pulse closely, the overshoot of V_d is nearly equal and opposite to the voltage drop across the series resistance, IR_s . The depolarizing overshoot at the left end observed under the combined influence of R_s plus R_i (*G*) contrasts with a marked hyperpolarization seen in the presence of R_i alone (*K*). Both Fig. 1 *G* and *K* show a distinct longitudinal voltage gradient, due to axial current flow, after activation of the Na system. The voltage gradient, $(V_d - V_o)$, reaches ≈ 14 mV at $t = 1.8$ ms in (*G*) and ≈ 28 mV at $t = 1$ ms in (*K*). The smaller gradient in (*G*) is explained by a general reduction of clamp current by the series resistance as can be seen from a comparison of (*E*) to (*J*). (Concerning the voltage tracings in (*K*), it should be noted that the records were obtained with a low gain of the control amplifier. The positive voltage deflection at the V-end is the expression of an imperfect voltage control during inward current flow. The strong depolarization at the I-end is a part of a damped oscillation that occurs in the beginning of a clamp before Na activation sets in. Details are discussed in connection with Fig. 3 and 4 of a preceding paper (Solchenbach et al. 1986)). A comparison of Fig. 1 *D* (full trace) and *H* shows that the peak value of Na conductance is slightly increased by the cable properties. This is explained by a

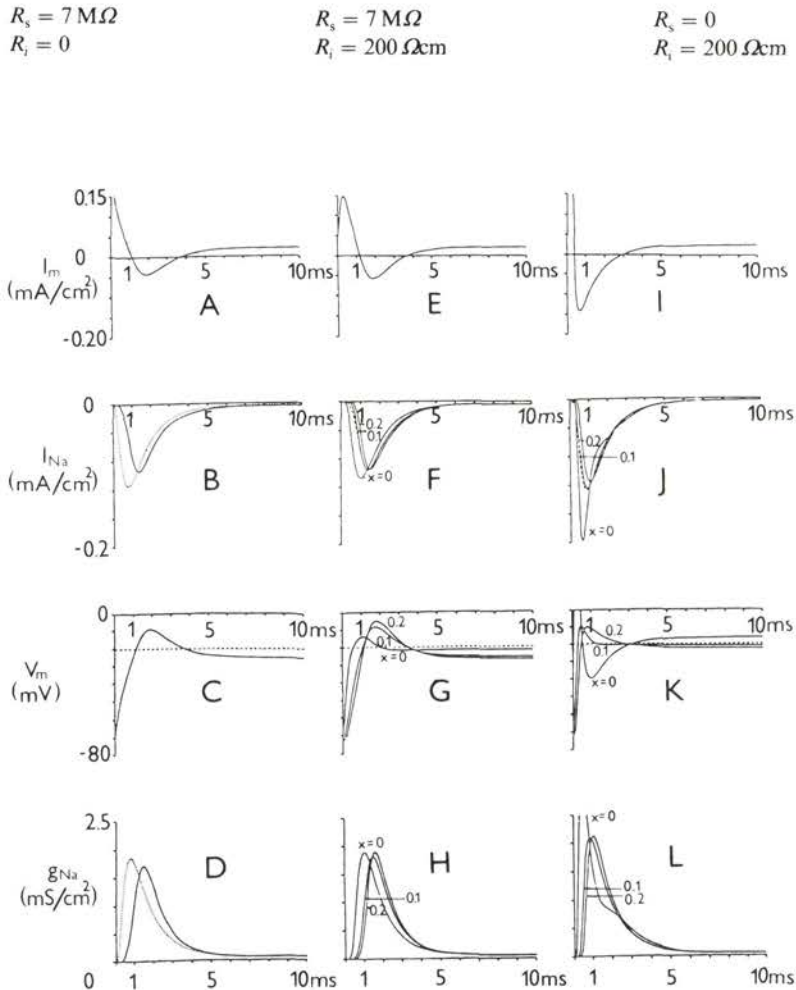


Fig. 1. Voltage clamp responses of an active, isolated fiber ($\bar{g}_{\text{Na}} = 10$; $g_{\text{K}} = 0.5 \text{ mS/cm}^2$) at various configurations of external (R_s) and internal resistance (R_i) as given at the top of each column. Clamp step from resting potential (-72 mV) to -20 mV applied at zero time. First row: clamp current I (expressed as current per cm^2 of membrane in the test node, I_m); second row: transient Na current; third row: transmembrane potential; fourth row: membrane Na conductance as functions of time. All records in a row refer to the same calibration. The curves shown in the right column are replotted from Fig. 4 of a preceding paper (Solchenbach et al. 1986). In calculations with $R_i = 200 \Omega\text{cm}$, different positions (in millimeters) along the fiber are labelled by $x = 0$ (left end), $x = 0.1$ (middle), and $x = 0.2$ (right end of the test node). Sodium current and conductance as expected under ideal clamp conditions ($R_i = R_s = 0$) are shown by the dotted curves in (B) and (D). The dashed curves in (F) and (J) represent the mean Na current density of the test node. The interrupted horizontal lines in (C), (G), and (K) indicate the command potential.

