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Quantitative Analysis of Activation and Inactivation of Asymmetry Currents in Biological Membranes, Based on a Conformational Transition Model

Gerhard Schwarz

Department of Biophysical Chemistry, Biocenter of the University of Basel,
Basel, Switzerland

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Summary. A basic voltage-dependent conformational transition mechanism is proposed. It comprises one relatively fast conversion between two individual states which are comparatively slowly coupled with a third state. Having introduced voltage as an additional parameter of state, standard methods of thermodynamics and rate theory are employed to describe the equilibrium and kinetic behavior of the system. In particular, a quantitative discussion is given regarding the asymmetrical displacement currents generated by switching on and off a voltage pulse. Effects of temperature, pulse duration, and application of a conditioning prepulse are examined. The results provide a comprehensive basis for a quantitative analysis of pertinent experimental work. The so far presented measuring data can indeed be very well described along these lines.

The sodium and potassium permeabilities through nerve membranes are controlled by the membrane potential, $V=\phi_{\text{in}}-\phi_{\text{ex}}$ (the ϕ referring to the electric potential in the cell interior and exterior, respectively). This gating effect has been quantitatively described by the phenomenological equations of Hodgkin and Huxley [1952]. Its molecular mechanism is so far not known. At any rate, however, it should involve virtual transverse displacements of charges associated with the gating structures. These imply small nonlinear capacitance currents when voltage-induced changes of the degree of permeability occur. Some years ago it has indeed been possible for the first time to detect such asymmetry currents under conditions where the much greater ionic currents are largely eliminated (Armstrong & Bezanilla, 1973; Keynes & Rojas, 1973). Although the now available data are inconsistent with a straight-forward interpretation according to the Hodgkin-Huxley approach, they nevertheless clearly indicate some kind of relation to the actual gating mechanism (*see*, e.g., Ulbricht, 1977).

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The gating processes as well as the asymmetry currents reflect fairly fast molecular events with distinct saturation in the accessible voltage range. As pointed out in detail in the preceding article (Schwarz, 1978), such properties are found to be quite unreasonable quantitatively if one assumes a purely electromechanical mechanism (i.e., a transverse motion of charged or dipolar particles directly caused by the field). They are well compatible, however, with field-induced structural rearrangements. In fact, these considerations suggest that the physical basis of any voltage-dependent transition in a biological membrane may primarily be seen in the influence of the field on a functional chemical conversion. The underlying equilibrium will generally be pushed towards the state of greater overall dipole moment parallel to the field (Schwarz, 1977). This chemical field effect consequently implies an inherent transfer of dipolar charge, thus giving rise to an asymmetry current.

Molecular models of voltage dependence along these lines generally have to be based on an appropriate reaction system. Depending on the dipolar properties of the chemical states involved, the respective equilibrium and rate constants can be expressed as functions of the electric field. The complete quantitative analysis may then be developed by means of standard approaches used in chemical thermodynamics and reaction kinetics. For a one-step transition this has already been fully demonstrated and shown to account for certain simple asymmetry current data (Schwarz, 1978).

There is an apparent diversity of voltage-dependent effects even in the same membrane. This indicates a variety of field-induced steps which could be independent or may be coupled with each other. A relatively uncomplicated situation was encountered in switching on short voltage pulses (around 1 msec) to squid axon membranes. The asymmetry currents could essentially be described by a single exponential time function and all the transferred charge, Q_{on}, is displaced back once the pulse is switched off, i.e., $Q_{\mathrm{off}}=Q_{\mathrm{on}}$ (Keynes & Rojas, 1974; Meves, 1974). A longer prepulse shortly before the measuring pulses depresses both current amplitude and charge transfer (Bezanilla & Armstrong, 1975; Armstrong & Bezanilla, 1977; Meves & Vogel, 1977*a*). These studies also revealed an apparently exponential decrease of Q_{off} at measuring pulse durations longer than 1 msec (*see* Fig. 1). It will be shown in the present article that both inactivation phenomena as well as an apparent temperature dependence of the saturating charge transfer can be quantitatively understood on the basis of transitions between three structural states. Two would have to convert into each other comparatively fast, whereas the third is much more slowly accessible. The latter will thus be virtually

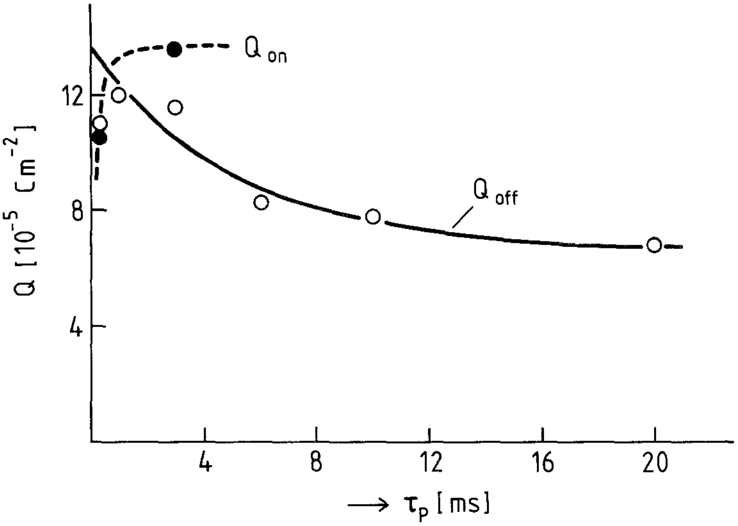

Fig. 1. Measured charge transfer in a squid axon membrane (V: $-70\,\mathrm{mV} \rightarrow +20\,\mathrm{mV}$; 8.5 °C) after Meves and Vogel (1977*a*). Depending on pulse duration, τ_p, the apparent on-charge per unit area, Q_{on} (filled points, dashed curves), very soon approaches a final value (relaxation time $\tau_1 \approx 0.2$ msec). The corresponding off-charge, Q_{off} (open points), equals Q_{on} only at about $\tau_p < 0.5$ msec and then decreases approximately according to the exponential relation $Q_{\mathrm{off}} = \{13.6 - 7(1 - e^{-\tau_p/\tau_2})\} \times 10^{-5}\,\mathrm{C\,m^{-2}}$ where $\tau_2 = 5$ msec (solid curve)

frozen during any short voltage pulse so that it does not seem to exist then. Once longer pulses or temperature variations are applied, a redistribution of "fast" and "slow" states will be induced. This naturally affects the amount of charge involved in the primarily observable fast current component.

