CONCERNING NEGATIVE CONDUCTANCE IN THE SQUID AXON*

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It is generally agreed that the experimental observation most relevant for both fundamental problems of axon behavior and molecular problems of excitation is the negative slope conductance region of the current-voltage characteristic of axons. Cole,\textsuperscript{1} for example, has emphasized that the first and necessary goal of any membrane theory is to produce a region of steady-state or quasi-steady-state negative conductance. The quantitative and empirical description of squid axon behavior developed by Hodgkin and Huxley\textsuperscript{2} has proved to be valuable, but attempts to get at the underlying fundamental variables have not been successful. Most theoretical models have been based on the liquid junction theory of Planck,\textsuperscript{3} but in none of these models does a negative conductance region appear. This difficulty has done much to delay progress in understanding axon behavior.

The purpose of this communication is to present the elements of a very simple but apparently not unreasonable electrochemical hypothesis which produces in a quantitative way both a negative slope conductance for the sodium ion and a high rectification for the potassium ion. Transient phenomena are not yet included, but the apparent agreement with a wide range of steady-state and short time observations suggests that the ideas may be potentially useful. The aim has been to isolate those assumptions which appear necessary to account for experimental observations.

Theory.—An electric current, whether it be a total current or a partial current for a particular ion species, is defined as the product of a charge density and a velocity. If current is restricted to one dimension, all terms are scalar, and for a positively and singly charged species:

$$I_i = FC_i v_i$$

where $I_i$ is a partial electric current (coul/cm$^2$ sec), $F$ is the Faraday constant (coul/mol), $C_i$ is a concentration (mol/cm$^3$), and $v_i$ is a velocity (cm/sec). It is sometimes convenient to decompose the velocity into the product of a mobility (velocity/unit driving force) and a driving force and to describe the current as

$$I_i = -FC_i \bar{u}_i \frac{dU}{dx},$$

where $U$ is an energy term (joule/mol) and $\bar{u}_i$ is a generalized mobility (cm$^2$ mol/sec joule). Equation (2) has been termed a generalized flux equation.\textsuperscript{4} The simplest possible assumption is that the current is linear in the driving forces, and for the electrochemical systems of interest to biologists, these driving forces are taken as the gradients of the chemical potential and the electric potential:

$$I_i = -FC_i \bar{u}_i \frac{d}{dx} \{\mu_i + F\psi\},$$

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where $\mu_i$ is a chemical potential (joule/mol) and $\psi$ is an electric potential (joule/coul). Equation (3) is familiar and appears often in the literature. A significant and implicit assumption, however, is that the velocity per unit driving force is the same for all driving forces, i.e., that there is only one migration mechanism available to the ions. In condensed phases this assumption is often not appropriate. If more than one migration mechanism exists, equation (3) must be further decomposed:

$$I_i = -FC_i \left\{ \bar{u}_i^a \frac{d\mu_i}{dx} + \bar{u}_i^b F \frac{d\psi}{dx} \right\}.$$  

(4)

The product $F\bar{u}_i^m$ has dimensions of cm$^2$/sec volt or cm$^2$ coul/sec joule, and can be replaced by $u_i^m$:

$$I_i = -C_i \left\{ u_i^a \frac{d\mu_i}{dx} + Fu_i^b \frac{d\psi}{dx} \right\}.$$  

(5)

In dilute ideal solutions, the chemical potential is given by:

$$\mu_i = RT \ln a_i + \mu_i^0,$$  

(6)

where $\mu_i^0$ is a reference energy/mol which depends on the interaction energy with the solvent, $a_i$ is an activity or mole fraction, $R$ is the gas constant (joule/mol deg), and $T$ is the Kelvin temperature. Although appropriate for dilute solutions, equation (6) may not be appropriate for other systems. In a condensed phase, for example, in which the number of places (charged or uncharged) available to ions is limited and each place can be occupied by only one ion, the appropriate expression for the chemical potential is (cf. Appendix):

$$\mu_i = RT \ln \left( \frac{C_i}{\omega_i - C_i} \right) + \mu_i^0,$$  

(7)

where $\omega_i$ is the total density of available places (mol/cm$^3$). It will be seen that equation (7) appears to be appropriate for the axon membrane.

Combining equations (5) and (7), with $\omega_i$ constant,

$$I_i = -RTu_i^a \left( \frac{\omega_i}{\omega_i - C_i} \right) \frac{dC_i}{dx} - Fu_i^b C_i \frac{d\psi}{dx}.$$  

(8)

If $\omega_i \gg C_i$, equation (8) reduces to the familiar electrodiffusion equation with a decomposed mobility.

