DIFFUSION OF IONS IN MYELINATED NERVE FIBERS

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ABSTRACT The diffusion of ions towards or away from the inner side of the nodal membrane in preparations, the cut ends of which are placed in various media, was investigated. The ion concentration changes were calculated by numerical solution of the unidimensional electrodiffusion equation under a variety of media compositions, axoplasmic diffusion coefficients, and internal anionic compositions. The potassium and cesium ion diffusion along the axon towards the node was determined experimentally by two different electrophysiological methods. On the basis of comparison between the experimental data and the computational predictions the axoplasmic potassium ion diffusion coefficient was determined to be almost equal to that in free aqueous solution, while that of cesium ion was close to one half of that in aqueous solution. Utilizing the values of diffusion parameters thus determined, we solved the electrodiffusion equation for a number of common experimental procedures. We found that in short fibers, cut 0.1–0.2 cm at each side of the node, the concentration approached values close to the new steady-state values within 5–30 min. In long fibers (over 1 cm long) steady-state concentrations were obtained only after a few hours. Under some conditions the internal concentrations transiently overshot the steady-state values. The diffusion potentials generated in the system were also evaluated. The ion concentration changes and generation of diffusion potential cannot be prevented by using side pools with cation content identical to that of the axoplasm.

INTRODUCTION

Most electrophysiological studies on myelinated nerve fibers are carried out on excised fibers placed in artificial media. In such experiments, which include voltage clamp procedures, the cut ends of the fiber are in direct contact with an electrolyte solution whose composition is different from that of the axoplasm. The concentration differences between the axoplasm and electrolyte solutions around the cut ends result in diffusion of constituents into and out of the fiber. Thus the axoplasmic composition changes as a function of time and distance from the cut ends. Obviously, these concentration changes affect the electrical activity of the nodal membrane and must be considered in any analysis of membrane properties and activity studied with excised fibers.

The magnitude of the diffusion effect is a function of fiber length. While some single-fiber experiments used long preparations (over 1 cm) (Frankenhaeuser, 1957*a*;

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Stämpfli, 1952), recent voltage clamp techniques use fibers only 0.2–0.5 cm long (Frankenhaeuser, 1962; Nonner, 1969; Hille, 1971).

In a typical voltage clamp experiment the node under investigation is externally perfused with the test solution, while the two cut ends are immersed in side pools filled with a different solution. The most common solutions used in the side pools are: isotonic KCl (117–120 mM) (Frankenhaeuser, 1962; Nonner, 1969; Hille, 1971), amino acids, etc. However, sometimes Ringer's solution containing local anesthetics was also used (Dodge and Frankenhaeuser, 1958; Frankenhaeuser, 1957*b*; Stämpfli, 1969).

Recently, a number of investigators have purposefully used the above diffusion process to introduce substances such as Na^+, Ca^{++}, tetraethylammonium ion (TEA^+), Cs^+, IO_3^- (Koppenhöfer and Vogel, 1969; Hille, 1971; Armstrong and Hille, 1972; Stämpfli, 1974; Dubois and Bergman, 1975*a*; Frankenhaeuser and Århem, 1975; Århem, 1976), from the side pools to the inner side of the nodal membrane. However, detailed quantitative attempts to determine the penetration rates, taking into consideration all factors affecting the diffusion, have not been made.

It is the purpose of this study to investigate experimentally and by appropriate computations the axoplasmic ion concentration changes resulting from the diffusion of electrolytes into and out of the cut ends of myelinated nerve fibers. The ion concentration changes, at the inner side of the nodal membrane, will be computed by using the electrodiffusion equations for the given multionic system. An attempt will be made to determine experimentally the values of those parameters required for the computations that are not well known, mainly the ionic diffusion coefficients in the axoplasm.

METHODS

Analytical

The diffusion of substances into and out of the axoplasm, through the cut ends of a myelinated fiber, is assumed to be a longitudinal movement of solutes in an isotropic cylinder with impermeable walls, cut at both ends. Radial diffusion is neglected since the internodal myelinated membrane is practically impermeable to solutes, and the flux through the nodal membrane is also negligible because of its relatively low permeability and its small surface area. Such a movement of electrolytes is described properly by the unidimensional electrodiffusion equation. Note that the diffusion may result in an osmotic gradient along the axoplasm. However, the calculated water movement, due to this effect, is negligible because of its relatively low permeability and the small surface area.

The electrodiffusion equation can be solved numerically by means of the finite differences approximations method (Crank, 1957). In such a procedure the diffusion path, X, i.e. the fiber length, is divided into equal segments, δX.

The flux of ion i, J_i, from segment $n\,\delta X$ to segment $(n+1)\delta X$, due to electrochemical driving force, is:

$$J_{i(n\delta X \to (n+1)\delta X)} = D_i \frac{C_{i(n+1)} - C_{i(n)}}{\delta X} + \frac{Z_i F \bar{C}_i}{RT} \cdot \frac{\Delta\phi}{\delta X}, \qquad (1)$$

where D_i is the ion diffusion coefficient, $C_{i(n)}$, $C_{i(n+1)}$ are the ion concentrations at segments $n\,\delta X$ and $(n+1)\delta X$, respectively, Z_i is the ion charge, F is Faraday's constant, $\bar{C}_i$ is the average concentration between the two segments (assuming that the concentration changes linearly

between any two adjacent segments), R is the gas constant, T is the absolute temperature, and $\Delta\phi$ is the potential difference between the two segments ($\Delta\Phi \equiv \Phi_{(n+1)} - \Phi_{(n)}$).

