AN APPROACH TO THE CURRENT-VOLTAGE CHARACTERISTICS OF NERVE MEMBRANES BASED ON ADSORPTION PHENOMENA

M. AMIN

From the Department of Toxicology, Gesellschaft für Strahlen- und Umweltforschung, Munich, West Germany

ABSTRACT Using Stern's double-layer adsorption model for the density of cations in the membrane pores, a quantitative approach to the stationary current-voltage characteristic of nerve membranes is developed. The interaction of mobile cations with the negative fixed charges, located inside the membrane, constitutes a resistance for the current through the membrane. The stepwise increase in the resistance for the hyperpolarization is ascribed to a stronger interaction accompanying a depletion of the adsorbed cations from the interior. Thermodynamic treatment of flows and forces is adapted to the situation, to give a current voltage relation amenable to experimental check. The value of the resting potential thus obtained gives a deviation from Nernst equation applied to the ion for which the membrane is mainly permeable. The effect of the membrane double-layer potential on the potential range in which the transition from low to high resistance takes place, is explicitly incorporated. Finally, a comparison of the theory with the experimental results for the squid axon and frog nerve fibers is made.

INTRODUCTION

The importance of steady-state membrane behavior, in its bearing on the phenomena of excitation, is well recognized (1, 2). The transient negative conductance for the sodium current, observed during excitation (3), is analogous to the negative conductance observable in steady state for potassium current (4, 5). A region of negative conductance in the current-voltage relation of nerve membrane, poisoned with veratridine and with sodium substituted for potassium (6), points to the generality of this membrane property for all ionic transport. Since the occurrence of a region of negative conductance in the *I-V* characteristic of a physical system is connected with a number of factors involved in exitation phenomena (7–9), it is important to gain insight in this membrane property.

The differential negative conductance in the *I-V* relation is equivalent to a step change in the membrane conductance which occurs in a short range of the membrane potential. Since the membrane resting potential, under normal physiological

conditions, lies close to this potential range (which may be characterized by $V_{1/2}$ i.e., the potential at the average value of the two conductances (10), the membrane acquires the property of a rectifier. Shifting the resting potential in the direction of depolarization gives rise to the N-shaped curves, with the slant bar representing the region of negative conductance.

The failure of the classical theory of electrodiffusion (11) to describe the strongly nonlinear nerve membrane I-V characteristic led to several attempts of varying success and relevancy. Some of the notable models may be read in reviews (12–14). The most successful approach to the physical basis of negative conductance known to us is the lattice gas model of Agin (15), in which the stepwise increase in the resistance is caused by a depletion of the mobile cations from the membrane, when the external field exceeds the interionic forces of the cations with the membrane fixed charges. A rather strong and unfounded assumption is that the ionic distribution, after taking its equilibrium value, becomes exclusively potential dependent. Without this assumption a depletion of ions from the membrane, signalizing potential dependent conductivity change, would not be possible. Unless the depletion is accompanied by some simultaneous morphological change the depleted ions would be replenished by ions entering from the outside solution. In the following we shall take over the idea of depletion, adopting the standpoint that whatever morphological changes accompany the change in the membrane conductivity are triggered by the onset of depletion and, in their turn, make further depletion possible.

Apart from this conceptual difficulty a shortcoming of Agin's approach is its inability to predict the resting potential. Also, the theory leads to zero conductance for higher polarizations whereas experiments show a nonzero constant conductance (10). In spite of these drawbacks the current-voltage relation obtained by Agin can be made to fit quite accurately the experimental curves in the regions of negative slope and of high conductivity by a proper choice of parameters (16). The aim of the present study is to improve and extend Agin's quantitative results and to point out certain possible connections among the stationary parameters.

A constant field in the membrane shall be assumed with the justification provided by Agin (15): the Debye length for the membrane is much greater than the membrane thickness. Under such conditions a constant field may exist in the membrane even if microneutrality is violated there. We have also relied on the morphological assumption of cation selective pores (13) and postulate a single file transport mechanism in them (17).

