J. Membrane Biol. 43, 127–148 (1978)

On the Physico-Chemical Basis of Voltage-Dependent Molecular Gating Mechanisms in Biological Membranes

Gerhard Schwarz

Department of Biophysical Chemistry, Biocenter of the University of Basel, Basel, Switzerland*, and Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania

Received 3 March 1978

Summary. The possible nature and theoretical treatment of electric field-induced molecular processes in a membrane are examined. Special attention is given to fairly fast switching phenomena as reflected by asymmetry currents as well as ionic gating in squid axon and similar systems. The apparent charge displacement associated with the underlying mechanisms is argued to be brought about by conformational transitions of integral macromolecular structures. Under these circumstances, voltage changes can actually control the functional state of membranes by direct interference with conformational equilibria. A basic model is quantitatively discussed and shown to account for certain observed asymmetry currents. Effects due to temperature, pressure, or chemical interactions can be readily described. It is indicated how more complicated voltage-dependent membrane processes may be approached along these lines.

Certain biological as well as synthetic membrane systems exhibit permeabilities which depend on the membrane potential, V, i.e., the voltage measured between the two bulk electrolyte compartments (with the reference electrode in the exterior one). Employing the voltage-clamp technique (Cole, 1949), this was especially well studied in nerve membranes by measuring the voltage dependence of the sodium and of the potassium conductance, respectively. In these cases all the experimental findings, including the action potential, could be very satisfactorily described by a number of fairly simple empirical relations (Hodgkin & Huxley, 1952). The sodium conductance, for instance, takes the form

$$g = g_o m^3 h \qquad (0 \leqq m, h \leqq 1) \tag{1}$$

* *Permanent address.*

0022-2631/78/0043-0127 $04.40

involving an upper limit, g_o, and two time and voltage dependent functions m and h which reflect the activation and inactivation processes of the sodium permeability, respectively. They are each subject to first order kinetic equations, i.e., with x being either m or h

$$\frac{dx}{dt} = \alpha(1-x) - \beta x \tag{2}$$

where α, β are the respective voltage-dependent rate constants. At constant membrane potential any transient change of x thus follows a single exponential time course with a reciprocal relaxation time of

$$\frac{1}{\tau} = \alpha + \beta. \tag{3}$$

In addition, the potassium conductance can be expressed in terms of the fourth power of an analogous function n.

This behavior could most simply be attributed to the existence of three different species of respective molecular gating structures in the membrane which can occur in "closed" and "open" states, depending on voltage. The "open" states would interact with each other in an appropriate way to let a certain ion pass through (for instance three of type m and one of type h would interact in the sodium case). Then the total fractions of the "open" states are directly given as functions m, h, or n, respectively. There is some evidence that the observed field-dependent gating mechanism consists of channel formation while the selectivity of permeation is due to an unaffected filter at a different location (*see*, e.g., Ulbricht, 1977). Although the actual molecular nature of these phenomena is not known so far, our present knowledge of membrane structure (Singer & Nicolson, 1972) indicates that they are associated with some integral macromolecules, presumably lipoproteins.

In any event, as already pointed out by Hodgkin and Huxley (1952), the voltage dependence of a gating mechanism must give rise to a corresponding displacement current when the membrane potential is suddenly altered. This gating current is, however, usually not detectable experimentally because of the much larger currents due to recharging the overall membrane capacitance of the system and due to the ion fluxes, respectively. Only recently have suitable methods been developed to eliminate these, the latter by substituting nonpermeable ions as well as applying channel blocking drugs, and the former by canceling out the ordinary symmetric contributions to the measured displacement current.

In the millisecond range this resulted in a comparatively small remaining so-called asymmetry current which may be a gating current (Armstrong & Bezanilla, 1974; Keynes & Rojas, 1974; Meves, 1974; Nonner, Rojas & Stämpfli, 1975; Kniffki & Vogel, 1976).

In some cases the asymmetry current exhibits a remarkably pronounced, purely exponential time dependence. This indicates a single molecular relaxation process. Quantitatively it is quite consistent with the estimated number of "m-structures" and their τ-value. Frequently, however, evidence of more than one relaxation process has also been found. Moreover, a special study of sodium current activation together with asymmetry currents in frog myelinated nerve showed no direct correlation (Neumcke, Nonner & Stämpfli, 1976). Nevertheless, whatever the relation between the actual gating and the measured asymmetry currents may be, both must be based on voltage-dependent molecular processes in the membrane. The inherent displacement currents allow definite basic properties to be evaluated, namely, the charge transfer involved and the respective relaxation time(s). In the following, a molecular interpretation of such phenomena and its theoretical analysis are to be developed. An accompanying article (Schwarz, 1978) demonstrates the practical merit of the proposed concept. It presents a quantitative interpretation of the inactivation of asymmetry currents in squid axon membranes as observed when various experimental conditions are altered.

Fundamental Aspects

An appropriate molecular picture of the biological membrane has been described in detail by Singer and Nicolson (1972). This so-called fluid mosaic model essentially comprises a lipid bilayer in a largely fluid state with integral protein molecules embedded in it. The nonpolar hydrocarbon chains of the lipids make up a hydrophobic core, while their polar head groups form the interfaces with the outside aqueous regions. The integral proteins must to some extent consist of hydrophobic parts which are intercalated within the hydrocarbon core so that a strong association is brought about through the hydrophobic effect (Tanford, 1973).

The overall membrane potential is composed of the voltage drop inside the membrane and the voltage steps associated with the two membrane/electrolyte interfaces. These latter steps are due to the re-

spective ionic double layers (as determined by fixed charges and electrolyte concentrations) and possible nonzero surface densities of permanent dipole moments. A constant electric field, E, may be assumed in the hydrophobic membrane interior as follows from basic electrostatic theory because of zero charge density in that region. We thus have

$$V = E \cdot d + \Delta\psi \tag{4}$$

with d denoting the thickness of the hydrophobic region of low electrical conductivity which makes up the ionic permeation barrier. The $\Delta\psi$ stands for the appropriate difference of the outer and inner potential steps at the interfaces. Thus with ordinary membrane potentials around ± 100 mV and a d of about 50 Å, field strengths of the order of 100 kV/cm can be estimated.

