Proc. Natl. Acad. Sci. USA
Vol. 73, No. 12, pp. 4492–4496, December 1976
Biophysics

Physical-chemical approach to the transient change in Na ion conductivity of excitable membranes

(voltage clamp/kinetic analysis/nerve excitation/acetylcholine receptor)

PHILIP K. RAWLINGS AND EBERHARD NEUMANN*

Max-Planck-Institut für Biochemie, D-8033 Martinsried bei München, West Germany

Communicated by David Nachmansohn, August 23, 1976

ABSTRACT **A new method is proposed for analyzing the rapid transient current component (Na ions) in voltage clamp experiments on excitable membranes. The method is based on only two very general assumptions: the Na ion conductivity of an excitable membrane is determined by some general membrane parameter, the kinetic behavior of which is consistently described by the sum of only two simple exponential terms. A least square computer analysis for the data by L. Goldman and C. L. Schauf on *Myxicola* axons is described [(1973) *J. Gen. Physiol.* 61, 361–384]. The method gives (as a result) the relationship between conductivity and membrane parameter. A physically plausible, chemical model (cycle of three states) is proposed for a dissipative control of the Na ion conductivity. The rate constants for the specific model are calculated from kinetic parameters derived only from the general analysis. These rate constants reproduce the original voltage clamp data in *every* feature which includes peak current ratios (h_∞)-shift with test potential. By allowing for differences in the experimental conditions, we derive essentially the same rate constants for the voltage clamp data of A. L. Hodgkin and A. F. Huxley on squid giant axons.**

An essential characteristic of nerve excitation is a rapid transient current component which is usually carried by sodium ions across excitable membranes, probably through localized permeation zones (1). These permeation sites are dynamic membrane pathways (or flexible channels). Under resting conditions with a large (negative) membrane potential (potential difference across the membrane; outside potential zero), the pathways are practically closed, but they open for a short period if the membrane is depolarized. This opening and closing cycle of membrane permeation sites is the most important feature of the action potential.

General Voltage Clamp Analysis. Basic physical information of nerve excitation is derived from voltage clamp experiments (1–3) which yield electric current (across the membrane) as a function of time at constant voltage. The driving force causing, e.g., Na ions to flow passively across the membrane during the short time of a voltage clamp experiment is essentially constant. Therefore, this current contribution is directly proportional to the membrane's Na ion conductivity or permeability. Physically and mathematically, the least complicated description is to associate the membrane conductivity, g_{Na}, with a single membrane parameter, ρ, in some (unknown) function $g_{Na} = f(\rho)$ that is affected by membrane and environment properties including membrane potential, concentration of various ions in the experimental bath solution, etc., but is independent of time. With very limited information available about the nature of the control system, the simplest possible equation necessary to describe the time dependence of ρ is the solution of a second order differential equation for ρ as a function of time, t:

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

The τ's are the normal mode time constants, and are functions only of the clamping conditions (notably ion concentrations and membrane potential). The β's are constant coefficients determined by the conditions at the start of the voltage clamp experiment and also by the previous history of the membrane.

A set of 10 normalized conductivity (equivalent to normalized membrane permeability) versus time curves are plotted in Fig. 1. These curves have been calculated from data given by Goldman and Schauf in terms of Hodgkin-Huxley parameters (4). As such, these curves are certainly good approximations to the actual experimental data which are not published in the necessary details. The curves in Fig. 1 show short lag times. Eq. 1 has no lag time and we conclude that ρ represents an element in some higher order structure in the membrane. Because g_{Na} first increases and then decreases, there are thus two different times for which the membrane permeability parameter has the same value. Also, ρ must reach its maximum value at exactly the same time that the membrane conductivity has a maximum. Based on this correlation of $g_{Na}(t)$ and $\rho(t)$, a special computer program was developed for obtaining the normal mode time constants τ_1 and τ_2 and the amplitude ratio β_2/β_1 which describe each of the given conductivity curves with Eq. 1. With these three constants, it is possible to *experimentally* obtain the relationship between membrane conductivity g_{Na} and ρ by comparing $\rho(t)$ with the measured $g_{Na}(t)$ at the same time. It was found that the Hodgkin and Huxley parameters used for the Goldman and Schauf data can be *exactly* described by Eq. 1. Only the curves for −23 mV and −13 mV showed slight deviations ($\leq 5\%$). The best possible functional relationship between Na conductivity and the membrane parameter was *found* to be the proportionality:

$$g_{Na}(t) \propto [\rho(t)]^3. \qquad [2]$$

Only for the two cases of −23 mV and −13 mV is this relationship a more complicated cubic polynomial. However, to a good approximation, the relationship [2] can be used for *all* the curves shown in Fig. 1. Because our analysis is very sensitive to curve shape, the very original data of Goldman and Schauf would probably produce a somewhat different relationship than Eq. 2. The simple cubic dependence is mainly caused by the data being presented by Goldman and Schauf in terms of Hodgkin and Huxley parameters.

It must be emphasized that the results so far, the τ's, β_2/β_1, and the g_{Na}–ρ relationship have been obtained with an absolute minimum number of assumptions. Any more specific description of the permeability parameter ρ requires chemical information about the control system which is beyond the scope of voltage clamp measurements.

Physical-Chemical Model. Details of the chemical (membrane) reactions which control the operation of the permeation pathways in excitable membranes are not known. The large heat changes accompanying the opening-closing cycle (5)

* To whom correspondence should be addressed.

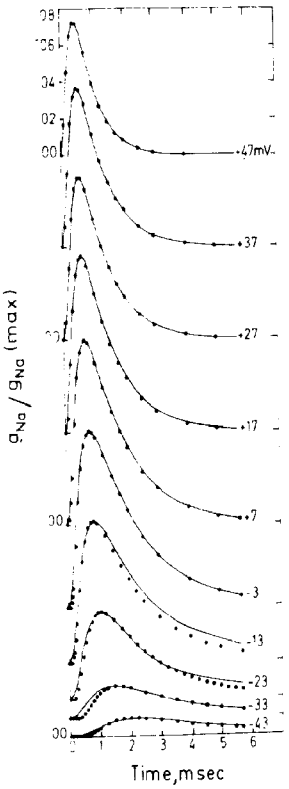

FIG. 1. Normalized Na ion conductivity versus time for a *Myxicola* axon. Solid lines, Goldman and Schauf data calculated from Hodgkin and Huxley parameters published in ref. 3; ●, calculated from our physical chemical model. Initial membrane potential is −115 mV and final membrane potential is labeled. Maximum peak conductivity at +7 mV. Same scale for all curves. For −43 mV and (+47 mV): $\beta_2/\beta_1 = C_2/C_1 = -0.930$ (−0.960); $\tau_1^{-1} = 0.129$ msec^{-1} (0.529 msec^{-1}); $\tau_2^{-1} = 0.995$ msec^{-1} (5.848 msec^{-1}).

suggest that some dissipative chemical process is responsible for the permeability changes (6–8). Probably a control system functioning under conditions which are far removed from chemical equilibrium accounts for the observed electrical conductivity changes. By considering the present stage of knowledge on chemical composition and functional organization of excitable membranes, we can justify only a minimum parameter model for the electrical excitation process. Any detailed model for the permeability changes should be specified in a form that allows the individual aspects of the mechanism to be independently determined by other techniques. Recent voltage clamp experiments at synaptic parts of excitable membranes show that the time and the voltage (V) dependence of the ion conductivity $g(t, V)$ of frog neuromuscular end plates are similar to $g_{Na}(t, V)$ of squid giant axons (9). Together with other arguments (6–8), it seems possible that the molecular mechanisms for the control systems are very similar in both cases. We therefore use a control principle that has features in common with the proposal of Nachmansohn (6, 7) now widely applied to synaptic membrane reactions.

