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Kinetic Properties of Electrostatic Pores with Orientable Dipoles, for Na^+ and K^+ Transport through Biological Membranes

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Summary. The model, used previously to account for the transport of K^+ ions through squid axon membranes under steady-state conditions, is extended to the description of the kinetic behavior of Na^+ and K^+ currents, for sudden variations of the applied potential. Theoretical curves are obtained by numerical integration of the electrodiffusion equation for ions within pores, with variable boundary conditions resulting from a progressive reorientation of dipoles at the pore surfaces. The pores are supposed to be selective and the dipole parameters are allowed to be different for Na^+ and K^+ pores.

The K^+ current varies with time, in agreement with the K^+ dipole parameters deduced from the steady-state results of Gilbert and Ehrenstein (1969). The dipole parameters for the Na^+ current are deduced from the steady-state results of Armstrong, Bezanilla & Rojas (1973), where the inactivation phase of the Na^+ current is suppressed by introducing pronase in the inside solution. The dipole reorientation is relevant to explain the sigmoid shape of the activation phase of the Na^+ current, while the inactivation phase seems to resort to another physical mechanism. The predictions based on this model agree with the experimental results for the steady-state *negative resistance* and the *gating current*, associated both with a reorientation of surface dipoles, as well as the activation phase of the Na^+ current using a consistent set of parameters for all these comparisons.

Hodgkin and Huxley (1952*a*) already succeeded in providing a quantitative description of the ionic events, responsible for the action potential of electrically excitable biological membranes, in terms of K^+ and Na^+ currents. The need of a distinction between Na^+ and K^+ pores has been underlined in the meantime by various discoveries, and in particular by the specific blocking action of tetrodotoxin (TTX) on the Na^+ current (Narahashi, Moore & Scott, 1964) and of tetraethylammonium (TEA) on the K^+ current (Tasaki & Hagiwara, 1957). Since the "gates" controlling the ionic fluxes in these pores seem to function differently, it

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is now urgent to search for an understanding of the underlying gating mechanisms. As an example of such endeavors, we mention the recent results concerning the Na^+-gating current, subsisting even when the ionic currents are suppressed (Armstrong & Bezanilla, 1973; Keynes & Rojas, 1974). This peculiarity suggests that the gating currents are displacement currents, resulting from a small displacement of charged particles, situated at the entrance of the Na^+ pores and controlling their opening.

The aim of this paper is to analyze the kinetic properties of a simple physical model for Na^+ and K^+ pores, in order to compare the resulting predictions with known experimental data. The chosen pore model takes into account previous work (Van Lamsweerde-Gallez & Meessen, 1974, 1975—referred to hereafter as VM) concerning the steady-state K^+ voltage-current characteristics of some biological membranes, displaying two regions of negative resistence. On one hand, it has been shown that one has to assume the existance of *dielectric pores*, since the high potential barrier encountered by ions at the interface of the normal phospholipid membranes—of low dielectric constant—and the adjacent electrolytic solutions prohibits the passage of the ions through the membrane. The pores, postulated long ago, do thus not have to correspond to holes, but can simply result from the known fact that biological membranes contain easily polarizable proteins as local inclusions. On the other hand, it was possible to account for the negative resistance regions, by considering the orientation of *electric dipoles* at the interface between the pore material and the electrolytic solutions, at both sides of the membrane. This model provided, indeed, a satisfactory quantitative agreement with the experimental results of Gilbert and Ehrenstein (1969). These considerations were limited however to K^+ currents in a time-independent situation. We considered it therefore necessary to verify if the same model is also able to account for more complicated processes.

In section I, we recall the basis assumptions and the essential analytical expressions associated with the chosen model, that should now be extended to the description of kinetic effects, displayed in voltage-clamp experiments. The *gating current* should be explained, in particular, by a change of orientation of the dipoles at the pore-water interface.

An analytical expression can also be given for the *instantaneous current* (which is shown to be linear with the applied voltage change) and for the final *steady-state current*. The complete set of *kinetic electrodiffusion equations* is then integrated numerically, with the assumption of a constant field in the membrane, and for the case of time-dependent dipole barriers at the pore surfaces. The case of the electrodiffusion of ions in a homogeneous membrane without dipoles has already been treated by Arndt & Roper (1972).

In sections II and III, we compare our theoretical predictions with the experimental results of Hodgkin and Huxley (1952) for K^+ currents, measured after sudden changes of the potential difference across the membrane, according to the voltage-clamp method. For Na^+ currents, we chose the experimental results of Armstrong et al. (1973) which indeed provide the advantage of a separate display of the activation and inactivation phase of the Na^+ current. The activation phase of both K^+ and Na^+ currents may be explained by an adequate reorientation of the external dipole barrier (voltage-controlled opening of the pores). However, the inactivation phase of the Na^+ current cannot be adequately explained in the framework of this dipole model.

I. Theoretical Model

Basic Assumptions

a) The proteins which are embedded in the phospholipid bilayer of biological membranes, according to the mosaic membrane model of Singer & Nicolson (1972), are considered as *electrostatic pores*, since the ions can penetrate more easily into a region whose dielectric constant is close to that of the electrolytic solutions (VM, 1975). The dielectric constant of proteins is indeed of the order of 20 for dry synthetic polypeptides (Tredgold & Hole, 1976) and can reach the value of 80 as the water content of the proteins increases (Takashima & Schwan, 1965).

b) The pores are relatively large (diameter of the order of 80 Å) since they correspond to protein inclusions of this size. Each pore can contain, of course, many narrow channels (of the order of a few angströms) which control the individual motions of the ions, including "single file" effects. To put forward the essential general features of pore permeability, we will use, however, averaged local values for the ionic concentration and electric field within the pore, so that we can use the basic equations of the electrodiffusion.

c) The *electric potential profile* is represented schematically in Fig. 1. It takes into account the applied potential difference V, the effect of fixed charges, α_0 and α_i, and the discontinuities, V_0 and V_i, resulting from the existence of dipole layers at the outside and inside surface of a nerve

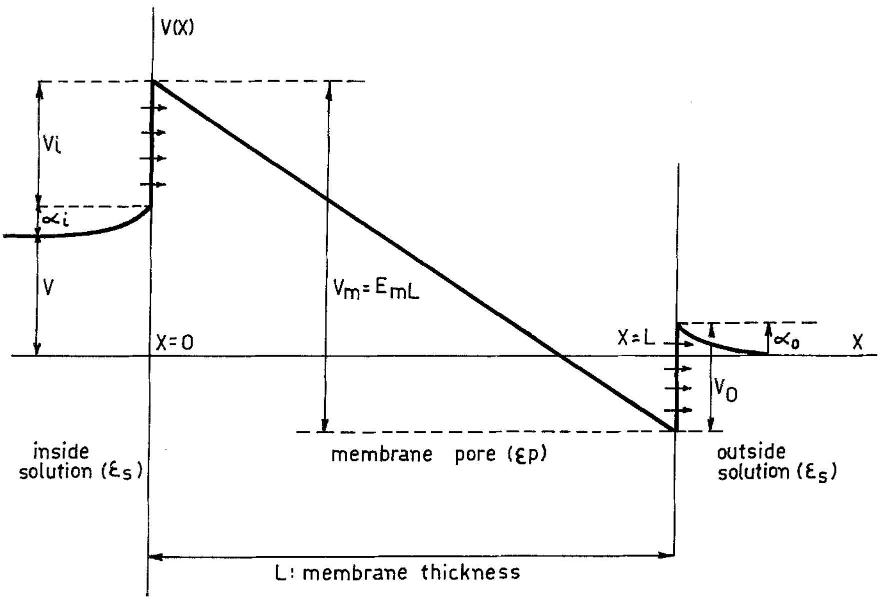

Fig. 1. Potential profile $V(x)$ across a pore. The signs of α_i and α_0 are positive for positive surface charges and the signs of V_i and V_0 are positive when the dipoles are oriented towards the right. $V=0$ in the outside solution

axon. The values of V_0 and V_i can be modified by reorienting the dipoles through a variation of V. The values of α_0 and α_i depend on the concentration of divalent ions in the electrolytic solutions and its ionic strength, but can be considered to be practically independent of V (VM, 1974).

d) The *orientable dipoles* are assumed to have two prefered orientations, so that they can point towards the electrolytic solutions or towards the inside of the pores. Recent experiments suggest that such dipoles must have more than two field orientations because, at large depolarizations, gating currents are observed even after the activation of the Na pores (Neumcke, Nonner & Stämpfli, 1976).