in (G) as compared to (C). The observation that the voltage overshoot increases, or the driving force ($V_m - E_{Na}$) decreases with increasing distance from the I-end (G) is reflected by a decrease of peak I_{Na} along the test node (F). The normalized sum of the Na currents from all segments (dashed curve in Fig. 1 F) is essentially the same as the current curve in (B) (full line) and thus smaller than the control (dotted curve in (B)). This is in contrast to the results shown in Fig. 1 J where, in the presence of internal resistivity alone, normalized I_{Na} exceeds the control current. Similar effects of R_s and R_i on current size are seen when inward component of clamp current rather than the pure Na current is considered: peak inward current is distinctly smaller than the control ($88 \mu A/cm^2$ referred to zero line) in (A); it is slightly increased but still below the control in (E) and far above the control in (I).

Voltage clamp simulations as shown in Fig. 1 were performed at clamp potentials between -50 and 50 mV. In Fig. 2 A the clamp current records obtained in the presence of both external and internal resistance are shown. In all records a smooth downward deflection is seen resembling an Na inward current wave. At first sight, the configuration of the current curves looks similar to what one would expect from an adequate voltage control. A detailed inspection, however, reveals typical aberrations from ideal space clamping. Fig. 2 B shows the peak inward current-voltage relation taken from Fig. 2 A together with the corresponding relation obtained for R_s alone and a plot of peak I_{Na} under ideal clamp conditions. With $R_i = 0$, a strong depressing effect of series resistance on peak inward current, I_p , is seen at any clamp potential below 40 mV. Over the ascending limb of the $c-v$ relation, the reduction is about 50% as compared to the controls. This is the same order of reduction as observed in the continuous model of a fiber bundle (Haas and Brommundt 1980, Fig. 9 B). A further typical effect of series resistance is to shift the maximum I_p in the hyperpolarizing direction compared to the ideal $c-v$ relation (cf. Kootsey and Johnson 1972). When the external resistance is combined with internal resistivity, peak inward current increases at any potential between -40 and 40 mV but it still is distinctly smaller than the control one. An increase by about 20% in I_p by R_i in the presence of a series resistance is less than expected since internal resistivity alone, in the absence of a series resistance, causes a marked increase in I_p against the control at all potentials tested (e.g. 185 vs. $122 \mu A/cm^2$ at $E_c = -10$ mV). Thus the alteration of I_p due to a combination of external and internal resistance is not a simple superposition of the respective separate effects. In both $c-v$ relations with $R_s = 7 M\Omega$, the apparent reversal potential of I_p is somewhat above the Na equilibrium potential, $E_{Na} = 43$ mV. The main reason for this error is that, in the presence of a series resistance, depolarization of the fiber membrane develops with a distinct delay after the onset of a clamp (cf. Fig. 1 C and G). Since time to peak Na conductance decreases with increasing

clamp steps, peak inward current at $E_c = 43$ mV is to be measured at a time when the driving force, $(V_m - E_{Na})$, is negative.

Fig. 3 shows a set of records analogous to those shown in Fig. 1, the only modifications being a 5-fold increase of Na conductance ($\bar{g}_{Na} = 50$ mS/cm²) and

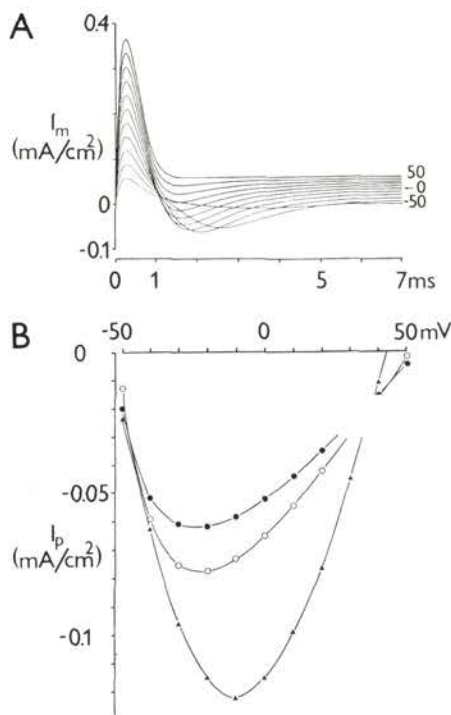


Fig. 2. (A) Family of clamp currents associated with various depolarizations (10 mV steps); simulation of a fiber with external and internal resistance; $R_s = 7$ M Ω ; $R_i = 200$ Ω cm; $\bar{g}_{Na} = 10$ mS/cm². Abscissa: time after onset of clamp; ordinate: clamp current divided by membrane area of test node. Numbers at the curves indicate the clamp potential (in millivolts). (B) Current-voltage diagrams for peak early inward current, I_p , of a fiber with series resistance in the presence (○) or absence (●) of internal resistivity and for peak I_{Na} of a free patch of membrane (▲). I_p data refer to the respective steady outward currents. The case of a fiber with $R_s = 0$ and $R_i = 200$ Ω cm was omitted; in this case, peak inward currents were larger than the control values (cf. Fig. 5 B of a preceding paper (Solchenbach et al. 1986)).