One fundamental characteristic of this control model is the association of a small neuro-activator molecule (A) with receptor macromolecules (R) embedded in the excitable membrane:

$$A + R \rightleftharpoons AR \rightleftharpoons AR'. \qquad [3]$$

The permeability change (Na activation) is caused by the structural transition of the receptor complex AR (closed state) of the pathway to a configuration AR' corresponding to a high permeability arrangement (open state). The temporal limitation of the permeability change is accounted for by a removal mechanism for A, for example, accomplished by a practically irreversible decomposition catalyzed by an enzyme. When the activator removal process is coupled to the reaction $AR' \rightleftharpoons A + R'$, the formation of AR' by the direct association of activator and receptor is suppressed, and the pathway does not reopen by this step. Thus, the opening-closing cycles of the pathway are controlled by the flow of activator through the control system. This flow is small under normal steady state (resting) conditions, but is strongly increased during excitation. It is evident from the millisecond time range in which the conductivity changes occur, that the conformational changes $AR \rightleftharpoons AR'$, as well as the removal of A, must be at least as rapid as the electrical changes. Because the measured conductances depend on the membrane potential, at least one of the reactions involved in the permeability change must depend on the electric field across the membrane.

For reasons of efficiency, it seems unlikely that the removal mechanism for A is directly competing with the R form of receptors for the activator molecules. Thus, the reaction space where the receptor–activator association occurs is assumed to be separated in some manner from the region where the removal reaction for A is active.

To summarize, our model basically assumes that *some* kind of correlation exists between the measured conductivity changes $g_{Na}(t, V)$ and the concentration of activator–receptor $[AR']$:

$$g_{Na}(t,V) = F([AR']) \qquad [4]$$

based on the cyclic three-state reaction system:

$$A_1 + R \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} AR \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} AR' \underset{k_{13}}{\overset{k_{31}}{\rightleftharpoons}} R + A_2. \qquad [5]$$

In Eq. 5, R represents unbound receptor macromolecules, AR and AR' symbolize, respectively, the receptor–activator complex in the low and high permeability configurations, while A_1 and A_2 stand for activator molecules located in different regions of the membrane. (The rate coefficient k_{31} accounts for the transition $R' \rightarrow R$.) Storage binding sites for the activator molecules are probably located near the receptors (6–8), thereby assuring that activator is (always) available. Because details of activator supply are not known, we assume that the concentration of A_1 is buffered at some constant level. Consequently, k_{12} can be replaced by the apparent rate constant $k'_{12} = k_{12}[A_1]$. If an effective removal mechanism keeps the concentration of A_2 very low, then $k_{13}[A_2]$ can be replaced by $k'_{13} = 0$. Due to mass conservation, the total receptor concentration, $[R_T]$, in Eq. 5 is constant, $[R_T] = [R] + [AR] + [AR']$ with $[R'] \ll [R]$ and only activator molecules are consumed. With the definitions of the fractions of occupied receptors: $f = [AR]/[R_T]$, $f' = [AR']/[R_T]$, and $[R]/[R_T] = 1 - f - f'$, the time course of concentration changes for Eq. 5 is described by the linear differential equations:

$$df/dt = -(k_{21} + k'_{12} + k_{23})f + (k_{32} - k'_{12})f' + k'_{12} \qquad [6]$$

$$df'/dt = (k_{23})f - (k_{31} + k_{32})f' \qquad [7]$$

Setting Eqs. 6–7 equal to zero gives the time independent (steady state) fractions, f_{ss} and f'_{ss} of bound receptors. Integration of Eqs. 6–7 yields:

$$f(t) = B_1 \cdot \exp(\lambda_1 t) + B_2 \cdot \exp(\lambda_2 t) + f_{ss} \qquad [8]$$

$$f'(t) = C_1 \cdot \exp(\lambda_1 t) + C_2 \cdot \exp(\lambda_2 t) + f'_{ss} \qquad [9]$$

B_1, B_2, C_1, and C_2 are constants dependent on the initial conditions, while the normal mode decay constants $\lambda_1 = -1/\tau_1$

