A DIPOLE-ASSISTED MECHANISM FOR ION TRANSPORT ACROSS NERVE CELL MEMBRANES

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THERE are two major unsolved problems in the biophysics of excitable cell membranes, for example, the membranes of nerve cells[1-5]. The first problem concerns the origin of the selective Na^+ and K^+ permeabilities necessary to explain the following two characteristic features of the resting state of these membranes: (1) the unequal distribution of ions between the intra- and extracellular electrolytes, in particular, the extrusion of Na^+ from the cell interior in opposition to an electrochemical potential gradient of the order of 10^5–10^6 volts cm^{-1}, and (2) the existence of a resting potential, V_r, ($\sim$ 50–100 mV) which is not given by simple concentration cell formulae. The second problem pertains to the mechanism of the ionic permeability changes responsible for the electrical excitation of the membrane during which action potentials ($\sim$ 100 mV) are initiated by an early Na^+ influx current and a late K^+ efflux current. Both these problems ultimately involve the deeper question of the mechanism of ionic transport across excitable membranes. Pending an answer to this fundamental question, the alternative has been to resort to empirical descriptions[6], metaphorical language[3] (*e.g.*, 'pumps' keeping Na^+ outside resting cells, and 'sodium gates' opening during action potentials), and analogies (*e.g.*, with passive iron wires[7,8], transistors[9] and electrode-electrolyte interfaces[10]). This communication, however, describes a direct attempt to understand the mechanism of ionic migration through excitable cell membranes.

The model proposed here involves three assumptions:

(1) ionic migration through the membranes of nerve and other excitable cells occurs by jumps from one polar group to another:

$$P - N \cdot\cdot \oplus \overset{\curvearrowright}{} N - P \rightarrow P - N \oplus \cdot\cdot N - P \qquad (1)$$

where P and N specify the positive and negative ends of the dipoles representing the polar groups;

(2) these jumps are preceded by the 'flipping', *i.e.*, rotation through an angle π, of an 'ion-dipole pair' which is the term used to describe the electrostatic association of an ion with a polar group in the membrane matrix:

$$\ominus \cdot\cdot N - P \rightarrow P - N \cdot\cdot \ominus, \qquad (2)$$

and by the favourable orientation of the dipole which receives the jumping ion:

$$P - N \cdot\cdot \ominus \; P - N \rightarrow P - N \; \cdot\cdot \ominus N - P; \qquad (3)$$

(3) the polar groups (dipoles) are associated with an anisotropy energy (Δ_A) so that, even in the absence of an electric field across the membrane, they tend to align preferentially, for example, with the positive and negative ends facing the interior and exterior of the membrane respectively.

These assumptions require that the membranes of excitable cells should possess some very general structural features. Firstly, they should not have solution-filled pores large enough to permit the conventional Stokes' mechanism of migration of ions. Secondly, the membranes should have polar groups with such a spatial distribution that the ionic jumps from group to group define ionic pathways or channels through the membrane. Thirdly, the polar groups should be bound to the membrane matrix so that, in the absence of sufficiently strong electric fields, they do not function as free dipoles; instead, steric factors and/or interactions should normally hinder the rotation of the dipoles and confer on them an anisotropy energy, Δ_A. These requirements imposed by the model upon the membrane structure appear to be sufficiently realistic[1,5,11] to warrant processing of the model. Since, however, a detailed treatment is being presented elsewhere, only a brief account of the results of the analysis will be given below.

Assuming that the flipping of the 'ion-dipole pair' is the rate-determining step in the elementary act of migration, (2) $\rightarrow$ (3) $\rightarrow$ (1), the flipping frequency, $\vec{\nu}_i$, for singly-charged positive ions† of species i moving in the influx direction is given by

$$\vec{\nu}_i = \nu_0 \exp\,[-2\beta\Delta_i] \qquad (4)$$

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† For negative ions, $\Delta_i = -\Delta_A - \mu X_m \mp z_i e_0 X_m (r \mp \delta)$.

where

$$\Delta_i = \Delta_A + \mu X_m - z_i e_0 X_m (r_i + \delta)$$

where $\beta = 1/k_B T$, μ and δ are the moment and half-length associated with the dipoles, $z_i e_0$ and r_i are the charge and crystallographic radius of the ions, and $X_m = V \epsilon_m / d_m$, the electric field in the membrane. Before the ion of such a flipped 'ion-dipole pair' can jump to the adjacent dipole, the latter must be suitably oriented [*cf.*(3)], *i.e.*, the dipole gate in the ionic channel must be open. The probability, $\vec{p_i}$, of the dipole gate being open for an inflowing positive ion‡ is

$$\vec{p_i} = \frac{\exp[\beta\{\Delta_A + \mu X_m + \Delta_{i-D}\}]}{2\cosh[\beta\{\Delta_A + \mu X_m + \Delta_{i-D}\}]} \quad (5)$$

where

$$\Delta_{i-D} = \frac{z_i e_0 \mu}{(r_i + \delta)^2},$$

the ion-dipole interaction energy.