a lowered series resistance ($R_s = 3$ rather than 7 M Ω). With the series resistance alone (left column) a pronounced voltage overshoot of ≈ 34 mV relative to the command level is observed during the phase of transient inward current; it is followed by a very small hyperpolarization during outward current flow (Fig. 3 C). Actually, the voltage overshoot means an abortive action potential (cf. Kootsey and Johnson 1972, Fig. 2). Time course of Na conductance increase is slightly delayed, and peak Na conductance is elevated as compared to the control (12 vs. 9.2 mS/cm², Fig. 3 D). The excess in Na conductance, however, is overbalanced by a decrease in driving force, $(V_m - E_{Na})$, so that peak Na inward current is distinctly smaller than the control one (B). These effects are in principle the same as, but more accentuated than, those seen in Fig. 1 A–D. With $R_i = 200$ Ω cm (Fig. 3 E–L) the higher level of Na conductance results in a stronger manifestation of cable complications, i.e. membrane current and

voltage inhomogeneities along the test node. Clearly, the voltage, current, and conductance tracings in the middle column of Fig. 3 look much like those in the right column. (This is different from Fig. 1 where the middle column resembles the left rather than the right one.) Most of the features seen with R_i alone are also encountered, in an attenuated and smoothed fashion, in the presence of R_i plus R_s , e.g. the damped voltage oscillations associated with the onset of the clamp (G , K) or the occurrence of two transients in the Na conductance change (H , L) and in the Na current (F , J) at the I-end of the test node. A second inward current wave is also seen in the clamp current record (I) but is barely discernible in (E).

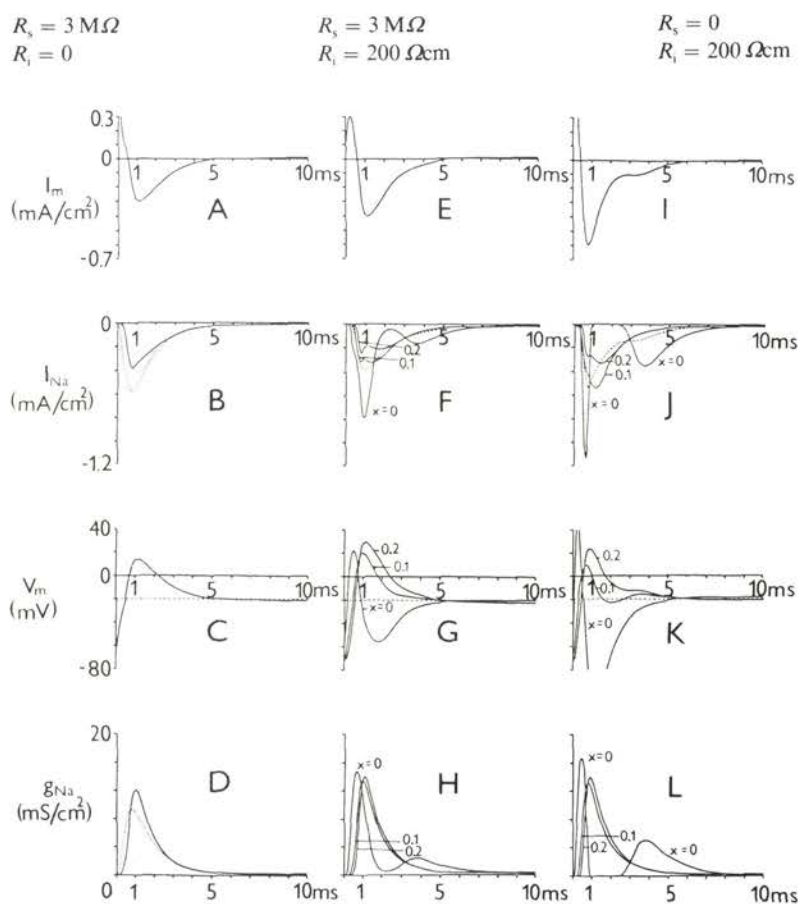


Fig. 3. Voltage clamp responses of a fiber calculated for a membrane Na conductance $\bar{g}_{Na} = 50 \text{ mS/cm}^2$. Same arrangement of the records as in Fig. 1. The curves in (I – L) were taken from Fig. 7 of a preceding paper (for details, see Solchenbach et al. 1986). A portion of the downward voltage deflection related to $x = 0$ in (K) is off scale.