We note that externally produced variations of V will hardly affect $\Delta\psi$ but will change the voltage drop in the low conductivity region. Therefore, it is E which generally determines the relevant behavior of voltage dependent structures. Consequently, these must to an essential degree be located in the hydrophobic part of the membrane. This would, of course, be only natural if they really mediate ionic permeation.

Let us return now to asymmetry currents measured after applying a voltage pulse which starts at such a negative potential that a further

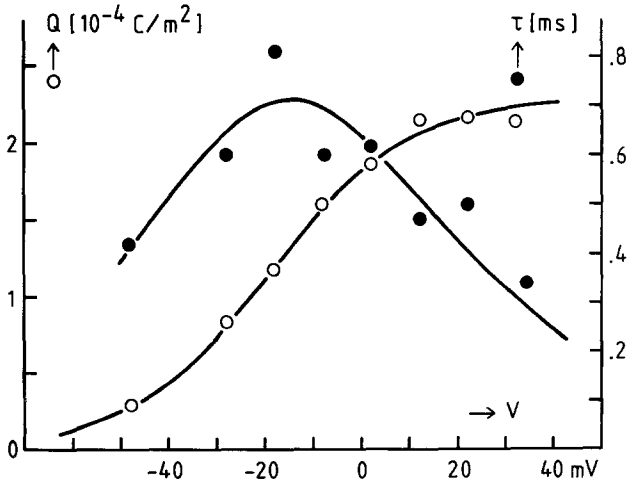

Fig. 1. Experimentally measured values of transferred charge, Q, and relaxation time, τ, of asymmetry currents in a squid axon membrane after Meves (1974). Theoretical curves have been calculated with $Q_\infty = 2.3 \times 10^{-4}$ C m^{-2} (dashed line), $V_{1/2} = -19$ mV, $b = 0.069$ (mV)$^{-1}$, $\tau_{1/2} = 715$ μsec, $\xi = 0.44$ (*see* text)

decrease has no additional effect. By integration of the current-time curve we can then evaluate the total charge, Q, transferred upon the implied change of state in the field-sensing structural elements. A saturating value, Q_∞, corresponding to a complete transition is asymptotically reached at sufficiently high membrane potential (*see* Fig. 1). The fraction Q/Q_∞ can be set equal to the degree of transition, θ, at the respective value of V (during the pulse).

We shall first discuss the general question as to which kind of structural changes in a membrane can possibly serve as sufficiently fast field induced switching devices, i.e., can proceed in the millisecond range and also allow saturation of a transition between different states by means of voltage variations on the order of 100 mV. Such properties are not only reflected by asymmetry currents, but by the actual ionic gating processes. Although the latter appears to be comparatively more complex, the basic problem is evidently also relevant to their field-sensing individual steps.

The Case of Field-Induced Molecular Switching in a Membrane

Let us start with some points about the energetics of hydrophilic and hydrophobic interactions involved in the stabilization of membrane structures. We note that a free energy of about 20 kJ/mol is necessary in order to bring a small nonpolar molecule at room temperature from its pure liquid (i.e., hydrophobic) state into water (*see*, e.g., Tanford, 1973). On the other hand, even more free energy is required for the transfer of charges or dipoles from a polar (i.e., hydrophilic) medium into a nonpolar one. Taking a monovalent ion of 2.5 Å radius from water to benzene is associated with an expenditure of more 100 kJ/mol (*see*, e.g., Moore, 1972). On the molecular level this can be interpreted in terms of the removal of dipolar solvent molecules from the immediate surroundings of the charged particles. For an analogous transfer of dipoles, the energy needed may certainly be smaller but generally remains of considerable magnitude.

In principle, this implies a substantial activation energy with regard to the penetration of the hydrophobic interior of a membrane by even a small charge or hydrophilic structure. Nevertheless, there are many well-known examples of ions which pass through membranes in a markedly short time. This is obviously brought about by appropriate structural arrangements which screen unfavorable interactions so that the or-

dinarily inhibitory activation barrier is sufficiently reduced. Analogous possibilities apply to hydrophilic molecules in general or to the inverse case of hydrophobic groups being moved through polar regions.

On the other hand, the creation and destruction of such favorable conditions for fast permeation must still involve changes of hydrophilic and hydrophobic interactions with high energy expenditure. This has to be taken into account if the degree of permeability is *altered.* Thus it plays a decisive role in the voltage dependent processes under consideration which are completely switched on or off by simply changing the membrane potential. Consequently, the corresponding energy can only be taken from the simultaneous gain in electrical energy.

It has been suggested that asymmetry currents may be due to field-induced orientation of permanent macromolecular dipoles in the membrane. In some cases, results from Debye's classical theory have been applied. However, this implies a rotational mechanism of randomly distributed dipoles in a homogeneous medium. Such an assumption is hardly reasonable with regard to the highly heterogeneous and anisotropic membrane. In this system rotational orientation involving only a few equilibrium states would be more appropriate. A two-state model already describes an observed transition curve for asymmetry currents (Kniffki, 1975) and, in addition, can explain pure exponential relaxation behavior (whereas Debye's theory predicts a more complex time dependence close to saturating conditions). In any event, it follows according to Boltzmann's distribution law that with realistic molecular dipoles of about 100–1000 D (i.e., debye units) the given membrane field changes can cause saturation effects only if the dipoles undergo quite pronounced transverse rotation between the states of equilibrium orientation.

As far as the available energy to drive these transitions is concerned, we note that a permanent molecular dipole of 500 D can gain a maximum of 10 kJ/mol in electrical energy after a complete flip-flop (i.e., a turn through 180°) if the field changes by 100 kV/cm. In homogeneous media (e.g., ordinary solvents) this would well suffice for a fast saturation of orientational equilibrium because only thermal energy has to be overcome ($kT \approx 2.5$ kJ/mol at room temperature). A different situation, however, must be expected in a membrane.

