BRIEF COMMUNICATION

DISCRETE MEMBRANE SURFACE CHARGE DISTRIBUTIONS

EFFECT OF FLUCTUATIONS NEAR INDIVIDUAL CHANNELS

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ABSTRACT Each gating mechanism controlling permeability in a membrane may be influenced by only a few charge binding sites on the membrane surface, so that fluctuations in the occupancy of these sites are important. Two extreme cases arise. (*a*) The time scale of these fluctuations is much shorter than the gating time constant. Then the gating mechanisms are subject to a rapidly varying electric field. If the gating in the absence of these fluctuations obeys exponential kinetics, so does the gating in the presence of the fluctuations. Changes in surface charge do not simply shift the gating variable curves on the voltage axis, but also change their shape. Such effects are seen experimentally and cannot be explained in terms of conventional surface charge theory. If the activation curve in the absence of any surface charge binding is symmetric about the half-activation point, when some of the surface charge sites are occupied the activation curve is in general asymmetric. (*b*) The fluctuations occur much more slowly than the gating reaction. There are several pools of channels present with different time constant and activation curves. Again the activation curve is asymmetric about the half-activation point, and its shape is changed by alterations in the surface charge. The kinetics of gating of the whole population of channels are multiexponential.

Conventional analyses of the effects of surface charge agents on membrane channel gating variables assume that the gating mechanism of each channel experiences an average field due to the charge over a large area of membrane (Chandler et al., 1965; Gilbert and Ehrenstein, 1969). However, the density of charge calculated using such treatments (about one charge per $(15\text{Å})^2$) is consistent with the idea that each gating mechanism may in fact sense a field that reflects quite strongly the discrete nature of the surface charge (Cole, 1969; Brown, 1974), perhaps glutamic and aspartic residues on the protein forming the channel (Cotman and Levy, 1975, p. 202). Because some

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of the surface charge present in excitable membranes can be titrated with H^+ and perhaps Ca^{2+} ions (e.g. Hille, 1968), if the discrete nature of the charge distribution is important then the effects of fluctuations of the surface charge around individual channels, due to binding and unbinding of these ions, can be significant. To demonstrate this, we take the simplified case of each channel having one surface charge site near it, titrated for a fraction f of the time, and untitrated for a fraction $1-f$ of the time. (At high Ca^{2+} and/or H^+ concentrations, f will be close to unity; at low $[Ca^{2+}]$ and $[H^+]$, f will be near zero.) Initially we assume that the time scale of the fluctuations in surface charge is much less than the time constant of the gating variables studied. This is reasonable for H^+ titration: the average time H^+ stays bound to many carboxyl groups (when pH = pK for the reaction) is in the range 10^{-6}–10^{-5}s (at least in free solution; Eigen, 1963, p. 107; Hague, 1971, p. 83), whereas gating time constants are $\gtrsim 10^{-4}$ s. The Ca^{2+} exchange rate may be similarly high (Eigen and de Maeyer, 1963, p. 1041). We define τ_1, as the gating time constant in the untitrated state; τ_2 as the gating time constant in the titrated state; $X_{1\infty}$ as the steady-state fraction of gates open for a population of channels all in the untitrated state, and $X_{2\infty}$ as the steady-state fraction of gates open for channels in the titrated state. X is the fraction of *all* the gates open at any instant in time. In a time δt the change in X is

$$\delta X = (1 - f)(X_{1\infty} - X)\delta t/\tau_1 + f(X_{2\infty} - X)\delta t/\tau_2$$

assuming that the gating variables obey first order reaction kinetics (Hodgkin and Huxley, 1952) even on time scales as short as 10^{-6} s. Thus, if the initial value of X is X_0, $X = X_\infty + (X_0 - X_\infty)e^{-t/\tau}$, where the effective value of X_∞ is

$$X_\infty = \frac{(1 - f)X_{1\infty}/\tau_1 + fX_{2\infty}/\tau_2}{(1 - f)/\tau_1 + f/\tau_2} \tag{1}$$