Fig. 4 *A* shows the clamp current records obtained with step commands to potentials between -50 and 50 mV in the presence of R_s plus R_i . Again, the early inward currents resemble the conventional pattern. A small, second inward current wave is observed with depolarizations up to -30 mV. Fig. 4 *B* illustrates the influence of external and internal resistance on peak early inward current-voltage relation. The alterations due to R_s are similar to, but more pronounced than, those seen in Fig. 2 *B*. As compared to the control curve, I_p is reduced to about 40 % at strong depolarizations and maximum I_p is strongly shifted in the hyperpolarizing direction. Because of this shift there is a small range of potentials at which I_p is larger than the respective controls. When R_s is in conjunction with R_i , I_p increases as compared to currents observed with R_s alone, but it still is distinctly smaller than in the controls at potentials between -30 and 40 mV.

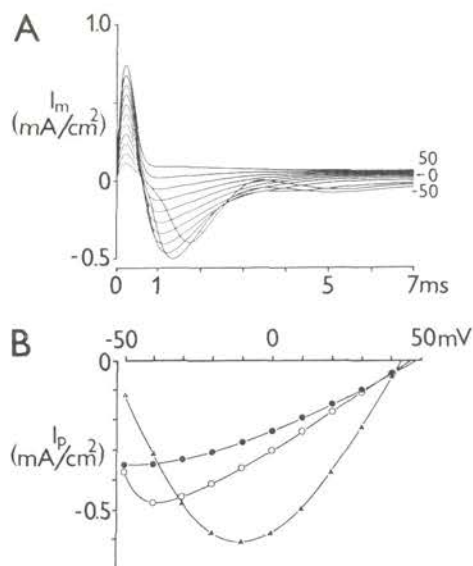


Fig. 4. (*A*) Family of clamp currents, or membrane currents, based on simulations with $R_s = 3 \text{ M}\Omega$, $R_i = 200 \text{ }\Omega\text{cm}$, $\bar{g}_{\text{Na}} = 50 \text{ mS/cm}^2$. (*B*) Current-voltage diagrams for early inward current; same arrangement as in Fig. 2 *B*.

In Fig. 5 records obtained with $\bar{g}_{\text{Na}} = 120 \text{ mS/cm}^2$ and $R_s = 1.8 \text{ M}\Omega$ are shown. The errors introduced by the series resistance (*A*—*D*) are readily understood. Voltage overshoot during inward current flow is ≈ 42 mV (*C*) and peak inward current is reduced to about half the control value (*B*). Cable complications are manifest in an extreme manner (*E*—*L*). As expected, axial resistivity causes a very strong hyperpolarization at the left end of the fiber ($x = 0$) during the flow of a large inward current. The right end ($x = d$) undergoes a depolarization which approaches the Na equilibrium potential, E_{Na} ; the time course of V_d during the first three milliseconds is essentially an uncontrolled action potential (*G* and *K*). (It should be noted that a loss of membrane voltage control is of different origin in the two cases: In the presence of a series resistance (*G*) the

monitored potential E is well controlled but the transmembrane potential V_d strongly deviates from E because of a voltage drop across R_s whereas in the absence of R_s (K) the feedback control itself is imperfect.) In a formal sense, a given time course of V_d may be considered as a specification of the boundary values for the cable equation which describes the electrical behavior of the fiber in the test node. The boundary values of membrane potential, together with the inherent boundary condition $\partial V_m / \partial x = -V_d q/b$, determine the response of the fiber in the segments away from the V-end (cf. eqs. (1) and (2) of the preceding paper (Solchenbach et al. 1986)). This explains the observation that the tracings shown in ($E-H$) are almost identical to the respective curves in ($I-L$).