We recall that the voltage-dependent structures must to a great extent be intercalated within the nonpolar core of the membrane. On the other hand, they are required to have polar groups in order to build up a sufficient dipole moment for the field effect. From a thermodynamic point of view, the necessary stabilization in the membrane is achieved by

minimization of free energy through the most favorable hydrophobic and hydrophilic interactions. Accordingly, the hydrophobic parts of the structures under consideration are largely in the nonpolar membrane interior, whereas charged or dipolar parts protrude into the polar lipid head regions or even the aqueous exterior. This is, of course, just the same situation as pointed out for integral membrane proteins according to the fluid mosaic model. Presumably, we are actually dealing here with protein molecules of this general type. Appreciable transverse rotation of any such amphiphatic molecular structure in the membrane must automatically result in the transfer of some of its nonpolar parts into polar regions and vice versa. This would certainly require much more energy than is gained by the simultaneous rotation of the molecular dipole in the field. Accordingly there remains a high activation barrier which can only be overcome by means of thermal energy. This in turn will slow down the process enormously. To be more quantitative, it may be noted that in water at room temperature a particle of about 30 Å length has a rotational relaxation time of an order of magnitude of 10^{-8} sec. Since the apparent viscosity in a membrane is at least two orders larger (Edidin, 1974), hardly less than a microsecond can be expected here even without any additional constraints (it can be estimated that the torque exerted by the applied field does not suffice to speed up the rotational process appreciably). Thus only another 15 kJ/mol of extra free activation energy could be tolerated for rotations within a millisecond.

On the other hand, regarding flip-flops of certain phospholipids, half-times of a few hours have been measured (Kornberg & McConnell, 1971). This is equivalent to an extra activation energy of about 60 kJ/mol. For the voltage-dependent structures in question with their greater number of polar groups, still higher activation barriers should exist for ordinary transverse rotation. In other words, structurally stable macromolecules involved in the field-sensing event can be expected to have a practically constant orientation parallel to the field (i.e., normal to the membrane). In fact, this is in perfect agreement with the fluid mosaic model as already seen by Singer and Nicolson (1972), who stated that functional integral proteins "would maintain their molecular orientation and their degree of intercalation in the membrane." Experimental evidence about rotational diffusion apparently refers to rotation around the normal axis (Cherry *et al.*, 1976), which does not necessarily involve appreciable changes of hydrophilic and hydrophobic interactions. The same applies to translational diffusion.

Nevertheless, substantial transverse rotation of macromolecular di-

pole moments may well occur sufficiently fast in the course of appropriate structural changes which proceed in such a way that unfavorable energetic constraints are largely neutralized. However, this kind of indirect orientation is better described as a conformational transition associated with change of dipole moment. As we shall see in detail, the given electric field can indeed strongly displace equilibria of such macromolecular processes at a satisfactory rate.

Quite analogous argumentation can be put forward regarding mechanisms which assume a translational motion of charged particles between stable sites across the membrane (Keynes & Rojas, 1974). The same energetical shortcomings arise if field-induced motion of conformationally stable structures is considered. On the other hand, a shift of charge may well be effected by an appropriate structural rearrangement which can proceed fast enough. The displacement of charge will be equivalent to an overall change of dipole moment.

By reason of the above discussion it appears to be most appropriate to conceive voltage-dependent membrane processes as being generally based on conformational transitions of integral macromolecules associated with a dipolar change, at least in any case of fairly fast saturating phenomena. The great flexibility of this concept and how it is approached theoretically will be discussed in the following sections.

The Chemical Field Effect

According to thermodynamic principles, any chemical equilibrium will be displaced by an electric field if the underlying reaction changes the overall dipole moment of the system. As derived in more detail elsewhere (Schwarz, 1967; 1977), we have generally (in analogy to the well-known van't Hoff relation of the temperature effect)

$$\frac{\partial \ln K}{\partial E} = \frac{\Delta M}{RT} \tag{5}$$

where the quantity K is a practical equilibrium constant obtained with equilibrium concentrations instead of activities, and ΔM denotes the molar change of the total dipole moment parallel to the field (R = gas constant, T = absolute temperature).

Quantitative calculations of the change in equilibrium concentrations induced by an electric field can be directly made by means of Eq. (5). The magnitude of the effect is found to be much too small to be detected for

ordinary chemical reactions in solution and usual field strengths. Only in certain special cases was experimental evidence observed at extremely high fields using a special dielectric measuring technique (Bergmann, Eigen & De Maeyer, 1963; Hopmann, 1975). Macromolecular reactions are more apt to exhibit significant effects even at low fields (Schwarz, 1967). Corresponding dielectric effects have been measured (Schwarz & Seelig, 1968; Wada, Tanaka & Kihara, 1972; Schwarz & Bauer, 1974). A more direct indication of the field-induced displacement of a conformational equilibrium of a macromolecule was reflected in the electric birefringence data of polypeptides in solution registered over the range of the helix-coil transition (Schwarz & Schrader, 1975). It can be inferred in this case that a transition between two states with dipole moments differing by some hundred D can be almost completely induced by fields of the order of 100 kV/cm. We note that membrane proteins may indeed easily build up permanent dipoles of hundreds of D (Petersen & Cone, 1975). Such transitions are therefore also conceivable in a membrane.

Conformational changes of biopolymers are well-known to regulate many processes in molecular biology. Thus it is a rather straightforward and natural idea that such events are involved in the molecular mechanism of voltage-dependent functions of biological membranes. In fact, this was occasionally considered in the literature. With regard to the gating phenomenon, it has been taken into account as a possibility (Hill & Chen, 1972) and some corresponding thermodynamic calculations are available (Levitan & Palti, 1975). Nevertheless, the underlying conception was widely neglected so that its inherent potentialities remained unexploited.

As pointed out in the present article, chemical field effects, particularly field-induced conformational changes of macromolecules, may be indeed proposed to constitute the principal basis of any molecular voltage-dependent mechanism in a membrane. Elaboration of this appears worthwhile. Accordingly, one can develop respective models in terms of chemical reaction systems and treat them by pertinent application of chemical thermodynamics and rate theory. Consequently, for instance, effects due to temperature, pressure, or interaction with certain chemical agents are to be interpreted as resulting from corresponding changes of equilibrium and rate constants which may be described by standard methods. The theoretical procedure will be demonstrated here for a basic one-step model mechanism. This already permits a satisfactory quantitative analysis of some simple, experimentally observed asymmetry currents. Subsequently, further possibilities will be pointed out

bearing on the approach to more complex systems, among them the actual gating processes.