THE ADSORPTION MODEL

The study of bilipid membranes has shown that biological membranes owe their selectivity and nonohmic behavior to some special proteins which build tunnel-like structures in an otherwise highly resistive lipid phase (18, 19). The cation-cation selectivity has its origin, probably, in the intricate protein structure. The cation-anion

selectivity arises, at least partially due to the negative charge carried by the membrane surfaces. Counterionic adsorption and screening at the outer membrane surface, specially that of Ca has been shown to play a vital role in influencing the threshold potential, the membrane permeability for different ions, the limiting values of the conductance and the activation and inactivation parameters (20–23). Our aim is to take into account the influence of negatively charged groups in the pores for the ionic transport in the membrane. Their presence provides a definite number of sites which can be occupied by the mobile cations for which the pore is selective. In this way the charge carrier density is increased in the membrane. Further, a displacement or shift mechanism functions in a filled pore in which the ions of the side solutions do not traverse the whole length of the pores but cause a shift of an array of ions whose net result is the uptake of the ion from the solution at one end and ejection of the ion at the other end of the pore. For the density of ions permanently occupying the sites Stern-Langmuir adsorption relation (24) shall be used, the interaction potential of the cations with the sites, denoted by U_p, being taken as Stern's potential. In this case pores are supplied with counterions by the electrolyte on both sides of the membrane.

An extremely important and crucial property of the membrane is that the increase in its conductivity upon depolarization is much more rapid than would be the case if the membrane ionic concentration were governed by Boltzmann statistics. In other words the diffusion process by which the sites in the membrane are occupied differs from the normal diffusion obeying Nernst-Einstein relation. This so-called "gating current" is a long standing puzzle awaiting experimental confirmation and theoretical explanation (25). In the present treatment we shall not venture to discuss this aspect, but density fluctuations seem to be the cause for the departure from the normal statistical mechanics for the extremely thin membrane and pores of atomic diameters.

The presence of the fixed charges in the pores constitutes a resistance for the membrane current. The cations fall into the potential dips of the fixed charges, losing their momentum to the membrane frame, and have to be activated in order to make them jump over the saddlepoints separating two potential dips. The work which has to be done to dissociate a cation from a fixed charge will of course depend upon whether or not a substitute for the dissociating cation is available at the site of the fixed charge group. In other words the field of the fixed charges resisting the ionic transport will depend on the number of cations in the pore. Our basic idea is that the stepwise decrease in the membrane conductance is caused by an increase in the electric field of the fixed charges due to a depletion of the adsorbed ions from the pores. The cause of this depletion is the external field, i.e., the combined effect of the field corresponding to the measured membrane potential and of the field due to the difference in the effective surface charge density of the two membrane surfaces.

Apart from the process of conduction mediated by the array of adsorbed ions of density c_p, there will be ions which effectively traverse the whole length of the

pores. The time average density c_q of these ions is responsible for the conductivity of the depleted pores. In the depleted state of the pores the ions interact with the sites with a stronger potential U_q so that their mobility u_q is less than the mobility u_p for the filled pores. Without introducing a large error we shall include the contribution of c_q for all states of the pores taking for their mobility the lower value u_q. For the conduction through empty pores we introduce unequal electric and diffusional mobilities, u_q^e and u_q^d, respectively. Since the occupation of the adsorption sites comes about by the same diffusion process as the diffusion through empty pores, the same ratio $u_q^e : u_q^d = \alpha$ shall be used for both processes.

We now proceed to derive the current-voltage relation on the lines laid out above. For the potential dependent concentration of cations in the membrane we write a modified Stern-Langmuir relation:

$$c_p(x) = \frac{w}{1 + \gamma \exp\left[\frac{F}{RT}(U_p - \alpha V(x))\right]}. \tag{1}$$

γ $N/c_0 M$.
w Density of sites.
N Loschmidt number.
M Molecular weight of water.
F Faraday constant.
R Gas constant.
T Absolute temperature.

For c_0, the bulk concentration of the concerned ion, an average value $(c_0^o + c_0^i)/2$ should be appropriate. The modification lies in the introduction of the parameter $\alpha = u_q^e : u_q^d$ containing the deviation of the ionic distribution in the membrane from the Boltzmann statistics.

The total current is separated into two parts, j_p and j_q. The former is mediated by the ions of density c_p, permanently held in the pores, whereas the latter does not involve these. j_q consist in an actual passage of ions through the pores, involving the density c_q. The membrane of thickness d lies between $x = 0$ and $x = d$ separating the inside from the outside electrolyte. Only a single ion species, i.e. potassium, for which the stationary membrane is mainly permeable, shall be considered in the following. Apart from the ionic double-layer potential at the membrane surfaces all other quantities shall relate to this ion only.

