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Asymmetric displacement currents in giant axons and macromolecular gating processes

(gating current/conformational changes/charge-charge interaction)

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ABSTRACT **An electrical-chemical gating model is proposed that describes basic observations on asymmetric displacement currents and transient Na^+ conductivity changes in squid giant axons. A previously developed single-parameter analysis of primary voltage clamp data yields normal mode relaxation times that agree well with the time constants of asymmetric capacitative currents, suggesting these currents as gating currents associated with charge displacement in a subunit of a complex gating system. The physical-chemical approach correlates the opening of Na^+ channels with charge-charge interactions amongst displaceable membrane charges or dipoles and conformational changes in gating macromolecules. The model covers the close correspondence between the voltage dependence of the peak value of the Na^+ conductance change and that of the square of the total displaced charge for small depolarizing voltage steps. The quadratic charge relationship also describes the two-mode relaxation of asymmetric displacement currents; the transiently inhibited return transition of two-thirds of the displaced charge after a prolonged depolarization is interpreted to reflect a dissipative chemical gating process.**

Impulse transmission in nerves and skeletal muscle fibers is founded on transient permeability changes in excitable membranes, dominantly for Na^+ and K^+. The molecular gating processes responsible for the permeability changes depend on membrane voltage, suggesting the involvement of charged or dipolar structures in the regulation of the ionic pathways. Considerable effort has been expended to analyze capacitative components of membrane current so as to isolate displacement currents attributable to gating processes (1, 2).

The kinetics of ionic permeability changes in squid axon membranes were first described by Hodgkin and Huxley (H–H) (3). When the voltage-clamped membrane is suddenly depolarized, i.e., when the normally more negative potential of the cell interior is raised to a more positive value, there is first a brief capacity current. This is normally followed by an inward current carried dominantly by Na^+, which peaks rapidly but then diminishes more slowly, plus an outward K^+ current, which rises slowly (msec time range) to a steady-state value. The specific effects of pharmacological agents such as tetrodotoxin, which blocks the Na^+ current but leaves K^+ current unaffected (4), suggest that Na^+ and K^+ cross the membrane at different and discrete sites.

In the classical H–H formulation, the transient increase in the membrane's Na^+ conductance, $g_{Na}(t,V)$, is expressed in terms of two independent variables, m and h, as a function of time, t, and voltage, V, at which the current or conductance relaxation is measured. Alternatively, the transient opening of Na^+ channels can be described in terms of a single linear second-order variable (see, e.g., refs. 3, 5, and 6). The single parameter must then represent the "open" configuration of a gating subunit or an elementary "opening" process in a complex gating system (6). A general analysis of voltage-clamp data shows that $g_{Na}(t,V)$ is proportional to a cubic function of this single parameter for squid and *Myxicola* giant axons (6).

Displacement currents that correlate well with the opening of Na^+ channels have been reported for voltage-clamped squid (1, 2) and *Myxicola* (7) giant axons and for nodes of Ranvier in myelinated fibers (8). These capacitative currents reflect the displacement of membrane components that respond asymmetrically with respect to depolarizing compared to hyperpolarizing voltage steps, and are detectable after the much larger ionic currents have been eliminated and the linearly voltage-dependent capacitative current has been subtracted out by averaging techniques. This asymmetric displacement current, $I_g(t,V)$, measured as the response to a depolarizing voltage step at voltage V, appears to be associated with the opening transitions of Na^+ channels (1, 2).