Basic Model of a Field-Induced Conformational Change in a Biological Membrane

A certain species P of macromolecular structure is to be somehow distributed in the membrane. We assume that this P occurs in two conformations. They are subject to the transition

$$P_1 \rightleftharpoons P_2. \tag{6}$$

According to our pertinent argumentation above, the dipole components parallel to the normal of the membrane are taken as being fixed for both conformations. The field effect on the conformational equilibrium [Eq. (6)] is then easily evaluated by appropriate application of Eq. (5). We can write here

$$K = \frac{\bar{\theta}}{1-\bar{\theta}}, \qquad \Delta M = N_A \mu \tag{7a, b}$$

where θ is the fraction of P in state 2. Equilibrium conditions are indicated by the bar. μ stands for the *difference* of the dipole moments contributed by the two conformations parallel to the membrane field ($\mu = \mu_2 - \mu_1$), and N_A is Avogadro's number. The field dependence of K is then immediately obtained as

$$K = K_o \exp\{(\mu/kT)\, E\} \tag{7c}$$

with K_o referring to the value of K at zero field (k = Boltzmann's constant). Expressing E in terms of V according to Eq. (4) finally leads to

$$\bar{\theta} = \exp\{b(V - V_{1/2})\}/[1 + \exp\{b(V - V_{1/2})\}] \tag{8}$$

involving the two parameters

$$b = \frac{(\mu/d)}{kT}, \qquad V_{1/2} = \Delta\psi - \frac{1}{b} \ln K_o. \tag{9a, b}$$

(A graphical representation of $\bar{\theta}$ *vs.* $V - V_{1/2}$ is given in Fig. 2.) We note that $V_{1/2}$, i.e., the membrane potential which determines the midpoint of transition, can take any nonzero value in this model even at $\Delta\psi = 0$

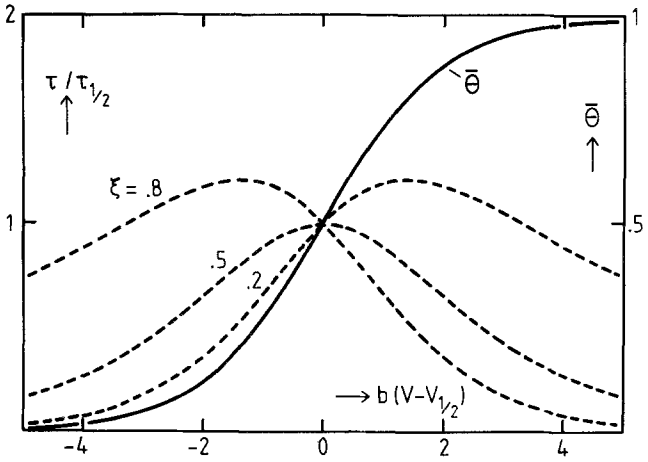

Fig. 2. Theoretical voltage dependences of the degree of conformational transition, $\bar{\theta}$, according to Eq. (8), and of the relaxation time, τ, according to Eq. (15) (dashed curves) $(1/\tau_{1/2} = 2k_{1/2})$

(depending on the thermodynamic properties of the conformational transition at $E=0$).

Regarding the kinetics of the model, the simplest case would be that of first order reactions in both directions of the conformational conversion. With rate constants k' and k'' for the forward and reverse reactions in Eq. (6), respectively, this implies

$$\frac{d\theta}{dt} = k'(1-\theta) - k''\theta; \qquad k'/k'' = K = \exp\{b(V - V_{1/2})\}. \qquad (10\,\text{a, b})$$

Because of the macromolecular nature of P, the process $P_1 \rightarrow P_2$ (as well as $P_2 \rightarrow P_1$) is not likely to be a single elementary step. We must rather expect quite a number of intermediate steps (though all the states involved except P_1 and P_2 must be negligibly populated). If one of them is comparatively slow, the overall reaction can be formulated (*cf.* Fig. 3)

$$P_1 \underset{<}{\overset{(K^*)}{\rightleftharpoons}} P^* \xrightarrow{k^*} P_2. \qquad (11)$$

Here a rate-limiting elementary step is supposed to originate at an intermediate state P^* (k^* being the corresponding rate constant) so that the latter state is virtually at equilibrium with P_1, as described by the equilibrium constant K^*. Then we have

$$k' = k^* \cdot K^*. \qquad (12)$$

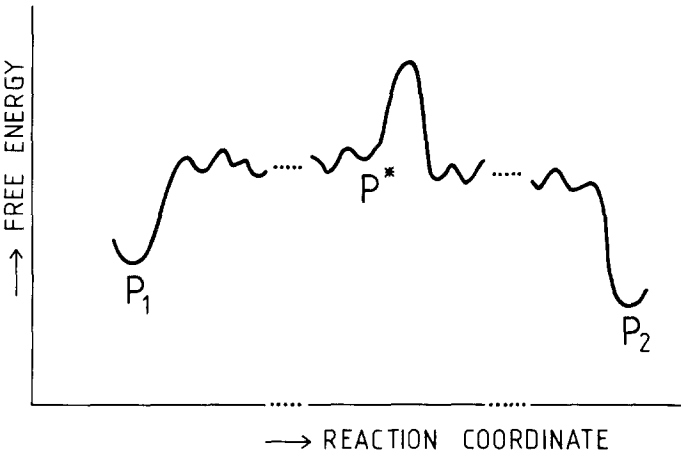

Fig. 3. Schematic free energy profile for the conformational transition, $P_1 \rightarrow P_2$, in case only one intermediate step has a comparatively high activation barrier (*see* text)