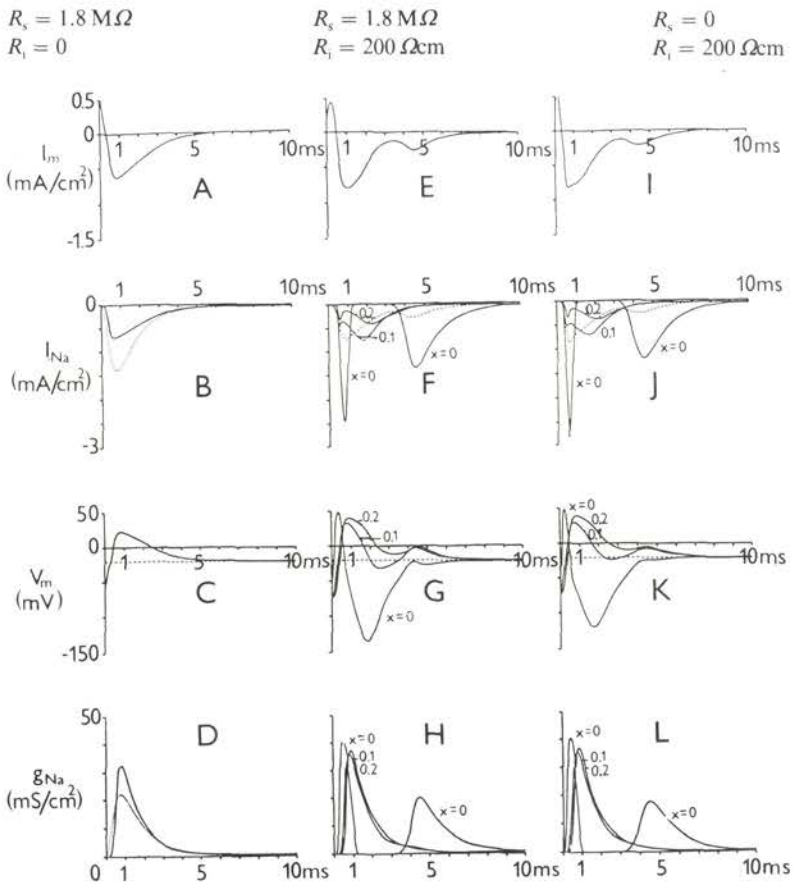


Fig. 5. Voltage clamp simulations for a fiber with $\bar{g}_{Na} = 120 \text{ mS/cm}^2$. Same arrangement of the curves as in Fig. 1. The records in ($I-L$) were re-plotted from Fig. 8 of a preceding paper (Solchenbach et al. 1986).

Discussion

In the present study a single fiber with an appropriate external resistance in series to the fiber membrane was used as a model for voltage-clamped multifiber trabeculae. The unavoidable imperfections of voltage clamping due to cable properties and series resistance are best recognized by considering a preparation with passive properties only, e.g. a fiber subjected to small de- or hyperpolarizations from the resting level which do not significantly alter the state of the membrane. In this case, the current response of the fiber can be followed by analytical methods. For simplicity, we assume a perfect external insulation in the two sucrose regions of the voltage clamp circuit shown in Fig. 2 of the preceding paper (Solchenbach et al. 1986), i.e. we consider the fiber in the test node as a finite cable with sealed ends with current injected at one end, $x = 0$. The steady values of clamp current I and monitored potential E are then related by

$$I = \frac{E - E_{\text{rev}}}{R_s + r_i \lambda / \sinh(d/\lambda)} \quad (1)$$

where E_{rev} is the reversal potential of the membrane, r_i the internal resistance per unit length, $\lambda = \sqrt{r_m/r_i}$ the length constant of the fiber, and d the length of the test node. Eq. (1) is directly derived from the cable equation and appropriate boundary conditions. The denominator of the right-hand side of eq. (1) may be understood as the effective resistance of the preparation. It is the sum of the external resistance, R_s , plus the term $r_i \lambda / \sinh(d/\lambda)$; the latter depends on cable properties only and may be considered as the intrinsic resistance of the fiber, R_{int} . Obviously the intrinsic resistance decreases with increasing values of r_i or decreasing values of r_m . For sufficiently low values of r_i , R_{int} approaches the membrane resistance of the test node, $\bar{R}_m = r_m/d$, so that

$$I = (E - E_{\text{rev}})/(R_s + \bar{R}_m). \quad (1a)$$

This is the well-known c - v relation for an isopotential membrane patch with a resistance in series to the membrane (cf. Kootsey and Johnson 1972). With increasing values of r_i , R_{int} becomes smaller than the membrane resistance. The decrease of R_{int} is equivalent to the development of an axial voltage gradient which increases the driving force for membrane current in the fiber segments distant from the V-end. For large values of r_i , i.e. small values of λ , $R_{\text{int}} \rightarrow 0$ and

$$I \rightarrow (E - E_{\text{rev}})/R_s. \quad (1b)$$

(Note that the dependence of the intrinsic resistance on r_i is just opposite to the behavior of the input resistance as defined by conventional cable theory. The difference is due to the fact that R_{int} refers to the potential at the far end of the

preparation, $x = d$, while the input resistance is related to the potential at the point of current injection, $x = 0$.)