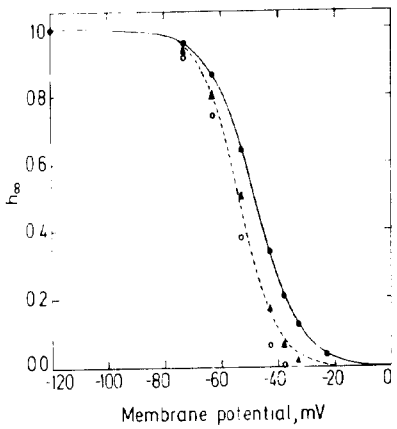

FIG. 2. Peak current ratio, h_∞, versus conditioning membrane potential, calculated by applying our physical chemical model to Goldman and Schauf data, at three different test potentials: +17 mV (●), −23 mV (▲), and −33 mV (○). Voltage clamp at the holding potential for ≃ 80 msec, next by a clamp to the conditioning potential for a period of time (conditioning time), then by a change to a new potential (test potential) yields the ratio, h, of peak current with conditioning step and peak current without conditioning step.

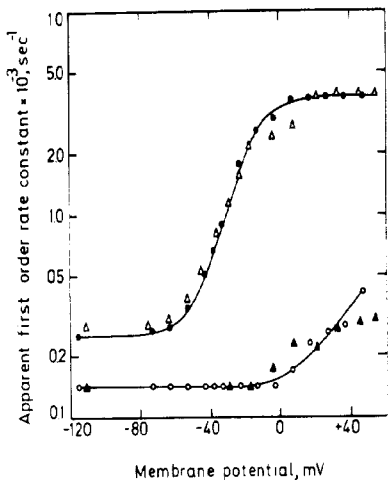

FIG. 3. Semilogarithmic plot of the apparent rate constants k_{23} (upper curve) and k_{31} (lower curve) for our physical chemical model; (○, ●) determined from Goldman and Schauf data (3); (△, ▲) calculated from Hodgkin and Huxley data (1). For scaling, k_{31} from Goldman and Schauf data is multiplied by 1.316.

and $\lambda_2 = -1/\tau_2$ are given by the roots of the quadratic polynomial:

$$\lambda^2 + (k'_{12} + k_{21} + k_{23} + k_{32} + k_{31})\lambda + (k'_{12} + k_{21} + k_{23})\cdot(k_{31} + k_{32}) - k_{23}(k_{32} - k'_{12}) = 0. \quad [10]$$

The time to reach the maximum value of f' is

$$t_{max} = (\lambda_1 - \lambda_2)^{-1}\cdot\ln(-C_2\lambda_2/C_1\lambda_1). \quad [11]$$

RESULTS

Determination of the Rate Constants. The physical model developed in the previous section is a chemical specification of the general analysis discussed earlier. Consequently, all the information derived from the general analysis of voltage clamp experiments *must* also apply to this specific physical-chemical model. For instance, the comparison of Eq. 1 with Eq. 9, establishes the relationship:

$$\rho(t) \propto [f'(t) - f'_{ss}]. \quad [12]$$

It is the *difference* $|f'(t) - f'_{ss}|$, rather than the quantity $f'(t)$ itself, that has the same kinetic behavior as the general membrane parameter introduced in the first section. Thus, Eq. **12** is the *result* of a general analysis and is not itself a new assumption. Also, Eq. **10** shows that the τ's previously obtained from voltage clamp experiments are functions solely of the apparent rate constants of the chemical model. At present, the rate constants can be determined from the normal modes alone, only if a few assumptions are introduced: the rate constants in Eq. **5** are independent of membrane potential, unless it is absolutely necessary to introduce a potential dependence. By using the identity $k'_{13} = 0$, we account for the experimental fact that $g_{Na}(t)$ increases only when the membrane potential is made more positive (depolarization) and not when it is made more negative (hyperpolarization). In a hypothetical equilibrium situation (i.e., no removal mechanism for activator), the intrinsic rate constants for formation of either AR or AR' directly from activator and receptor molecules are equal (probably diffusion controlled). By applying the principle of detailed balance to the receptor reactions, we imply that $k_{21} = k_{23}\cdot k_{31}\cdot(k_{32})^{-1}$. The apparent rate constant k'_{12} determines the amount of material that "moves around the cycle." Due to the relative smallness of k'_{12}, this constant doesn't affect the ability of the model to fit the τ's. Therefore, k'_{12} was set to a "reasonable" value, so that $f'(t_{max})$ is approximately 0.384, and then held constant. The uncertainty in the value of $f'(t_{max})$ is present because there is as yet no method of experimentally determining the constant of proportionality in Eq. **9**. Within the framework of the above assumptions, the rate constants k_{23}, k_{32}, and k_{31} are uniquely determined from the τ's obtained from voltage clamp experiments.