The K^* will be a function of E as follows in the same way as for K. Accordingly,

$$K^* = K_o^* \exp\{(\mu^*/kT)E\}$$

with the parameter μ^* representing the change of apparent dipole moment (parallel to E) when converting P_1 to P^*. The k^* on the other hand is an elementary rate constant to which absolute rate theory may be directly applied. Since the dipole moment of the transition state can hardly differ more than a few D from that of P^*, any field dependence of k^* is to be neglected. This yields immediately

$$k' = k'_o \exp\{(\mu^*/kT)E\} \quad (\text{where } k'_o = k^* K_o^*).$$

Introducing V instead of E finally leads to

$$k' = k_{1/2} \exp\{\xi b(V - V_{1/2})\} \quad (\text{where } k_{1/2} = k'_o K_o^{-\xi}) \tag{13}$$

with a dimensionless parameter defined as

$$\xi = \mu^*/\mu.$$

Taking into account Eq. (10b), we obtain

$$k'' = k_{1/2} \exp\{-(1-\xi) b(V - V_{1/2})\}. \tag{14}$$

The relaxation time τ can therefore be expressed according to

$$\frac{1}{\tau}=k'+k''=k_{1/2}[\exp\{\xi b(V-V_{1/2})\}+\exp\{-(1-\xi)b(V-V_{1/2})\}]. \quad (15)$$

Because of Eq. (8), this may be alternatively formulated as

$$\tau=\frac{1}{k_{1/2}}(1-\bar{\theta})^{\xi}\bar{\theta}^{(1-\xi)}. \quad (16)$$

Graphs of τ at various ξ *vs.* $V-V_{1/2}$ are also shown in Fig. 2. A symmetric course of the τ-curve around $V_{1/2}$ is obtained only for $\xi=1/2$ which corresponds to a rate-determining intermediate state having a dipole moment exactly half way in-between those of P_1 and P_2.

Displacement Currents

Changing the dipole moment M of a system in the course of time will always be associated with a displacement current. Its density is

$$j=\frac{1}{v}\frac{dM}{dt} \quad \text{(at fixed volume } v\text{)}.$$

Applying this to our special model where

$$M=M_1+N\mu\theta$$

(M_1 = dipole moment with all P in state P_1, N = number of all P-structures) thus results in a current density of

$$j=(\mu/d)c_p\frac{d\theta}{dt} \quad (17)$$

(c_p is the number of P-structures per unit area of the membrane). Experiments are usually conducted in the way that an equilibrium state at $t=0$ is suddenly perturbed by applying a voltage pulse of constant amplitude. Solving Eq. (10*a*) under these conditions yields

$$\theta=\bar{\theta}-\delta\bar{\theta}\cdot e^{-t/\tau} \quad \text{so that} \quad \frac{d\theta}{dt}=\frac{\delta\bar{\theta}}{\tau}e^{-t/\tau} \quad (18)$$

where $\bar{\theta}$ refers to the equilibrium value at the voltage which is effective during the pulse, while $\delta\bar{\theta}$ stands for the total change of θ produced by the pulse under equilibrium conditions.

In asymmetry current experiments, successive depolarizing and hyperpolarizing voltage pulses are applied in such a way that the symmetric recharging currents of the membrane capacitance exactly cancel out each other. The remaining asymmetry current density due to the induced conformational changes during the "on" periods accordingly follows as

$$j_{\text{on}} = (\mu/d)\, c_p\, \Sigma_i \frac{\delta \bar{\theta}_i}{\tau_i} e^{-t/\tau_i} \tag{19}$$

with the index i indicating values determined by the individual pulse voltages $V_i = V_o + \delta V_i$ (V_o being the original holding potential), the time t is always taken relative to the onset of the respective pulse. The analogously evaluated current density after switching off the pulses is found to be

$$j_{\text{off}} = -(\mu/d)\, c_p\, \Sigma_i\, \delta\theta_i\, e^{-t/\tau_o}/\tau_o. \tag{20}$$

The $\delta\theta_i$ are the changes of θ actually produced by the pulses; the τ_o refers to the holding potential.

These relations become simpler for extreme values of the holding potential. Experiments have especially been performed at a V_o so negative that the corresponding $\bar{\theta}$ practically equals zero. Then with regard to one depolarizing pulse

$$j_{\text{on}} = (\mu/d)\, c_p (\bar{\theta}/\tau)\, e^{-t/\tau} \tag{21}$$

where θ as well as τ correspond to $V = V_o + \delta V$ effective during that pulse. By integration the charge transfer per unit area may be evaluated. We find

$$Q = \int_0^\infty j_{\text{on}}\, dt = (\mu/d)\, c_p\, \bar{\theta} \tag{22}$$

provided the pulse is sufficiently wide in comparison with τ.

It should be noted in this context that a displacement current as an effect in the time domain must be paralleled by a dielectric polarization in the frequency domain. Therefore, asymmetry currents in excitable membranes can be predicted to give rise to changes in the membrane capacitance. This phenomenon may be treated on the basis of the present model. Taking advantage of the pertinent derivation given elsewhere (Schwarz, 1977), the corresponding increment of the dielectric constant is evaluated as

$$\Delta\varepsilon = \bar{\theta}(1-\bar{\theta})\frac{\mu^2/d}{\varepsilon_o kT} c_p \cdot \frac{1}{1+\omega^2\tau^2} \tag{23}$$

(ω = angular frequency of the polarizing field, $\varepsilon_o = 8.85 \cdot 10^{-12}\,\mathrm{CV^{-1}m^{-1}}$). We note that there should be a maximum at the mid-point of transition and a dispersion at frequencies $\omega \gtrapprox 1/\tau$.

Let us now compare our results with some experimental data. According to Eq. (22), the charge transfer is bound to approach an asymptotic value of

$$Q_\infty = (\mu/d)\, c_p \tag{24}$$

for low V_o and sufficiently large amplitudes δV. As mentioned before, such a limiting value is indeed observed for asymmetry currents (*see* Fig. 1). This permits an examination of the relation

$$\ln\left\{\frac{Q}{Q_\infty - Q}\right\} = b(V - V_{1/2}) \tag{25}$$

which follows from Eqs. (8) and (22). Rojas and Keynes (1975) have derived a formally identical equation and shown that their squid axon data can be quite well fitted to it. This is also true for the results reported by Meves (1974) as illustrated in Fig. 4. In that case one finds $V_{1/2} = -19\,\mathrm{mV}$ and $b = 0.069\ (\mathrm{mV})^{-1}$. The latter leads to

$$\mu/d = 8.0\,\mathrm{D/Å}.$$

Apparently no absolute value of the change in the dipole moment can be determined. The membrane thickness involved here may be estimated as 50 Å (remember that it describes the low conductivity hydrophobic part only). This yields a μ of 400 D (which will be commented on below). According to Eq. (24), the reported Q_∞ of $2.3 \cdot 10^{-4}\ \mathrm{C/m^2}$ implies a

$$c_p = 860\ \text{per}\ \mu\mathrm{m}^2$$

as the underlying density of reacting macromolecules [which is on the order of magnitude estimated for the hypothetical *m*-structures (Ulbricht, 1977)].

