ORIGIN OF THE RISING PHASE OF GATING CURRENTS

ROSALIE C. HOYT
Physics Department, Bryn Mawr College, Bryn Mawr, Pennsylvania 19010

ABSTRACT It is suggested that the delayed rise of gating currents may result from a dielectric relaxation process within the membrane, not of the gating systems themselves but of molecules in the neighborhood of the gating systems. Such dielectric relaxation may prevent the electric fields at the locations of the gates from rapidly attaining their final, "clamped," values. Calculations based on this concept, using the Hodgkin Huxley *m*-process as an example, are shown to lead to gating currents whose shapes are similar to those observed experimentally. One possible interpretation in terms of membrane structure is proposed.

In most published records of the asymmetry currents in nerve fibers there is a clearly defined rising phase, for both the ON and the OFF processes. The time to peak of the gating currents is typically 40–100 μs after the voltage clamp has been applied. Although the time required for the voltage clamp itself to reach its final value may contribute to this delay, the delay is still present when the settling time of the voltage clamp is 5 μs or less (Armstrong and Gilly, 1979; Kimura and Meves, 1979). A number of experimenters believe that this delayed rise is not an artifact either of clamping system or of the procedure of substracting out the linear part of the displacement current through the use of control pulses (Bezanilla and Taylor, 1978; Armstrong and Gilly, 1979).

If the delayed rise is indeed due to the sodium gating systems, the question of whether it represents an intrinsic aspect of the gating systems or the response of these gating systems to a separate membrane phenomenon arises. The latter is a very plausible hypothesis, because it is the local electric field, not the overall potential difference across the membrane, that exerts an influence on a gating system. Relaxation processes within the membrane can cause these local electric fields to vary with time even after the membrane voltage, as measured by external electrodes, has attained its final value. Transversely through the membrane, if a dielectric relaxation process takes place at one layer within a voltage-clamped membrane, the electric field will increase with time across some layers, and across others it will decrease. Assuming that this relaxation process is linear, the accompanying displacement currents will be symmetric for positive and negative voltage clamps. Later subtraction of these symmetric displacement currents through the use of control pulses enables the asymmetric gating currents to be observed. However, these gating currents will not be the same as those that would have been observed were the symmetric component not originally present.

The concept outlined above is of importance to investigators attempting to develop models to account for the gating of the sodium conductance channels. If there is a finite settling time of the electric fields within the membrane, then it is not necessary to invoke a complicated gating mechanism to account for the delayed rise. Rather, the voltage dependent rate constants of a given model, such as the α's and β's of Hodgkin and Huxley (1952), HH, should be treated as time varying parameters during the time that it takes for the field at the location of the gating systems to change from its initial to its final value. The effect on model simulations of conductance vs. time experiments should, in large part, be merely a delay of the order of 40–100 μs. The effect on simulation of gating currents should be the presence of a delayed rise.

As a test of the above concept we have computed gating currents according to one model. In this analysis it has been assumed for simplicity that the series resistance has been completely compensated so that the voltage clamp across the membrane attains its final value instantaneously, but that the electric fields at the location of the gating systems follow an exponential time course,

$$E = E_\infty - (E_\infty - E_o)e^{-t/\tau_R}, \tag{1}$$

in passing from their initial value, E_o, to their final value, E_∞. τ_R is the time constant of the membrane relaxation process. Eq. 1 can not be applied directly. In fitting a model for Na conductance to the results of clamp experiments, the model parameters, such as the α's and β's of HH, can be determined as function of the membrane voltage, not as functions of the electric field at the locations of the conductance gating systems. However, it is not unreasonable to assume that the initial and final clamp values of these fields, E_0 and E_∞, are linearly related to the initial and final values of the membrane voltage, V_0 and V_∞. This assumption allows one to predict the time variation of a given parameter, p, of the model: It will be the same as that which would obtain if there were no relaxation process, but if instead the membrane voltage followed the time course

$$V' = V_\infty - (V_\infty - V_0)e^{-t/\tau_R}. \tag{2}$$

Note that the voltage V' of Eq. 2 is not the actual clamped voltage. The latter is assumed to have a negligible time constant compared to τ_R.