The potential difference ("diffusion potential") between any two adjacent segments each containing ions with different mobilities is expressed, for multi-ionic systems, under the above described conditions, by Henderson's equation (Plonsey, 1969; Robinson and Stokes, 1959):

$$\Delta\phi = -\frac{RT}{F} \cdot \frac{\sum_{i=1}^{m} \frac{U_i}{Z_i}}{\sum_{i=1}^{m} U_i} \frac{(C_{i(n+1)} - C_{i(n)})}{(C_{i(n+1)} - C_{i(n)})} \ln \frac{\sum_{i=1}^{m} U_i C_{i(n+1)}}{\sum_{i=1}^{m} U_i C_{i(n)}}, \quad (2)$$

where m is the number of ions in the system and U_i is the mobility of ion i.

The diffusion process was calculated for fibers of various lengths and various initial values. Since the anionic content of the axoplasm has not been experimentally determined, the computations were carried out for two possible cases: in one the axoplasmic anions were assumed to be organic acids (e.g. amino acids, $D_{20,w}$[1] = 0.7 × 10^{-5} cm^2/s; see Conway, 1952) while in the other they were assumed to be polyelectrolytes (e.g. proteins, $D_{20,w}$ = 0.05 × 10^{-5} cm^2/s; see Sober, 1970). As the values of ionic diffusion coefficient in the axoplasm quoted in literature range from those in an aqueous solution ($D = D_w$) to half of those values ($D = D_w/2$) (Hodgkin and Keynes, 1953; Koppenhöfer and Vogel, 1969; Gilbert, 1975*b*; Frankenhaeuser and Århem, 1975), the computations were carried out with diffusion coefficient values in this range.

The diffusion coefficients in an infinitely diluted solution were determined from the limiting equivalent conductivities at the specific temperature (Robinson and Stokes, 1959), while the summated effects of ionic concentration, and the presence of other ions was estimated to amount to a 10% reduction in these values.

Experimental

Single myelinated fibers (12–18 μm diameter) were isolated from the frog *Rana esculenta*, mounted and voltage-clamped as described by Stämpfli (1952, 1969) and Nonner (1969).

Both the holding potential and command pulses were generated by a digital-to-analog converter under computer program control (Nonner et al., 1975). The node was externally perfused by Ringer's solution containing 300 nM tetrodotoxin (TTX). pH was adjusted to 7.4 by trishydroxymethyl aminomethane buffer (5 mM). The total length of the preparation before cutting was over 10 mm. Both ends of the fiber were cut while in isotonic KCl solution. The final total length of the cut fiber was 1.5–2 mm. The experimental temperature was held constant at 15°C.

After the fiber was cut, 10–15 min were allowed for equilibration before membrane currents were sampled at 20-μ s intervals by means of a 12-bit analog-to-digital converter operating also under program control.

Before each series of pulses, the membrane leakage conductance, g_L, was determined by means of a hyperpolarizing pulse. All currents were corrected for leakage, assuming a linear leakage current-voltage relationship that crosses the current axis at $V = 0$.

DETERMINATION OF POTASSIUM ION DIFFUSION The membrane was held at its initial resting potential value, V_H, as determined before the beginning of external perfusion with TTX-Ringer's solution, as follows: after a strong conditioning membrane hyperpolarization, the maximal peak sodium current $I_{Na_{max}}$, as well as the membrane depolarization that produces it, were determined. The membrane holding potential, V_H, was fixed at a value such that for the above depolarization (without a conditioning pulse) I_{Na} was 75% of $I_{Na_{max}}$

[1] $D_{20,w}$ is the diffusion coefficient in an aqueous solution at 20°C (Robinson and Stokes, 1959).

(Nonner, 1969). V_H is defined as zero and all values of the pulses are given relative to V_H. Depolarization is positive while hyperpolarization is negative. At intervals of 2 s a pair of pulses was applied: a conditioning prepulse (V_{pp}) and a test pulse (V_p). The prepulse amplitude, V_{pp}, was 120 mV and its duration 5 ms. The amplitude of the test pulses, V_p, which immediately followed it, varied from −60 to +100 mV, and their duration was 6 ms.

The current analysis focused on the so-called "tail currents" generated upon stepping membrane potential from a depolarizing V_{pp} to V_p. Since an adequate amount of TTX was used, the measured currents, when corrected for leak currents, were assumed to represent potassium currents, I_K. The initial value of the tail current, I^0_{tail}, was obtained by extrapolating the exponential portion of tail current, up to the instant of the step (V_{pp} to V_p). Plotting I^0_{tail} as a function of V_p gives essentially a linear current-voltage relationship (Fig. 5 C). The potassium current reversal potential values, V_K, were determined from the zero current crossing point of this current voltage relationship (Adelman et al., 1973). The potassium reversal potential values were monitored every 1–5 min up to 2 h after cutting the fiber.

The fibers investigated were first cut in side pools filled with isotonic KCl solution (phase 1); then the KCl solution was replaced by a test solution, such as Ringer's solution without calcium (phase 2) (maximal calcium concentration in the solutions was 2 μM), and finally the side pools were refilled with isotonic KCl solution (phase 3). The side pools were thoroughly washed with the new solution upon each solution change. Each phase lasted 20–40 min.