Our two-state model must thus be generalized to allow multiple transitions of each gating dipole, but the fundamental conclusions of this paper remain valuable. Moreover, it is assumed, to explain the negative resistance in the steady-state potential current characteristics, that the dipoles tend to be oriented towards the solution (lower potential well) unless they are oriented towards the pore under the influence of a sufficiently strong and adequately oriented electric field. In addition, we consider the amplifying effect of the Lorentz field, resulting from the relatively high polarizability of the pore material. The dipole-dipole interactions, which are opposed to the orienting effect of the applied field, may be neglected if the dipole separation exceeds a certain value (approximately two times the dipole length) (VM, 1975).

e) The pores are assumed to be *selective*, i.e., Na^+ and K^+ ions diffuse through different pores. A molecular interpretation of this selectivity lies beyond the scope of this article (*see*, for instance, Hille, 1972, 1973).

Kinetic Properties of the Orientable Dipoles

Let $U^c(\theta)$ be the configurational energy of an electric dipole moment p, as a function of the angle θ between the dipole moment and the normal to the pore surface in the direction of the solution. This function only takes into account the effect of the immediate surrounding of the dipole (interactions and steric hindrance). We assume a double potential well, allowing the dipoles to point towards the solution (s) or towards the inside of the pore (p), so that a reorientation of the dipoles requires a thermal activation to overcome a barrier of given height. One could imagine other models, and in particular a progressive increase of $U^c(\theta)$, without an intermediate potential barrier. This would lead, however, to considerably smaller time constants for the dipole reorientation (Rojas & Keynes, 1975).

We assume that the two preferred orientations correspond to dipoles pointing towards the solution ($j=s$ or $\theta=o=\theta_s$) or towards the inside of the pore ($j=p$ or $\theta=180°=\theta_p$). The actual minima of the potential wells are given by

$$U_j=U^c(\theta)-pE_j^*\cos\theta \tag{1}$$

where $j=s$ or p, while E_j^* is the effective electric field acting on the dipole moment p. This field is, of course, not the same within the pore and the adjacent solution. We set

$$E_j^*=E_j+E_j^L$$

when E_j is the field resulting from the potential profile and E_j^L the Lorentz field in the medium $j=s,p$. When the dielectric constant of the pore is relatively high ($\varepsilon_p \gg 1$) one can get indeed a considerable amplification of the action of the applied field, since $E_j^L=\varepsilon_p E_j/3$ (VM, 1975). A schematic representation of the potential wells is given in Fig. 2, where

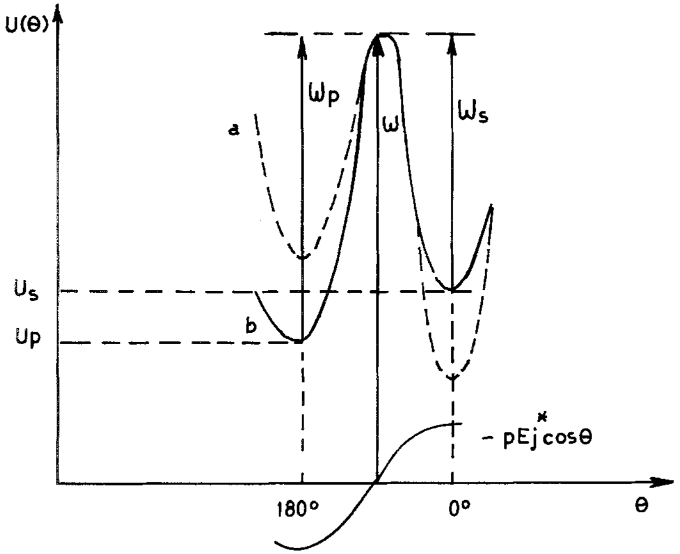

Fig. 2. Potential energy $U(\theta)$ of an electric dipole moment situated at the membrane surface for different values of the local electric field $E^*\cdot\theta$ is the angle between the dipole and the normal to the surface, pointing towards the exterior of the axon

the curves (*a* and *b*) correspond to two different values of the applied potential difference V across the membrane.

If N_j represents the average number of dipoles per unit area in the configuration $j=s$ or p, for a given value of the potential difference, we can describe the variations of these populations by the first order rate equation

$$\frac{dN_p}{dt}=N_sP_s-N_pP_p, \quad \text{with} \quad N_s+N_p=N. \tag{2}$$

The probability P_j for a dipole to undergo a transition from the configuration j to the other one is given by

$$P_j=f_j\exp(-W_j/kT) \tag{3}$$

where f_j is practically equal to the frequency of oscillation of the dipole in the potential well j; while the height of the potential well that has to be overcome in this transition is

$$W_j=W-U^c(\theta_j)=W-U_j+p\,E_j^*\cos\theta_j \tag{3'}$$

where p is the dipole moment.

For any fixed potential difference, there will exist an equilibrium distribution, so that $N_s P_s = N_p P_p$. But when the potential difference V is suddenly modified, one gets a sudden variation of E_j^*. At the outer surface, we get, for instance, the linear relation (VM, 1975):

$$E_{op}^* = E_{op} + E_{op}^L = \left(1 + \frac{\varepsilon_p}{3}\right)(V + V_i + \alpha_i - \alpha_0)/L \tag{4}$$

where L is the membrane thickness and ε_p the dielectric constant of the pore material. It follows, thus, from Eqs. (3) and (3′) that P_j varies suddenly, so that N_p changes exponentially according to Eq. (2):

$$N_p(t) = N_p(0) + \Delta N_p[1 - e^{-t/\tau_R}]. \tag{5}$$

$N_p(0)$ and $N_p(\infty) = N_p(0) + \Delta N_p$ are the initial and final densities of dipoles oriented towards the pore before and after the application of the potential step V. The relaxation time for the dipole reorientation is

$$\tau_R = 1/(P_s + P_p) \tag{6}$$

where the probabilities P_s and P_p correspond to the final potential difference. The reorientation of the dipoles leads of course to a modification of the height of the potential barrier. For the barrier at the outside surface we would get the relation (VM, 1975):

$$V_0 = \frac{4\pi\gamma}{\varepsilon_s} p[(1+q)N_p - N] \tag{7}$$

where $\gamma = 1$ in gaussian units and $\gamma = 1/4\pi\varepsilon_0$ in MKS units (meter, kilogram, second), while $q = \varepsilon_s/\varepsilon_p$ is the the ratio of the dielectric constants of the solution and the pore material. According to Eqs. (5) and (7), the dipole potential difference V_0 varies as a result of a sudden variation of V as

$$V_0(t) = (V_{oh} - V_{oc})\, e^{-t/\tau_R} + V_{oc}. \tag{8}$$

V_{oh} and V_{oc} are the potential barriers in the initial and final states of equilibrium assuming that V varies abruptly from V_h ("holding potential") to V_c ("clamp potential"). These variations are exponential with a time constant τ_R. The dipole potential barrier $V_i(h)$ at the inside surface of the axon varies, of course, in a similar way,

$$V_i(t) = (V_{ih} - V_{ic})\, e^{-t/\tau_R} + V_{ic}. \tag{9}$$

Gating current

This readjustment of the dipole population after a voltage step will induce a "displacement current" which is simply due to the displacement of the dipolar charges. The existence of this current was already mentioned by Hodgkin & Huxley (1952*d*) and has been detected experimentally, for the Na^+ system. It has been called "gating current" since its time and voltage dependence seems to correspond to the opening and closing of the Na^+ gates. These properties will be discussed below.