For the current densities j_p and j_q we write the usual expressions (26):

$$j_p = u_p c_p(x)\left\{RT \frac{d \ln c(x)}{dx} + F \frac{dV}{dx}\right\} \tag{2}$$

$$j_q = u_q^e c_q \left\{\frac{RT}{\alpha} \frac{d \ln c(x)}{dx} + F \frac{dV}{dx}\right\}. \tag{3}$$

Applying steady-state condition, $\partial j(x)/\partial x = 0$ to the two current densities separately, and using the constant field approximation, the above equations are integrated over the x-variable to give:

$$j_p = G_p(\bar{V})(RT \ln c^o/c^i + FV) \tag{4}$$

$$j_q = G_q([RT/\alpha] \ln c^o/c^i + FV), \tag{5}$$

where $V = V^o - V^i$, $\bar{V} = \Delta\psi + V$,

$$G_p^{-1}(\bar{V}) = \int_0^d \frac{dx}{u_p c_p(x)} = \frac{dRT}{\alpha\omega u_p F\bar{V}} \left(\frac{\alpha F\bar{V}}{RT} + e^{F\phi/RT}(1 - e^{-\alpha F\bar{V}/RT}) \right), \tag{6}$$

$$G_q^{-1} = \frac{d}{u_q^e c_q}. \tag{7}$$

In the expression for $G_p^{-1}(\bar{V})$, $\phi = (RT/F) \ln \gamma + U_p$ and $\Delta\psi$ is the difference of the Stern's potential at the outer and inner membrane surfaces. The maximal value of $G_p(\bar{V})$ is found to be wu_p/d, for $V \gg \phi/\alpha - \Delta\psi$.

Putting the total current $J = j_p + j_q = 0$, we obtain an implicit equation for the resting potential:

$$V_0 = -\frac{RT}{F} X^{-1}(V_0) \ln c^o/c^i, \tag{8}$$

with

$$X(V_0) \equiv \frac{G_p(V_0) + G_q}{G_p(V_0) + G_q/\alpha}. \tag{9}$$

Since $G_p(V)_{\max} \gg G_q$, $X^{-1}(V)$ takes the maximal value of about 1 for $V \gg \phi/\alpha - \Delta\psi$, and the minimal value of $1/\alpha$.

The transition $G_p(V) \to 0$ occurs at the potential value

$$V_{tr} = \phi/\alpha - \Delta\psi, \tag{10}$$

so that this value may be identified with $V_{1/2}$ of Mozhayeva and Naumov (10). The transition in $X(V)$ takes place for the same value of the membrane potential.

In terms of V_0 the current voltage relation may be written as:

$$J = FG_p(\bar{V})(V - X(V_0)V_0) + FG_q(V - X(V_0)V_0/\alpha). \tag{11}$$

For $V_0 > V_{tr}$ a simplified equation for the current voltage relation may be used:

$$J = F\, G_p(\bar{V})(V - V_0) + F\, G_q\, (V - V_0/\alpha), \tag{12}$$

where the maximal value of 1 has been taken for $X^{-1}(V_0)$.

DISCUSSION

The current voltage relation (12) differs from Agin's results by the term linear in V, by the value of V_0, and in containing $\Delta\psi$ in the expression for $G_p(\bar{V})$. Agin's I-V relation is known to fit the experimental curves in the high conduction region and in the region of transition from high to low conductivity, if V_0 is taken as the experimental value. Our equation is an improvement on Agin's result in that it contains the low conduction region as well. The inclusion of the $\Delta\psi$ term giving the shift of the conductivity function with the changes in the Stern's potential (almost equal to the electrokinetic potential) with the outer electrolytic concentration according to Grahame's equation (27) makes the relations (11, 12) valid for nonisotonic solutions as well.

The most interesting departure from the previous treatments is the value of the resting potential V_0. As $G_p(V_0)$ goes to zero, that is, as the transition from high to low conductivity takes place, the steepness

$$\Delta V_0/\Delta \ln (c^o/c^i)$$

decreases from RT/F to $RT/\alpha F$. This transition takes place around -70 mV for the squid axon and -60 mV for frog nodal membrane under isotonic conditions. We may thus interpret the result of Baker et al. (28) on the internally perfused axon in the following way. For internal concentrations of potassium $c^i(K)$ up to around 50 mmol/liter the membrane is in the high conduction region in which the Nernst-Einstein relation holds. As $c^i(K)$ is increased further $G_p(V_0)$ decreases so that ultimately, the steepness decreases to $RT/\alpha F$ according to Eq. 8 for $G_p(V_0) = 0$.