Thus, if the functional relationship, $p = f[V]$ is known for clamped values of the membrane voltage after the relaxation process is complete, the time dependence of p during the relaxation process will be given by

$$p \ f[V'(t)] \tag{3}$$

where V' is given by Eq. 2.

Although experiments have shown a number of differences between the behavior of gating currents and those predicted by the m-system of HH (Armstrong and Bezanilla, 1974; Meves, 1974; and Ulbricht, 1977), an analysis of that model provides a simple illustration of the method. Thus we shall assume that the gating current, I_g, is given by

$$I_g = K\,(\mathrm{d}m/\mathrm{d}t) \tag{4}$$

where K is a scaling factor determined by the charge/area carried by the m-particles. m and $\mathrm{d}m/\mathrm{d}t$ are determined from the equation

$$\mathrm{d}m/\mathrm{d}t = \alpha_m(1 - m) - \beta_m m \tag{5}$$

where α_m and β_m are now time-dependent rates. Thus, using today's voltage convention and a resting potential of −60 mV,

$$\alpha_m = 0.1(V' + 35)/[1 - \exp 0.1(V' + 35)] \tag{6}$$

$$\beta_m = 4 \exp - (V' + 60)/18 \tag{7}$$

where V' is given by Eq. 2. Eq. 5, with the subsidiary Eqs. 2, 6, and 7, has been solved by numerical integration for a number of different clamping situations. Typical results are shown by the solid curves in Fig. 1 *A* and *B* for τ_R's of 13 and 40 μs, respectively. The similarity between the solid curves and experimental I_g curves is obvious. The dashed curves give the responses to be expected where the relaxation process not present, but have been shifted to the right by the time t_d, so that they overlap the falling phase of the computed $\mathrm{d}m/\mathrm{d}t$ values.

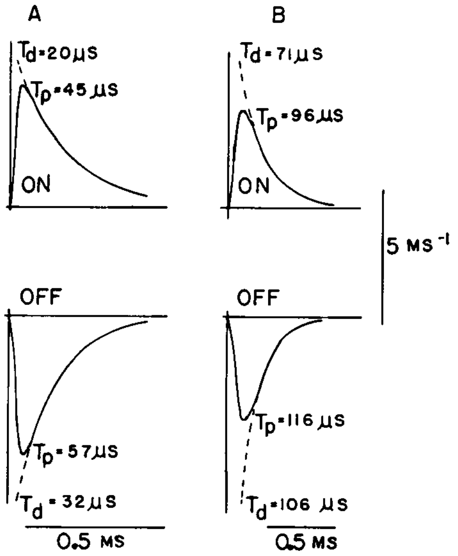

FIGURE 1 Model calculations of $\mathrm{d}m/\mathrm{d}t$ assuming the absence of any relaxation process (----) and the presence of a relaxation process (———) where τ_r is 13 μs in *A* and 40 μs in *B*. t_p gives the time to peak, and t_d gives the delay time required to fit the dashed curves to the falling phase of the solid curves. A 6° fiber is assumed. For the ON process, $V_0 = -70$ mV, $V_\infty = +20$ mV. For the OFF process $V_0 = 20$ mV with m assumed at steady state, $V_\infty = -70$ mV. Note difference of time scale in curves *A* and *B*.