The theoretical expression of this current can be obtained as follows: the electric field in the membrane $E_m(t)$ will vary with time after a voltage step V according to its definition (VM, 1975)

$$E_m(t)=\frac{V_m(t)}{L}=\frac{V+V_i(t)+V_0(t)+\alpha_i-\alpha_0}{L}. \tag{10}$$

This temporal variation of the electric field in the membrane, due to dipole reorientation, will produce a displacement current (Jackson, 1962):

$$i_{D1}(t)=4\pi\gamma\varepsilon_p\frac{\partial E_m(t)}{\partial t}=4\pi\gamma\frac{\varepsilon_p}{L}\frac{\partial V_m(t)}{\partial t}$$

which varies according to Eqs. (8), (9) and (10) as

$$i_{D1}(t)=\frac{4\pi\gamma\varepsilon_p}{L\tau_R}S(V_{oh}-V_{oc}+V_{ih}-V_{ic})\,e^{-t/\tau_R} \tag{11}$$

where S is the fraction of the membrane surface occupied by pores. This predicted current thus decreases exponentially, with a time constant τ_R which is the dipole relaxation time. This is in agreement with the observed exponential gating current. The current i_{D1} depends on the value of the dipolar barriers $V_{0,i}$, which will be determined by fitting with steady-state current-voltage curves, and on the value S, the fraction of the membrane surface occupied by Na^+ pores, since the gating current is produced only by dipole reorientations at the pore surfaces, and not on the entire membrane surface. The values of these parameters will be discussed later, but it may already be mentioned that the observed gating current is about 100 times smaller than the observed ionic currents.

Numerical Integration of the Kinetic Electrodiffusion Equations

The ionic current density for an ion of type k at a point x in a pore is given by the Nernst-Planck equation:

$$i_k^I(x,t) = z_k u_k RT\left(\beta z_k c_k(x,t) E_m(x,t) - \frac{\partial c_k(x,t)}{\partial x}\right) \tag{12}$$

with $\beta = F/RT$, where F is the Faraday's constant, R the perfect gas constant, T the absolute temperature, u_k the ionic mobility in the pore and c_k the ionic concentration in the pore (in mM/liter H_2O). The current is assumed to be due only to monovalent ions ($z_k = \pm 1$) and the electric field E_m is assumed to be constant within the pore, but varies exponentially with time, after an applied voltage step, according to Eq. (10).

Using the *equation of continuity*,

$$\frac{\partial i_k^I(x,t)}{\partial x} = -F z_k \frac{\partial c_k(x,t)}{\partial t} \tag{13}$$

and Eq. (12), we get a *partial differential equation* in $c_k(x,t)$:

$$\frac{\partial c_k(x,t)}{\partial t} = \frac{u_k}{\beta}\left(\frac{\partial^2 c_k(x,t)}{\partial x^2} - \beta z_k E_m(t) \frac{\partial c_k(x,t)}{\partial x}\right). \tag{14}$$

Its solution $c_k(x,t)$ defines the concentration of ions of type k at the point x, at time t. To solve this equation, we need only one *initial condition*, which is actually the solution of the Nernst-Planck Eq. (12) in the initial steady-state at time $t=0$:

$$c_k(x,0) = c_k(0,0) + [c_k(L,0) - c_k(0,0)]\,\frac{1 - e^{\beta z_k E_m(0) x}}{1 - e^{\beta z_k E_m(0) L}}. \tag{15}$$

The *boundary conditions* at $x=0$ and $x=L$, under thermal equilibrium, are now time dependent, since

$$\begin{aligned} c_k(0,t) &= c_k^i \gamma_k e^{-\beta z_k(\alpha_i + V_i(t))} \\ c_k(L,t) &= c_k^o \gamma_k e^{-\beta z_k(\alpha_o - V_o(t))} \end{aligned} \tag{16}$$

where c_k^o and c_k^i are respectively the ionic concentrations in the outer and inner solutions, and γ_k the partition coefficient for the ions of type k. The concept of a flux control by dipolar potential barriers is thus clearly apparent: when the potential barriers $V_o(t)$ and $V_i(t)$ are varying after a sudden variation of the applied voltage difference, the ionic concentrations at $x=0$ and $x=L$ will immediately be modified by these variations, so that the electrodiffusion current changes essentially as a result of the time-dependent boundary conditions.

The total current density is the sum of the total ionic current density $i_k^I(x,t)$ and of the displacement current density:

$$i_k(x,t)=i_k^I(x,t)+\frac{1}{4\pi\gamma}\,i_{D1}(x,t)+\frac{1}{4\pi\gamma}\,i_{D2}(x,t). \tag{17}$$

The displacement current density is due to the time variation of the electric field in the membrane and may be written as the sum of a displacement current i_{D1} due to the time variation of the imposed electric field and of a displacement current i_{D2} due to the time variation of the electric field produced by ions moving with time. In the expression [Eq. (10)] for the "gating current" we used only expression i_{D1} because there was no ionic current. Using Poisson's equation and continuity equation, it can be demonstrated (Arndt & Roper, 1972) that the total current density [Eq. (17)] is independent of x, because any increase in ionic current density at x will be compensated by an increase in displacement current, and

$$i_k(x,t)=i_k(t)=\frac{1}{4\pi\gamma}\,i_{D1}(t)+\int_0^L i_k^I(x,t)\,dx$$

or using Eqs. (11) and (12)

$$i_k(t)=\frac{\varepsilon_p}{L}\,\frac{\partial V_m(t)}{\partial t}+\frac{z_k u_k RT}{L}\left[c_k(0,t)-c_k(L,t)+\beta z_k E_m(t)\int_0^L c_k(x,t)\,dx\right]. \tag{18}$$

With this expression, which may be identified with the measurable current in the external circuit, we are now able to describe the ionic current which will follow the application of a voltage clamp. In this case $\partial V/\partial t=0$, but $\partial V_m(t)/\partial t\neq 0$ according to Eq. (10). The problem then is to solve Eq. (14) for $c_k(x,t)$ with the initial condition [Eq. (15)], and to introduce this solution in Eq. (18).

We recall that an analytic solution of Eq. (12) has been obtained by Arndt & Roper (1972) for a homogeneous membrane, without surface dipole layers, since the field E_m is then independent of t, so that the equation becomes separable in x and t, while the boundary conditions [Eq. (11)] are also time-independent. The solution $c_k(x,t)$ and the total current density are expressed in this case by functions, decreasing exponentially with time. In the more general case, we have to integrate Eq. (14) numerically. We used an IBM 370/158 computer, adopting a subroutine [*origin*: P. Hallowel, Atlas Lab. Chilton, Berks.; *language*: FORTRAN; *date*: September, 1965; *version*: DPO1A]. This subroutine provides the solution of the parabolic partial differential equation

$$\frac{\partial c}{\partial t} = A\frac{\partial^2 c}{\partial x^2} + B\frac{\partial c}{\partial x} + C\,c + D, \tag{19}$$

where A, B, C, D are functions of x and t, with boundary conditions of the form

$$P\frac{\partial c}{\partial t} = Q + R\,c + S\frac{\partial c}{\partial x} \tag{20}$$

where P, Q, R, S can be functions of t. The coefficients P, Q, R, S can be determined for given boundary conditions of the form (16). This program will be called $C1$. All variables are expressed in nondimensional form:

$$\begin{aligned} &x^* = x/L \text{ where } L \text{ is the membrane thickness;}\\ &c^* = (c - c(L,0))/\tilde{c} \quad \text{with} \quad \tilde{c} = c(0,0) - c(L,0). \end{aligned} \tag{21}$$

Given an initial solution $c^*(x^*, t^*)$ and an interval ∂t^*, the subroutine executes the integration over t^* for one step, yielding $c^*(x^*, t^* + \partial t^*)$. When the values $c^*(x^*, t^* + \partial t^*)$ are known, they are integrated for $0 < x^* < 1$ by Simpson's rule, with an even number of intervals ∂x^*. The total current density is calculated from Eq. (18). We then express this current density in mA/cm^2, for comparison with the experimental curves.

Instantaneous and Steady-State Current-Voltage Relations

It is already possible to get an analytical expression for the *instantaneous current* densities, i.e., current density for $t = 0$, without this numerical integration. We can then show that the instantaneous current density predicted by the present model will vary linearly with the applied voltage difference V, as it has been shown experimentally for the squid axon in sea water (Hodgkin & Huxley, 1952b).