The membrane was held at the said V_H throughout the experiment, except during the process of side pool solution change. Special care was taken to minimize and monitor the voltage clamp feedback currents, which, if of sufficient amplitude, may induce ion concentration changes. Initially the currents were set to a negligible value by adjusting the bias potential at electrode E (Nonner, 1969) such that the potential at the output of the amplifier was zero. Note that the feedback current does not change upon switching from voltage to current clamp mode of operation. Hereafter the currents were monitored and, except during the clamp pulses, were in the reported experiments always less than $5 \cdot 10^{-11}$ A or $5 \cdot 10^{-16}$ mol/s. Taking into consideration the above value and the small difference between the fraction of inward current carried by potassium ions through the nodal membrane and the corresponding fraction in the axoplasm, we calculated that error introduced at the node by this ionic flow is negligible (less than 2% of the concentration change induced by the diffusional current in the first 30 min). Currents of an opposite direction are encountered only in "unnatural" side pool solutions, such as high-NaCl, low-KCl solutions. They result in a moving boundary leading the advance of NaCl-rich solution from the cut ends towards the node. However, even in a fiber cut 1 mm away from the node, it takes this boundary about 30 min to reach the nodal area. By this time the diffusional driven concentration changes studied here have practically reached steady state.

It has been reported that during membrane depolarization potassium ions accumulate at the external surface of the membrane (Adelman, et al., 1973; Dubois and Bergman, 1975*b*). However, since the conditioning prepulse amplitude and duration were the same for all the current measurements, the potassium ion concentration external to the node, $[K^+]_o$, was assumed to be the same during all test pulses. The actual $[K^+]_o$ value was determined experimentally by measuring $E_k(V_k)$ using the Nernst relationship (see Results). Obviously, for this determination one has to know the value of internal potassium ion concentration, $[K]_i$. These values were determined experimentally by a similar procedure when the effective $[K^+]_o$ was known; i.e. after a weak (20–50 mV) and brief (1–2 ms) depolarizing pulse. Under such conditions external potassium ion accumulation is negligible and thus the effective $[K^+]_o$ is practically equal to that of the external solution. Hereafter, the internal potassium concentration, $[K^+]_i$, was calculated by Nernst's equilibrium potential equation, using the experimentally determined values for E_K and $[K^+]_o$.

DETERMINATION OF THE DIFFUSION OF CESIUM IONS Cesium ions, when applied internally, attenuate outward-going potassium currents, I_K (Chandler and Meves, 1965; Dubois and Bergman, 1975). The magnitude of this attentuation is a function of both Cs^+ concentration

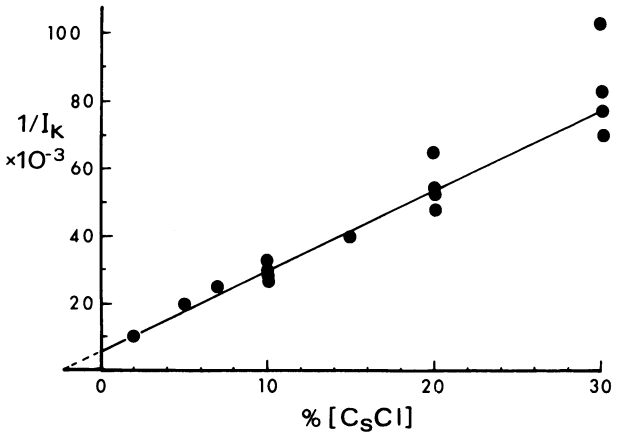

FIGURE 1 The reciprocal of potassium current relative amplitude as a function of the percentage of cesium in the axoplasm. Ordinate: $1/I_K$, where I_K is defined as the ratio between steady-state I_K measured in the test solution and I_{K_o} determined before the test solution was applied. Points represent pooled data obtained from 10 preparations.

and membrane potential. Given a I_K – Cs^+ dose-response relationship (for a given potential), one can determine the Cs^+ concentration at the inner side of the nodal membrane by measuring I_K.

Fig. 1 illustrates that the reciprocal of the steady-state I_K generated by a 150 mV depolarizing pulse is a linear function of $[Cs^+]$. Each point reflects the steady-state Cs^+ effect. This steady-state was determined by following the I_K attenuation by depolarizing test pulses given every 30–60 s. Usually the Cs^+ effect was complete within 10–15 min. This dose-response curve is typical of noncompetitive inhibition.

The diffusion of Cs^+ along the fiber from the side pools to the node was determined by measuring the steady-state potassium currents generated by the above depolarizing pulse at various time intervals after the isotonic KCl solution in the side pools was replaced by isotonic mixtures of KCl and CsCl. These I_K vs. time relationships were translated into $[Cs^+]$ (at the inner surface of the nodal membrane) vs. time relationships by means of the I_K vs. $[Cs^+]$ curve relationship of Fig. 1.

RESULTS

Computations

The ion concentrations at the inner side of the nodal membrane were computed as a function of time after cutting the fibers for various conditions which correspond to some of the commonly used experimental procedures. The side pool solutions chosen were: Ringer, isotonic KCl, and isotonic mixtures of KCl + CsCl and KCl + NaCl. As the lengths of preparations used varies, and as the axoplasmic composition and the ionic diffusion coefficients in the axoplasm were not accurately known, the computations were repeated for a wide range of these values. In all computations the ionic concentrations in the side pools were assumed to be constant, and the temperature 15°C. The node referred to in the calculations is assumed to be in the center of the cut fiber, unless noted otherwise.