Strictly speaking, it appears that actually more than three states take part in the voltage-dependent behavior of a squid axon membrane (Armstrong & Bezanilla, 1977; Bezanilla & Armstrong, 1977). Nevertheless, the as yet presented main features of the asymmetry currents are rather well consistent with our basic model. More states may, however, be introduced later if necessary for refinements.

General Approach to Structural Transitions Involving Three States

We assume a macromolecular entity P whose individual states may undergo conversions according to the most general scheme

$$\begin{tikzcd} P_1 \arrow[rr, shift left] \arrow[dr, shift left] & & P_2 \arrow[ll, shift left] \arrow[dl, shift left] \\ & P_3 \arrow[ul, shift left] \arrow[ur, shift left] & \end{tikzcd} \qquad (1)$$

The individual degree of transition, i.e., the relative amount of P_i, is to be denoted θ_i $(=[P_i]/[P])$. Introducing K_{ji} as the apparent equilibrium constant for $P_j \rightleftharpoons P_i$, we obviously have at equilibrium

$$\bar{\theta}_i = \frac{K_{1i}}{1+K_{12}+K_{13}} \tag{2}$$

(the bar indicating the equilibrium value; note that $K_{11}=1$, $K_{13}=K_{12}K_{23}$). Any equilibrium behavior of the system can therefore be quantitatively analyzed in terms of the respective effects on K_{12} and K_{13}.

Every individual K_{ji} depends on external conditions by which it may be manipulated. Of particular interest are voltage, V, temperature, T, and the concentration, c_A, of some free chemical agent A as far as this binds differently to the respective states. How such effects are quantified has been discussed in detail elsewhere (Schwarz, 1978). Let us first recall the influence of voltage. If P_j and P_i have different dipole moments parallel to the acting field, the K_{ji} will become a function of the field strength, E. In a biological membrane, integral macromolecular particles have practically fixed orientations normal to the surfaces (Singer & Nicolson, 1972). Thus the relevant changes of dipole moment, μ_{ji}, when going from P_j to P_i may be taken as constant parameters. It then follows

$$K_{ji} = K_{ji}^o \exp\{\mu_{ji}E/kT\} = \exp\{b_{ji}(V - V_{ji})\} \tag{3}$$

where

$$b_{ji} = (\mu_{ji}/d)/kT, \qquad V_{ji} = \Delta\psi - \frac{1}{b_{ji}} \ln K_{ji}^o \tag{4 a, b}$$

(K^o refers to the value at $E=0$, d is the thickness of low conductance region of the membrane, $\Delta\psi$ stands for the voltage contributed by the two interfacial potentials). Note that V_{ji} represents that voltage at which equal amounts of P_j and P_i would exist under equilibrium conditions.

The principal contribution to the temperature effect must be expected to arise from K_{ji}^o according to van't Hoff's relation. Taking into account also the interference of binding and structural equilibria the most essential points regarding external effects can be summarized explicitly in the expression

$$K_{ji} = \frac{1+K_i c_A}{1+K_j c_A} \exp\left\{\frac{\mu_{ji}/d}{kT}(V - \Delta\psi) - \frac{\Delta H_{ji}^o}{R}\left(\frac{1}{T} - \frac{1}{T_{ji}^o}\right)\right\} \tag{5}$$

(K_i is the binding constant of A to P_i; the reaction enthalpy, ΔH^o_{ji}, and entropy, ΔS^o_{ji}, at zero-field and equal concentration of the involved states, are assumed to be independent of temperature; $T^o_{ji} = \Delta H^o_{ji}/\Delta S^o_{ji}$). The temperature dependence of $\Delta\psi$ and of the K_i should ordinarily be negligible against that determined by ΔH^o_{ji}. Possible voltage effects on the K_i will only be appreciable if the binding leads to an unusually great change of dipole moment.

Expressing K_{12} and K_{13} according to Eq. (5) and introducing this into Eq. (2) thus permits us to describe the $\bar{\theta}_i$ as functions of the variables T, V, and c_A. This may be too involved, however, if only the effect of comparatively small changes of a certain variable x is to be examined. In this case we conveniently employ partial derivatives. Generally we have

$$\frac{\partial \bar{\theta}_i}{\partial x} = \frac{\partial \bar{\theta}_i}{\partial \ln K_{12}} \cdot \frac{\partial \ln K_{12}}{\partial x} + \frac{\partial \bar{\theta}_i}{\partial \ln K_{13}} \frac{\partial \ln K_{13}}{\partial x} \tag{6}$$

with

$$\frac{\partial \bar{\theta}_i}{\partial \ln K_{1i}} = \bar{\theta}_i(1-\bar{\theta}_i); \qquad \frac{\partial \bar{\theta}_2}{\partial \ln K_{13}} = \frac{\partial \bar{\theta}_3}{\partial \ln K_{12}} = -\bar{\theta}_2 \bar{\theta}_3. \tag{7a, b}$$

Regarding voltage we easily gather from Eq. (3) that

$$\frac{\partial \ln K_{1i}}{\partial V} = b_{1i}. \tag{8}$$

Furthermore, it follows

$$\frac{\partial \ln K_{1i}}{\partial T} = \frac{\Delta H_{1i}}{RT^2} \tag{9}$$

where this ΔH_{1i} includes some comparatively small terms in addition to ΔH^o_{1i} (Schwarz, 1978). Finally

$$\frac{\partial \ln K_{1i}}{\partial c_A} = \frac{K_i - K_1}{(1+K_i c_A)(1+K_1 c_A)} \tag{10}$$

as is easily derived from Eq. (4).