If it can be assumed that the mobilities are constant, then from equation (8):

$$I_i = -\frac{RT\omega_i}{\alpha_i F} \int \frac{dC_i}{C_i(\omega_i - C_i)} - (\psi' - \psi) \left( \frac{1}{F_{u_i}^b} \int \frac{dx}{C_i} \right) \alpha_i \equiv u_i^b / u_i^a,$$  

(9)

$$I_i = \left( V_i^0 - V \right) Fu_i^b \int \frac{dx}{C_i},$$  

(10)

$$I_i = \frac{(V_i^0 - V) Fu_i^b \int \frac{dx}{C_i}}{\alpha_i},$$  

(11)
\[ V = \psi', \quad \psi' = 0, \quad (12) \]

\[ V_{s}^\alpha = -\frac{RT}{\alpha F} \ln \left[ \frac{C_i^\alpha \left( \omega_i - C_i \right)}{C_i^\prime \left( \omega_i - C_i^\prime \right)} \right], \quad (13) \]

where the double prime refers to the outside solution. If \( \omega_i > C_i \), equation (13) reduces to the familiar Nernst equilibrium potential. If \( I_i \) is small, i.e., if

\[ |I_i| \ll \left| RTu_i \left( \frac{\omega_i}{\omega_i - C_i} \right) \frac{dC_i}{dx} \right| \quad (14) \]

\[ |I_i| \ll \left| F_{u_i} \frac{d\psi}{dx} \right| \quad (15) \]

then from equation (8):

\[ \frac{RT}{\alpha F} \frac{dC_i}{\omega_i \left( \omega_i C_i - C_i^\prime \right)} \approx -d\psi \quad (16) \]

\[ \frac{C_i}{C_i - \omega_i} = ae^{-\alpha F\psi/RT}, \quad (17) \]

where \( a \) is an integration constant. For \( \psi = 0 \), \( C_i = C_i^\prime \), and

\[ \frac{C_i}{C_i - \omega_i} = -\frac{C_i^\prime e^{-\alpha F\psi/RT}}{\omega_i - C_i^\prime} \quad (18) \]

\[ C_i = \frac{\omega_i \beta_i}{e^{\alpha F\psi/RT} + \beta_i} \quad (19) \]

\[ \beta_i = \frac{e^{\alpha F\psi/RT} + \beta_i}{\omega_i - C_i^\prime} \quad (20) \]

The charge density \( \rho \) (coul/cm\(^3\)) is given by the Poisson equation:

\[ \frac{d^2\psi}{dx^2} = -\frac{\rho(x)}{\epsilon} \quad (21) \]

where \( \epsilon \) is the dielectric constant (coul\(^2\)/joule \cdot cm). Let \( \delta \) be the thickness of the membrane and let

\[ \psi \equiv \frac{RT}{F} \psi^* \quad (22) \]

\[ x \equiv \delta y \quad (23) \]

\[ \rho \equiv |\beta| \rho^* \quad (24) \]

where \( \psi^* \) is a reduced electric potential, \( y \) is a reduced position coordinate, \( \rho^* \) is a reduced charge density, and \( |\beta| \) is the absolute value of the mean charge density. One then has

\[ \frac{d^2\psi^*}{dy^2} = -\delta^2 \frac{F |\beta| \rho^*}{RT \epsilon} \quad (25) \]
where $\lambda$ is a Debye length (cm). Therefore, if the membrane thickness, $\delta$, is small compared to a Debye length, the electric field will be essentially constant even when the charge density is not small, a result also noted by Cole. It will be assumed that the electric field is constant. It might be noted that given the alternative condition, $\lambda \ll \delta$, microscopic electroneutrality is an appropriate approximation.

Direct integration of equation (8) yields an implicit transcendental expression in $I$ and $V$ which will be dealt with elsewhere.