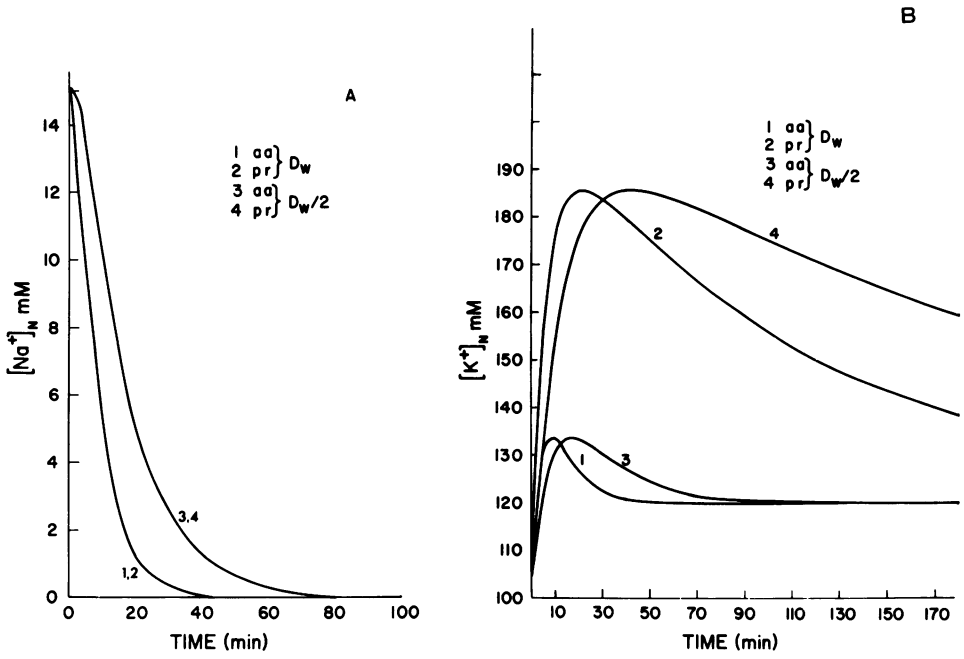

FIGURE 2 Sodium ion (A) and potassium ion (B) concentrations at the inner side of the nodal membrane computed as a function of time after the nerve is cut in isotonic KCl solution. The numbers along each curve refer to a different combination of internal ion composition and diffusion coefficient: *1.* the internal organic anions have properties typical for amino acids (aa) and the diffusion coefficient $D = D_w$; *2.* the internal organic anions have properties typical for proteins (pr) and $D = D_w$; *3.* the internal organic anions have properties typical for amino acids (aa) and the diffusion coefficient $D = D_w/2$; *4.* the internal organic anions have properties typical for proteins (pr) and the diffusion coefficient $D = D_w/2$. Note that in *A* curves 1 and 2, and curves 3 and 4 superimpose. Fiber length was 0.2 cm.

In the first set of computations the effects of axoplasmic ion diffusion coefficient values (relative to aqueous solution) and axoplasm anion composition on the internal ion concentration changes were investigated.

Fig. 2 illustrates the changes in sodium and potassium concentrations at the inner side of the nodal membrane ($[Na^+]_N$, $[K^+]_N$), as a function of time after the cut ends are exposed to isotonic KCl (in side pools). The figure gives the computed ion concentrations in a 0.2-cm fiber, i.e. the length commonly used today in voltage clamp experiments. The computations were repeated for a number of different conditions: (*a*) the diffusion coefficient (D) equals that in aqueous solution (D_w); (*b*) $D = D_w/2$; (*c*) the axoplasm is composed of amino acid-like anions (aa); (*d*) the axoplasm is composed of protein-like anions (pr). The internal anion valency was taken as unity: $Z = -1$. The initial internal sodium ion concentration, $[Na]_i$ (at $t = 0$) was assumed to be 15 mM. The choice of this value is based on Frankenhaeuser and Huxley's (1964) estimation that $[Na]_i$ = 13.7 mM, the Hille's (1972) and Koppenhofer and Vogel's (1969) estimation that internal sodium activity is about 11 mM. The initial internal potassium ion concentration $[K]_i$ was assumed to be 105 mM (see Stämpfli, 1952, and

Frankenhaeuser and Huxley, 1964). It is seen that the concentration changes are quite rapid: significant changes are obtained within 10 min. The rate of decrease of $[Na^+]_N$ is independent of the type of internal anions, but is markedly affected by the diffusion coefficient (Fig. 2 A). The rate of potassium concentration change is also strongly affected by the diffusion coefficient. But in contrast to the monotonously decreasing nodal sodium concentration, potassium concentration increases towards its steady-state value (which equals the side pools concentrations, i.e. 120 mM) with a marked overshoot (Fig. 2 B). The overshoot, which appears only initially after cutting the fiber, results from the large difference between the mobility of chloride ions and the mobilities of the internal organic anions; chloride ions move into the axoplasm much faster than organic anions move out of it. This increase in $[Cl]_i$ "drags" cations from side pools into the axoplasm and locally increases their concentration above the concentration of the side pools. Therefore, the larger the internal anions and the smaller their mobilities, the more cations will move into the axoplasm and the higher the amplitude of overshoot. (The overshoot is inversely proportional to the valency, z, of the internal organic anion.) This driving of cations against their concentration gradient is expressed by the flux equation (Eq. 1) when the electrical component is larger in magnitude than the chemical component and is of an opposite sign. This phenomenon does not reappear as the axoplasm is depleted of its organic anions and as the $[Cl]_i$ values approach steady state.