The kinetics of the system is most simply based on first order rate constants, k_{ji} for $P_j \rightarrow P_i$. Accordingly, we derive along standard lines

$$\frac{d\theta_2}{dt} = -(k_{12}+k_{21}+k_{23})(\theta_2-\bar{\theta}_2)+(k_{32}-k_{12})(\theta_3-\bar{\theta}_3) \tag{11a}$$

$$\frac{d\theta_3}{dt} = (k_{23}-k_{13})(\theta_2-\bar{\theta}_2)-(k_{13}+k_{32}+k_{31})(\theta_3-\bar{\theta}_3). \tag{11b}$$

Such a set of two inhomogeneous linear differential equations is of the ordinary type encountered in chemical relaxation kinetics. It can always be solved by means of well-known procedures (*see*, e.g., Schwarz, 1968). For time-independent equilibrium conditions the solution may be formulated as

$$\theta_i = \bar{\theta}_i - \delta\theta_{oi}\{\alpha_i e^{-t/\tau_1} + (1-\alpha_i)\, e^{-t/\tau_2}\} \tag{12}$$

where $\delta\theta_{oi} = \bar{\theta}_i - \theta_{oi}$ (θ_{oi} being the initial value, i.e., the actual value at time $t=0$). The relaxation times τ_1, τ_2 turn out to be the negative reciprocal eigenvalues of the matrix associated with Eq. (11). Thus they are generally somewhat involved functions of all the rate constants. The amplitude parameters α_1, α_2, on the other hand, are determined by the initial and final values of the θ_i and, in addition, by the ratios of the rate of different steps.

This implies a time dependence of the over-all dipole moment

$$M = M_1 + N(\mu_{12}\theta_2 + \mu_{13}\theta_3)$$

(M_1 being the value of M when all P are in state P_1; N denotes the total number of P). Transitions between the P-states thus will result in a displacement current. Its density becomes

$$j = \frac{1}{v}\frac{dM}{dt} = \frac{c_p}{d}\left\{\mu_{12}\frac{d\theta_2}{dt} + \mu_{13}\frac{d\theta_3}{dt}\right\}$$

(v = volume of the system, c_p = number of P per area) which is readily evaluated once the appropriate expressions for the θ_i according to Eq. (12) have been calculated.

Slow Coupling with the Third State

If only one of the three steps in the cyclic scheme [Eq. (1)] is comparatively slow the two faster ones will essentially determine the actual time dependence (since the slow step can be easily by-passed). Therefore, two clearly separated fast and slow phases must arise from two states which are connected via a fast step but are *both* coupled fairly slowly with the third one.

We may assume that the fast step is $P_1 \rightleftharpoons P_2$. Thus the rate constants are subject to

$$k_{12}, k_{21} \gg k_{13}, k_{31}, k_{23}, k_{32}.$$

This permits a rather straightforward solution of the relaxation Eq. (11). During a first phase after $t=0$, only θ_2 changes while θ_3 practically remains equal to θ_{03}. Hence

$$\frac{d\theta_2}{dt} = -\frac{1}{\tau_1}(\theta_2 - \bar{\theta}_2) + k_{12}\,\delta\theta_{03}$$

with

$$\frac{1}{\tau_1} = k_{12} + k_{21}$$

yielding

$$\theta_2 = \bar{\theta}_2 - \left(\delta\theta_{02} + \frac{K_{12}}{1+K_{12}} \cdot \delta\theta_{03}\right) e^{-t/\tau_1} + \frac{K_{12}}{1+K_{12}} \cdot \delta\theta_{03}.$$

When $t \gg \tau_1$, however, P_1 and P_2 are virtually at equilibrium, i.e., $\theta_2/\theta_1 = K_{12}$. This (together with $\theta_1 + \theta_2 + \theta_3 = 1$) can be used to express the second differential equations in terms of θ_3 only. The result is

$$\frac{d\theta_3}{dt} = -\frac{1}{\tau_2}(\theta_3 - \bar{\theta}_3)$$

where

$$\frac{1}{\tau_2} = k_{13} \cdot \frac{1}{1+K_{12}} + k_{31} + k_{23}\,\frac{K_{12}}{1+K_{12}} + k_{32} \ll \frac{1}{\tau_1}.$$

Evidently τ_1 and τ_2 are the two relaxation times which describe the kinetic behavior at any time. The amplitude factors are seen to be

$$\alpha_2 = 1 + \frac{K_{12}}{1+K_{12}} \cdot \frac{\delta\theta_{03}}{\delta\theta_{02}}, \qquad \alpha_3 = 0.$$

Thus

$$\theta_2 = \bar{\theta}_2 - \left(\delta\theta_{02} + \frac{K_{12}}{1+K_{12}} \cdot \delta\theta_{03}\right) e^{-t/\tau_1} + \frac{K_{12}}{1+K_{12}} \cdot \delta\theta_{03}\, e^{-t/\tau_2} \tag{13a}$$

$$\theta_3 = \bar{\theta}_3 - \delta\theta_{03}\, e^{-t/\tau_2} \tag{13b}$$

is the general and complete solution in case of comparatively slow access to P_3. The corresponding displacement current density can then be derived as

$$j = (Q_1/\tau_1)\, e^{-t/\tau_1} + (Q_2/\tau_2)\, e^{-t/\tau_2} \tag{14}$$

where

$$Q_1 = \left\{\delta\theta_{02} + \frac{K_{12}}{1+K_{12}} \cdot \delta\theta_{03}\right\} (\mu_{12}/d)\, c_p \tag{15a}$$

$$Q_2 = \left\{-\frac{K_{12}}{1+K_{12}}(\mu_{12}/d) + (\mu_{13}/d)\right\} \delta\theta_{03}\, c_p. \tag{15b}$$

The τ_1, τ_2 as well as K_{12} refer to the effective equilibrium conditions. By integration up to $t \to \infty$ we find that Q_1, Q_2 represent the final amounts of displaced charge per unit area which are associated with the fast and slow components of j_{on}, respectively.

Let us now turn to the displacement currents generated by switching on and off a rectangular voltage pulse described by

$$V_0 \xrightarrow[\text{on}\,(t=0)]{} V \xrightarrow[\text{off}\,(t=\tau_p)]{} V_o .$$

In other words, the holding potential V_o is suddenly increased to V which will be maintained for a time τ_p until V_o is restored.