The H–H model of independent m and h variables, suggesting that independent processes are responsible for the rise and fall, respectively, of $g_{Na}(t,V)$ during application of a depolarizing pulse, does not appear to be an adequate representation of more recent voltage-clamp data, incuding the observation that preceding depolarizing pulses appear to "inactivate" the same gating processes that are involved in opening Na^+ channels. Therefore, a formal description in which $g_{Na}(t,V)$ is a function of only a single variable may lead to a better characterization of the molecular events underlying Na^+ permeability changes. As justified previously (6), such a single membrane variable, $\rho(t,V)$, has to be the solution of at least a second-order differential equation,

$$\rho(t) = \beta_1 \exp(-t/\tau_1) - \beta_2 \exp(-t/\tau_2), \qquad [1]$$

where the dependence of the relaxation amplitudes, β_1 and β_2, and the normal-mode time constants, τ_1 and τ_2, on membrane voltage is implicit. Since $g_{Na}(t,V)$ is considered as a *functional* of $\rho(t,V)$, τ_1 describes the reactions gating the "opening" phase of the permeability change. It is consistently found that $\tau_1 < \tau_m$, the corresponding time constant of the H–H two-parameter model. For the data (10) on *Myxicola* axons, the approximate relationship between τ_1 and τ_m is given by $\tau_m \approx 1.3\ \tau_1$ over a wide voltage range (6).

In Fig. 1, the τ_m values of H–H (3), assuming a resting potential of −60 mV, are represented along with the τ_1 values derived from the original voltage-clamp data of ref. 3 by a normal-mode analysis using Eq. 1. In addition, Fig. 1 contains the relaxation time constant, τ_g, of the fast component of the two-phase displacement current, I_g, measured in squid giant axons (11). For large values of V, where the resolution of I_g into its two distinct components is most accurate, $\tau_g(V)$ is closer to $\tau_1(V)$ of the single-parameter model than to $\tau_m(V)$ of the H–H

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Abbreviation: H–H, Hodgkin–Huxley.

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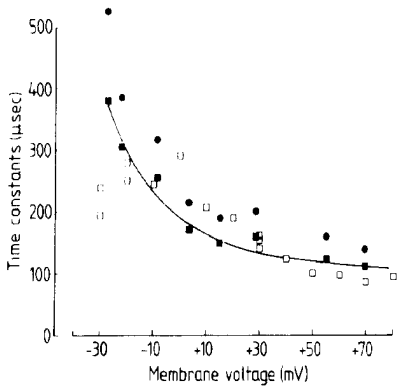

FIG. 1. Values of τ_m (●) reproduced from table 2 of ref. 3, taking the resting potential as −60 mV, τ_g (□) from figure 6 of ref. 11, and τ_1 (■) from the normal-mode analysis reported in ref. 6. The solid curve is fit by eye to the τ_1 values.

model. Conversely, the time constant τ_g of the faster asymmetric displacement current may be identified with the normal-mode relaxation time constant τ_1, representing the structural transitions of the gating subunits from a closed, R, to an open, R', conformation. It is thus justifiable to associate this component of the measured displacement current with an intrinsic gating reaction $R \rightarrow R'$ and to identify the accompanying asymmetric charge movement as the "gating current."

Suggestion of a quadratic charge dependence

In this study, the notion of macromolecular conformational changes, originally proposed by Nachmansohn for the chemical control of rapid ion flows across excitable membranes (12, 13), is extended in a model in which charge interactions are coupled to structural changes leading to the open conformation of a Na^+ channel; these interactions are proposed to be amongst the charges, or dipoles, contributing to the asymmetric capacitance of the axon membrane.

Fig. 2 illustrates the voltage dependence of the peak value, $g_{Na}(t_{max},V)$, of $g_{Na}(t,V)$ in squid giant axon membranes. Since the $g_{Na}(t_{max},V)$ values should be correlated with the total gating charge, $Q_g(\infty,V)$, displaced during identical pulses, where

$$Q_g(\infty,V) = \int_0^\infty I_g(t,V)\,dt, \qquad [2]$$

these values are also introduced in Fig. 2 for comparison.