On the basis of the above and the comparison of the computed concentration changes with those determined experimentally (see below), most of the results selected for this presentation focus on computations based on the assumption that the axoplasmic ion diffusion coefficients of K^+, Na^+, and Cl^- are equal to those in aqueous solution ($D = D_w$) and internal anions behave like amino acids. The dependency of the nodal ion concentration on fiber length is given in Fig. 3. It is seen that for fibers cut close to the node (as commonly used now in voltage clamp systems), $[Na^+]_N$ decreases or increases rapidly when side pools contain isotonic KCl or Ringer's solution, respectively. Steady-state values are approached within about 20 min. In contrast, in a 1.0 cm fiber $[Na^+]_N$ reaches half its steady-state value only after about three h. The rates of changes in $[K^+]_N$ are also strongly affected by the fiber length: in isotonic KCl, $[K^+]_N$ peaks after 10 min and approaches steady state within 1 h in a 0.2-cm fiber, while 3 h pass before $[K^+]_N$ reaches its peak value in a 1.0-cm fiber. However, the amplitude of the overshoot is independent of fiber length. Similarly, when fibers are placed in Ringer's solution, internal KCl concentration is halved in less than 10 min in a 0.2-cm-long fiber, while 3 h pass before the concentration reaches similar values in a 1-cm-long fiber.

In the computations illustrated here, it was assumed that the internal organic anions are univalent. This assumption is reasonable for amino acids but may introduce an error if proteins are present to an appreciable extent, since the valency of the internal anions may affect the diffusion. The valency effect was investigated for proteins in the valency, Z, range of -1 to -10. It appears that the only effect of increasing Z was lowering the amplitude of the overshoot of $[K^+]_N$ when isotonic KCl solution was in the side pools and of $[Na^+]_N$ when Ringer's solution was in the side pools. For exam-

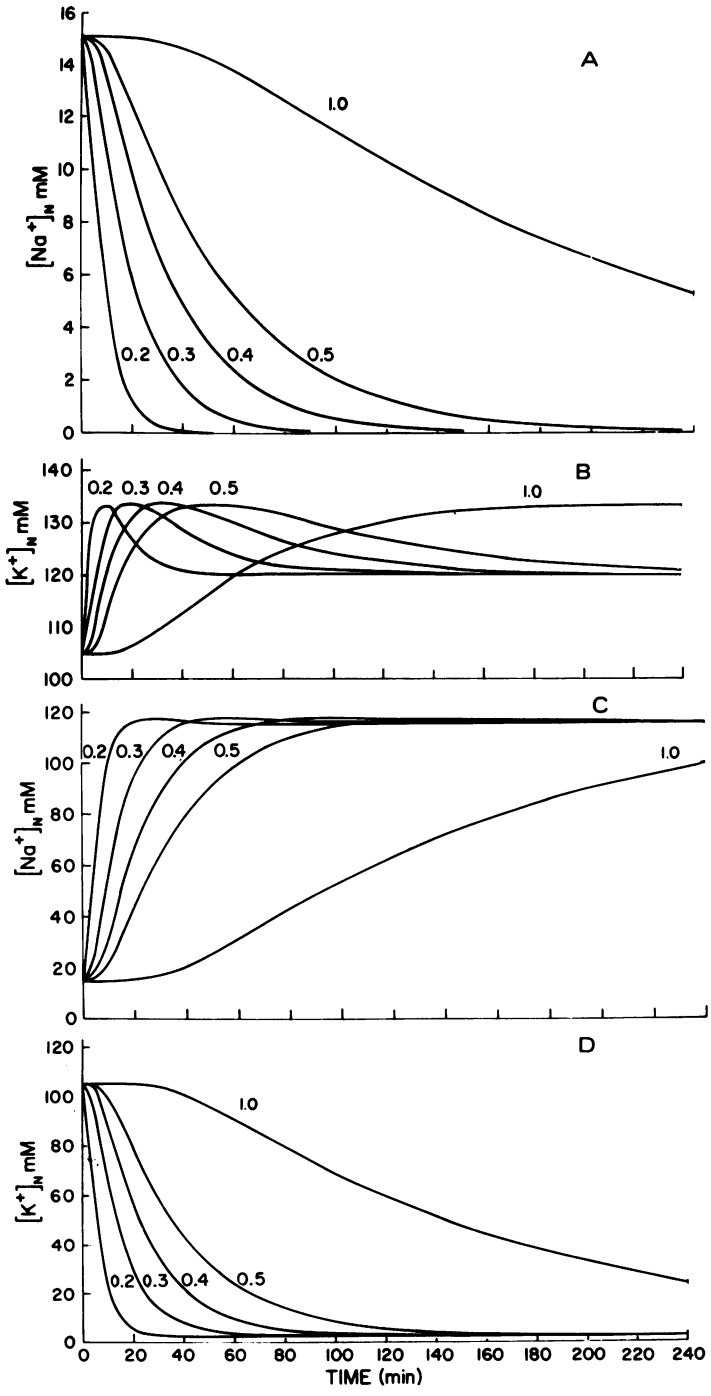

FIGURE 3 Sodium ion (A and C) and potassium ion (B and D) concentrations at the inner side of the nodal membrane computed as a function of time after cutting the fiber in isotonic KCl (A and B) or Ringer's solution (C and D). The numbers above each curve indicate fiber length (0.2 – 1.0 cm). The computations are based on the assumptions that the diffusion coefficients of ions in the axoplasm equal those in aqueous solution ($D = D_w$) and the internal organic anions behave like amino acids.

ple, computations using $Z = -10$ predict peak values 15% lower than those predicted using $Z = -1$.