and the effective time constant for changes of the gating variable is τ where

$$1/\tau = (1 - f)/\tau_1 + f/\tau_2. \tag{2}$$

Note that the gating variable still obeys first order kinetics. Changing the H^+ or Ca^{2+} concentration over a range large enough to go from complete lack of binding ($f = 0$) to complete titration ($f = 1$) will give a simple shift of the X_∞ and τ curves along the voltage axis, as is expected in the absence of the fluctuations considered here. However, if the titration is incomplete, the curves will not only be shifted but in addition their shape will be changed by the surface charge agent. A similar change of shape is in general expected if the ionic strength (screening) is changed at a value of $[H^+]$ or $[Ca^{2+}]$ giving incomplete titration of the H^+ or Ca^{2+} binding sites. Furthermore, even if the X_∞ curves for the extreme cases of all sites titrated or no sites titrated, are symmetrical about the half-activation point, the measured activation curve will not be symmetric in this way when measured with the $[H^+]$ or $[Ca^{2+}]$ in the range to partially titrate the sites.

Changes in gating variable curve shape, with changes in pH, pCa, or ionic strength,

have been seen in the squid by Frankenhaeuser and Hodgkin (1957; Fig. 8), Chandler et al. (1965, Fig. 1), and Shoukimas (1978; Figs. 2 and 5), and in the Ranvier node by Hille (1968; Fig. 3, τ_h: small effect at positive potentials), Moore (1971; Figs. 4–6), and Brismar and Frankenhaeuser (1975; Fig. 1). Vogel (1974; Figs. 3 and 4), on the other hand, does not find such a shape change. Some of these effects may be explicable in terms of the mechanism discussed above thus avoiding the need to invoke any more complicated explanation. To give an example of the changes in shape of the gating variable curves predicted by Eqs. 1 and 2 above, in Fig. 1*a* and *b* we show the $\tau_h(V)$ and $h_\infty(V)$ curves expected from the Frankenhaeuser and Huxley (1964) equations for the Ranvier node. We assume that on going from no titration (of e.g. H^+ sites) to complete titration, there is a (pure) shift of these curves by 60 mV. Plotted are the curves expected when various fractions (f) of the sites are titrated on average. Note that on going from no titration to half-titration, there is a significant reduction in the amplitude of the $\tau(V)$ curve. This is especially apparent for changes in the concentration of the surface charge agent near the ends of the dose-response curve for binding, where the change in magnitude of $\tau(V)$ greatly outweighs the shift of the curve. Striking experimental support for this prediction comes from the work of Shoukimas (1978; Fig. 2 B). The similarity between his τ_h curves for $[Ca^{2+}]_0$ = 10 and 100 mM, and the curves for f = 0.9 and 1 in our Fig. 1*b*, is quite remarkable. On the basis of the theory above (for complications see below), we would predict that lowering $[Ca^{2+}]_0$ to (say) <0.5 mM, would change the τ_h curve to the shape shown for low values of f in Fig. 1*b*. Testing experimentally that the amplitude of the τ_h curve increases again as $[Ca^{2+}]_0$ is lowered toward zero would rule out the alternative hypothesis that lowering $[Ca^{2+}]_0$ produces a simple decrease of τ_h by altering the intramembrane structure directly. Ehrenstein and Gilbert (1973) found similar changes in the magnitude of the τ_n curve in squid giant axon, with changes in surface charge.

The $\tau(V)$ curves for different f all cross at one point in Fig. 1*b*, whereas experimental $\tau(V)$ curves in different concentrations of surface charge agents cross at different points (Moore, 1971). Extending the model presented in this paper to the case where each channel is influenced by more than one surface charge binding site (but still a small number, so that the fluctuations are significant), gives $\tau(V)$ curves that cross over at different points. Note that the additional sites can be identical or different to the first one.