First we shall consider the case in which the system has attained equilibrium before the pulse is applied. The on-current density j_{on} can then be expressed by the corresponding charge parameters $\bar{Q}_1, \bar{Q}_2$. These are directly obtained from Eq. (15) if the $\delta\theta_{oi}$ are set equal to the equilibrium changes $\delta\bar{\theta}_i = \bar{\theta}_i - \bar{\theta}_i^{(o)}$ with the superscript (o) marking the value applicable at the holding potential. The total electric charge density transferred during the pulse becomes

$$\bar{Q}_{\text{on}} = \bar{Q}_1 (1 - e^{-\tau_p/\tau_1}) + \bar{Q}_2 (1 - e^{-\tau_p/\tau_2}).$$

Regarding the off-current, we must take into account that generally the system has not yet reached the new equilibrium at the moment of switching off. The appropriate actual degrees of transition, $\theta_i^{(p)}$, can be calculated by means of Eq. (13). The over-all changes upon return to $\bar{\theta}_i^{(o)}$ are deduced to be

$$\begin{aligned}
\delta\theta_2^{(p)} = \bar{\theta}_2^{(o)} - Q_2^{(p)} = {} & -\left(\delta\bar{\theta}_2 + \frac{K_{12}}{1+K_{12}}\delta\bar{\theta}_3\right)(1 - e^{-\tau_p/\tau_1}) \\
& + \frac{K_{12}}{1+K_{12}}\,\delta\bar{\theta}_3 (1 - e^{-\tau_p/\tau_2}) \\
\delta\theta_3^{(p)} = \bar{\theta}_3^{(o)} - \theta_3^{(p)} = {} & -\delta\bar{\theta}_3 (1 - e^{-\tau_p/\tau_2}).
\end{aligned}$$

Substituting them for the $\delta\theta_{oi}$ (and $K_{12}^{(o)}$, i.e., the equilibrium constant at V_o, for K_{12}) in Eq. (15) yields the individual displaced charge densities during the off-current:

$$\bar{Q}_1^{(o)} = -\bar{Q}_1 (1 - e^{-\tau_p/\tau_1}) + \bar{Q}_c (1 - e^{-\tau_p/\tau_2}) \tag{16a}$$

$$\bar{Q}_2^{(o)} = -(\bar{Q}_2 + \bar{Q}_c)(1 - e^{-\tau_p/\tau_2}) \tag{16b}$$

with a coupling charge parameter

$$\bar{Q}_c = \delta\left(\frac{K_{12}}{1+K_{12}}\right)\delta\bar{\theta}_3(\mu_{12}/d)\,c_p \tag{16c}$$

(the δ-operator on the K_{12}-term standing for the difference of the values effective at pulse voltage and holding potential, respectively). If $t=0$ is now assigned to the end of the pulse, we have

$$j_{\text{off}} = (\bar{Q}_1^{(o)}/\tau_1^{(o)})\,e^{-t/\tau_1^{(o)}} + (\bar{Q}_2^{(o)}/\tau_2^{(o)})\,e^{-t/\tau_2^{(o)}}$$

(note that the relaxation times $\tau_1^{(o)}$, $\tau_2^{(o)}$ are determined by the holding potential). Accordingly, all the displaced charge (for $t \to \infty$) amounts to

$$\bar{Q}_1^{(o)} + \bar{Q}_2^{(o)} = -\bar{Q}_{\text{on}}.$$

In other words, the total off-charge cancels the total on-charge, as naturally would be expected. With regard to the on- and off-charges carried by the individual fast and slow current components, however, significant differences may be observed.

Evaluation and Interpretation of Experimental Data

In practice, the slow component of the displacement current may be difficult to detect. Its amplitude would become very much smaller than that of the fast component if Q_2 is of the same order of magnitude as Q_1 or smaller. This is clearly demonstrated by the general expression [Eq. (14)] when taking into account the condition $\tau_2 \gg \tau_1$.

Under these circumstances the current appears largely as a purely exponential time function. It will, accordingly, make up all the measured asymmetry current, provided only the depolarizing pulse induces a fast transition. This complies with the findings in the case of the previously mentioned squid axon system of Meves and Vogel (1977*a*). From the respective data thus only the relaxation time τ_1 is directly accessible. Consequently, the apparent charge transfer is essentially given by the Q_1-term. Armstrong and Bezanilla (1977), on the other hand, have under certain conditions in addition observed a slow current component.

Let us assume for the moment that essentially only the fast component is encountered. Then the apparent on-charge density when starting from equilibrium follows on the basis of Eqs. (15a) and (2) as

$$\bar{Q}_{\text{on}} = \delta\left(\frac{K_{12}}{1+K_{12}}\right)(1-\bar{\theta}_3^{(o)})\frac{\mu_{12}}{d}\,c_p(1-e^{-\tau_p/\tau_1}).$$

With regard to the effect of pulse duration, τ_p, and voltage, V, this naturally reflects the same behavior which has been ascertained for a single transition step. The fast relaxation time, τ_1, as well as the displaced charge thus display the respective dependences on V as they have been derived before and found to agree with squid axon data (Schwarz, 1978). For $\tau_p \gg \tau_1$ and sufficiently negative holding potential so that $\bar{\theta}_2^{(o)} = 0$, we may accordingly write

$$\bar{Q}_{\mathrm{on}} = \frac{\exp\{b_{12}(V - V_{12})\}}{1 + \exp\{b_{12}(V - V_{12})\}} Q_\infty \tag{17a}$$

where

$$Q_\infty = (1 - \bar{\theta}_3^{(o)})(\mu_{12}/d)\, c_p. \tag{17b}$$

Obviously the parameters b_{12} and V_{12} as well as τ_1 are solely determined by the fast step. Its saturating charge transfer, Q_∞, on the other hand, evidently depends also on the fraction of P locked up in the slowly coupled third conformational state. This is indeed easily intelligible since only P_1 and P_2 participate in the observed process (note that $1 - \theta_3 = \theta_1 + \theta_2$).