The total current density for an ion of type k is indeed given by Eq. (17) immediately after a voltage clamp ($t = 0$):

$$i_k(0) = \frac{\varepsilon_p}{L}\frac{\partial V_m(0)}{\partial t} + \frac{1}{L}\int_0^L i_k^I(x,0)\,dx.$$

The first term can be neglected since it is equal to expression (11) for the displacement current, which appear to be about 100 times smaller than the ionic current given by the second term. It is thus sufficient to show that this second term varies linearly with V_c, i.e., that each $i_k(x,0)$ is linear with V.

Let us write the Nernst-Planck equation (12) for the ionic current density $i_{kh}(x)$ before the voltage clamp $(V=V_h)$ and for the current density $i_k(x,0)$ just after the voltage clamp:

$$\begin{aligned} i_{kh}(x) &= z_k u_k RT\left(\beta z_k c_{kh}(x) E_{mh} - \frac{\partial c_{kh}(x)}{\partial x}\right) \\ i_k(x,0) &= z_k u_k RT\left(\beta z_k c_{kh}(x) E_m(0) - \frac{\partial c_{kh}(x)}{\partial x}\right). \end{aligned} \tag{22}$$

The same concentrations $c_{kh}(x)$ are used in both expressions, since the ionic concentrations do not vary instantaneously in the pore after application of a voltage clamp. The electric fields are expressed by Eq. (10):

$$\begin{aligned} E_{mh} &= (V_h + V_{oh} + V_{ih} + \alpha_i - \alpha_o)/L \\ E_m(0) &= (V + V_{oh} + V_{ih} + \alpha_i - \alpha_o)/L. \end{aligned} \tag{23}$$

Substracting the two parts of Eq. (22) and using Eq. (23), we obtain the instantaneous current density at x:

$$i_k(x,0) = i_{kh} + z_k^2 u_k F c_{kh}(x) \frac{V-V_h}{L}.$$

The total instantaneous current density is:

$$i_k(0) = i_{kh} + \frac{1}{L^2} z_k^2 u_k F\left(\int_0^L c_{kh}(x)\,dx\right)(V-V_h), \tag{24}$$

which is *linear in V*.

On the other hand, the *steady-state current* density for an ion of type k is given by (VM, 1974):

$$i_k = \frac{F^2}{RT} P_k e^{\beta(V_o(V)-\alpha_o)} \frac{c_k^o - c_k^i e^{\beta V}}{1-e^{\beta V_m}} V_m \tag{25}$$

where the permeability coefficient is defined by

$$P_k = \frac{u_k \gamma_k}{L} \tag{26}$$

while the potential drop across the membrane is given by

$$V_m = V + V_i(V) + V_o(V) + \alpha_i - \alpha_o. \tag{27}$$

The voltage variations $V_{o,i}(V)$ of the dipolar barriers being described in a model with two preferred orientations, it follows from Eq. (7) that

$$V_o(V) = \frac{4\pi\gamma N_o}{\varepsilon_s} p_o[(1+q)F - 1] \tag{28}$$

where

$$F(V) = \frac{N_p(V)}{N} = \frac{1}{1+\exp[(E' - E_{po})A\,p/kT]}.$$

E' is the value of the critical field where the dipoles have equal chances to be in one of the two possible states of orientation. $E_{po} = (V + V_i + \alpha_i - \alpha_o)/L$ is the applied field, and $A = \frac{2}{3}(\varepsilon_p + 1 + \varepsilon_p/\varepsilon_s)$ is the amplification factor due to the Lorentz field (VM, 1975). Since $V_o(V)$ does not vary linearly with V and appears in an exponential factor in Eq. (25), it is clear that i_k will not be a linear function of V.

II. Comparison with Experimental Results for K$^+$ Currents

Experimental Curves

The pore model with orientable dipoles was already able to explain the two negative resistance regions appearing in the experimental steady-state current-voltage curves for K$^+$ ions given by Gilbert & Ehrenstein (1969) for the squid axon. This required the use of Eq. (25) for the current density (VM, 1975) and allowed us to determine several parameters by a fitting procedure: the *height* $\tilde{V}_{o,i}$ of the dipolar potential barriers (which determines the dipole density $N_{o,i}$), the *critical potentials* $V'_{o,i}$ (which depend on the values of the critical fields $E'_{o,i}$) and the *widths of the transition* regions $\Delta V_{o,i}$ (which determine the values of the dipole moments $p_{o,i}$). The values of these parameters are recalled in Table 1, assuming that $q = \varepsilon_s/\varepsilon_p = 2$, and $L = 50$ Å.

It is to be outlined here that the negative resistance region in the positive quadrant of the $I_k - V$ curves may be due to internal Na$^+$ ions, blocking the outflow of K$^+$ ions (Bezanilla & Armstrong, 1972). Two interpretations are possible: (i) the two mechanisms (Na$^+$ blocking and variation of the *internal* dipolar barrier V_i) could act together to produce

Table 1. Parameters for K^+ pores determined by fitting with curve *II* of Gilbert and Ehrenstein (1969)

V_0' mV	V_i' mV	ΔV_0 mV	ΔV_i mV	$\tilde{V}_0$ mV	$\tilde{V}_i$ mV	p_0 eÅ	p_i eÅ
—53.5	120	29	85	42	41	6.4	2.2

negative resistance region for $V>0$, or (ii) the internal barrier could be constant with V, and even be absent, when the negative resistance is only attributed to Na^+ blocking. In either case, it does not alter the subsequent kinetic treatment of the K^+ current, since the kinetic behavior of this current is essentially influenced by the temporal variation of the *external* dipolar barrier $V_0(t)$.

To test the kinetic properties of the K^+ system, we have chosen the voltage clamp data of Hodgkin and Huxley (1952*a*) for the squid axon, when the temporal variation of the K^+ current is observed separately (the Na^+ current being eliminated by varying the external Na^+ concentration). The ionic concentrations in the external and internal solutions are, respectively, $c_K^o = 10$ mM/liter H_2O and $c_K^i = 400$ mM/liter H_2O so that the Nernst potential for K^+ is given by:

$$V_K = \frac{RT}{F} \ln \frac{c_K^o}{c_K^i} = -92\,\text{mV}.$$

These ionic current curves display a sigmoid shape, tending to a voltage-dependent saturation value, for the final steady state. Their time course was determined by two factors:

1) the time needed by the ions to redistribute themselves in the pore after a voltage clamp, i.e., the "diffusion time"

2) the time needed by the dipoles to reorient themselves after a new voltage clamp, i.e., the "relaxation time".

For the diffusion of K^+ ions in K^+ pores, we have no information at present about the relative values of these two times, since no gating current has been observed for the K^+ system, allowing us to evaluate the K^+ dipolar relaxation time. Two extreme possibilities have thus to be considered:

1) the "rate-limiting step" could be the dipolar conformational change, while the ions would redistribute themselves nearly instantaneously within the membrane. This possibility must be excluded, however, since

the instantaneous current density would not be a linear function of V in that case.

2) The configurational change of the dipoles could be so fast that the "rate-limiting step" is the diffusion of the ions, resulting from their finite mobility within the pores. It is known (Arnd & Roper, 1972) that the current would vary exponentially with time in that case.

The time variation of the observed K^+ currents corresponds, however, to a sigmoid shape. We conclude, therefore, that the dipole reorientation introduces a *delay* in the variation of the K^+ current, and that this is the cause of the slow increase at the origin. The first possibility has thus to be modified by taking into account a finite redistribution time for the ions.

Simulation of the K^+ Currents for Different Depolarizations

To simulate the experimental curves of Hodgkin & Huxley for K^+ after a voltage clamp, we use the program *C*1, for different depolarizations starting at the resting potential $V_{RP} = -70\,\mathrm{mV}$ with the dipolar parameters of Table 1. The theoretical current density curves obtained in this way are represented in Fig. 3. We adopt the following conventions: the external potential is zero and the outward current is positive. For all values of the depolarization steps only the external potential barrier is assumed to be varying. For each depolarization, we have chosen the dipolar relaxation time which gives the best fit with the experimental curves of Hodgkin & Huxley. This relaxation time is of the order of 1 msec, depends on the applied voltage, and corresponds approximately to the $\tau_n(V)$ curve of the Hodgkin & Huxley theory. We require thus that, if experimental gating currents should be measured in the future, their time constants have to display such a voltage dependence.