There are several reasons why the theory above cannot be applied quantitatively to fit data already in the literature. Firstly, it is uncertain how many surface charge binding sites influence each channel. Secondly, even if it were shown that only one such site affects each channel, so that the theory presented above should be applicable, there is a lack of data in the literature giving both activation curves and time constant curves over a wide range of surface charge agent concentrations. In particular, it is necessary to have such information at extremes of concentration of the agent under study, and in the absence of fluctuations of the surface charge due to other agents present. Studying surface charge effects of H^+, for example, gating variable curves must be obtained both with no Ca^{2+} or H^+ present, and also with no Ca^{2+} present,

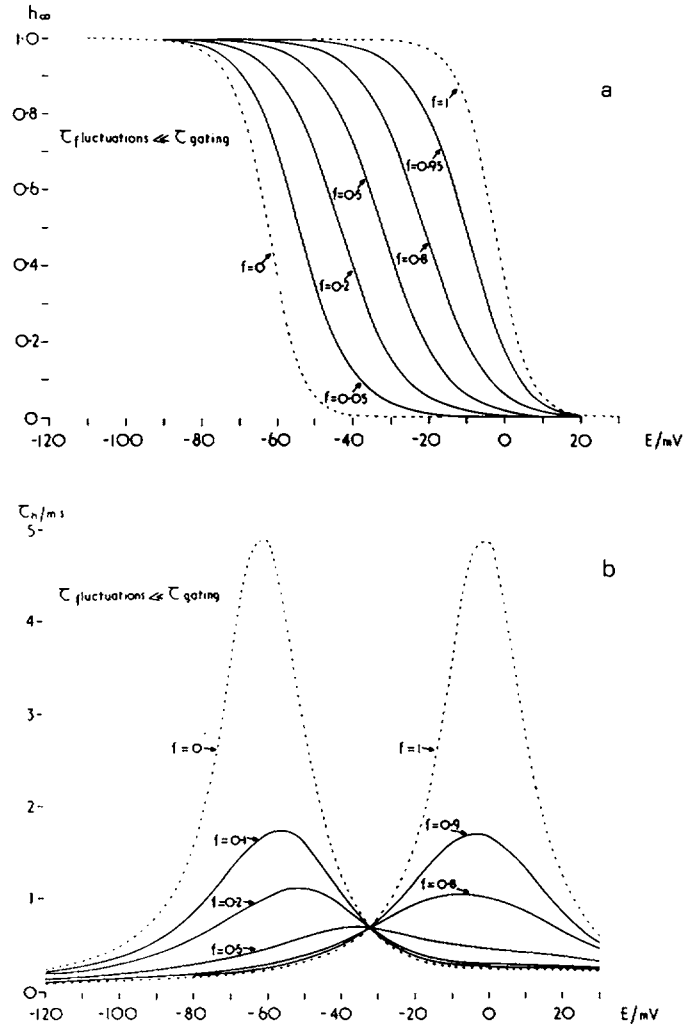

FIGURE 1 (a) Changes in shape of activation curve for a population of ion channels affected by one surface charge binding site, with changes in the mean fraction (f) of the time that site is occupied. Fluctuations in site occupancy assumed to occur on a time scale much faster than the gating time constant. The exact changes in shape depend on the distance apart of the $f = 0$ and $f = 1$ curves, the shape of those curves, and the shape of the $\tau(V)$ curve for $f = 0$ and $f = 1$ (see Eq. 1). Computed from Eq. 1 with $X_{1\infty}$ and τ_1 being the h_∞ and τ_h curves of Frankenhaeuser and Huxley (1964), and $X_{2\infty}$ and τ_2 as the same curves shifted 60 mV positive on the voltage axis. (b) Effects of surface charge fluctuations on time constant curves. As in *a*, but changes in shape of the $\tau(V)$ curves are not affected by the activation curve shape (calculated from Eq. 2, as in *a*).

but a high $[H^+]$. Furthermore even if such manipulations are applied to one side of the membrane, fluctuations in the surface charge on the other side of the membrane prevent the simple application of the quantitative predictions above. It seems, therefore, that this paper can only provide a qualitative explanation of the observed changes in shape of gating variable curves.

Chiu (1977) has drawn attention to the asymmetry of the h_∞ curve in the Ranvier node, about the point $h_\infty = \frac{1}{2}$. Because the physiological $[Ca^{2+}]$ (unlike the $[H^+]$) is apparently not close to one end of the dose-response curve for Ca^{2+} binding (Moore, 1971; Hille, 1968), it is possible that fluctuations in the number of calcium ions bound could contribute to this asymmetry. However, the derivation given above does not lead to the nonexponential kinetics observed by Chiu for the time dependence of h.