The membrane of axons undoubtedly has a complex structure, both laterally and transversely, and it is impossible at present to speculate in precise terms as to the nature of the membrane component responsible for the proposed relaxation process that affects the electric fields within the membrane. The diagrams shown in Fig. 2 are intended to make this concept more plausible, but do not represent the only possibility. As a first approximation, all capacitances in Fig. 2 are assumed to have phase angles of 90° and no dielectric saturation is considered. Transversely through one section (Fig. 2 *a*), the dielectric properties of the membrane can, to a first approximation, be thought of as being composed of a number of capacitive elements in series. The overall static capacitance, per unit area, is given by $C_\mathrm{m} = \epsilon_0/\Sigma\,(d_\mathrm{i}/\kappa_\mathrm{i})$ where κ_i and d_i are the relative dielectric constant and thickness of the *i*th structure

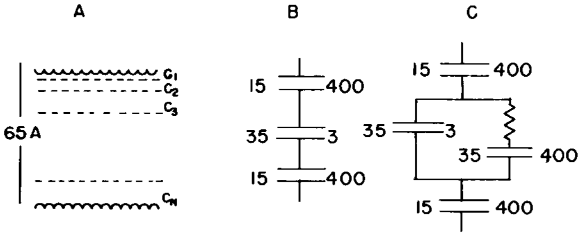

FIGURE 2 (A) Division of the membrane into a series of capacitors. (B) Pure phospholipid region of the membrane, with two polar head regions and a central carbon chain region. (C) Equivalent circuit representation of the inclusion, in the central region, of a polarizable protein with a finite relaxation time constant. The numbers shown at the left of each capacitor give the thickness in angstroms, those at the right give the relative dielectric constant.

respectively, and $\epsilon_0 = 8.85 \times 10^{-12}$ F/m. Far away from any specialized regions, such as those for active transport or gating, it is plausible to divide the membrane into three regions (Fig. 2 *b*): phospholipid head group, carbon chain tails, phospholipid head group. We assume that the relaxation time constants of these three regions is very fast (<1 μs), and that this structure constitutes the major part of the average membrane capacitance. The numbers shown in Fig. 2 *b* yield a value of $C_m = 0.76$ μF/cm^2. Further, 99.4% of a membrane potential difference then occurs across the carbon tail region, and only 0.3% across each of the head regions.

Next we assume that in regions close to the sodium gating system, and probably in many other regions of the membrane, the normal carbon tail region is replaced by a (or part of a) protein molecule, of large polarizability and with a relaxation time on the order of 13 μs (Fig. 2 *c*). Using the numbers shown, the capacitance of this region is 0.76 μF/cm^2 at $t = 0$ and 54.7 μF/cm^2 after the system has relaxed. During the relaxation process there will be an observable displacement current, the symmetric part that is later subtracted out through the use of control pulses. Further, and most important to our argument, the fraction of the total change in membrane potential that appears across a head region relaxes from 0.3% immediately following a voltage clamp, to a final value of 23.1%, a 72 times greater change. We may conclude that if the field sensors for the Na gating systems lie in regions where the local potential change increases with dielectric relaxation of the membrane, such as in or near the linner head group region, the rising phase of gating currents will result. The gating systems themselves may lie in a neighboring, nonlipid region of the membrane.

The values chosen for the dielectric constants in Fig. 2 *c* are consistent with the known dielectric properties of phosopholipid head groups and of protein moities (Almers, 1975; Urry et al., 1975). If the area involved in structure *c* is 1/400 of the total area, an average total static capacitance of 0.84 μF/cm^2 results, not an unreasonable value. The gating systems themselves are not shown in Fig. 2*c*, but they will add a nonlinear, asymmetric component to the membrane capacitance.