According to Eq. (17b) any change of the initial θ_3-value must yield a corresponding change in the measured Q_∞. Such an effect is particularly to be expected upon variation of temperature. In fact, warming up a squid axon system appears to lead to an increase of the saturating on-charge (Kimura & Meves, 1977, and *unpublished results*). A quantitative analysis of this temperature effect is immediately carried out in the framework of our approach. It follows directly from Eq. (17b) that (at constant V_o)

$$\frac{dQ_\infty}{dT} = -\frac{d\bar{\theta}_3^{(o)}}{dT} \cdot Q_\infty^o$$

with $Q_\infty^o = (\mu_{12}/d)\, c_p$ denoting the upper bound of Q_∞, i.e., the amount displaced in the fast step in case of $\theta_3^{(o)} = 0$. Taking into account Eqs. (6), (7), and (9) and $\bar{\theta}_2^{(o)} = 0$, we find

$$\frac{dy}{dT} = -y(1-y)\frac{\Delta H_{13}}{RT^2} \quad (\text{where } y = Q_\infty/Q_\infty^o). \tag{18}$$

A 1% increase of Q_∞ per Kelvin is thus given if the product $\bar{\theta}_3^{(o)} \Delta H_{13}$ equals -7 kJ/mol (at 280 K). By integration of Eq. (18) it follows

$$Q_\infty/Q_\infty^o = \frac{1}{1 + B\exp\{-\Delta H_{13}/RT\}} = 1 - \bar{\theta}_3^{(o)}$$

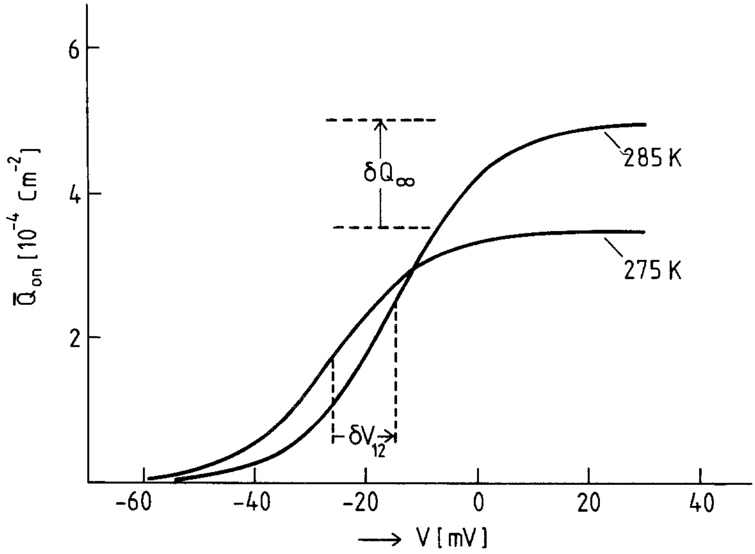

Fig. 2. Temperature effect on the final Q_{on} *vs.* V curve (*see* text). It is assumed that $V_{12} = -20$ mV, $\bar{\theta}_3^{(o)} = 0.5$ for $T = 280$ K, $\mu_{12}/d = 8$ debye/Å, $\Delta H_{12} = \Delta H_{13} = -50$ kJ/mol, $c_p = 3 \times 10^{15}$ m^{-2}. Under these conditions the indicated substantial increases of Q_∞ and V_{12} are induced by raising the temperature from 275 K to 285 K

with B being independent of temperature (but still a function of V_o). For an evaluation of the three unknown parameters Q_∞^o, B, and ΔH_{13}, measurements at a minimum of three temperatures are required.

In addition to the effect on the amplitude of the $\bar{Q}_{on}$ *vs.* V curve, there is also a temperature-induced shift of the curve along the voltage axis. According to our previous discussion of this point (Schwarz, 1978), we have here

$$\frac{dV_{12}}{dT} = \left(\frac{dV}{dT}\right)_{K_{12}=1} = -\frac{(\partial \ln K_{12}/\partial T)_V}{(\partial \ln K_{12}/\partial V)_T} = -\frac{c_p}{N_A Q_\infty^o} \cdot \frac{\Delta H_{12}}{T}.$$

A graphic illustration of both effects can be found in Fig. 2.

Naturally the external pressure as well as the concentration of a chemically interacting agent may likewise affect the value of $\bar{\theta}_3^{(o)}$. Therefore, variations of these physical quantities could also lead to analogous changes of Q_∞ and V_{12}.

We turn now to the current observable after switching off the pulse. The apparent charge density displaced back is to be defined as $\bar{Q}_{off}$. In case this is evaluated from the fast current component only, we obtain in general according to Eq. (16a)

$$\bar{Q}_{off} = \bar{Q}_{on} - \bar{Q}_c(1 - e^{-\tau_p/\tau_2}).$$

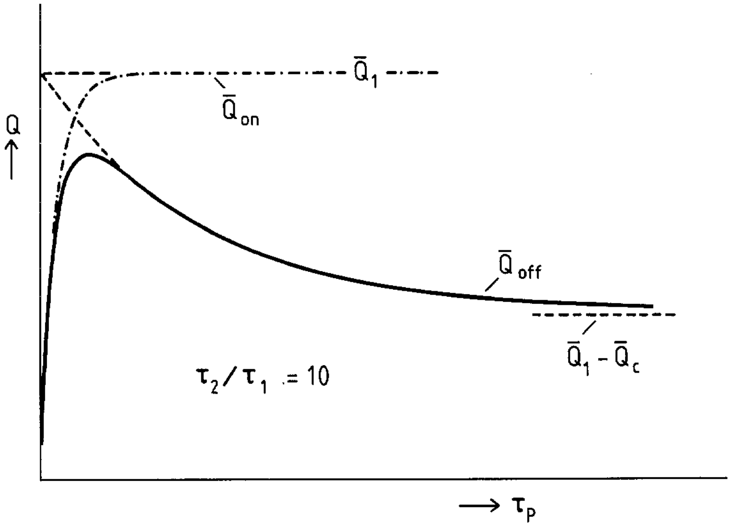

Fig. 3. Theoretical course of $\bar{Q}_{\mathrm{off}}$ as a function of pulse duration, τ_p, in comparison with $\bar{Q}_{\mathrm{on}}$ ($\tau_2 = 10\tau_1$; the dashed curve is the exponential extrapolation towards $\tau_p = 0$). This reflects the general situation represented by the experimental case of Fig. 1