If the electric field is constant, and equations (14) and (15) are obeyed, then from equation (19):

$$\int \frac{dx}{C_i} = \frac{\delta}{V \omega_i \beta_i} \int \{ e^{a_i F \psi / RT} + \beta_i \} d\psi,$$

and equation (11) becomes:

$$I \delta = \frac{-\left(F/R\right)^2 (V - V_0) V \alpha \beta_i}{e^{a_i F V / RT} - 1 + \alpha \beta_i F V / RT}$$

$$I_i = \frac{-F U_i \omega_i (V - V_0) V \beta_i}{\alpha_i F} \{ e^{a_i F V / RT} - 1 \} + \delta \beta_i V.$$

From equation (8) it is apparent that the relation between the classical diffusion coefficient $D_i$ and $u_t^a$ is

$$RT u_t^a = F D_i.$$

Therefore,

$$\alpha_i = \frac{u_t^b}{u_t^a} = \frac{RT u_t^b}{FD_i}. $$

There are three possibilities for the numerical value of $\alpha_i$: $^6, 7 \quad \alpha_i = 1; \ \alpha_i < 1; \ \alpha_i > 1$. For independent transport in dilute solutions and vacancy transport in solids, $\alpha_i = 1$ is appropriate, and for cases where ions may form neutral complexes with diffusing species, $\alpha_i < 1$ should apply. However, for interstitialy transport in solids, particularly collinear interstitialcy migration, it appears both theoretically $^9$ and experimentally $^8$ that $\alpha_i = 3$, and this is the value which will be used. The other possibilities yield unsatisfactory results. It will be assumed that $\alpha_i$ is the same for all ion species, in accordance with the Townsend relation. $^{10}$
Given $\alpha_t$, it is possible to use equation (13) to derive an expression for $\omega_t$:

$$\omega_t = \frac{C_t^* \left[ e^{-3FV^o/RT} - 1 \right]}{\left[ e^{-3FV^o/RT} - C_t^* \right] C_t^{*'}}$$

(33)

A numerical value may be obtained by using the following approximate experimental values for the squid axon:

- $V_K^o = +50 \text{ mV}$.
- $V_{Na}^o = -50 \text{ mV}$.
- $C_{Na}^* = 5.0 \times 10^{-4} \text{ mol/cm}^3$.
- $C_{K}^* = 1.3 \times 10^{-5} \text{ mol/cm}^3$.
- $C_{Na}^{*'} = 4.4 \times 10^{-5} \text{ mol/cm}^3$.
- $C_{K}^{*'} = 3.7 \times 10^{-4} \text{ mol/cm}^3$.

These values and equation (33) yield:

- $\omega_{Na} = 5.13 \times 10^{-4} \text{ mole/cm}^3$.
- $\omega_K = 3.73 \times 10^{-4} \text{ mol/cm}^3$.

In the squid axon the limiting slope resistance for both Na$^+$ at short times and K$^+$ in the steady state, for $V$ large and negative, is approximately 35 ohm$\cdot$cm$^2$. From equation (30) it is apparent that the theoretical limiting slope resistance is given by:

$$R_t = \frac{\delta}{F u_t^b \omega_t}$$

(34)

Assuming the experimental value for $R_t$, it is possible to obtain a numerical estimate for $u_t^b$:

- $u_{Na}^b = 6 \times 10^{-10} \text{ cm}^2/$sec volt.
- $u_K^b = 8 \times 10^{-10} \text{ cm}^2/$sec volt.

These values are not uncommon for ion mobilities in solids.$^6$

$\beta_t$ represents the partition function for the ion species when $\psi = 0$, and there does not seem to be any a priori basis for its estimation. It seems reasonable to assume that for both ions, when $\psi = 0$, the density of occupied sites equals the density of empty sites, i.e., $\beta_t = 1$.

With these parameters and equation (30), the $I$-$V$ curves for the two ion species are conveniently computed according to the following dimensionless equation:

$$A_t = \frac{- (\xi - \xi^o) \alpha \beta_t \xi}{e^{\alpha \xi} - 1 + \alpha \beta_t \xi}$$

(35)

$$A_t = \frac{I_t^o}{RT u_t^b \omega_t}$$

(36)

$$\xi = \frac{FV}{RT}$$

(37)
Results.—The results are shown in Figure 1. For $V^\circ = -50$ mv there is a pronounced negative slope conductance region, and for $V^\circ = +50$ mv this has essentially vanished and there appears an $I-V$ curve with considerable rectification. In the squid axon, of course, the $Na^+$ curve is transient and not stationary. In agreement with experimental observations, both ion currents essentially vanish to the left of $V = +50$ mv in Figure 1, and a small leakage current is required to account for observations with hyperpolarizing applied potentials.