The calculated curves exhibit the same sigmoid shape as the experimental curves. For each theoretical curve, we have also calculated the maximum rate of rise of the K^+ conductance $g_K = I_K/(V - V_K)$, which corresponds also to the results of Hodgkin & Huxley.

We conclude that the progressive reorientation of the dipoles of the K^+ pores after a depolarization can be considered as being responsible for the sigmoid shape in the K^+ current. It is also to be outlined that the dipole relaxation is the rate-limiting step of the overall process. Indeed, the measured Q_{10} of the maximum ion conductances are of the order of 1.2 to 1.3, suggesting that ions move through the pores almost as easily

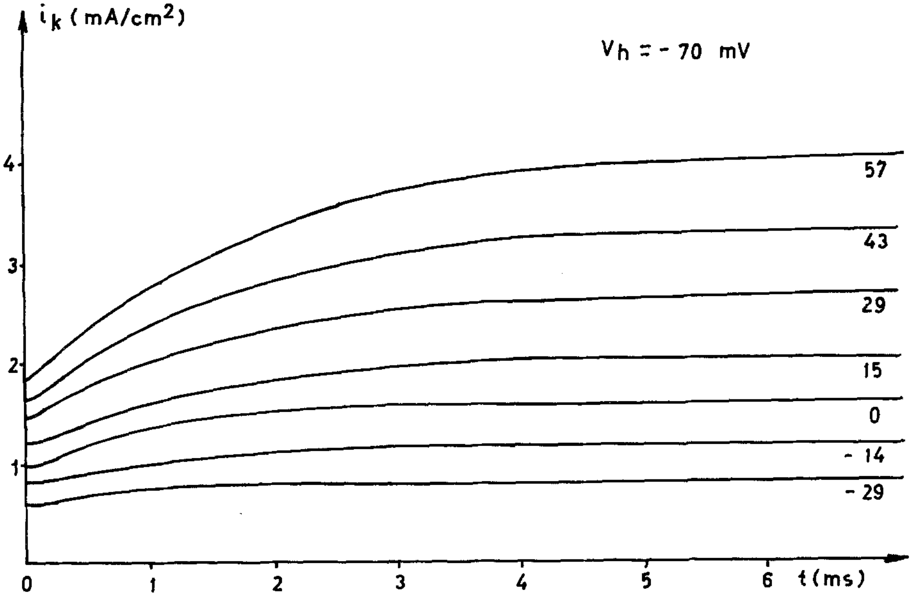

Fig. 3. Theoretical K^+ current density calculated for different depolarization steps (in mV)

as through water. In such a case, the mobility of K^+ ions in the pore must be close to that of K^+ ions in water (of about $10^{-4}\,\mathrm{cm}^2/\mathrm{sec} \times V$). Lacking quantitative information concerning the K^+ ionic pore mobility, we adopt a value of the order of $10^{-6}\,\mathrm{cm}^2/\mathrm{sec} \times V$. Using the electrodiffusion theory (Cole, 1968), the time distribution $\tau_D = L^2/kTu(eV/kT + \pi^2) \simeq 10^{-3}$ msec for a membrane width $L \simeq 50\,\text{Å}$ and $V \simeq 50$ mV. The redistribution of the ions in the pores is thus faster than the dipole relaxation, but not instantaneous, to ensure the linearity of the instantaneous current curve.

On the other hand, it can be verified that the calculated K^+ current at $t=0$ is indeed linear (*see* Fig. 4) and that the saturation values of the K^+ current after 6 msec show marked rectification effects, in agreement with the values given by Hodgkin & Huxley (1952*a*). The same values would be obtained using Eq. (24) for the instantaneous K^+ current and Eq. (25) for the steady-state K^+ current at saturation. However, the theoretical values of the initial current are higher than the experimental values. This point cannot be explained in the framework of the electrodiffusion theory, and would therefore require a more detailed description of events related to the fast charge motion of the capacitive current associated with a potential change.

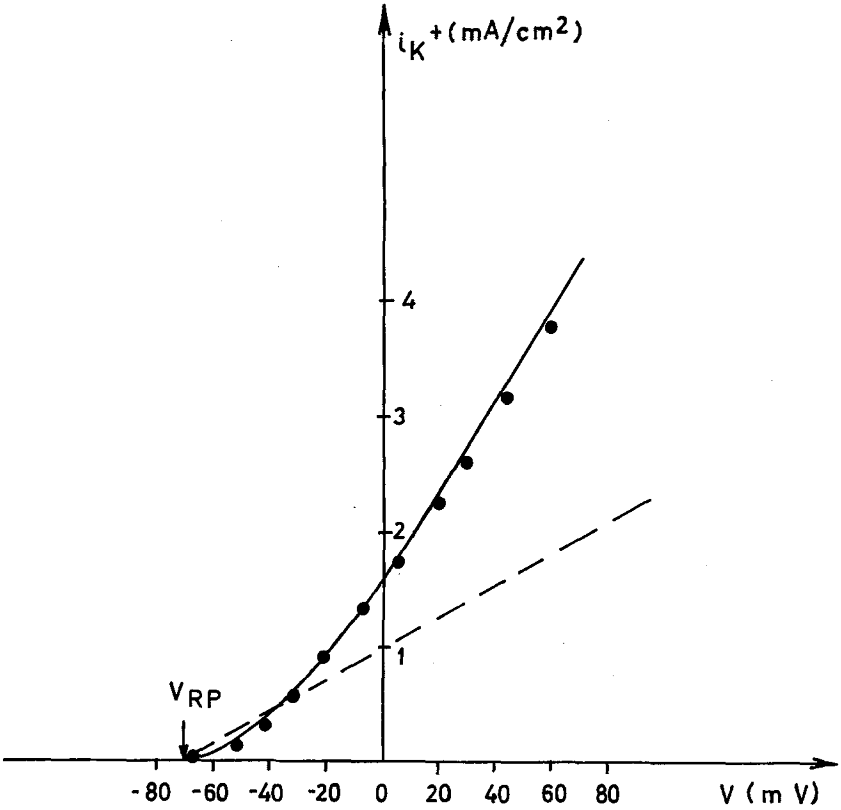

Fig. 4. K^+ current density as a function of *V*. ●, steady-state current density following Hodgkin and Huxley (1952*a*); —, theoretical steady-state current density [Eq. (25)]; ---, theoretical instantaneous current density [Eq. (24)]

III. Comparison with Experimental Results for Na^+ Currents

Sodium Inactivation

The kinetic behavior of the Na^+ current after a voltage-clamp shows two successive phases: an *activation phase* where the current increases following a sigmoid shape, and an *inactivation phase* where it decreases exponentially.

To study this kinetic behavior of the Na^+ currents we have chosen the results of Armstrong, Bezanilla and Rojas (1973) for the squid axon. These authors have shown that the inactivation phase of the Na^+ current can be suppressed by internal perfusion of the proteolytic enzyme pronase. They observed the following properties:

Table 2. Ionic concentrations following Armstrong *et al.* (1973) (in mM/literH_2O)

	Na^+	K^+	Ca^{++}	Mg^{++}	TEA^+	Cl^-	F^-	Br^-	Sucrose
External solution	440	0	10	50	0	560	0	0	0
Internal solution	50	275	0	0	15	0	275	25	325

The Nernst potential for Na^+ ions is given by:

$$V_{Na} = \frac{RT}{F} \ln \frac{C^0_{Na}}{C^i_{Na}} = 55\,\text{mV}.$$

– the time and voltage dependence of the activation phase are not affected by pronase;

– the K^+ current remains unchanged;

– external application of TTX does not affect pronase action;

– the voltage dependence of the peak value of Na^+ current before pronase application and the voltage dependence of the steady-state value of Na^+ current after pronase application are approximatively the same. Table 2 gives the ionic concentrations used in the experiments of Armstrong *et al.* The observed currents are now only Na^+ currents, since K^+ currents are blocked by TEA application.