The relationship between $Q_g(\infty,V)$ and the peak value, $g_{Na}(t_{max},V)$, is more complicated than a simple power law would allow (11). The data in Fig. 2 suggest, however, that at

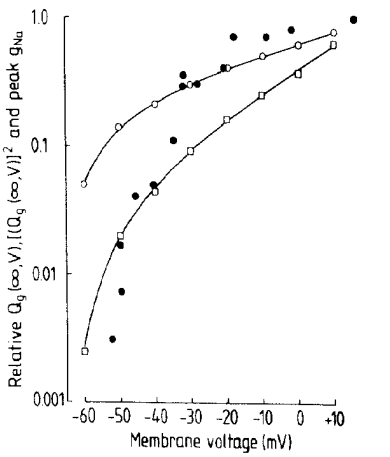

FIG. 2. Peak values of g_{Na} (●) during various clamp voltages from figure 9 of ref. 14, compared with $Q_g(\infty,V)$ (○) and $[Q_g(\infty,V)]^2$ (□) from figure 10 of ref. 15.

low voltages the (approximately exponential) dependence of $g_{Na}(t_{max},V)$ on membrane voltage is essentially identical to that of $[Q_g(\infty,V)]^2$; thus,

$$g_{Na}(t_{max},V) \propto [Q_g(\infty,V)]^2. \qquad [3]$$

Such a quadratic dependence is also indicated when $g_{Na}(t_{max},V)$ is compared with the data of Bezanilla and Armstrong (11) for the total charge displaced in the rapid component of I_g (figure 6 of ref. 11). At large values of V, $g_{Na}(t_{max},V)$ is an approximately linear function of $Q_g(\infty,V)$; this suggests that if $I_g(t,V)$ is indeed a reflection of the molecular processes underlying the transient changes in $g_{Na}(t,V)$, then different elementary steps may dominate the opening of Na^+ channels at small and large depolarizations. For the voltage range where $g_{Na}(t_{max},V) \propto [Q_g(\infty,V)]^2$, electrostatic interactions are indicated amongst flexible charged groups of the gating system or other mobile charges, while $g_{Na}(t_{max},V) \propto Q_g(\infty,V)$ is predicted if the rise in $g_{Na}(t,V)$ is caused by the interaction of charged or dipolar structures with the transmembrane electric field. The comparison in Fig. 2 suggests that the measured polarizations may have contributions from both mechanisms, with charge-charge interactions dominating at small depolarizations.

Kinetic analysis

An extensive comparison of the asymmetric displacement current, $I_g(t,V)$, and Na^+ current, $I_{Na}(t,V)$, has been given (15). $I_{Na}(t,V)$ was expressed in terms of H–H parameters, and a single time constant, τ, was judged to describe the relaxation of the total time course of $I_g(t,V)$; Keynes and Rojas (15) found that $\tau \approx \tau_m$ for the entire voltage range, $-140 < V < +50$ mV. Although, in the light of recent experimental evidence suggesting a coupling between activation and inactivation, such a direct identification of $I_g(t,V)$ with the hypothetical "m-particle" current of the H–H model is questionable (5, 9), one may investigate the observation that $g_{Na}(t_{max},V) \propto [Q_g(\infty,V)]^2$ from a kinetic standpoint.

The time course of $g_{Na}(t,V)$ shows a rising and a falling phase, while a rising phase for $I_g(t,V)$ has not been clearly resolved: the finding of Keynes and Rojas (15), that $\tau \approx \tau_m$, suggests that $I_g(t,V)$ is associated with the rising phase of $g_{Na}(t,V)$. If, as in the H–H model, the processes responsible for the rising and falling phase of $g_{Na}(t,V)$, respectively, are assumed to be decoupled and independent, one may define a quantity, $g'_{Na}(t,V)$, in terms of the H–H time constant τ_m:

$$g'_{Na}(t,V) = g'_{Na}(\infty,V)\,[1 - \exp(-t/\tau_m)]^3. \qquad [4]$$

$g'_{Na}(t,V)$ rises from an initial value of zero to a "steady-state" value $g_{Na}(\infty,V)$, concurrent with the rise of $Q_g(t,V)$ from zero to $Q_g(\infty,V)$, and thus provides at least a formal basis for comparison of $g_{Na}(t,V)$ with $Q_g(t,V)$. Of course, this comparison is only meaningful if $g'_{Na}(t,V)$ satisfactorily reflects that part of the reaction sequence which leads to the opening of Na^+ channels. The corresponding expression for the buildup of total asymmetric charge is Eq. 5:

$$Q_g(t,V) = Q_g(\infty,V)\,[1 - \exp(-t/\tau)]. \qquad [5]$$

Obviously, if $\tau = \tau_m$, a comparison of Eqs. 4 and 5 rules out a direct proportionality between $g'_{Na}(t,V)$ and $[Q_g(t,V)]^2$. With $\tau = \tau_m$, $[1 - \exp(-t/\tau_m)]^3$ will always have a value less than $[1 - \exp(-t/\tau)]^2$; consequently, the relative rise of $g'_{Na}(t,V)$ will be slower than that of $[Q_g(t,V)]^2$. However, interesting conclusions follow if $g'_{Na}(t,V)$ is postulated to be proportional to the square of some fraction $Q(t,V)$ of $Q_g(t,V)$. For the following arguments, it is useful to define a time-dependent parameter, $\lambda(t,V)$ such that

$$\frac{g'_{Na}(t,V)}{g'_{Na}(\infty,V)} = \left[\frac{Q_g(t,V) - \lambda(t,V)}{Q_g(\infty,V) - \lambda(\infty,V)}\right]^2, \qquad [6]$$

with $Q(t,V) = Q_g(t,V) - \lambda(t,V)$. This defines $\lambda(t,V)$ as the "residual" fraction of $Q_g(t,V)$, i.e., that fraction not involved in the suggested quadratic dependence of $g_{Na}(t,V)$ on displaced charge.

Defining normalized quantities $p(t,V)$ and $q(t,V)$ from

$$p(t,V) = Q_g(t,V)/Q_g(\infty,V)$$
$$q(t,V) = g'_{Na}(t,V)/g'_{Na}(\infty,V), \quad [7]$$

and also rewriting $\lambda(t,V)$ in terms of a normalized parameter $s(t,V)$, where

$$s(t,V) = \lambda(t,V)/Q_g(\infty,V), \quad [8]$$

Eq. **6** can be transformed into

$$q(t,V) = \{[p(t,V) - s(t,V)]/[1 - s(\infty,V)]\}^2. \quad [9]$$

Solving for $s(t,V)$ yields two roots:

$$s(t,V) = p(t,V) \pm [q(t,V)]^{1/2}[1 - s(\infty,V)]. \quad [10]$$

The more negative root satisfies the required condition that $s(t,V) \to s(\infty,V)$ as $t \to \infty$, and yields the time dependence of $s(t,V)$ in terms of τ and τ_m. However, $s(\infty,V)$ is an unknown parameter, so that the absolute value of $s(t,V)$ cannot be determined from Eq. **10.**

This difficulty can be overcome by recognizing that for $t \gg \tau$, one may put $s(t,V) = s(\infty;V)$. Defining a new variable, $s_{t,V}$, from the expression

$$q(t,V) = \{[p(t,V) - s_{t,V}]/(1 - s_{t,V})\}^2, \quad [11]$$

one may calculate values of $s_{t,V}$ at all times, with $s_{t,V}$ corresponding to $s(\infty,V)$ at times large enough that $s(t,V)$ can be assumed to have reached a steady state. The exact solution for $s_{t,V}$ is

$$s_{t,V} = \frac{p(t,V) - q(t,V) \pm [q(t,V)]^{1/2}[p(t,V) - 1]}{1 - q(t,V)} \quad [12]$$