A similar interpretation may be given for the active state of the membrane. A closer inspection of Fig. 1, 3, and 5 $E-L$ shows that the delay time for propagation of Na conductance increase along the test node is short relative to the duration of the activated state itself, and the size of activation does not drastically differ in different fiber segments. This is true for the greater part of the test node with the exception of the very I-end. In spite of a distinct voltage gradient along the fiber, the nodal membrane has a nearly uniform conductance, g_p , at a time when the inward component of clamp current, or total membrane current, reaches its peak. (At first sight, an appreciable amount of Na conductance at $x = 0$ would seem to conflict with the hyperpolarization that develops during a strong inward current flow. It should be noted, however, that a hyperpolarization at the I-end is preceded by an excess depolarization which gives rise to a strong activation of the Na system, and that the deactivation of Na conductance due to hyperpolarization is not complete at peak time of total inward current, cf. Fig. 3 E and H .) For simplicity, we neglect a capacitive component of clamp current which seems justified with higher values of Na conductance (Fig. 3 and 5). With these assumptions, current and voltage distribution along the fiber at peak time will be similar to the steady response of a finite cable with constant membrane conductance, g_p , and eq. (1) is expected to hold again, with appropriate values of E_{rev} and λ . To check the validity of this concept, let us consider the records shown in Fig. 3 $I-L$ ($\bar{g}_{Na} = 50 \text{ mS/cm}^{-2}$; $R_s = 0$; $E_c = -20 \text{ mV}$). From the time courses of Na conductance related to the midpoint and the right end of the test node (L) peak Na conductance may be assigned a mean value of $\simeq 13 \text{ mS/cm}^2$ which, together with $g_K = 0.5$, gives $g_p \simeq 13.5 \text{ mS/cm}^2$ and $E_{rev} \simeq 39 \text{ mV}$. With $a = 3 \times 10^{-4} \text{ cm}$; $r_i = 708 \text{ M}\Omega/\text{cm}$ length constant $\lambda_p = \sqrt{1/(2\pi a r_i g_p)}$ reaches $\simeq 0.0075 \text{ cm}$. The membrane potential at the V-end is poorly controlled and E is $\simeq 23 \text{ mV}$ at peak time (K). With the above data and $d = 0.02 \text{ cm}$, eq. (1) yields a theoretical value of $I_p \simeq 22 \text{ nA}$ which is close to the actual value (peak inward current density shown in Fig. 3 I , 0.59 mA/cm^2 , multiplied by the area of nodal membrane, $3.77 \times 10^{-5} \text{ cm}^2$). An approximate coincidence between theoretical and actual values of peak inward current is also obtained when the records of Fig. 3 $E-H$ ($R_s = 3 \text{ M}\Omega$) are analyzed. Thus the current-voltage relation predicted by eq. (1) would seem to hold for an active fiber with a reasonable accuracy.

In particular, eq. (1) may be applied to strong depolarizations underlying the positive limb of the peak inward current-voltage relation. Under ideal clamp conditions, the slope conductance of the positive region is essentially equivalent to the limiting Na conductance, g'_{Na} , i.e. the maximum value of Na conductance that can be reached by depolarization from the resting level. Assuming that in

our single fiber model all segments of the fiber undergo a rapid, sufficiently strong depolarization, any segment will reach a peak Na conductance near g'_{Na} . Neglecting the non-Na currents, g'_{Na} may be taken as an approximative value for g_p . In the presence of a large series resistance, the monitored potential E is well controlled throughout a clamp so that $E \simeq E_c$ and a conventional peak inward current-voltage diagram may be constructed. According to eq. (1), then, the apparent positive slope resistance for peak inward current density is

$$R_{s1} = A\{R_s + r_i\lambda'/\sinh(d/\lambda')\} = A\{R_s + R'_{\text{int}}\} \quad (2)$$

where A is the area of nodal membrane, λ' the length constant, and R'_{int} the intrinsic resistance of the cable related to the fully activated state of the membrane. This formulation allows a simple interpretation of the current-voltage relations shown in Fig. 2 *B* and 4 *B*. With $\bar{g}_{\text{Na}} = 50 \text{ mS/cm}^2$ (Fig. 4 *B*) the limiting membrane resistance, $1/g'_{\text{Na}}$, as derived from H-H kinetics is $\simeq 61 \Omega\text{cm}^2$. A lumped resistance (R_s) of $3 \text{ M}\Omega$ corresponds to a specific resistance (AR_s) of $113 \Omega\text{cm}^2$. For $R_i = 0$ the intrinsic resistance of the cable (AR'_{int}) approaches the limiting resistance of the membrane (cf. eq. (1a)). With $R_s = 3 \text{ M}\Omega$; $R_i = 0$, then, the apparent slope conductance will be related to the true conductance, g'_{Na} , by $61/(113 + 61) = 0.35$. With $R_i = 200 \Omega\text{cm}$, the intrinsic resistance is reduced to $\simeq 16 \Omega\text{cm}^2$ so that the expected conductance ratio is $\simeq 61/(113 + 16) = 0.47$; that is, the depressing effect of the series resistance is attenuated by the cable properties but the slope conductance still is no more than about half the control value. This estimation roughly agrees with the actual c - v relations shown in Fig. 4 *B*. In experiments on a real fiber or fiber bundle, eq. (2) may be used to determine λ' from the measured slope conductance ($1/R_{s1}$) provided that the values of A , R_s , and r_i are known and the intrinsic cable resistance is not too small compared to the series resistance. (The value of series resistance may be estimated e.g. from the voltage jump in response to a sudden release of voltage clamp at the time of peak inward current; cf. Tarr and Trank 1971.) In a second step g'_{Na} may be calculated from λ' .