In the potential range between −115 mV and −43 mV, there is no measurable current response in *Myxicola* giant axons. In this range, the value of k_{23} was estimated from conditioning experiments reported by Goldman and Schauf; see also Fig. 2. The resulting peak current ratios, h, are equal to the ionic permeability ratios. For very long conditioning times, $h = h_\infty$ (see ref. 1). The solid curve in Fig. 2 shows h_∞ versus conditioning membrane potential, providing information about the rate constants in the potential region where $h_\infty > 0$. Eqs. **2** and **12** are used to calculate h_∞ from the behavior of the physical chemical model.

The Hodgkin and Huxley equations used by Goldman and Schauf to report their data (Fig. 1) contain assumptions. These assumptions imply slightly different values for the ratio β_2/β_1 than would be found for the mechanism proposed in the earlier section and small distortions in the τ's. The time constant that is most affected is the one associated with the rapid increase in Na permeability. Therefore, this normal mode time constant may be replaced in the rate constant determination by the experimental quantity, t_{max}, which is the time required to reach the maximum Na conductivity. The values of k_{23} and k_{31} obtained in this way from the Goldman and Schauf data are plotted in Fig. 3. In summary, the apparent rate constants in Fig. 3 reflect one normal mode time constant and t_{max} in the potential region where these quantities are experimentally available, and they reflect the value of h_∞ (with a test potential of + 17 mV) in the potential region where the normal mode time constants cannot be measured. The rate constants reproduce these experimental quantities with an average error of about 3%. It was found that $k_{32} = 1058\ sec^{-1}$, independent of membrane potential, and k'_{12} is $56.77\ sec^{-1}$. If one assumes for the data of a squid giant axon (1) the same value for k'_{12} (= $56.77\ sec^{-1}$), one obtains $k_{32} = 704.8\ sec^{-1}$ and $f'(max) = 0.267$.

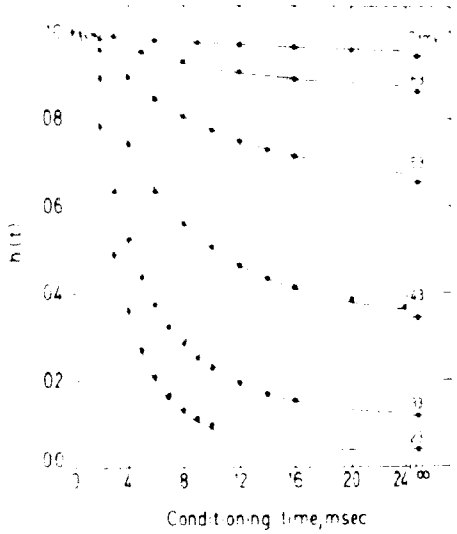

FIG. 4. Peak current ratio, $h(t)$, as a function of conditioning time at the indicated conditioning potential. The lag times vary from 1 msec (−73 mV) to 0.5 msec (−23 mV). Test potential, +17 mV.