A reduced ion conductance is given by equation (35) as

$$\bar{G}_t = \frac{\alpha \beta \xi}{e^{\alpha t} - 1 + \alpha \beta \xi'}$$  \hspace{1cm} (39)

and its relation to $\xi$ is shown in Figure 2. The relation between $V$ and $\psi$ is

$$\psi = \frac{V_x}{\delta},$$  \hspace{1cm} (40)

so that as $V$ goes positive $\psi$ goes positive, and according to equation (28), the integral resistance will rapidly increase as the membrane becomes depleted of charge and the current will fall toward zero. For values of $V$ beyond $+50$ mv, i.e., hyperpolarizing membrane potentials, the current carried by any positively charged species will be vanishingly small. A pronounced negative slope conductance region will appear when the equilibrium potential for the ion is at a negative value. The theoretical result that no parameter other than the equilibrium potential need be changed in order to produce such markedly different current-voltage behavior is perhaps of some interest agrees with a suggestion by Cole.\textsuperscript{14}

For an axon with a limiting slope resistance of 35 ohm·cm\textsuperscript{2}, equation (30) pre-
dicts a peak $\text{Na}^+$ current of 720 $\mu$A/cm$^2$, which is reasonably close to the experimental transient value of 600 $\mu$A/cm$^2$ for the same limiting resistance.$^2$

When the squid axon is exposed to isoosmotic KCl, the resting potential falls to zero and a steady-state negative slope conductance appears for $\text{K}^+$. Setting $V_K^0 = 0$ in equation (30) results in a shift of the $\text{K}^+$ curve to the right in Figure 2, and the emergence of a negative slope conductance which is in reasonable agreement with experimental observations and agrees with a suggestion by Cole.$^{11}$

**Conclusion.**—The purpose of this investigation was to identify those assumptions which appear necessary to account for three important properties: a steady-state negative slope conductance, high rectification for one ion species, and a linear $I$-$V$ curve for large depolarizations.

It seems that electrochemical systems which exhibit negative slope resistance (current-controlled) or negative slope conductance (voltage-controlled) can be divided into two types. In the first class this kind of nonlinearity is associated with quantum-mechanical tunneling processes, and the available theory is not complete but nevertheless adequate. The second class involves systems for which tunneling processes have been ruled out for one reason or another, but for which there is no satisfactory theory. It is not always a simple matter to distinguish between electronic and ionic conduction, but for the squid axon the available evidence indicates that ions are the charge carriers$^2$ and that ion tunneling is very improbable.$^5$ The existence in the squid axon of both a linear instantaneous $I$-$V$ relation and a steady-state negative slope conductance seems to imply depletion of charge carriers somewhere in the system rather than space charge effects. It is possible, however, that the time-dependent negative slope conductance for $\text{Na}^+$ is due to a space charge transient, and such transients ought to be of interest to biologists. The electrochemical hypothesis which has been presented here essentially describes a depletion system. It seems to account for a number of experimental observations, but whether it can account for others remains to be seen. At the moment it may provide a framework with which to interpret old experiments and design new ones. A detailed analysis will be given elsewhere.$^{12}$

**Appendix.**—The entropy of the system is given by

$$S = k \ln \Omega,$$

(1)

where $k$ is the Boltzmann constant and $\Omega$ is the number of possible configurations. If a site can be occupied by only one ion and there is no interaction between ions, then $\Omega$ is given by:

$$\Omega = \frac{N!}{n!(N - n)!},$$

(2)

where $N$ is the number of sites and $n$ is the number of particles. The total energy of the system is

$$E = U_1 + nU_2,$$

(3)

where $U_1$ is the intrinsic energy and $U_2$ is the interaction energy per ion. The Helmholtz free energy is defined by

$$A = E - TS,$$

(4)
and the chemical potential is defined as

$$\mu' = \left(\frac{\partial A}{\partial n}\right)_{T,V}$$

(5)

and,

$$\left(\frac{\partial A}{\partial n}\right) = U_2 - kT \frac{\partial}{\partial n} \ln \left\{ \frac{N!}{n!(N-n)!} \right\}.$$  

(6)

Using the Stirling approximation:

$$\ln \left\{ \frac{N!}{n!(N-n)!} \right\} 
= N \ln N - n \ln n - (N-n) \ln (N-n)$$

(7)

$$\frac{\partial}{\partial n} \ln \left\{ \frac{N!}{n!(N-n)!} \right\} 
= -\ln n + \ln (N-n).$$

(8)

Therefore,

$$\mu' = U_2 + kT \ln \left( \frac{n}{N-n} \right)$$

(9)

$$\mu = U_2' + RT \ln \left( \frac{c}{\omega - C} \right).$$

(10)

An extended discussion is given by Fowler and Guggenheim.13

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10 Townsend, J. S., Phil. Trans., A193, 129 (1900).