Finally, it may be interesting to compare the double sucrose gap arrangement considered in this study with another technique which has been developed in recent years, i.e. whole-cell clamping by means of a patch pipette (for review, see Marty and Neher 1983). In the whole-cell configuration, one pipette may be used for both voltage recording and current injection on an isolated cell when a membrane patch is broken and a "gigaseal" has been established between the pipette rim and the membrane surface. The electrical resistance of a patch pipette is of the order of several $\text{M}\Omega$, depending on the taper angle and the diameter of the tip opening, and may largely be compensated for electronically, say to 90 %. The residual resistance, e.g. $200 \text{ k}\Omega$, is in series to the cell membrane and it still is sufficient to cause an appreciable voltage drop when the clamp

current exceeds 10 nA. Currents of that size are expected to occur in many types of excitable cells. Fig. 6 shows current and voltage responses calculated for a thin fiber (6 μm diameter, which applies e.g. to frog atrial cells) and for a thicker fiber (20 μm diameter, corresponding to myocytes from guinea-pig ventricle). Obviously, the current and voltage traces exhibit much less distortion than seen with the double sucrose gap (Fig. 5). In the case of the thin fiber (left panel of Fig. 6), voltage control is acceptable at the site of pipette impalement ($x = 0$);

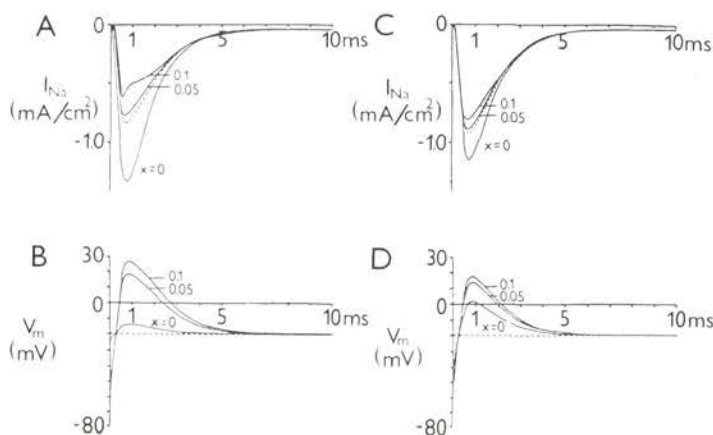


Fig. 6. Voltage clamp of an isolated cylindrical cell (an active fiber) using a patch pipette. Cell length 200 μm ; cell diameter 6 μm (A, B) or 20 μm (C, D). The ends of the cell are thought to be perfectly sealed. Site of impalement is the center of the cell. Effective pipette resistance is taken to be 200 $\text{k}\Omega$. Membrane parameters are $C_m = 2 \mu\text{F}/\text{cm}^2$; $g_K = 0.5$, $\bar{g}_{Na} = 120 \text{ mS}/\text{cm}^2$; $E_K = -74.5$, $E_{Na} = 43 \text{ mV}$. Internal resistivity of the fiber was set to 200 Ωcm ; extracellular resistance has been neglected. Clamp step from resting potential (-72 mV) to -20 mV . *Upper row:* transient Na current at the center of the fiber ($x = 0$) and at distances of 0.05 or 0.1 mm from the center (full lines); mean Na current density of the fiber (dashed line). *Lower row:* time course of membrane potential at different positions.

the membrane potential overshoots the command level by about 6 mV. Much larger voltage deviations are seen in the remote segments of the fiber, indicating a large voltage drop along the fiber due to axial current flow. The voltage deviations are equivalent to a decrease of the driving force for Na ions, ($V_m - E_{Na}$), compared to a proper clamp, and are reflected by a decrease of peak I_{Na} with increasing distance from the pipette. Mean inward current density reaches 0.83 mA/cm^2 which is about 60 % of peak inward current under ideal clamp conditions. The thicker fiber (right panel), with larger clamp currents, shows a distinct loss of voltage control at $x = 0$. On the other hand, cable complications are less pronounced than with the thin fiber, i.e. the voltage gradient along the fiber during the phase of inward current flow is reduced. This

is what one might expect from a larger "space constant" of the thicker fiber. In conclusion, even the patch clamp method, though much better defined than the double sucrose gap arrangement, does not ideally suit for measurements of fast Na inward current.