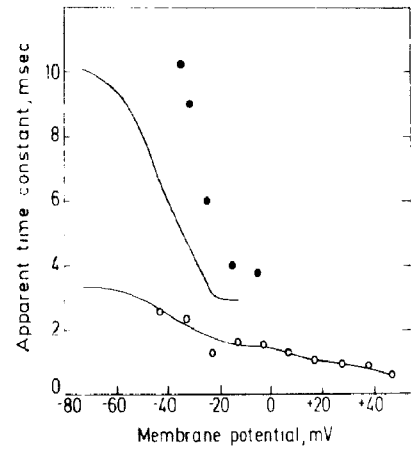

FIG. 5. "Apparent decay time constants" τ_h (lower curve) determined from curves in Fig. 1 and τ_c (upper curve) measured by conditioning experiments as simulated in Fig. 4, by using the physical chemical model applied to Goldman and Schauf data; (○, ●) experimental values from Goldman and Schauf.

Calculation of Conductance Changes. Fig. 1 shows a comparison between the (reproduced) curves of Goldman and Schauf and the conductivity changes produced by the physical chemical model with the apparent rate constants derived from the general analysis of voltage clamp experiments. The constants of proportionality in Eq. **12** are obtained at t_{max}, where both the experimental and calculated conductivities are equal. The most pronounced deviation from an otherwise good agreement is the longer lag times that appear for the smaller membrane potentials. In ref. 3 (Fig. 9), there is some suggestion of *actually* longer lag times than those resulting from the Hodgkin and Huxley parameterized Goldman and Schauf data.

Calculation of the h_∞-Parameter. In the Hodgkin and Huxley model, the parameter h is associated with the closing of membrane pathways (Na-inactivation), the time independent value being h_∞. Experiments proposed by Hodgkin and Huxley to measure h_∞ were simulated with the rate constants in Fig. 3. The results are shown in Fig. 2. The h_∞ values for a test pulse potential of +17 mV were used to infer the value of k_{23}. For the other test pulse potentials, the h_∞ curves are shifted in a manner similar to that found experimentally by Goldman and Schauf; (see Fig. 6 in ref. 10). The shape of the calculated h_∞ curve changes slightly as a function of the test potential, as observed experimentally. This shift is an *intrinsic* feature of our physical chemical model.

Simulation of Conditioning Experiments. Fig. 4 shows the results of a number of conditioning experiments simulated with our chemical model. The solid curves are calculated for an exponential process with a short delay (the curves do not extrapolate back to one at zero time) as found experimentally (10). The decay constant, τ_c, for the curves in Fig. 4 are plotted in Fig. 5. The values of τ_c from the experiments of Goldman and Schauf are greater than the τ_c's calculated from our model, but, they both have a very similar shape (see Fig. 1 in ref. 10). However, as was found experimentally by Goldman and Schauf are greater than the τ_c's calculated from our model, but, constant τ_h obtained from the declining portions of the curves in Fig. 1.

Conditioning experiments similar to those in Fig. 4, only with a membrane holding potential near 0 mV, have been reported by Schauf (11). The lag times were found to be much longer than those shown in Fig. 4, but for long times the results could also be described by an exponential function.

DISCUSSION

The general analysis proposed in the first section involves reasonable physical assumptions to reproduce the voltage clamp data, without being specific about details of the mechanism actually involved. The analysis of voltage clamp experiments in this manner *produces* several important results: the normal mode time constants τ_1 and τ_2, the amplitude ratio β_2/β_1, and the relationship between g_{Na} and ρ. It is these fundamental quantities, rather than only the voltage clamp curves themselves, that must be covered by any molecular model. Consequently, it is not advisable to *begin* the analysis of voltage clamp data with a particular molecular model.

There is a large number of schemes in the literature which attempt to describe the Na ion behavior of excitable membranes under voltage clamp conditions (e.g., 1, 10–16). So far every specific reaction scheme reported involves restrictive assumptions which could be replaced by others and are often made *before* the actual experimental data are analyzed. It is thus difficult to decide objectively why some models reproduce the results of experiments better than others.