A membrane relaxation time constant of 13 μs is consistent with the results of Taylor et al. (1981) on the dielectric properties of squid axon membranes at low frequencies ($\tau_R = 13$ μs corresponds to a frequency of 12 khz). Furthermore, it leads, for an HH fiber, to a time to peak of 40–60 μs for gating currents (Fig. 1 *a*), consistent with the recent results of Bezanilla et al. (1982). If there is a residual, uncompensated series resistance, the overall effective time constant for the local field would be expected to increase. This may account for the longer times to peak that are often observed, on the order of 100 μs, and the larger average relaxation time constants, $\sim$40 μs, required to simulate them (Fig. 1 *b*). For example, Keynes and Rojas (1976) show total displacement currents ($I_S + I_G$) with time constants of $\sim$40 μs, while the time to peak for their separated I_G is $\sim$100 μs, consistent with the simulation shown in Fig. 1 *b*. A useful test of the concept proposed in this communication could be made by using the gating currents, in their turn, to obtain the symmetric component of the displacement current, I_S. Integration of I_S from $t = 0$ to $t = t$ should then indicate the time course of V' of Eq. 2.

If the concept outlined above has validity, the characteristics of the rising phase should be dependent in part on the relaxation time constant, τ, and in part on the voltage dependence of the rate parameters of the system responsible for the gating current. For a given relaxation time constant, such characteristics of the rising phase as time to peak and magnitude of peak should therefore be dependent on the test voltage, conditioning voltage and recovery interval. Such effects have been observed experimentally (eg. Armstrong and Bezanilla, 1975; Bezanilla and Armstrong, 1976; Bezanilla et al., 1982) and models of the gating systems can be tested for their ability to reproduce the observed experimental effects. While it seems unlikely that our explanation of the rising phase can account for its complete absence under the particular conditions reported by Armstrong and Gilly (1979), techniques for observing the earliest part of the rising phase are still open to question.

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REFERENCES

Almers, W. 1975. Some dielectric properties of muscle membrane and their possible importance for excitation-contraction coupling. *Ann. N. Y. Acad. Sci.* 264:278–292.

Armstrong, C. M., and F. Bezanilla. 1974. Charge movement associated with the opening and closing of the activation gates of the Na channels. *J. Gen. Physiol.* 63:533–552.

Armstrong, C. M., and F. Bezanilla. 1975. Currents associated with the ionic gating structures in nerve membrane. *Ann. N. Y. Acad. Sci.* 264:265–277.

Armstrong, C. M., and W. F. Gilly. 1979. Fast and slow steps in the activation of sodium channels. *J. Gen. Physiol.* 74:691–711.

Bezanilla, F., and C.M. Armstrong. 1976. Properties of the sodium channel gating current. *Cold Spring Harbor Symp. Quart. Biol.* 40:297–304.

Bezanilla, F., and R. E. Taylor. 1978. Temperature effects on gating currents in the squid axon. *Biophys. J.* 23:479–484.

Bezanilla, F., R. E. Taylor, and J. M. Fernandez. 1982. Distribution and Kinetics of Membrane Dielectric Polarization. *J. Gen. Physiol.* 79:21–40.

Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.).* 117:500–544.

Keynes, R. D., and E. Rojas. 1976. The temporal and steady-state relationships between activation of the sodium conductance and movement of the gating particles in the squid giant axon. *J. Physiol. (Lond.).* 255:157–189.

Kimura, J. E., and H. Meves. 1979. The effect of temperature on the asymmetrical charge movement in squid giant axons. *J. Physiol. (Lond.).* 289:479–500.

Meves, H. 1974. The effect of holding potential on the asymmetry currents in squid giant axons. *J. Physiol. (Lond.).* 243:847–867.

Taylor, R. E., J. M. Fernandez, and F. Bezanilla. 1981. Squid axon membrane low-frequency dielectric properties. *In* The Biophysical Approach to Excitable Systems. W. J. Adelman and D. E. Goldman, Editors. Plenum Publishing Corp., New York. 97–106.

Ulbricht, W. 1977. Ionic channels and gating currents in excitable membrane. *Annu. Rev. Biophys. Bioeng.* 6:7–31.

Urry, D. W., M. M. Long, M. Jacobs and R. D. Harris. 1975. Conformation and Molecular Mechanisms of Carriers and Channels. *Ann. N. Y. Acad. Sci.* 264:203–220.