It is worth considering whether the first order kinetics, used above to describe the changes in X, are valid on a time scale of the order of 10^{-6} s. Suppose there is a finite time needed for the gating mechanism to change state. Although this time must be much less than the time constant of the gating reaction (so that on the normal time scale the first order equations are appropriate), it might not be negligible on the time scale of the fluctuations discussed above. In this case the derivation we give loses its quantitative predictions but remains valid qualitatively. The derivation above is also quantitatively invalid if the fluctuation frequency is so high that the Debye-Hückel screening layer cannot equilibrate with the changes in surface charge.

Having considered the case where surface charge fluctuations occur much more quickly than gating changes, we now assume the reverse, i.e., the surface charge is essentially fixed but is different around different channels. This may be a reasonable approximation for experiments in which large charged molecules are incorporated into the membrane as a surface charge agent (if these molecules enter and leave the membrane only slowly). The same considerations apply if, in the absence of externally added surface charge agents, different channels in a membrane experience different local surface charges. Again for simplicity we assume that there is one binding site near each channel, which is either occupied or vacant. Neglecting differences in the instantaneous I-V relationships of the channels experiencing different surface charges, the fraction of gates open in the steady state is given by

$$X_\infty = (1 - f)X_{1\infty} + fX_{2\infty} \tag{3}$$

(where again $X_{1\infty}$ and $X_{2\infty}$ are the steady-state activation or inactivation curves for channels in the absence or presence, respectively, of the surface charge agent). In Fig. 2 we plot this curve using the Hodgkin-Huxley (1952) equations for $h_\infty(V)$. We assume a (simple) shift of 30 mV for the change between no titration and complete titration of the sites, and show the predicted $h_\infty(V)$ curve for when a fraction f = 0.2, 0.5, or 0.8 of the sites is titrated (on average).

The $X_\infty(V)$ curve will again in general be asymmetric about the point $X_\infty = \frac{1}{2}$, if the $X_{1\infty}(V)$ and $X_{2\infty}(V)$ curves are symmetric. Furthermore this model will give biexponential kinetics for the time-course of X, reflecting the two pools of channels with different time constants. Chiu (1977) found biexponential kinetics for the decay of h

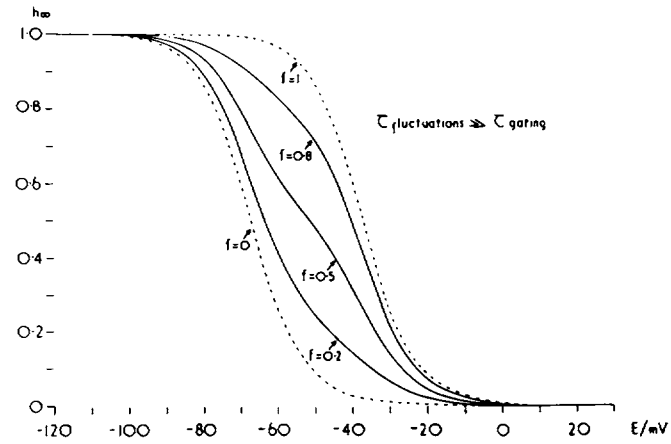

FIGURE 2 Effects of nonhomogeneity of surface charge, due to the time scale of surface charge fluctuations being much slower than the gating time constant, on the apparent h_∞ curve in squid giant axon. Computed from Eq. 3 using the Hodgkin-Huxley equations for h_∞ to describe $X_{1\infty}$, and the same curve shifted 30 mV positive on the voltage axis to describe $X_{2\infty}$.

in the node of Ranvier, and attributed this to a gating mechanism governed by second order kinetics. However, Chiu's results cannot be simply reinterpreted in terms of nonhomogeneity of the surface charge distribution, because the initial delay (or sigmoid onset) that Chiu observed at the start of removal of inactivation is not explained by our model. During a clamp pulse, if X is initially zero, we have

$$X = (1 - f)X_{1\infty}(1 - e^{-t/\tau_1}) + fX_{2\infty}(1 - e^{-t/\tau_2})$$

(again ignoring differences in the instantaneous I-V relationship with different surface charges). Thus we predict that d^2X/dt^2 should always be negative, whereas in Fig. 2 of Chiu (1977) d^2h/dt^2 is positive.