The kinetics of the system is accessible experimentally from the time course of the on- and off-currents. With the above special choice of sufficiently negative holding potential, our model predicts a purely exponential behavior of j_{on} as is, in fact, observed in the quoted experiments. The τ is directly obtained as a function of V. Taking into account

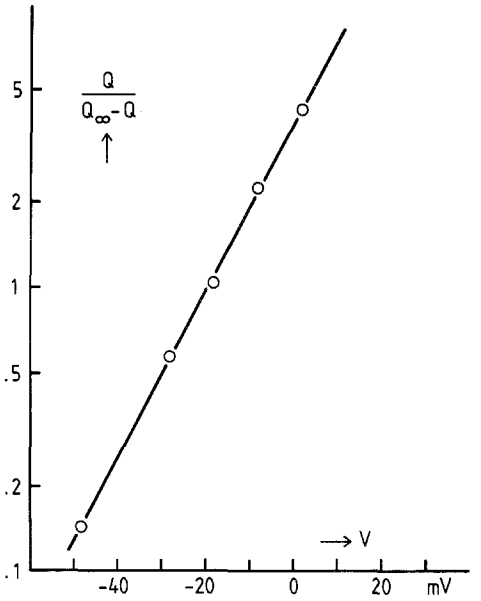

Fig. 4. Semilogarithmic plot of $Q/(Q_\infty - Q)$ *vs.* V using the experimental data in Fig. 1 yields $V_{1/2} = -19\,\mathrm{mV}$ and $b = 0.069\ (\mathrm{mV})^{-1}$

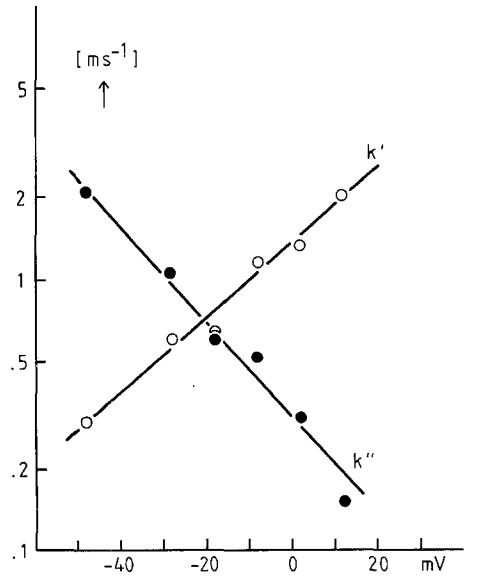

Fig. 5. Semilogarithmic plots of the rate constants k' and k'' *vs.* V using the experimental data in Fig. 1 (evaluated by means of the relations $k'/k'' = Q/(Q_\infty - Q)$ and $k' + k'' = 1/\tau$) yields $k_{1/2} = 0.7\ \mathrm{msec}^{-1}$ and $\xi \approx 0.44$

measured $\bar{\theta}$-values, individual rate constants k' and k'' may be determined. Their logarithms satisfactorily follow the linear voltage dependence to be expected on the basis of Eqs. (13) and (14) (*see* Fig. 5). We find for our parameter ξ a value of about 0.44. This means that the course of τ *vs.* V is slightly asymmetric, displaying a maximum at a membrane potential somewhat above $V_{1/2}$ (*see* Fig. 1).

Measurements of the membrane capacitance of excitable membranes have resulted in some experimental evidence for dielectric effects which apparently reflect respective asymmetry currents. The theoretical capacitance increment due to our model is apparently given as $\Delta C = \varepsilon_o \Delta \varepsilon / d$ with the $\Delta \varepsilon$ of Eq. (23). A maximum at a certain voltage as predicted by the theory has indeed been observed in a pertinent study on muscle membranes (Adrian & Almers, 1976). Quantitative calculations can be carried out using the above squid axon data. This yields a maximum capacitance increment for the limit of $\omega \to 0$

$$\Delta C^o_{\max} = \frac{(\mu/d)^2}{4kT} c_p \approx 0.4\,\mu\mathrm{F/cm}^2$$

and a dispersion frequency, $f_m = 1/(2\pi\tau)$, in the range of 1 kHz. This agrees rather well with experimental results of Takashima and Schwan (1974) if their higher temperature is considered, which should appreciably decrease the τ-value.

Discussion

We recall that any chemical transition which is associated with an increase of dipole moment will be driven by an electric field. As was pointed out, a few hundred debye units are required for conformational changes of an integral macromolecule giving rise to experimentally observed asymmetry currents. This magnitude (of the μ-value) is in principle well possible as can be inferred from measured absolute values for protein molecules, although little is actually known about their changes upon a structural transition. By all means the dipole moment of any molecule is given as $Q_p \cdot \delta$, i.e., the product of the total amount of positive charge and the distance between the centers of gravity of the positive and negative charges, respectively. Since even a comparatively small macromolecule certainly comprises more than 1000 elementary charge units of either kind, a change of less than 0.1 Å in δ may already result in a dipole moment difference of about 500 D.

Furthermore, it should be emphasized in this context that a much smaller dipolar change would really suffice if it is amplified by appropriate molecular interactions. Let us, for instance, assume that the transition of *P*-structures discussed above actually represents a cooperative all-or-none conversion of a bunch of n equivalent macromolecular subunits. In one of these the dipole moment has then to be altered merely by μ/n. Accordingly, cooperative mechanisms may not only reduce the necessary μ-value in the membraneous switching units but could also bring about functional effects by means of much smaller voltage changes than are encountered in gating. Such phenomena, in fact, occur in the nervous system (*see* Schmitt, Dev & Smith, 1976). Since this appears still more difficult to be explained otherwise, it lends further support to the general concept of voltage controlled structural rearrangements.