The net steady-state current density, *i.e.*, the difference between the influx ($\vec{i}$) and efflux ($\overleftarrow{i}$) current densities, can be expressed thus:

$$i_i = z_i F d [C_i^o \vec{k}_i (V) - C_i^I \overleftarrow{k}_i (V)] \quad (6)$$

where C_i^o and C_i^I are the ionic concentrations in the exterior and interior solutions,

$$\vec{k}_i = \tfrac{1}{2} \vec{\nu}_i \vec{p}_i [1 + \{\overleftarrow{\nu}_i \overleftarrow{p}_i / \vec{\nu}_i \vec{p}_i\}^{-(m-1)}] \quad (7)$$

and

$$\overleftarrow{k}_i = \tfrac{1}{2} \overleftarrow{\nu}_i \overleftarrow{p}_i [1 + \{\overleftarrow{\nu}_i \overleftarrow{p}_i / \vec{\nu}_i \vec{p}_i\}^{m-1}] \quad (8)$$

(m is the number of elementary acts required for an ion to traverse the membrane).

Expression (6) for the net ionic current density has many implications with regard to the electrical and transport phenomena in excitable membranes.

(1) In the resting state of the membrane, the net current density can be set equal to zero provided that leakage can be ignored. Then,

$$[C_{Na}^o / C_{Na}^I] = [C_K^o / C_K^I] \exp[4me_0 V_m \epsilon_m \times (r_K - r_{Na}) / d_m k_B T]. \quad (9)$$

Inserting the following data for the squid axon:

$C_K^o / C_K^I = 0{\cdot}025$,

$V_m = -V_R = 0{\cdot}060$,

$\epsilon_m / d_m = 36\pi.10^{11}$. $C_m = 1{\cdot}13 \times 10^7$,

$r_K - r_{Na} = 0{\cdot}33 \times 10^{-8}$ cm and $m = 15$,

the result is $C_{Na}^o / C_{Na}^I \approx 10$. This result depends upon the choice of m; hence, what should be stressed here is not the agreement with the observed[3] concentration ratio of 9·2, but the fact that the transport mechanism proposed here leads automatically to the smaller ion, Na^+, being extruded from the cell interior and the larger cation, K^+, being accumulated inside the cell. From this point of view, therefore, the so-called 'sodium pump' is an unnecessary hypothesis.

(2) An expression for the resting potential, V_R, follows from equation (9):

$$V_m = -V_R = \frac{RT}{F} \left[\frac{d_m}{4m\epsilon_m (r_K - r_{Na})}\right] ln \left[\frac{C_{Na}^o C_K^I}{C_{Na}^I C_K^o}\right]. \quad (10)$$

The observed concentration ratios for the squid axon yield a value of $V_R = E^I - E^o = -V_m$ of −60 mV which is in agreement with the observed value[3].

Equation (10) can also be used to show that a ten-fold dilution of K^+ within the axon should produce a decrease of 8 mV in the resting potential, in comparison with the observed[5] value of 10 mV.

The use of Gauss's law in combination with expressions for the concentration ratios, (C_i^o / C_i^I), leads to another expression for the resting potential:

$$V_m = -V_R = \frac{2\pi F d_m d}{\epsilon_m} \sum_i C_i [1 + z_i \tanh a_i (V_R)], \quad (11)$$

where d is the thickness of the solution outside the membrane,

$$C_i = C_i^o + C_i^I,$$

$$a_{Na, K} (V_R) = 2m\beta\Delta_{Na, K}$$

$$a_{Cl} (V_R) = m\beta\Delta_{Cl} = m\beta [\Delta_A + \mu X_m - e_0 X_m \times (r_{Cl} + \delta) + \Delta_{Cl-D}].$$

The transcendental form of the expression(11) shows that the resting potential is a self-generated and self-sustained quantity arising uniquely from the mechanism of ionic transport through the membrane.