The more negative root predicts $s_{t,V} \approx 1$ for times on the order of τ, which would imply that all of $Q_g(\infty,V)$ is residual; this root must be disregarded. Substituting $p(t,V)$ and $q(t,V)$ from Eq. **7** into the more positive value of Eq. **12**, and setting $\tau = \tau_m$, one obtains the following approximate expression for $s_{t,V}$ at $t \gg \tau$:

$$s_{t,V} \approx 1 - 2\{3[1 - \exp(-t/\tau)] + \exp(-t/\tau)\}^{-1}. \quad [13]$$

$s_{t,V}$ will approach the value 1/3 asymptotically as t grows large relative to τ. Thus, the hypothesis that $g'_{Na}(\infty,V) \propto [Q_g(\infty,V)]^2$, along with the condition $\tau = \tau_m$, has led to the prediction of a slower component in $Q_g(t,V)$, saturating at one-third of $Q_g(\infty,V)$.

The above kinetic analysis suggests that the time constant τ is only an approximate characterization, i.e., the asymmetry current described by τ actually encompasses two kinetically distinguishable components, with the faster of these components associated with charge-charge interactions. However, if the opening and closing of Na^+ channels is accomplished via coupled reactions, then the experimentally resolvable kinetics would reflect only the normal modes of the overall reaction sequence. As a result of such coupling, the above analysis using the model parameter τ_m cannot be expected to reproduce the asymmetry current time constants even though the predictions of the model are in qualitative agreement with experimental results. First, the fraction of predicted residual charge is in accordance with the fraction of $Q_g(\infty,V)$ not influenced by depolarizations lasting several milliseconds, after which the immediate return transition of two-thirds of $Q_g(\infty,V)$ appears inhibited (9). In turn, this finding indicates that the events that are responsible for the closure phase, or "inactivation," of $I_{Na}(t,V)$ during a maintained depolarization, have a dramatic effect on only two-thirds of the total asymmetric capacitative charge displaced during the opening phase of the Na^+ permeability change. It may therefore be argued that the remaining one-third of $Q_g(\infty,V)$, which is apparently not affected by inactivation processes, may have *initially* not been involved in charge-charge interactions accompanying the opening of Na^+ channels; one-third of $Q_g(\infty,V)$ would thus be residual. Second, the predicted slower kinetics of this residual charge is confirmed by the experimental findings of Bezanilla and Armstrong (9). They resolved the time course of $I_g(t,V)$ into two kinetically distinct components and reported that the slower component does not correlate well with the opening of Na^+ channels.

It must be emphasized that because the relationship $g_{Na}(t_{max},V) \propto [Q_g(\infty,V)]^2$ does not hold over the entire voltage range, the present findings may be only fortuitous. However, the experimental results are reproduced by the model and suggest that at least some of the events associated with Na^+ channel gating may be intimately connected with electrostatic interactions between membrane components, the displacement of which is directly reflected in the measured asymmetry currents.

Another note of caution that must be attached to these arguments concerns the values of the experimental time constants τ and τ_m reported for clamp voltages more negative than −50 mV. These values were reported for repolarization experiments (15) and hence may not be representative of normal activation processes occurring during depolarization (11). The present analysis can therefore apply only to τ_m and τ values determined at larger depolarizations. The fact that the quadratic relationship between the peak value, $g_{Na}(t_{max},V)$, and $Q_g(\infty,V)$ does not hold at high voltages may then indicate that the "charge displacing" structures undergo further displacements during large depolarizations, but that these transitions have little influence on the opening phase of $I_{Na}(t,V)$.

It would have been preferable to have based the above analysis on the normal mode time constant τ_1. Unfortunately, in the extensive comparison of Na^+ and asymmetry current time constants given in ref. 15, the "inactivation" time constants τ_h of the H–H model are not provided, so the overall g_{Na} curves cannot be reproduced and a normal mode analysis could not be performed.