An inherent advantage of our approach can also be seen in the point that appropriate models may be quantitatively described on the basis of ordinary chemical thermodynamics and rate theory. This is to be demonstrated with regard to the temperature dependence of our basic one-step model (effects of pressure can be treated analogously). It follows immediately from Eq. (7*a*) and van't Hoff's relation that

$$\left(\frac{\partial \bar{\theta}}{\partial T}\right)_V = \frac{d\bar{\theta}}{d\ln K}\left(\frac{\partial \ln K}{\partial T}\right)_V = \bar{\theta}(1-\bar{\theta})\frac{\Delta H}{RT^2}$$

where ΔH represents the reaction enthalpy of the underlying conformational transition. This quantity generally depends on V. Starting from Eq. (10*b*) and using Eq. (9), we find

$$\Delta H = \Delta H_{1/2} - N_A(\mu/d)(V - V_{1/2})$$

where

$$\Delta H_{1/2} = \Delta H_o + RT\ln K_o - N_A(\mu/d)T\frac{\partial \Delta\psi}{\partial T}.$$

For our previous experimental example the implied electrical contribution to ΔH turns out to be on the order of 5 kJ/mol which is of minor significance in comparison with the usually expected magnitude of the purely chemical ΔH_o (i.e., ΔH at $E=0$).

Fundamentally, it follows that a change of temperature leads to a shift of the transition curve along the voltage axis. The corresponding effect on $V_{1/2}$ is derived as

$$\frac{dV_{1/2}}{dT} = -\frac{(\partial\bar{\theta}/\partial T)_{1/2}}{(\partial\bar{\theta}/\partial V)_{1/2}} = -\frac{\Delta H_{1/2}/T}{N_A\mu/d} = -\frac{c_p\Delta H_{1/2}}{N_A T Q_\infty}.$$

With the data of the asymmetry current system evaluated before, this means that $V_{1/2}$ will be decreased by 1 mV for a 1 K increase of T if $\Delta H_{1/2} = 50$ kJ/mol. In general, the measured charge transfer at given V must be increased (decreased) with temperature for a positive (negative) ΔH. However, its limit, Q_∞, has to remain constant. Nevertheless, an apparent effect on Q_∞ could occur if more than one voltage-dependent transition exists and at least one of them is comparatively sensitive to temperature (so that it may be moved into or out of the accessible voltage range) or slow (Schwarz, 1978).

Another important means of affecting voltage-dependent processes consists of adding certain chemical substances. This can be readily understood as being caused by differences in their interactions with the various conformational states involved. Let us assume a species A binds to the P_1, P_2 as described by the respective binding constants K_1 and K_2. The conformational equilibrium at constant T, V will then be pushed towards that state which has the stronger binding power. Application of the mass action laws for the two binding steps leads to

$$\frac{\bar{\theta}}{1-\bar{\theta}} = \left\{\frac{\bar{\theta}}{1-\bar{\theta}}\right\}_{c_A=0} \cdot \frac{1+K_2 c_A}{1+K_1 c_A}$$

where c_A means the molarity of free A. Alternatively, we have

$$\left(\frac{\partial \bar{\theta}}{\partial c_A}\right)_{T,V} = \frac{K_2 - K_1}{(1+K_1 c_A)(1+K_2 c_A)}.$$

Thus it is very well possible that a voltage-dependent process is inactivated due to ligand induced stabilization of unfavorable states.

In the framework of our approach, rate properties can be interpreted in terms of chemical reaction kinetics. Accordingly, transition-state theory permits the rate constant k' in Eq. (12) to be expressed as

$$k' = \frac{kT}{h} \exp\left\{\frac{\Delta S^{\neq}}{R}\right\} \exp\left\{-\frac{\Delta H^{\neq}}{RT}\right\}$$

(h = Planck's constant) where $\Delta S^{\neq}$ and $\Delta H^{\neq}$ stand for the activation entropy and enthalpy, respectively, of the rate-determining step relative to P_1. On the basis of Eqs. (15) and (10*b*), the Arrhenius activation energy of $1/\tau = k'(1+K^{-1})$ thus becomes

$$E_a = RT^2 \frac{d \ln(1/\tau)}{dT} = \Delta H^{\neq} + RT - (1-\bar{\theta})\Delta H.$$

The measured temperature dependence of τ for asymmetry currents ($Q_{10} \approx 2.5$) (Keynes & Rojas, 1974) yields an E_a of about 65 kJ/mol. The corresponding $\Delta H^{\neq}$ could apparently be even greater, which then necessitates a high $\Delta S^{\neq}$. For instance, if $\Delta H^{\neq} = 100$ kJ/mol, the $k' \approx 10^3$ sec^{-1} requires $\Delta S^{\neq} \approx 150$ J K^{-1} mol^{-1}. In case of intramolecular transitions, this has to be brought about by changes of the vibrational modes. The contribution of one such mode may be on the order of 0.1 J K^{-1} mol^{-1} (as can be estimated from fundamental relations in statistical mechanics). On the other hand, a macromolecule consisting of n atoms comprises about $3n$ of these modes so that a sufficient magnitude of $\Delta S^{\neq}$ is certainly possible if the molecule is big enough. In fact, similar kinetic properties are known among experimentally investigated conformational changes of proteins.

Naturally we must expect that there are systems of practical interest which comprise more than two voltage-sensitive conformational states. Then, the so far discussed one-step mechanism needs, of course, to be improved. It is an important feature of the concept presented that reasonably advanced models can be readily developed by introducing appropriate coupling of different transition steps. This is demonstrated, for example, in an accompanying article (Schwarz, 1978) in which various inactivation effects of asymmetry currents in squid axon membranes (Meves & Vogel, 1977; Armstrong & Bezanilla, 1977) are well described quantitatively on the basis of a three-state model.