To be able to simulate the kinetic variations of the Na^+ current, we have to provide a model for the activation and inactivation phase of the Na^+ current. These two phases are generally described as corresponding to the opening and closing of "gates" which control the Na^+ ion fluxes. Among the mechanisms that could be involved, one has mentioned "the movement of a blocking particle to a certain region of the membrane" (Hodgkin & Huxley, 1952*d*) as well as the presence of a third conformation of the Na^+ ionophore in addition to the resting state and active state (Hill, 1972; Gillespie, 1976). Some authors proposed, on the other hand (Tasaki, 1968; Hoyt & Strieb, 1971; Offner, 1972; Lefever & Deneubourg, 1975), that the inactivation could result from a limited diffusion of Na^+ on one or both sides of the pore. The assumption of a blocking particle thus requires some time-dependent structural mechanism in order to close the pore, and may be called an "uncoupled" system, where Na^+ activation and inactivation mechanisms are independent of one another. The second interpretation, on the other hand, requires only a fixed structural barrier, and is a so-called "coupled" system, since the Na^+ transport system must be activated before its inactivation can start. Hoyt and Adelman (1970) and Goldman and Schauf (1972) showed, however, that experimental measurements were consistent with a coupled model.

We decided to test this idea in the framework of the dipole model, developed so far. The two membrane surfaces seem to play quite different roles concerning the control of the Na^+ fluxes. While the activation phase seems to be controlled by the outer surface, there are, however, several experimental arguments to indicate that the inner surface is controlling Na^+ inactivation. The experiments of Armstrong et al. (1973) indicate that pronase applied at the inner membrane surface destroys the inactivation phase. More recent experiments of Rojas and Rudy (1976) show also that a protein extract of pronase (alkaline proteinase *b*) dissolved in a KF solution and perfused inside a squid axon also suppresses the process of fast inactivation.

Two types of inactivation processes were then plausible at the inner surface: First, a reorientation of dipoles at the inner surface. This possibility has to be excluded, since dipoles would normally reorient themselves in order to lower the potential barrier (to open the pore and not to "close" it); moreover, activation and inactivation would not necessarily be coupled in this model.

Secondly, we could also consider a limited diffusion of Na^+ at the inner surface. The potential barrier at the outside surface would then be lowered by dipole reorientation so as to initiate the activation phase of the Na^+ current. The Na^+ ions are now able to flow into the pore, but at the inside surface they encounter a potential barrier which reduces their diffusion. The Na^+ current is then reduced to the small value allowed by the diffusive barrier. The inactivation phase of the Na^+ current thus follows automatically the opening of the pore and can only be removed by removing the barrier at the inside surface. Unfortunately, two major problems arise in this interpretation. First, the model is unidirectional, i.e., the inactivation process will occur only for inward Na^+ current and not for outward current. Second, a quantitative comparison with experimental results implies too low values for the Na^+ mobility in the pore.

The interpretation of the inactivation thus seems to resort to another type of mechanism. Nevertheless, as Armstrong et al. (1973) provide evidence that activation and inactivation phases may be separated, it remains useful to test the dipole model on the activation phase of the Na^+ current.

Steady-State Current-Voltage Curves for Na^+

The properties of Na^+ dipole system can be determined by fitting theoretical expressions to the observed steady-state *I–V* curves, in the

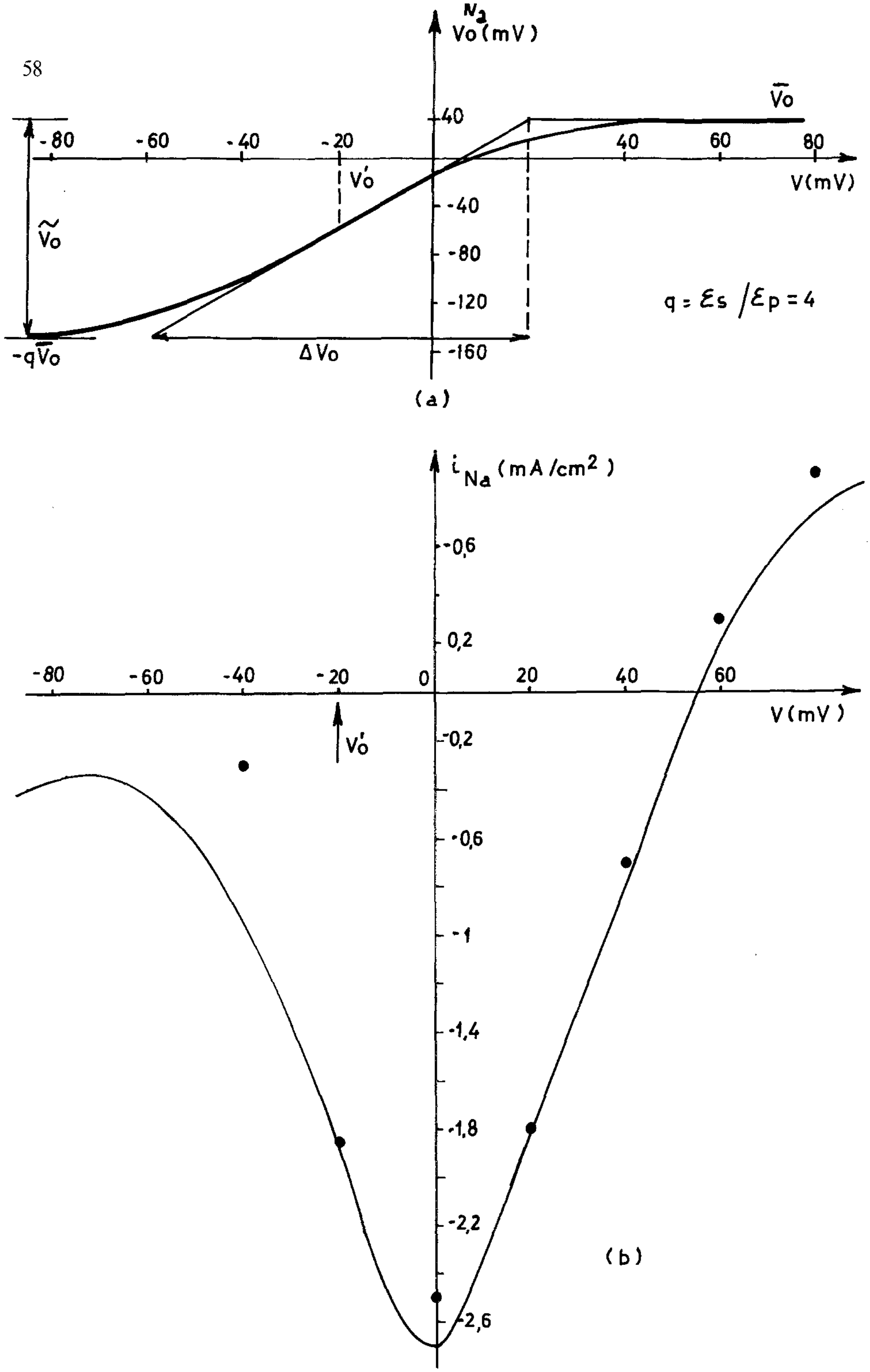

Fig. 5. (*a*): Variation of the external dipole barrier V_0 for Na^+ pores, *vs.* the applied potential difference *V*. (*b*) Na^+ current *vs.* *V*: ●, experimental data of Armstrong *et al.* (1973); —, theoretical steady-state current density [Eq. (25)]

Table 3. Dipole parameters for the Na^+ system determined by a fit with the steady-state I–V curve of Armstrong *et al.* (1973)

V_0' mV	ΔV_0 mV	$\tilde{V}_0$ mV	p_0/e Å
−20	80	185	4.7

same way as we did for the K^+ dipole system (VM, 1975). The theoretical Na^+ current-voltage relation [Eq. (25)] is compared to the experimental current-voltage curves of Armstrong et al. (1973) obtained after application of pronase, suppressing the inactivation phase. This fitting procedure allows us actually to determine the voltage dependence of $V_o(V)$ for the Na^+ system (*see* Fig 5). The corresponding parameters are given in Table 3, assuming that $\varepsilon_p = 20$ and $L = 50$ Å. The range of applied voltages does not seem to allow a reorientation of dipoles at the inside surface (if such dipoles exist in this case), since there appears only one negative resistance region. For simplicity, we thus assume that $V_i = 0$.