From the above computed values the time limit for dissection and mounting of fibers of different lengths can be evaluated.

In most side pool solutions chloride is the major anion. However, it is generally accepted that most of the internal anions are amino acids or organic anions (Gilbert, 1975*b*). Therefore, there must be an exchange of anions across the cut ends. The chloride concentration changes were computed for a variety of experimental conditions. The changes in concentration with time were found to be similar to those of sodium under the same set of conditions. Therefore, one can get a reasonable approximation of the chloride ion changes in short fibers (up to 0.5 cm long) from the data presented in Fig. 3 for sodium. Thus, if we assume amino acids are the major internal anions, the chloride concentration approaches steady state within 10–60 min, depending on fiber length. If proteins were the major internal negative charges, the rates of diffusion would be negligible.

Normal Ringer's solution contains about 2 mM calcium ions while the axoplasm is practically free of Ca^{++}. Obviously, when side pools are filled with Ringer's solution, Ca^{++} diffuses into the fiber. Such Ca^{++} accumulation within the fiber may affect the membrane (Feltz et al., 1972; Meech, 1974; Frankenhaeuser and Århem, 1975). Were there no calcium binding or buffering elements in the axoplasm, the calcium concentration changes would be rapid; assuming $D = D_w$ and amino acids as the internal anions, $[Ca^{++}]_N$ increases above 1 mM within 3 min in short fibers (0.2 cm) and within 60 min in long fibers (1.0 cm).

Experimental

Fig. 4 illustrates changes in E_k, the potassium reversal potential (in absolute units, i.e. $E_k = V_k - 70$), as a function of time during a typical voltage clamp experiment.

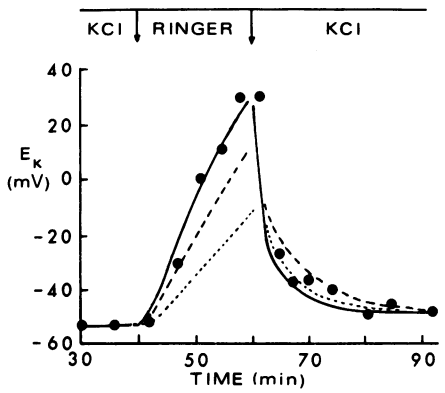

FIGURE 4 The computed and measured E_K values (symbols) as a function of time after changing the solution in the side pools. Each curve corresponds to a computation using a different diffusion coefficient value: $D = D_w$ – continuous curve, $D = \frac{3}{4} D_w$ coarsely interrupted curve, $D = \frac{1}{2} D_w$ finely interrupted curve. It is assumed that $[K^+]_o = 15$ mM (see Fig. 5B) and that the internal anions are a mixture of amino acids and protein-like anions in a 1:1 ratio. Initially the axon was in isotonic KCl for 40 min, then side pools were filled the Ringer's solution (for 20 min), and finally the side pools were refilled with isotonic KCl (Exp. 31/75).

In this experiment (75/31) the cut ends of the fiber were first placed in isotonic KCl for 40 min (phase I), then in Ringer's solution (phase II), and finally returned to isotonic KCl (phase III). The fiber length was 1.9 mm, the node being 0.65 mm and 1.25 mm from the cut ends. The computational predictions were carried out for this special case of asymmetric dimensions. The points are the experimentally determined E_k values (see Methods).

The continuous line gives the E_k values computed under the assumption that the ionic diffusion coefficient values (D) are those in an aqueous solution (D_w). The coarsely and finely interrupted lines are the E_k values computed under the assumption that $D = 0.75\ D_w$ and $D = 0.5\ D_w$, respectively.

On the whole the experimental points are seen to agree well with the E_k changes computed assuming $D = D_w$. However, both at the initial segment of phase II and during phase III the experimental results seem to agree best with $D = 0.5\ D_w - 0.75\ D_w$. Note, however, that in these regions the differences between the three computed curves are minimal. Note also that phase I is equivalent to the tail end of phase III.

Fig. 5A illustrates that the experimental data obtained upon cutting the nerve in isotonic KCl reasonably fit computations based on the assumption that the axoplasmic anions behave like amino acids rather than proteins.

Since the overshoot appears only immediately after cutting the fiber, it is barely noticeable in the short preparations used by the time the experimental initiation proce-