Appendix

Leakage current in a sucrose gap

In the sucrose gap model used in this study the access to the test compartment is represented by two resistive pathways in parallel, R_{ax} and R_{sh} , and leakage current (= that part of clamp current which does not cross the membrane of the test node) is identical to the current which flows through the sucrose (cf. Fig. 2 of the preceding paper (Solchenbach et al. 1986)). The size of leakage current is mainly determined by the $R_{ax}/(R_{sh} + R_{ax})$ ratio which is $\approx 5\%$ in our case. A refinement of the model is obtained by assuming that leakage current and axial current are not separated but exchange across the fiber membrane in the sucrose regions (McGuigan 1974, Appendix by McGuigan and Tsien; Beeler and McGuigan 1978; Jirounek et al. 1981; Pooler and Valenzano 1983). For the sake of simplicity, the transmembrane current may be considered as a pure ionic current, neglecting the capacitive component. The characteristics of the membrane under the sucrose is unknown. As a rough approximation, a membrane patch may be represented by a constant resistance in series to a constant electromotive force, E_{suc} . The length constant of the fiber in the sucrose regions is $\lambda_{suc} = \sqrt{r_{m, suc}/(r_i + r_{suc})}$ where $r_{m, suc}$ is the membrane resistance and r_{suc} the external longitudinal resistance related to one unit length of the gap. Provided that $r_{m, suc}$ is of similar size as the resting membrane resistance under normal conditions, λ_{suc} will be considerably shorter than the length constant in the test compartment, $\lambda = \sqrt{r_m/r_i}$, and very small as compared to the length of the gap, b .

Another factor to be considered is a liquid junction potential at the sucrose-saline boundaries (Blaustein and Goldman 1966; Lammel 1981). It is due to a disparity between cationic and anionic mobilities. When Tyrode or Ringer solution is used for perfusion of the central pool, Na^+ and Cl^- are the predominant ions and, because of a larger mobility of the Cl^- ions, the liquid junction potential, ΔU_o , (taken as saline minus sucrose potential) will be positive. On the other hand, a liquid junction potential in the side pools will be negligibly small since K^+ and Cl^- ions do not differ much in their mobility.

When the cable parameters of the fiber in the sucrose regions (λ_{suc} , E_{suc}) and a liquid junction potential at the sucrose-test solution interface are introduced into the model, the boundary conditions for the fiber in the test node must be

modified against the original notation given in the preceding paper (Solchenbach et al. 1986). Now, we have

$$\partial V_m / \partial x_{(x=d-0)} = -(V_d + \Delta U_o - E_{suc})q / \lambda_{suc}; \quad (3)$$

$$\partial V_m / \partial x_{(x=0+0)} = (V_o + \Delta U_o - E_{suc})q / \lambda_{suc} - IR_p / b; \quad (4)$$

furthermore

$$I = \{\Phi - V_o(1 - q) + \Delta U_o q\} / (R_p + R_s); \quad (5)$$

$$E = IR_s + V_d(1 - q) - \Delta U_o q. \quad (6)$$

These relations are the substitutes for the original eqs. (2), (3), (4), and (7), respectively. Total leakage current is the sum the current injected into the extracellular pathway and the transmembrane current related to the two sucrose regions. The injected current is a fixed fraction of clamp current, $IR_{ax} / (R_{sh} + R_{ax})$. The transmembrane leakage current may be written as

$$I_{leak, tr} = (\lambda_{suc} / r_{m, suc}) \{V_o + V_d + 2(\Delta U_o - E_{suc})\} \quad (7)$$

(cf. eq. (20) in McGuigan 1974).

We ran a few calculations in order to check to which extent the above modifications of the model would affect the results. According to Blaustein and Goldman (1966) the liquid junction potential at the sucrose/Ringer interface is expected to be ≈ 10 mV. The equilibrium potential E_{suc} is likely to be more negative than the normal resting potential since the ratio of internal to external potassium is increased under the sucrose. Thus we took the term $(\Delta U_o - E_{suc})$ as 100 mV. Assuming that $R_{m, suc} \approx R_m = 2 \text{ k}\Omega \text{ cm}^2$ and $r_i / r_{suc} = 1/20$, the length constant λ_{suc} , calculated for a fiber of $6 \text{ }\mu\text{m}$ diameter, reaches $\approx 80 \text{ }\mu\text{m}$. The above values were used in voltage clamp simulations with $\bar{g}_{Na} = 10$ or 120 mS/cm^2 . A comparison with earlier computations showed that the dynamic response of the fiber was little affected by the modification of the model. Peak sodium inward current decreased by some few percent with a low sodium conductance, and remained practically unaltered with a high value of $\bar{g}_{Na}$. In contrast, the steady component of clamp current markedly increased in the modified model. This was due to a positive transmembrane leakage current which was of the same order as the potassium current flow across the nodal membrane. The size of leakage current is easy to check from eq. (7). With near zero level depolarizations, the leakage current is mainly determined by the voltage term $2(\Delta U_o - E_{suc})$. With the values given above, the term $2(\lambda_{suc} / r_{m, suc})(\Delta U_o - E_{suc})$ reaches $\approx 1.6 \text{ nA}$. On the other hand, the potassium current in the test node at $E_c = 0$ is $\approx 1.4 \text{ nA}$.

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