The height of the external dipole potential barrier $\tilde{V}_o$ is found to be about 4 times greater for the Na^+ system than for the K^+ system. This corresponds to a higher dipole density. This seems to indicate that the Na^+ pores are probably protein molecules penetrating into the phospholipidic bilayer from the outside surface without sticking through the whole membrane. In that case, all the ionic and polar groups of the protein would be situated indeed at the external surface of the membrane, as a result of their hydrophilic nature (reduction of electrostatic self energy).

Properties of the Gating Current

We can now use these dipole parameters to calculate the gating current predicted by Eq. (11) and to compare this expression with the experimental gating current curves of Keynes and Rojas (1974) for the squid axon. This procedure constitutes a rather sensitive test of the internal consistency of the proposed model.

Again, we consider only the variation of the external potential barrier $V_o(V)$ and we assume that $\varepsilon_p = 20$ and $L = 50$ Å. Figure 6 shows the resulting theoretical gating current curves obtained with the dipole parameters of Table 3, when the membrane is suddenly depolarized from $V_h = -100$ mV to different potentials, and then repolarized, the gating currents being expressed in $\mu A/cm^2$.

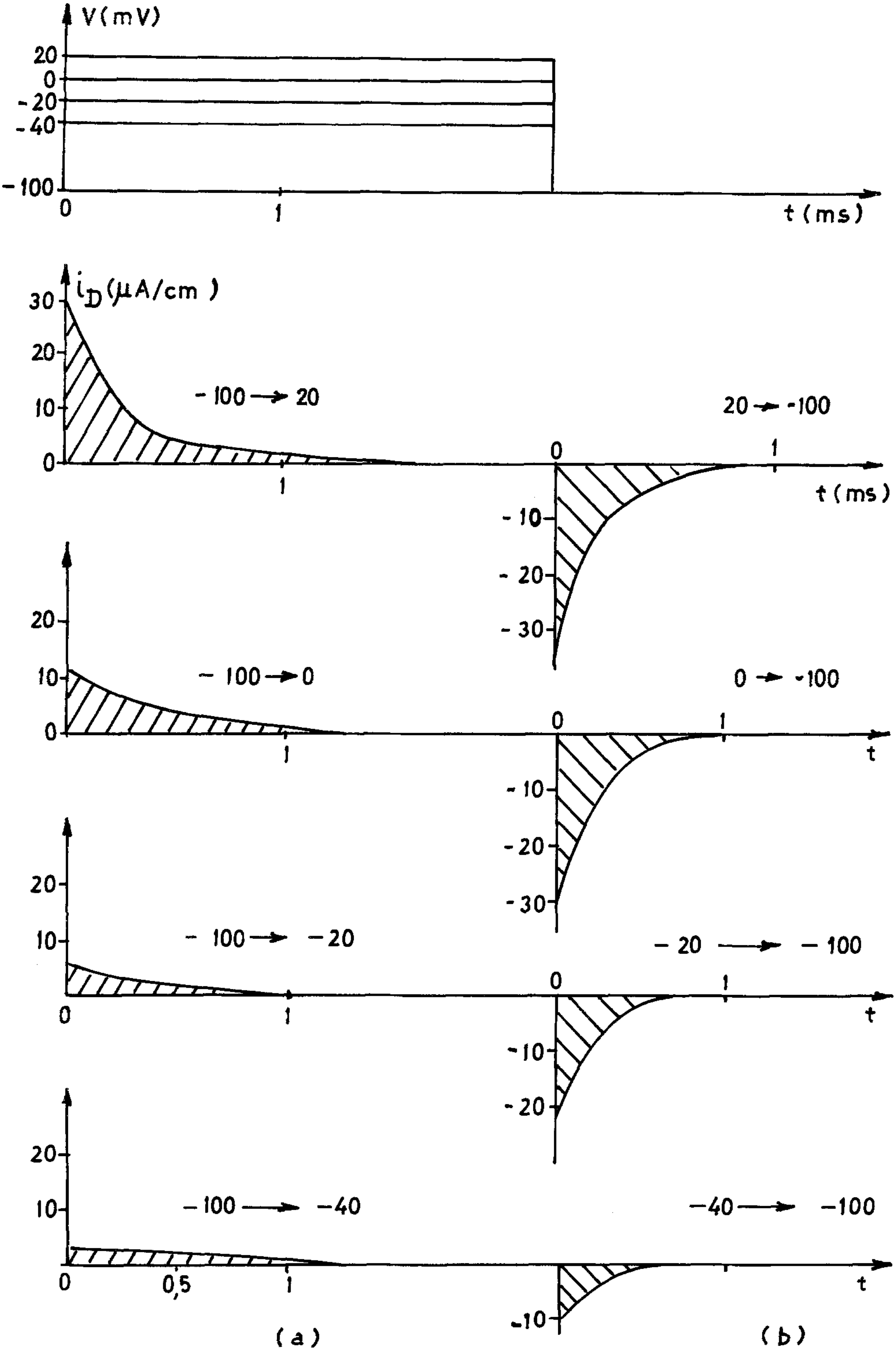

Fig. 6. Gating currents calculated by the dipole model for different depolarizations (*a*) and repolarizations (*b*)

To obtain gating currents of the order of the experimental gating currents, the fraction S_{Na^+} of the membrane surface occupied by Na^+ pores must be of the order of 1/100.

The predicted gating currents decrease exponentially and are positive for depolarization and negative for repolarization, as the experimental observed gating current. For the dipole orientation adopted in the framework of our basic assumption, a depolarization from −100 mV will reorient the dipoles, indeed, so that they are turned from the pore towards the solution at the external surface. The value of $V_{oc} - V_{oh}$ will be positive, as well as the gating current. For a repolarization, these values will be negative, eventually with a different time constant, but the total quantity of charge transferred has to be the same. We verified that this is the case by calculating the time integral of the current, using Eq. (11). The observed increases of membrane capacitance with depolarizations (Takashima, 1976) also points towards such a dipole reorientation.

The value of time constant τ_R is obtained as follows. The "dipole relaxation time" is rewritten explicitly as a function of the voltage, using Eqs. (3), (4) and (6), for $\varepsilon_s > \varepsilon_p \gg 1$:

$$\tau_R(V) = \frac{1}{A\,e^{-C(V-V'_R)} + B\,e^{D(V-V'_R)}} \tag{29}$$

with

$$\begin{aligned} A &= f_s \exp[(-\omega + U_p^c)/kT] \\ B &= f_p \exp[(-\omega + U_s^c) + kT] \\ C &= D = \varepsilon_p p/kTL \\ V'_R &= [3L(U_s^c - U_p^c)/\varepsilon_p p] - V_i + \alpha_i - \alpha_o . \end{aligned} \tag{30}$$

In order to compare the theoretical expression (29) of the dipole relaxation time and the experimental data of Keynes and Rojas (1974), we used the dipole parameters of Table 3 to determine C, D and V'_R, while the parameters A and B are determined by the best fit with experimental data.

Figure 7 shows the resulting "bell-shape" for the function $\tau_R(V)$. The continuous line is drawn according to Eq. (29) with

$$A = 1.15\ \mathrm{ms}^{-1}$$
$$B = 0.85\ \mathrm{ms}^{-1}$$
$$C = D = 0.025\ \mathrm{mV}^{-1}$$
$$V'_R = -40\ \mathrm{mV}.$$

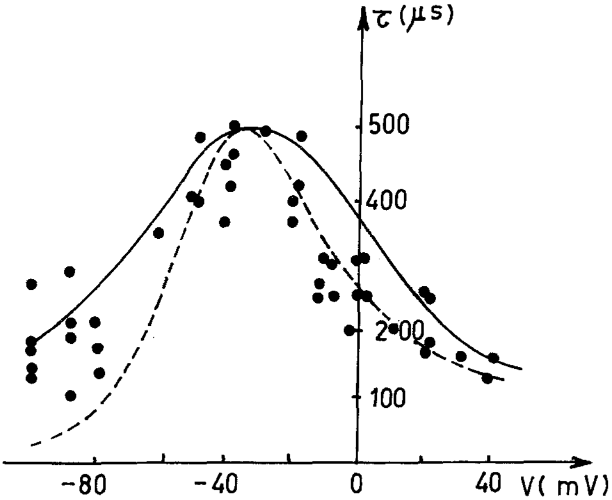

Fig. 7. Time constant τ_R as a function of V: ●, experimental data of Keynes and Rojas (1974); —, theoretical curves using Eq. (29) with parameters (31); ---, value of the activation time constant τ_m in the theory of Hodgkin and Huxley (1952)

The dashed line is the value of the activation time constant τ_m for Na^+ in the Hodgkin and Huxley theory (1952*d*). Except at large negative potentials, the agreement between the experimental data and the empirical curve τ_m is remarkably good, and thus confirms the interpretation of the gating current in terms of a reorientation of dipoles in order to assure the opening and closing of the Na^+ pores. It should be noted that the dipole relaxation times of the Na^+ system are about 10 times smaller than those of the K^+ system.