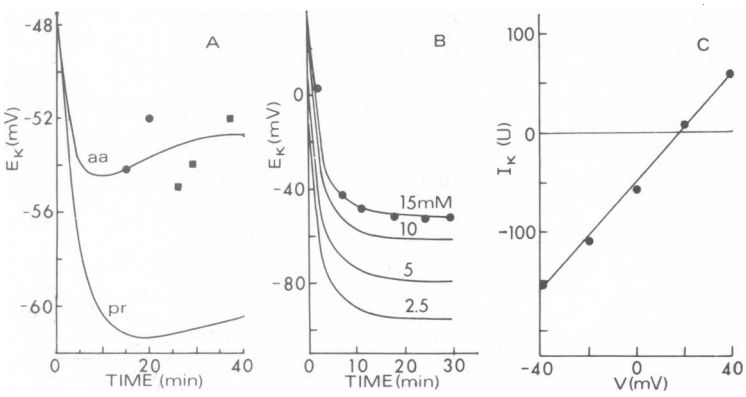

FIGURE 5 A. The computed (continuous curve) and measured (symbols) E_K values as a function of time after cutting the fiber in isotonic KCl. The two curves correspond to different internal anionic compositions: amino acids (aa) and univalent proteins (pr). It is assumed that the diffusion coefficient $D = D_w$ and $[K^+]_o$ = 15 mM. Circles, experiment 26/75; squares, experiment 31/75. B. The computed (continuous curve) and measured E_K values (symbols) as a function of time after changing the solution in the side pools from Ringer's to isotonic KCl (see Fig. 4). Each curve corresponds to a different K^+ concentration at the outer side of the nodal membrane. It is assumed that the diffusion coefficient $D = D_w$ and that the internal anions are a mixture of amino acids and proteins (1:1). Experiment 26/75. Note that the data best fits $[K]_o$ value of 14.8 mM, in agreement with the value of 15.5 mM as determined experimentally from V_K in Fig. 5C. C. The instantaneous membrane potassium current-potential relationship. The instantaneous current values (in arbitrary units) were obtained by extrapolating the exponential initial portions of the "tail currents" to the instant of the step. All currents were corrected for leakage and base line offset. Zero current crossing point is taken as the potassium reversal potential V_K, here V_K = 19 mV. Experiment 26/75.

dure is over. Note that the apparent scatter of the experimental points is due to the highly expanded E_k scale, the actual scatter being only 2–3 mV. Note also that the rates of ionic diffusion are strongly affected by the initial internal anionic composition only immediately after the fiber is cut, since as time goes on most of the internal anions are replaced by chloride ions.

Fig. 5 B illustrates that the experimental values are in best agreement with predictions based on $[K^+]_o$ = 15 mM. These relatively high $[K^+]_o$ values, compatible with those determined experimentally with the E_k values, result from K^+ accumulation during the conditioning prepulses. In the example given in Fig. 5C, the measured E_k value can be seen to correspond to $[K^+]_o$ = 14.8 mM. These results agree with those reported by Dubois and Bergman (1975*b*).

Fig. 6 compares the experimentally determined cesium ion diffusion along the axon towards the node (in four different axons) with computational predictions based on conditions corresponding to the experimental ones. The cesium concentrations were determined on the basis of the experimentally determined [Cs] vs. I_k dose-response

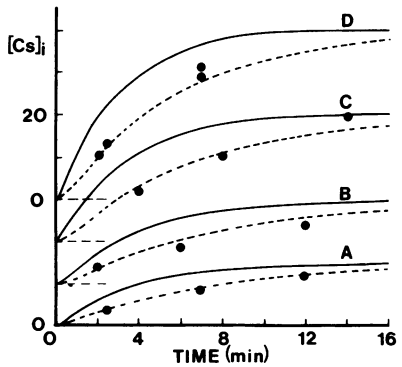

FIGURE 6 Computed and experimentally determined cesium ion concentrations (symbols) at the inner side of the nodal membrane as a function of time after the solution in side pools was changed from isotonic KCl to an isotonic mixture of KCl + CsCl. The CsCl content of the solutions was 15%, 20%, 30%, and 40% CsCl in A, B, C, and D, respectively. The data in each solution was obtained from a different preparation. In all cases initial $[Cs^+]$ was zero; however, the data for each new solution is shifted upwards along the ordinate by one unit. Ordinate: each division corresponds to 10% $[Cs^+]$. The continuous trace of each pair of curves corresponds to computations carried out assuming that the axoplasmic ionic diffusion coefficients equal those in water ($D = D_w$), while interrupted trace correspond to computations based on $D = D_w/2$.

relationship (see Fig. 1). In these experiments the cut ends of the fibers were first equilibrated with isotonic KCl and then placed in the test solution containing CsCl. Note that in all cases the initial [Cs] was zero; however, the initial points of traces *B-D* were shifted upwards to prevent superimposition of all curves and points.

It is seen that the diffusion of Cs^+, as manifested by the $[Cs]^+$ at the node, agrees with computations based on ionic diffusion coefficients close to half of those of cesium in water: $D = 0.5\ D_w$. As can be seen in Fig. 6, similar results were obtained for side pool cesium concentrations in the range of 10–40 mM.

DISCUSSION

To evaluate the ionic diffusion along the axoplasm, it is essential to have a good estimate of the axoplasmic ion diffusion coefficients. Comparison of our experimental and computed results indicates that the effective diffusion coefficient of potassium ions in the axoplasm is almost equal to that in aqueous solution. The comparison of the experimental and predicted potassium concentration changes is consistent with the assumption that the axoplasmic diffusion coefficients of sodium and chloride are also similar to those in aqueous solution. The corresponding coefficient for cesium ions is only about half of that in aqueous solution. The apparently slower rate of Cs diffusion in the axoplasm may be the result of interaction between Cs and some axoplasmic or membrane constituent. Note, however, that a part of the difference may result from the fact that the concentrations of K^+ and Cs^+ at the node were determined by two different electrophysiological methods. Note also that the error that may have been introduced by the feedback current, although very small, tends to increase the apparent axoplasmic potassium diffusion coefficient.