In other words, only at short pulse durations, $\tau_p \ll \tau_2$, the off-charge equals the on-charge; at longer pulses an exponential approach of $\bar{Q}_{\mathrm{off}}$ to a different value occurs (*see* Fig. 3). The deviation between the two extreme values can be written

$$\bar{Q}_c = \delta\left(\frac{K_{12}}{1+K_{12}}\right)(\bar{\theta}_3 - \bar{\theta}_3^{(o)})Q_\infty^o.$$

In comparison with the maximum on-current we then have

$$\bar{Q}_c/\bar{Q}_1 = (\bar{\theta}_3 - \bar{\theta}_3^{(o)})/(1 - \bar{\theta}_3^{(o)}).$$

The general quantitative behavior agrees very well with the quoted results of Meves and Vogel (1977*a*) where $\tau_2 \approx 5$ msec. In their case as a first approximation: $K_{12}^{(o)} = 0$ ($V_o = -70$ mV) and $K_{12} \gg 1$ ($V = +20$ mV). Thus we have at pulse voltage and 8.5 °C

$$k_{31} + k_{23} + k_{32} \approx 200\ \mathrm{sec}^{-1}.$$

Furthermore, from the pulse length dependence of $\bar{Q}_{\mathrm{off}}$ (Fig. 1)

$$\bar{Q}_1 = (1 - \bar{\theta}_3^{(o)})Q_\infty^o = 13.6 \times 10^{-5}\ \mathrm{C\,m}^{-2} \tag{19a}$$

and

$$\bar{Q}_c = (\bar{\theta}_3 - \bar{\theta}_3^{(o)})Q_\infty^o = 7.0 \cdot 10^{-5}\ \mathrm{C\,m}^{-2}. \tag{19b}$$

This clearly indicates that the fraction of P in the slowly coupled state P_3 increases at higher voltages. Absolute values of $\bar{\theta}_3$ can apparently not be evaluated as long as Q^o_∞ is not known. We have seen above that the latter quantity may be accessible from studies of the temperature dependence.

On the other hand, we could, in principle, also gather the necessary information by means of following the dependence on the holding potential. The effect of V_o in Q_∞ can be described analogously to that of T which has been treated above. This leads to the total differential of $y = Q_\infty/Q^o_\infty$ regarding both variables, namely,

$$dy = -\frac{y(1-y)}{RT^2}\{\Delta H_{13}\,dT + N_A(\mu_{13}/d)\,T\,dV_o\}. \tag{20}$$

(It must be emphasized that this applies to sufficiently negative holding potentials only where $\bar{\theta}_2^{(o)}=0$!) The apparent increase of y at higher temperatures implies a negative ΔH_{13} and a nonzero $\bar{\theta}_3^{(o)}$. Changing V_o will evidently support or counteract variations of the temperature depending on the signs of μ_{13} and δV_o. In particular, δV_o may be selected to cancel out the effect of T. In first approximation it follows from Eq. (20) in this special case

$$\delta V_o = -\frac{\Delta H_{13}}{N_A(\mu_{13}/d)}\cdot\frac{\delta T}{T}.$$

Thus, with a likely $\mu_{13}>0$ (*see below*), it needs an increase of V_o to decrease Q_∞ correspondingly, whereas more negative holding potentials would lead to higher saturating charge transfer. The same applies if V_o is held constant but the voltage of a long prepulse is varied accordingly, provided the time interval between the two pulses is sufficiently short, i.e., $\ll\tau_2^{(o)}$ (*see below*).

At a holding potential of -70 mV, Armstrong and Bezanilla (1977, Figs. 3 and 9 *l.c.*) have observed a pulse duration dependence of the off-on charge ratio essentially equivalent to that of Meves and Vogel. It only reflects a somewhat faster decrease and approaches a smaller limiting value. The latter may be due to the fact that in these experiments an appreciable slow on-current component was involved so that the actual $\bar{Q}_{\mathrm{on}}$ becomes greater than the charge density carried by the fast component alone. While the authors do not see a slow off-current in these experiments, they observe one after decreasing V_o to around -150 mV. In the scope of our model the occurrence of these slow current phases can be interpreted by the physically reasonable feature of τ_2 to decrease considerably at extreme voltages [*cf.* the V-dependence of τ in the one-step model (Schwarz, 1978)]. According to Eq. (16a) and with $\tau_p \gg \tau_1$, the fast off-charge density is

$$(\bar{Q}_{\mathrm{off}})_1 = \bar{Q}_1 - \bar{Q}_c(1 - e^{-\tau_p/\tau_2})$$

and because of Eq. (16b) the slow one becomes

$$(Q_{\mathrm{off}})_2 = (\bar{Q}_2 + \bar{Q}_c)(1 - e^{-\tau_p/\tau_2}).$$

In other words, $(Q_{\mathrm{off}})_2$ can be expected to increase from zero up to an asymptotic limiting value, reflecting the same exponential time constant as the decrease of $(\bar{Q}_{\mathrm{off}})_1$. Exactly this was found by Armstrong and Bezanilla (1977, Fig. 8. *l.c.*). The total off-charge density would then be given as

$$\bar{Q}_{\mathrm{off}} = \bar{Q}_1 + \bar{Q}_2(1 - e^{-\tau_p/\tau_2})$$

which explains the presented asymptotic *increase* of the off-on charge ratio (→1.2) (Fig. 9 *l.c.*) if the slow on-charge (at $V = 0$ mV) is not included completely in the measured $\bar{Q}_{\mathrm{on}}$.

The Effect of a Pulse Preceding the Measurement of Displacement Current

Instead of changing the characteristic parameters of the measuring pulse, those of a conditioning prepulse may be varied in order to analyze the behavior of the system. In particular, the voltage dependence of $\bar{\theta}_3$ can, in principle, be evaluated in this way.