In summary, a proportionality is evident between $g_{Na}(t_{max},V)$ and the square of the total displaced charge, $Q_g(\infty,V)$, for small depolarizations. $g_{Na}(t_{max},V)$ is regarded as a direct measure of the degree of activation, or the number of channels open, during a depolarizing pulse. On the other hand, the relationship $g_{Na}(t_{max},V) \propto [Q_g(\infty,V)]^2$ does not hold for large depolarizations, nor is it compatible with the experimentally observable similarity of time constants, i.e., $\tau = \tau_m$.

On the basis of arguments in ref. 11 (see above) and the scatter of τ values reported in figure 6 and 13 of ref. 15, the relation $\tau = \tau_m$ may be representative of gating only for large depolarizations, say $V > -30$ mV. In this voltage range, the hypothetical condition between two *hypothetical* quantities, $g'_{Na}(t,V) \propto [Q(t,V)]^2$, has been shown to imply that $Q(\infty,V) = (2/3)Q_g(\infty,V)$ and, furthermore, that the overall displaced charge $Q_g(t,V)$ must consist of two kinetically distinct components; the slower of these components would not reflect charge-charge interactions.

Cubic dependence from first principles

For squid and *Myxicola* giant axons $g_{Na}(t,V)$ can be adequately described as a *functional* of a single variable, $\rho(t,V)$. After

determination of the two normal-mode time constants of $\rho(t,V)$ (see Eq. 1) from the time course of $g_{Na}(t,V)$, the functional relationship between $g_{Na}(t,V)$ and the fraction, $f'(t,V)$, of "open" subunits can be derived. The opening and closing of Na^+ channels requires *at least* a three-state overall-unidirectional reaction cycle; solving the corresponding rate equation for $f'(t,V)$ (see equations 6–9 of ref. 6) and comparing the general form of $f'(t,V)$ with that of $\rho(t,V)$ (Eq. 1) dictates that $\rho(t,V) = f'(t,V) - f'(\infty,V)$, where $f'(\infty,V)$ is the steady-state fraction of open channels at the clamp voltage V. For both squid and *Myxicola* axons,

$$g_{Na}(t,V) \propto [f'(t,V) - f'(\infty,V)]^3. \quad [14]$$

Thus, the general analysis in terms of one membrane variable, $\rho(t,V)$, suggests that the *overall* $g_{Na}(t,V)$ curve can be represented by a cubic dependence on some elementary process.

If the quadratic charge dependence for small depolarizations, Eq. 3, reflects a simple coulombic force relationship between two charged groups on a macromolecule, the distance between the charged but flexible groups would be dictated by the balance of coulombic and structural-mechanical forces. If two groups, 1 and 2, carry net charges of z_1e and z_2e, respectively (e being the electronic charge and z the charge number, inclusively the sign), and assuming structural deformations to be small enough to be in the linear (Hooke's law) range, then the charge separation, r, will be determined by the equilibrium condition

$$(z_1z_2e^2)/(\epsilon r^2) - b(r - r_0) = 0; \quad [15]$$

ϵ is the effective dielectric constant of the medium, b is the "elastic" constant, and r_0 is the separation of the groups in the absence of coulombic interactions. Eq. **15** dicates that when $r = r_0$, either z_1 or z_2, or both, have to equal zero; consequently, when $r = r_0$, at least one of the two groups would have to be neutralized, e.g., by a "screening charge." Eq. **15** may be rewritten as

$$z_1z_2e^2 = \epsilon br^2(r - r_0); \quad [16]$$

with $r = r_0 + \delta r$, Eq. **16** is transformed into

$$z_1z_2e^2 = \epsilon b(r_0 + \delta r)^2\delta r. \quad [17]$$

In accordance with the previous assumption of small deformations, $\delta r \ll r_0$, and Eq. **17** predicts $z_1z_2e^2 \propto \delta r$.