The diffusion coefficient, D_K, for potassium in the axoplasm agrees well with the findings of Hodgkin and Keynes (1953) for the axoplasm of the giant axon of *Sepia*, and extracted axoplasm of *Myxicola* (Gilbert, 1975*a*).

Koppenhöfer and Vogel (1969) calculated sodium diffusion coefficient, D_{Na}, in the axoplasm of a toad's myelinated nerve fiber from E_{Na} measurements. They evaluated the coefficient to be about 50% of that in free aqueous solution. More recently Frankenhaeuser and Åhrem (1975) evaluated D_{Na} to be equal to that in a free aqueous medium. In both cases the calculations were based on approximations that may, under some conditions, introduce significant errors in the computed results. As the above workers used the free diffusion equation, they did not account for the electrical component generated in a multi-ionic diffusion system. However, this electric field may seriously affect the calculated results. Furthermore, Frankenhaeuser and Åhrem (1975) approximated the diffusion along the fiber by assuming that it can be described as diffusion in a semi-infinite system. However, this assumption may be too far from the actual experimental conditions.

The ionic diffusion obviously generates a diffusion potential (Eq. 2) along the axoplasm and between it and the side pool solution. Under normal experimental conditions, when side pools contain KCl, the initial value of this potential is −8 mV (assuming that the mobility of the internal anions is that typical of amino acids). Where the internal ions are larger (e.g. proteins), the initial diffusion potential would be larger. This diffusion potential approaches zero, after an overshoot of up to +3 mV, within 20 min in a 0.2 cm-long fiber.

The described diffusion potential is equivalent to an additional slow changing electromotive force (emf) between points *C* and *D* in the equivalent scheme of the voltage clamp (Nonner, 1969). This emf may induce one of the following errors in the voltage clamp operation: (*a*) In experiments where the holding potential is determined after the emf reaches a steady state, there may be a constant DC error in the measurement of membrane potential (potential difference between point *A* and ground). (*b*) When the holding potential is set before the diffusion emf reaches steady state,

on top of the time-dependent error in the measurement of membrane potential point *D* may not be at virtual ground. Under such a condition the measured nodal current is a function of both the potential at point *E* and the nodal resistance.

Note that as the ion concentration sometimes overshoots the steady-state values, the diffusion potential may also follow the same pattern. However, for the short fibers in current use the actual overshoot peaks within 5–10 min of the fiber cutting. Thus by the time the experimental initiation procedure is over, the recorded potential is usually already sloping downward towards its steady-state value. This initial unstable potential is well known.

In spite of our findings, the possibility that D_K and D_{Cs} values in the intact axoplasm are considerably lower cannot be completely excluded, as we evaluated the coefficients some 30 min after cutting the fibers, i.e. when the axoplasmic ionic composition has already been greatly altered. However, the diffusion coefficients, ion concentration changes, as well as potential shifts determined in this work, correspond well to those of normal voltage clamp and other experimental conditions, including internal perfusion of myelinated fibers.

The model on which the computational results described in this work are based does not take into account the ionic passive fluxes or active pumping across the nodal membrane. The passive diffusion across the nodal membrane is negligible since the nodal area is similar to the axon cross section, while its passive ionic permeability is about 10^4 times smaller. Unfortunately, the nodal ion pumping rate is not known. However, if we assume that the nodal sodium-potassium pumps and densities are similar to those found in giant axons, we can evaluate the magnitude of error introduced by the pumps. On the basis of the above, the potassium flux across the membrane will be of the order of $4 \cdot 10^{-11}$ mol/cm^{2}s (Sjodin, 1971). Since the nodal membrane area is approximately 30 μm^2 (Hille, 1971), the nodal flux will be 1.2×10^{-16} mol/s. Since the K^+ content of a 10 μm diameter fiber 1 mm long is approximately 10^{-1} mol, it would take about 20 h to deplete 50% of the fiber K^+ ions. In view of the relatively rapid diffusion rates computed in this work, the pumping effect is most likely to be negligible.

Internal perfusion of the node has recently been initiated to control the internal concentration of naturally occurring substances and to introduce new substances, electrolytes and nonelectrolytes, into the axoplasm (Koppenhöfer and Vogel, 1969; Armstrong and Hille, 1972; Århem and Frankenhaeuser, 1974; Stämpfli, 1974; Dubois and Bergman, 1975*a*; Frankenhaeuser and Århem, 1975; Århem, 1976). However, as the effect of any substance must be expressed in terms of its concentration, the various workers had to make some very rough concentration estimates (Hille, 1975) or wait, after initiating the perfusion, until they could assume the system had reached equilibrium. The data given in this work can be utilized to predict quantitatively the behavior of the system and thus enable one to use the perfusion procedure safely and efficiently. Moreover, the diffusion process from the side pools occurs under practically all experimental conditions. Even if the potassium and sodium content of the side pools is assumed to equal that of the axoplasm, there are transient changes in their concentrations, due to the anionic exchange between the axoplasm and side pools. Thus, on top of the above-mentioned potential errors, there are always changes in the

ion reversal potentials. Indeed, in some of the more recent experiments using short fibers (2–5 mm long), changes in equilibrium potentials during experiments were briefly mentioned and they agree well with our predictions (Koppenhöfer and Vogel, 1969; Hille, 1973; Frankenhaeuser and Åhrem, 1975). The information regarding these changes in reversal potentials is essential to evaluate correctly the ionic conductances and membrane ion kinetic parameters in any given experimental set of voltage clamp conditions.

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