A consistancy test for this is that the value of α of about 6 (16) is about the same as the ratio of the inclinations in the $V_0 - \log c^o(K)/c^i(K)$ relation in the internal perfusion experiment. Thus our approach offers a new way of explaining the deviation of the resting potential from the Nernst value for potassium diffusion potential. The previous attempts were based on the Goldman formula (29) or on the existence of an ionic double layer in the interior of the axon membrane (30).

To conclude we would say that, although our results, Eqs. 11, 12, and 8, describe the experimental curves quantitatively in the whole range of the voltage, the treatment is still empirical since the parameter α remains unexplained. The important contribution of the present work lies in pointing out the possibility of a connection in the deviation of the value of the resting potential from the K-diffusion potential for large (absolute) values of V_0 and the violation of the Nernst-Einstein relation for the gating current.

The author wishes to thank Prof. Stämpfli for his extremely helpful criticism and Doctors Barske and Engelhardt for useful discussions.

Received for publication 15 May 1972 and in revised form 21 June 1973.

REFERENCES

1. COLE, K. S. 1965. *Physiol. Rev.* **45**:340.
2. COLE, K. S. 1965. *In* Theoretical and Mathematical Biology. T. Waterman and H. J. Morowitz, editors. Blaisdell Publishing Company, New York.
3. HODGKIN, A. L., and A. F. HUXLEY. 1952. *J. Physiol.* (*Lond.*). **117**:500.
4. MOORE, J. 1959. *Nature* (*Lond.*). **183**:265.
5. STÄMPFLI, R., 1958. *Helv. Physiol. Acta.* **16**:127.
6. ULBRICHT, W. 1966. *Arch. Gesamte Physiol.* **289**:R9.
7. CUNNINGHAM, W. J. 1958. Nonlinear Analysis. McGraw-Hill Book Company, New York.
8. FITZHUGH, R. 1960. *J. Gen. Physiol.* **43**:867.
9. AGIN, D. 1970. *In* Treatise on Mathematical Biology. R. Rosen, editor. Academic Press, Inc., New York. Vol. 2.
10. MOZHAYEVA, G. N., AND A. P. NAUMOV. 1970. *Nature* (*Lond.*). **228**:164.
11. AGIN, D. 1967. *Proc. Natl. Acad. Sci. U. S. A.* **57**:1232.
12. ADRIAN, R. H. 1969. *Prog. Biophys. Mol. Biol.* **19**:341.
13. HILLE, B. 1970. *Prog. Biophys. Mol. Biol.* **21**:1.
14. KATCHALSKY, A. 1967. *In* The Neurosciences. G. C. Quarton, T. Melnechuk, and F. O. Schmitt, editors. The Rockefeller University Press, New York.
15. AGIN, D. 1969. *Biophys. J.* **9**:209.
16. DROUIN, H. 1971. 25th International Congress of Physiological Sciences. Munich.
17. HODGKIN, A. L., and R. D. KEYNES. 1955. *J. Physiol.* (*Lond.*). **128**:61.
18. MUELLER, P., D. O. RUDIN, H. TI TIEN, and W. C. WESCOTT. 1962. *Nature* (*Lond.*). **194**:979.
19. SINGER, S. J., and NICOLSON, G. L. 1972. *Science* (*Wash. D. C.*). **175**:720.
20. FRANKENHAEUSER, R., and A. L. HODGKIN. 1957. *J. Physiol.* (*Lond.*). **173**:217.
21. SCHMIDT, H. 1960. *Arch. Gesamte Physiol.* **271**:634.
22. GILBERT, D. L., and G. EHRENSTEIN. 1969. *Biophys. J.* **9**:447.
23. MCLAUGHLIN, S. G. A., G. SZABO, and G. EISENMAN. 1971. *J. Gen. Physiol.* **58**:667.
24. DAVIES, J. T., and E. K. RIDEAL. 1961. Interfacial Phenomena. Academic Press, Inc., New York.
25. CHANDLER, W. K., and H. MEVES. 1965. *J. Physiol.* **180**:788.
26. KIRKWOOD, J. G. 1954. *In* Ion Transport Across Membranes. H. T. Clark, editor. Academic Press, Inc., New York.
27. GRAHAME, D. C. 1967. *Chem. Rev.* **41**:441.
28. BAKER, P. F., A. L. HODGKIN, and T. I. SHAW. 1962. *J. Physiol.* (*Lond.*). **164**:330.
29. GOLDMAN, D. E. 1943. *J. Gen. Physiol.* **27**:37.
30. CHANDLER, W. K., A. L. HODGKIN, and H. MEVES. 1965. *J. Physiol.* (*Lond.*). **180**:821.