(3) By considering small departures, $\delta V = V - V_R$, from the resting potential, the following result can be derived.

$$\delta V = R_i (V) \delta I_i (V) + [1/C_i (V)] \delta Q_i (V) \quad (12)$$

where

$$\delta Q_i (V) = z_i F d \delta C_i (V),$$

$$R_i (V) = \left[A z_i e_0 d \left\{C_i^o \frac{\delta \vec{k}_i}{\delta V} - C_i^I \frac{\delta \overleftarrow{k}_i}{\delta V}\right\}\right]^{-1}, \quad (13)$$

‡ The numerator is exp $[-\beta\{\Delta_A + \mu X_m + \Delta_{i-D}\}]$ for negative ions.

and

$$[C_i(V)]^{-1} - R_i(V)\,[\vec{k}_i(V_R) + \overleftarrow{k}_i(V_R)], \quad (14)$$

showing that a change in the membrane potential can be decomposed into a resistive drop and a capacitative drop arising from each membrane-permeating species. This corresponds to an equivalent circuit in which the branch for each ionic species consists of a potential-dependent resistor and a potential-dependent capacitor in series. In so far as analytical expressions are available for these active circuit elements, the equivalent circuit presented here is perhaps an advance.

(4) The application of a voltage step (clamp) across the membrane leads to a characteristic current response which can be analysed in terms of the effect of the field on the dipoles, *i.e.*, on $\vec{k}_i$ and $\overleftarrow{k}_i$. Arguing that the transformation from resting values, $\vec{k}_i(V_R)$ and $\overleftarrow{k}_i(V_R)$, to final values, $\vec{k}_i(V)$ and $\overleftarrow{k}_i(V)$, involves a dipole relaxation process (with a time constant τ), one obtains a time-variation of the current, for $t \ll \tau$, given by:

$$i_i = (i_i)_{\text{peak}}\,(1 - e^{-t/\tau})^{\alpha}. \quad (15)$$

where

$$\theta \leqslant \alpha \leqslant m/2$$

For $t \gg \tau$, a quasi-steady state approach leads to

$$i_i = (i_i)_{\text{peak}} \exp\,[-\{\vec{k}_i(V) + \overleftarrow{k}_i(V)\}t]. \quad (16)$$

Except for the dipole relaxation time, τ, the other parameters, *e.g.*, $\vec{k}_i(V) + \overleftarrow{k}_i(V)$, can be derived from the resting state. In that sense, the present model possibly leads to a decrease in empirical content.

The expressions (15) and (16) correspond, under certain conditions, to the following observations in voltage-clamp studies[1-5]:

(*a*) the early transient current results from a Na^+ *influx* and the late current from a K^+ ***efflux,***

(*b*) the i_{Na} rises and falls rapidly in contrast to the slower rise to a constant value of i^+

(*c*) the initial rise of the currents is given by expressions of the form (15) and the decay by expressions of the form (16).

According to the model used here, the so-called 'opening of the Na^+ gates' corresponds to the anisotropically aligned and initially hindered dipoles being 'freed' as a result of an electric field of appropriate sign and magnitude. Such a 'freeing' of the dipoles during the action potential would be associated with changes in optical properties such as birefringence[12]—these changes have indeed been observed[1].

In conclusion: a theory has been presented here of what happens within an excitable membrane and of how ions are transferred through it. This theory of ionic permeability involves three types of parameters:

(1) an electrical parameter, *e.g.*, the membrane potential which is an independent variable in voltage clamp studies;

(2) parameters characteristic of the ions (ionic charge and ionic radius);

(3) parameters characteristic of the membrane.

The preliminary results appear sufficiently promising to encourage an examination of specific structural aspects of excitable membranes. What would perhaps be required is an identification of the polar groups, and their dipolar characteristics, spatial distribution, anisotropic energy, conformations and interactions with the matrix.

Note added in proof: Since the communication of this note, the authors became aware of a suggestion made by Onsager ["Physical Principles of Biological Membranes" ed. Snell, F. *et al.*, Gordon and Breach, Science Publishers, New York, (1970, pages 137–139] in which ions are postulated to migrate across a membrane by jumps along dipole chains. In this suggestion, however, Onsager has not proposed an inherent asymmetric alignment of the dipoles, without which threshold phenomena during action potentials and a fundamental distinction between the 'inside' and 'outside' of membranes, will not obtain.

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