In the single-parametric representation, the temporal characteristics of $g_{Na}(t,V)$ are described by $(f'(t,V) - f'(\infty,V))^3$ (see above). The present arguments imply that the primary effect of membrane depolarization is the inducement of an electrostatically unfavorable environment on flexible, similarly charged structures on a gating subunit; the opening of a subunit would then be induced by the unfavorable electrostatic interaction. Kinetic data show that $g_{Na}(t,V)$ must be a reflection of three such events, and hence that $g_{Na}(t,V) \propto (r - r_0)^3$. Accordingly, the quantity $f'(t,V) - f'(\infty,V)$ must be a direct reflection of the induced elastic deformation, and the membrane variable $\rho(t,V)$ represents a structural change induced by the separation of two interacting charges belonging to a gating subunit.

As already noted, during depolarizing steps to voltages above −30 mV, the dependence of $g_{Na}(t_{max},V)$ appears linear, rather than quadratic, on $Q_g(\infty,V)$ (see Fig. 2). One explanation of this finding is that $g_{Na}(t_{max},V)$ is influenced principally by the *initial* displacement of the structures responsible for $Q_g(\infty,V)$, with subsequent displacements not having a significant effect. The comparison of $g_{Na}(t_{max},V)$ with normalized $Q_g(\infty,V)$ can only be justified if $Q_g(\infty,V)$ reflects the same processes in all voltage ranges, If, for example, $Q_g(\infty,V)$ reflects the reorientation of interacting flexible charged groups, then Fig. 2 would suggest that these charged groups, or mobile charges, can be displaced in excess of the displacement needed to open Na^+ channels.

Within the framework of the molecular model suggested here, the predicted residual charge of one-third of $Q_g(\infty,V)$ may reflect the displacement of the "screening" charge, or charged group, implied by Eq. **17.** Since such a screening charge would not partake in the indicated charge-charge interactions, it would appear as "residual" in the analysis of the previous section. Alternatively, the residual charge may represent the late movement of the interacting charges themselves, as described in the preceding paragraph. Finally, there may be intermediate reactions between the processes represented by the left- and right-hand sides of Eq. **17.** If $r - r_0$ in Eq. **17** is a *direct* indication of the fraction of gating subunits in the "open" configuration, then intermediate reactions are suggested by the differential effects of hyperpolarization (15) and the presence of heavy water (^2H_2O) (16) on sodium compared to asymmetry currents. Hyperpolarization appears to induce a delay in the rise of $I_{Na}(t,V)$ and heavy water slows the overall kinetics of $I_{Na}(t,V)$; yet, in both cases, asymmetry currents appear to be unaffected.

Chemical gating scheme

The electrical, and some biochemical, properties of excitable membranes have been explored intensively, yet the molecular mechanisms underlying the dynamic permeability changes remain unknown. The theoretical approach here is presented as a working hypothesis, since it can, within the framework of certain assumptions, predict and reconcile disparate experimental results, as well as account physically for the *inherently* cubic dependence of $g_{Na}(t,V)$ on an elementary process occurring at the lowest organizational level. A more detailed molecular interpretation of the observed electrical properties would require chemical information on specific permeability-regulating structures. Existing physical-chemical approaches suggest that control of the rapid transient permeability change should be interpreted in terms of dissipative chemical models. The large heat changes accompanying the action potential can be realized if gating of ionic channels involves the dissipation of free energy due to "some substance being used up" during gating. It has been suggested (12, 13) that the activator-receptor concept, now widely accepted for synaptic control reactions, plays a key role in the regulation of all rapid ionic permeability changes. (A recent review of physical-chemical gating concepts is given in ref. 17.)