At any rate, the inherent chemical specificities as well as the potentialities of possible cooperative and noncooperative interactions between individual macromolecular reactions provide great flexibility towards a theoretical analysis of all kinds of voltage-dependent processes in the nervous system.

This study has been supported by grants from the Swiss National Science Foundation (No. 3.487-0.75) and the U.S. National Institute of Health (HL-1253-24). Part of it was done during a two months stay at the Department of Bioengineering of the University of Pennsylvania. The author is indebted to Drs. K.-D. Kniffky, H. Meves, H.P. Schwan, S. Takashima, and W. Vogel for many stimulating discussions.

References

Adrian, R.H., Almers, W. 1976. The voltage dependence of membrane capacity. *J. Physiol. (London)* **254**:317

Armstrong, C.M., Bezanilla, F. 1974. Charge movements associated with the opening and closing of the activation gates of the Na channels. *J. Gen. Physiol.* **63**:533

Armstrong, C.M., Bezanilla, F. 1977. Inactivation of the sodium channel. II. Gating current experiments. *J. Gen. Physiol.* **70**:567

Bergmann, K., Eigen, M., De Maeyer, L. 1963. Dielektrische Adsorption als Folge chemischer Relaxation. *Ber. Bunsenges. Phys. Chem.* **67**:819

Cherry, R.J., Bürkli, A., Busslinger, M., Schneider, G., Parish, G.R. 1976. Rotational diffusion of the band 3 proteins in the human erythrocyte membrane. *Nature (London)* **263**:389

Cole, K.S. 1949. Dynamic electrical characteristics of the squid axon membrane. *Arch. Sci. Physiol.* **3**:253

Edidin, M. 1974. Rotational and translational diffusion in membranes. *Annu. Rev. Biophys. Bioeng.* **3**:179

Hill, T.L., Chen, Y. 1972. On the theory of ion transport across the nerve membrane: VI. Free energy change and activation free energies of conformational change. *Proc. Nat. Acad. Sci. USA* **69**:1723

Hodgkin, A.L., Huxley, A.F. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (London)* **117**:500

Hopmann, R. 1975. Dielectric measurements at radio frequencies in the presence of a strong static field. *Rev. Sci. Instrum.* **46**:306

Keynes, R.D., Rojas, E. 1974. Kinetics and steady-state properties of the charged system controlling sodium conductance in the squid giant axon. *J. Physiol. (London)* **239**:383

Kniffki, K.-D. 1975. A model for intramembrane charge transfer based on statistical mechanics and relaxation. *Stud. Biophys.* **49**:67

Kniffki, K.-D., Vogel, W. 1976. Molecular concept of gating the ionic channels in the nerve membrane. *In:* Biophysics of Membrane Transport. J. Kuczera and S. Prestalski, editors. Vol. 2, pp. 71–116. Wroclaw, Poland

Kornberg, R.D., McConnell, H.M. 1971. Inside-outside transitions of phospholipids in vesicle membranes. *Biochemistry* **10**:1110

Levitan, E., Palti, Y. 1975. Dipole moment, enthalpy, and entropy changes of Hodgkin-Huxley type kinetic units. *Biophys. J.* **15**:239

Meves, H. 1974. The effect of holding potential on the asymmetry currents in squid giant axons. *J. Physiol. (London)* **243**:847

Meves, H., Vogel, W. 1977. Inactivation of the asymmetrical displacement current in giant axons of *Loligo forbesi*. *J. Physiol. (London)* **267**:377

Moore, W.J. 1972. Physical Chemistry. Chap. 10, Sect. 7, p. 430. Longman, London

Neumcke, B., Nonner, W., Stämpfli, R. 1976. Asymmetrical displacement current and its relation with activation of sodium current in the membrane of frog myelinated nerve. *Pfluegers Arch.* **363**:193

Nonner, W., Rojas, E., Stämpfli, R. 1975. Displacement currents in the node of Ranvier. Voltage and time dependence. *Pfluegers Arch.* **354**:1

Petersen, D.C., Cone, R.A. 1975. The electric dipole moment of rhodopsin solubilized in Triton X-100. *Biophys. J.* **15**:1181

Rojas, E., Keynes, R.D. 1975. On the relation between displacement currents and activation of the sodium conductance in the squid giant axon. *Phil. Trans. R. Soc. London B* **270**:459

Schmitt, F.O., Dev, P., Smith, B.H. 1976. Electronic processing of information by brain cells. *Science* **193**:114

Schwarz, G. 1967. On dielectric relaxation due to chemical rate processes. *J. Phys. Chem.* **71**:4021

Schwarz, G. 1977. Chemical transitions of biopolymers induced by an electric field and their effects in dielectrics and birefringence. *Ann. N.Y. Acad. Sci.* **303**:190

Schwarz, G. 1978. Quantitative analysis of activation and inactivation of asymmetry

currents in biological membranes, based on a conformational transition model. *J. Membrane Biol.* **43**:149

Schwarz, G., Bauer, P.J. 1974. Structural flexibility and fast proton transfer reflected by the dielectric properties of poly-L-proline in aqueous solution. *Biophys. Chem.* **1**:257

Schwarz, G., Schrader, U. 1975. The effects of cooperativity, finite chain length, and field induced changes of the conformational equilibrium on the electric birefringence of polypeptides in the range of the helix-coil transition. *Biopolymers* **14**:1181

Schwarz, G., Seelig, J. 1968. Kinetic properties and the electric field effect of the helix-coil transition of poly(γ-benzyl-L-glutamate) determined from dielectric relaxation measurements. *Biopolymers* **6**:1263

Singer, S.J., Nicolson, G.L. 1972. The fluid mosaic model of the structure of cell membranes. *Science* **175**:720

Takashima, S., Schwan, H.P. 1974. Passive electrical properties of squid axon membrane. *J. Membrane Biol.* **17**:51

Tanford, C. 1973. The hydrophobic effect: Formation of micelles and biological membranes. Wiley, New York

Ulbricht, W. 1977. Ionic channels and gating currents in excitable membranes. *Annu. Rev. Biophys. Bioeng.* **6**:7

Wada, A., Tanaka, T., Kihara, H. 1972. Dielectric dispersion of the α-helix at the transition region to random coil. *Biopolymers* **11**:587