In the past, changes in the shapes of gating variable-voltage curves seen on changing the surface potential have rarely received comment. If the fluctuations or nonhomogeneity of surface charge discussed here are ignored, the conventional theory cannot explain anything other than a pure shift of these curves. On that theoretical basis one might then try to explain the shape changes, in terms of surface charge agents like Ca^{2+} and H^+ changing the structure or energy levels of the gating mechanism, by penetrating into the membrane. The mechanism discussed above, in which agents like Ca^{2+} and H^+ need only alter the surface potential, provides a much simpler explanation of these phenomena. Furthermore, if the discrete nature of the surface charge distribution is important, then the phenomena discussed in this paper must have relevance to all experiments in which the surface charge is altered. However the effects of fluctuations in H^+ or Ca^{2+} binding to parts of the gating mechanism exposed to the solutions outside the membrane (Rojas and Rudy, 1976) are also worth considering.

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REFERENCES

BRISMAR, T., and B. FRANKENHAEUSER. 1975. Effects of ionic concentration on sodium permeability properties of myelinated nerve fibers of *Xenopus laevis*. *J. Physiol. (Lond.).* **249**:549–559.

BROWN, R. H. 1974. Membrane surface charge: discrete and uniform modelling. *Prog. Biophys. Molec. Biol.* **28**:343–370.

CHANDLER, W. K., A. L. HODGKIN, and H. MEVES. 1965. The effect of changing the internal solution on sodium inactivation and related phenomena in giant axons. *J. Physiol. (Lond.).* **180**:821–836.

CHIU, S. Y. 1977. Inactivation of sodium channels: second order kinetics in myelinated curve. *J. Physiol. (Lond.).* **273**:573–596.

COLE, K. S. 1969. Zeta potential and discrete vs. uniform surface charges. *Biophys. J.* **9**:465–469.

COTMAN, C. W., and W. B. LEVY. 1975. Membranes in nerve impulse conduction. *In* Biochemistry of Cell Walls and Membranes. MTP International Reviews of Science. Biochemistry Series 1, vol. 2. C. F. Fox, editor. Butterworths, London. 187–206.

EHRENSTEIN, G., and D. L. GILBERT. 1973. Evidence for membrane surface charge from measurement of potassium kinetics as a function of external divalent cation concentration. *Biophys. J.* **13**:495–497.

EIGEN, M. 1963. Fast elementary steps in chemical reaction mechanisms. *Pure Appl. Chem.* **6**:97–115.

EIGEN, M., and L. DE MAEYER. 1963. Relaxation methods. *In* Technique of Organic Chemistry. Vol. VIII. A. Weissberger, editor. John Wiley & Sons, Inc., New York. 895–1054.

FRANKENHAEUSER, B., and A. L. HODGKIN. 1957. The action of calcium on the electrical properties of squid axons. *J. Physiol. (Lond.).* **137**:218–244.

FRANKENHAEUSER, B., and A. F. HUXLEY. 1964. The action potential in the myelinated nerve fibre of *Xenopus laevis* as computed on the basis of voltage clamp data. *J. Physiol. (Lond.).* **171**:302–315.

GILBERT, D. L., and G. EHRENSTEIN. 1969. Effect of divalent cations on potassium conductance of squid axons: determination of surface charge. *Biophys. J.* **9**:447.

HAGUE, D. N. 1971. Fast Reactions. John Wiley & Sons, Inc., New York.

HILLE, B. 1968. Charges and potentials at the nerve surface. Divalent ions and pH. *J. Gen. Physiol.* **51**: 221–236.

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.

MOORE, L. E. 1971. Effects of temperature and calcium ions on rate constants of myelinated nerve. *Am. J. Physiol.* **221**:131–137.

ROJAS, E., and B. RUDY. 1976. Destruction of the sodium conductance inactivation by a specific protease in perfused nerve fibres from *Loligo*. *J. Physiol. (Lond.).* **262**:501–531.

SHOUKIMAS, J. J. 1978. Effect of calcium upon sodium inactivation in the giant axon of *Loligo pealei*. *J. Memb. Biol.* **38**:271–289.

VOGEL, W. 1974. Calcium and lanthanum effects at the nodal membrane. *Pflügers Arch. Eur. J. Physiol.* **350**:25–39.