The prepulse is to be applied to the system when it has attained equilibrium. The voltage will be raised from V_o to V' during the time interval τ'_p. Then it is switched back to V_o for a time τ'' until the actual measuring pulse with $V_o \rightarrow V$ is switched on. This is assumed to last for a time interval τ_p as before (*see* Fig. 4 for the entire pulse program). As far as the prepulse is concerned, we have the same situation as discussed above. Regarding the measuring pulse, however, we must take into account that the transitions induced by the prepulse may not have completely faded away during the interval between the pulses. Thus, the prepulse generally alters the initial conditions of the system at the moment of switching on the main pulse, i.e., $\delta\theta_{oi} \neq \delta\bar{\theta}_i$. The appropriate values of $\delta\theta_{oi}$ can, of course, be calculated by employing Eq. (13) with respect to the changes of θ_i during prepulse and subsequent fading.

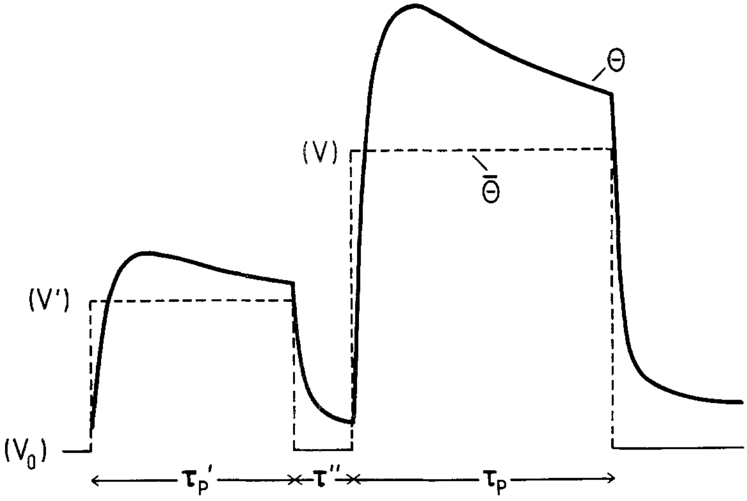

Fig. 4. Typical time course of a degree of transition, θ, as induced by two consecutive pulses (prepulse: $V_o \rightarrow V'$, measuring pulse: $V_o \rightarrow V$). It is assumed that the relaxation behavior involves a fast and a slow phase. The dashed lines represent the respective instantaneous equilibrium values, $\bar{\theta}$

Inserting them in Eq. (15) yields for the displaced charge components of the on-current

$$\begin{aligned} Q_1 = \bar{Q}_1 - \{\bar{Q}'_1(1-e^{-\tau'_p/\tau'_1}) - \bar{Q}'_c(1-e^{-\tau'_p/\tau'_2})\}\, e^{-\tau''/\tau_1^{(o)}} \\ - \hat{Q}_c(1-e^{-\tau'_p/\tau'_2})\, e^{-\tau''/\tau_2^{(o)}} \end{aligned}$$

$$Q_2 = \bar{Q}_2 - (\bar{Q}'_2 + \bar{Q}'_c - \hat{Q}_c)(1-e^{-\tau'_p/\tau'_2})\, e^{-\tau''/\tau_2^{(o)}}$$

where

$$\hat{Q}_c = \delta\left(\frac{K_{12}}{1+K_{12}}\right)(\bar{\theta}'_3 - \bar{\theta}_3^{(o)})\, Q_\infty^o$$

and all the dashed quantities refer to the values at prepulse voltage V'.

Let us again suppose that the measured on-charge density is essentially given by Q_1 only. If in addition the interval between the pulses is taken long enough so that $\tau_1^{(o)} \ll \tau''$ we find for $\tau_p \gg \tau_1$

$$Q_{\text{on}} = \bar{Q}_{\text{on}} - \hat{Q}_c(1-e^{-\tau'_p/\tau'_2})\, e^{-\tau''/\tau_2^{(o)}}.$$

By variation of τ'_p and τ'' it should be possible to evaluate the parameters $\bar{Q}_{\text{on}}$, $\hat{Q}_c$, τ'_2, and $\tau_2^{(o)}$. In such a way the slow relaxation time τ_2 and the fraction of slowly coupled state P_3 would be accessible as a function of the voltage (of the prepulse). The $\bar{\theta}_3$ can be obtained from $\hat{Q}_c$ determined as a function of V'. In particular, choosing once more V_o and V so that $K_{12} \gg 1$ and $K_{12}^{(o)} \ll 1$ apparently implies

$$\hat{Q}_c = (\bar{\theta}'_3 - \bar{\theta}_3^{(o)}) Q^o_\infty .$$

Inactivation of the displacement current is thus equivalent to an increase of $\bar{\theta}_3$ due to application of the prepulse voltage. According to the experimental data for squid axon this seems to be so in case of $V' > V_o$. It indicates that for hyperpolarizing conditioning pulses (i.e., $V' < V_o$) and $\bar{\theta}_3^{(o)} > 0$, a negative value of $\hat{Q}_c$ should be encountered, in other words, an activation of the displacement current.

We may extrapolate the on-charge towards $\tau'_p, \tau_p \gg \tau'_2, \tau_2^{(o)}$ and $\tau'' \to 0$ but take into account only measurements with $\tau'' \gg \tau_1^{(o)}$. This will, under the given circumstances, yield

$$Q_{\text{on}}^{(\text{ex})} = \bar{Q}_{\text{on}} - \hat{Q}_c = (1 - \bar{\theta}'_3) Q^o_\infty = \frac{1 + K'_{12}}{1 + K'_{12} + K'_{13}} \cdot Q^o_\infty$$

$$= \frac{1 + e^{b_{12}(V' - V_{12})}}{1 + e^{b_{12}(V' - V_{12})} + e^{b_{13}(V' - V_{13})}} Q^o_\infty .$$

The general course of the $Q_{\text{on}}^{(\text{ex})}$ *vs.* V' curve depends primarily on the magnitudes of the individual dipole moments in relation to each other. It can, of course, always be assumed that $\mu_{12} > 0$, but three different cases with respect to μ_{13} have to be considered:

1) if $\mu_{13} > \mu_{12}$ the curve decreases steadily, exhibiting saturating values Q^o_∞ (at $V' \to -\infty$) and 0 (at $V' \to \infty$);
2) if $\mu_{12} > \mu_{13} > 0$, the curve runs through a minimum with a saturating value of Q^o_∞ at both extremes of V';
3) if $\mu_{13} < 0$, the curves increase steadily exhibiting saturating values 0 (at $V' \to -\infty$) and Q^o_∞ (at $V' \to \infty$).