The opening and closing of Na^+ channels, when expressed in terms of a single membrane parameter, requires a cyclic reaction scheme including dissipation (6). In line with the traditional interpretation of voltage-clamp data (see, e.g., ref. 11), the reaction scheme of Fig. 3 may be proposed. In this scheme, channel permeability would be controlled by a basic excitation unit composed of three subunits, with only the R_3' configuration of the gating system representing the "open" configuration. The opening of a channel would be represented by the sequential transition along the pathway

$$R_3 \rightarrow R_2R' \rightarrow RR_2' \rightarrow R_3',$$

whereas the closure phase (inactivation) would be modelled by the practically irreversibly occurring reaction $R_3' \rightarrow R_3''$. Upon repolarization of the membrane before appreciable inactivation has occurred, the return transition to R_3 proceeds reversibly from R_2R', RR_2', and R_3'. Once the gating system is in the "pool" of inactivated conformations R_3'', however, the return to R_3 proceeds almost exclusively along the reaction path $R_3'' \rightarrow R_3$.

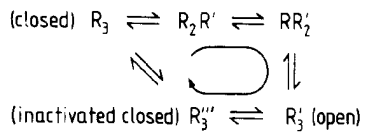

FIG. 3. Proposed chemical cycle for Na^+ channel gating.

Provided that the reaction steps from R_3 to R_3' (along the curved arrow in Fig. 3) occur on time scales of the same order of magnitude, the failure to resolve a definite rising phase for the asymmetry current would indicate that the first reaction step, $R_3 \rightarrow R_2R'$, is already associated with some charge displacement. In this gating scheme, the variable $\rho(t,V)$ would represent the intrinsic transition $R \rightarrow R'$; consequently, the charge-charge interactions must be such as to shift the reaction

$$R \underset{k'_b}{\overset{k'_f}{\rightleftharpoons}} R'$$

to the right when the membrane is depolarized, i.e., such that $k_f'(V) \gg k_b'(V)$.

According to arguments following the kinetic model presented above, charge-charge interactions are predicted to be mainly amongst structures contributing to the "fast" component of the squid axon asymmetry current. Furthermore, the proportionality between $g_{Na}(t_{max},V)$ and the square of the *total* displaced charge is evident only in a voltage range considerably more negative than the voltage range wherein $\tau_g(V)$, the time constant characterizing the *fast* component of the asymmetry current, has a maximum, namely, $-15 < V < 0$ mV (figures 5 and 6 of ref. 11), again suggesting that charge-charge interactions are mainly associated with the fast asymmetry current. Thus, the principal effect of the altered electrostatic interactions between displaceable charges must be such as to destabilize the R conformation relative to R' (by reducing k_b'). The forward transition, $R \overset{k_f'}{\rightarrow} R'$, may also be sensitive to membrane voltage, accounting for the approximately linear relationship $g_{Na}(t_{max},V) \propto Q_g(\infty,V)$ at more positive voltages. One possibility is that $k_f'(V)$ reflects the stability of a certain configuration of the same charges or dipoles responsible for the quadratic charge dependence exhibited at more negative voltages (small depolarizations).

The effects of a prolonged (5–10 msec) depolarization on asymmetry currents suggest that at least a part of the reaction sequence responsible for the observed charge displacement involves a slowed return transition. After termination of a long depolarizing pulse by repolarizing the membrane to −70 mV, only 1/3 of the charge displaced asymmetrically during depolarization returns with *measurable* kinetics (9). Although the remaining 2/3 of the charge returns with very slow kinetics when the repolarization voltage is lowered to values more negative than −70 mV (18), the apparent slowing of charge movement can be interpreted so as to suggest the involvement of (consumptive) dissipation in rapid Na^+ channel gating.

In summary, it appears that a single-parameter model can be consistently combined with first principles of electrostatic interactions in conformationally flexible gating molecules to describe basic observations on ionic and capacitative currents in excitable membranes. The measured charge displacement can be identified with an elementary gating reaction within an oligomeric gating system, and the dissipative element in the gating cycle is modeled by the inhibited mobility of asymmetric charge during the inactivation (closure) phase of the transient Na^+ conductance change.

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