Simulation of the Na^+ Current after Pronase Application

We also simulate the Na^+ current after pronase application, i.e., when the inactivation phase is suppressed. In that case, the ions are not blocked at the inner pore surface, and the boundary condition at $x^*=0$ takes the form Eq. (16). We then use the same program *C*1 as for the K^+ current simulation for the different depolarization steps given by Armstrong *et al.* (1973) from $V_h = -70$ mV. For different values of the depolarization steps, only the Na^+ external potential barrier V_o is assumed to vary, according to the $V_o(V)$ curve determined previously (Fig. 5*a*). We expect, indeed, that pronase dissolved in the internal solution does not affect the activation mechanism at the outside surface.

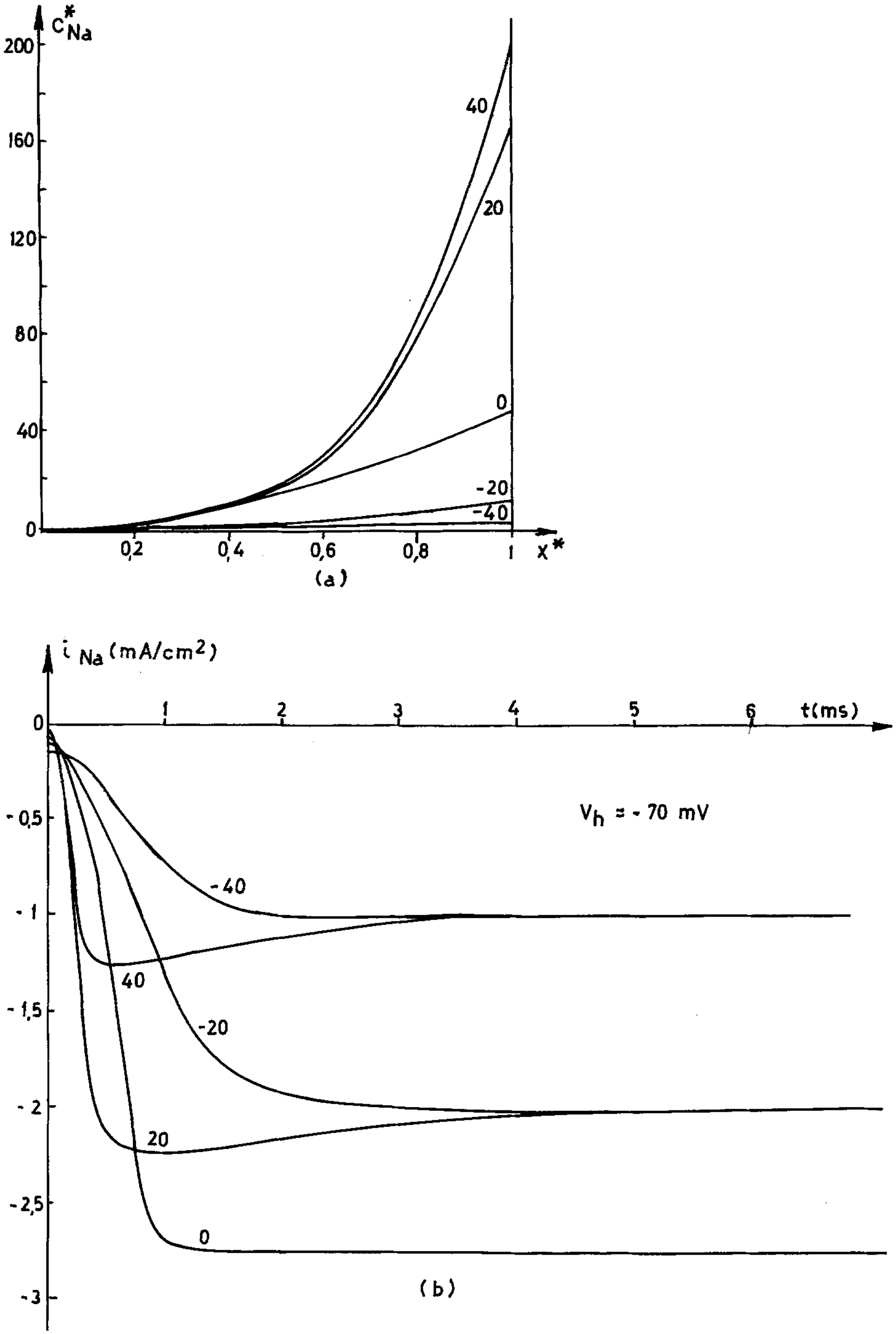

Fig. 8. (*a*): Normalized steady-state Na^+ concentrations for different depolarizations (in mV), after application of pronase. (*b*) Simulated Na^+ current density for different depolarizations, after application of pronase

The resulting theoretical curves are shown in Fig. 8. The instantaneous current is the same as before pronase application and the steady-state current is the same as that given by Eq. (25), and represented in Fig. 5*b*.

This steady-state current-voltage curve shows the same negative resistance as the experimental curve. Moreover, after Armstrong *et al.* (1973) this corresponds also to the peak current-voltage curve.

This negative resistance is the basic mechanism underlying excitability. When the applied voltage decreases to zero, the inward Na^+ current increases, indeed, depolarizing the membrane further, in a regenerative process. It was thus essential to demonstrate that the reorientation of dipoles at the external Na^+ pore surface induces a negative resistance in the Na^+ current-voltage curve and therefore a Na^+ permeability change, corresponding to a fast entry of Na^+ ions in the Na^+ pores at the beginning of the action potential.

Conclusions

1) The dipole model agrees with experimental curves for the increase of the *K^+ ionic current* after a sudden change of the applied potential (voltage clamp technique), obtained by Hodgkin and Huxley (1952*a*) for squid axons. The fitting was performed by using the dipole parameters deduced from a comparison with steady-state I–V curves for K^+ ions in squid axons, according to Gilbert and Ehrenstein (1969). The observed sigmoid-shape for the time variation of the K^+ current requires a relaxation time for the dipole reorientation of the order of 1 msec.

2) The *steady-state I–V curves for Na^+* ions in the squid axon, obtained by Armstrong *et al.* (1973) through the application of pronase, are fitted by theoretical curves. The large negative resistance regions are associated with a reorientation of the dipoles at the pore surface which is in contact with the outside solution. The determination of the dipole parameters shows that the dipole density is much larger for Na^+ pores than for K^+ pores, so that a reorientation of these dipoles produces quite different variations of the dipole potential barriers, controlling the penetration of the ions into the pores.

3) The observed "*Na^+ gating current*" after a sudden change of the applied voltage is attributed to a reorientation of the dipoles at the outside pore surface. The theoretical predictions agree qualitatively and quantitatively with the observed effects, using the same dipole parame-

ters and assuming that the portion of the membrane surface occupied by Na^+ pores is of the order of 1/100.

4) The *activation phase* of the Na^+ current may be well described by the orientation of dipoles at the outer surface (opening of the pore); the sigmoid-shape requires a dipole relaxation time about 10 times smaller than the K^+ dipole relaxation time.

On the other hand, we were not able to account for the inactivation phase of the Na^+ current by a limited diffusion of the Na^+ ions at the inner pore surface, especially for positive Na^+ outward current. This seems to indicate that the inactivation mechanism must be either a voltage-dependent process, coupled to the activation process, or a process of limited diffusion on *both* sides of the Na^+ pores.

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