A more detailed illustration is given in Fig. 5. A plot of experimentally determined $Q_{\text{on}}^{(\text{ex})}$ *vs.* V' would, therefore, permit a rather direct quantitative analysis of $\bar{\theta}_3$ and the involved parameters.

In this context we should return to some relevant data of Armstrong and Bezanilla (1977). The fact that they see a *positive* slow on-charge at positive V (where $K_{12} \gg 1$) requires $\mu_{13} > \mu_{12}$ in our model as can be concluded from Eq. (15b). This then implies a steady increase of $\bar{\theta}_3$ with voltage. Apparently it reaches its saturating value of unity at about +10 mV since there is no difference of the limiting values of the above discussed off-on charge ratio between $V = 10$ and 50 mV, respectively (*cf.* Fig. 3 *l.c.*). In the same article prepulse experiments have been reported

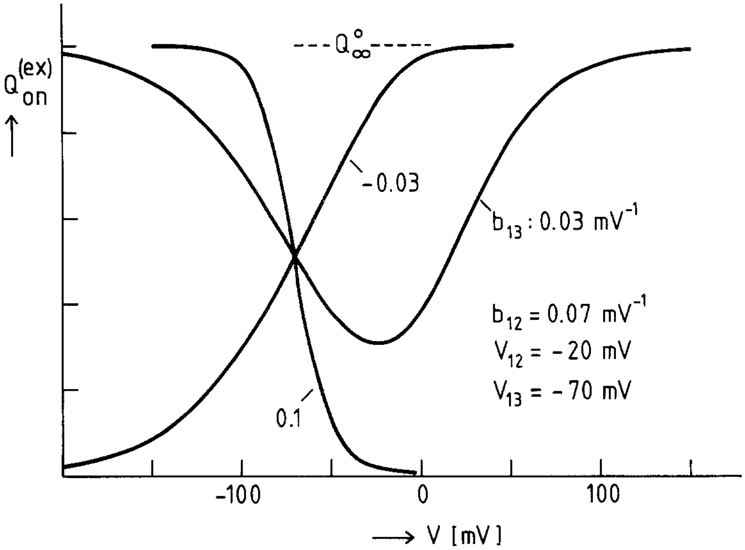

Fig. 5. Three typical examples illustrating the dependence of the extrapolated on-charge, $Q_{\text{on}}^{(\text{ex})}$ (*see* text) on the prepulse voltage V' (essentially reflecting $1-\bar{\theta}_3$ as a function of voltage)

(upper part of Fig. 6 *l.c.*) ($V=30$ mV). They seem to essentially satisfy our above conditions for extrapolation except that (i) τ'' ($=0.7$ msec) appears not to be negligible with regard to $\tau_2^{(o)}$ and (ii) the slow charge transfer component is included in the measured results. Under these circumstances the present model predicts

$$Q_{\text{on}}=(\mu_{13}/\mu_{12})\, e^{-\tau''/\tau_2^{(o)}}\, Q_{\text{on}}^{(\text{ex})}+(\mu_{13}/d)\, c_p(1-\bar{\theta}_3^{(o)}).$$

Accordingly, the charge transfer decreases steadily only until it takes at positive V' a nonzero limiting value just as exhibited by the experimental points in the quoted figure.

The displacement current after switching off the measuring pulse may be calculated analogously. It turns out that in the general case

$$\begin{aligned} Q_1 = &-\{\bar{Q}_1(1-e^{-\tau_p/\tau_1})-\bar{Q}_c(1-e^{-\tau_p/\tau_2})\} \\ &-\{\bar{Q}_1'(1-e^{-\tau_p'/\tau_1'})-\bar{Q}_c'(1-e^{-\tau_p'/\tau_2'})\}\, e^{-\tau''/\tau_1^{(o)}}\, e^{-\tau_p/\tau_1} \\ &-\hat{Q}_c(1-e^{-\tau_p'/\tau_2'})\, e^{-\tau''/\tau_2^{(o)}}(e^{-\tau_p/\tau_1}-e^{-\tau_p/\tau_2}) \\ Q_2 = &-(\bar{Q}_2+\bar{Q}_c)(1-e^{-\tau_p/\tau_2}) \\ &-(\bar{Q}_2'+\bar{Q}_c')(1-e^{-\tau_p'/\tau_2'})\, e^{-\tau''/\tau_2^{(o)}}\, e^{-\tau_p/\tau_2}. \end{aligned}$$

Simplified relations may naturally be obtained by appropriate choice of experimental conditions.

Concluding Remarks

The proposed three-state conformational transition model apparently explains very well the generally observed behavior of asymmetry currents in squid axon membranes if voltage pulses in the millisecond range are applied. However, for a complete quantitative examination of the theory in the light of the experimental data, more pertinent measurements are required. In particular, detailed temperature and voltage dependences with and without conditioning pulses would be desirable. Nevertheless, the great potential of the theoretical approach should already be obvious. Having introduced the voltage V (or the electric field strength, E, respectively) as an additional variable of state, standard methods of thermodynamics and reaction kinetics can be employed in order to develop a comprehensive quantitative picture of the system.

Up to now we have considered only one slowly coupled conformational state. This appears to suffice for the special experimental data so far considered. It may be noted that inactivation phenomena with much longer induction and recovery times in squid axon have been observed (Meves & Vogel, 1977*b*). These indicate the existence of further conformational states which are even more slowly coupled than the one discussed above. Our model could, in principle, be readily extended to include them.

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