The receptor–activator model (6–8) specified in Eq. **5** is not unique, but it is able to reproduce every feature observed by Goldman and Schauf in their extensive voltage clamp experiments and is in good quantitative agreement with almost all details. Furthermore, the application of our model to Hodgkin and Huxley data produces values for the kinetic coefficients which are comparable to those derived from Goldman and Schauf data. The potential dependence of the most important rate constant, k_{23}, is practically identical for both. The rather small differences in the other rate constants may be attributed to environmental factors such as the concentrations of various ions near the membranes, etc. Because both sets of data come from related species, the close similarity in the rate constants for the control system is not surprising. Goldman and Schauf present an extensive amount of evidence that suggests that the conductivity change is controlled by a single membrane parameter, as we have utilized in our model. With two *independent* parameters, the Hodgkin and Huxley model cannot describe some of the experiments by Goldman and Schauf (10, 11, 16).

The behavior of the membrane parameter ρ used in the general analysis reflects a process that is occurring at the lowest level of organization in the control system. Because the conductivity is found to be not directly proportional to ρ [see Eq.

2], there must be some structural organization at a higher level, a kind of basic excitation unit, which is *directly* responsible for the membrane conductivity (8). A detailed description of such a higher level structure, of course, depends on the physical quantity represented by ρ. We have associated ρ with a particular receptor–activator configuration AR'. Using Eqs. **2** and **12**, the dependence of membrane conductivity on the concentration of the high permeability configuration, f', can be calculated from the rate constants of the physical-chemical model. We *find that this functional dependence is strongly influenced by membrane potential.*

The sigmoidal dependence of k_{23} on membrane potential (Fig. 3) is certainly not produced by a simple dipole change or polarizability effect by itself. Some more complicated process must necessarily be present. One (*ad hoc*) possibility is that the closed state AR in Eq. **5** is replaced by the (very rapid) equilibrium $AR_S \rightleftharpoons AR_f$, being dependent on the membrane potential. If k_F and k_S are the rate constants for the transformation of AR_F and AR_S into AR', respectively, then k_{23} is replaced by $(k_F [AR_F] + k_S [AR_S])/([AR_F] + [AR_S])$. With $k_F \gg k_S$, the potential dependence of this expression is sigmoidal when a simple dipole mechanism controls the equilibrium between AR_S and AR_F.

It is known that the concentration of some ions, e.g., Ca^{2+}, strongly influences the voltage clamp data. A physical explanation of the Ca effects may be based on competition of Ca and activator molecules for the receptor binding sites (17). For instance, since k_{31} involves the breakdown of the receptor–activator complex, differences in the concentrations of ions competing with the activator would alter the magnitude of this rate constant, but would not affect its potential dependence. Direct comparison between the Hodgkin and Huxley and Goldman and Schauf data is difficult. The concentrations of Ca and Mg ions are about 10% higher for the axons used by Hodgkin and Huxley than for Goldman and Schauf's *Myxicola* axons. In addition, the bath solution used by Hodgkin and Huxley was 0.48 M in choline, and this ion may compete with activator molecules for receptor binding sites.

From the above discussion, it is apparent that the minimum parameter model and methods proposed in this study are powerful tools for analyzing voltage clamp experiments. However, a new approach that remains only a formalistic treatment of the data is not of much practical significance. Only a model whose assumptions and results may be checked by independent experiments offers a worthwhile advantage. For this reason we have stressed the properties of a receptor–activator reaction. In some excitable membranes, particularly of cholinergic synapses, components of a permeability control system can be identified (18). Thus, data of isolated receptor proteins may be compared with the properties calculated from our model. Some data already available are suggestive. For acetylcholine receptors, e.g., from *Torpedo* fish, a binding constant for the activator molecule acetylcholine is about 1 μM^{-1} (see e.g., ref. 18). If we assume a diffusion controlled rate constant k_{12}, then the voltage clamp data of Goldman and Schauf and Hodgkin and Huxley suggest an activator binding constant that is also about 1 μM^{-1}. Furthermore, the activator's steady-state concentration is then approximately 1 μM. Concentrations of acetylcholine in this range have been found to affect the permeability of some excitable membranes. Recent kinetic data show that the association of acetylcholine with isolated receptors is indeed close to diffusion controlled (17).

In view of the various similarities between synaptic and axonal parts of excitable membranes, it was suggested by Nachmansohn that the acetylcholine cycle is the general control system of bioelectricity (6, 7). This proposal is at present the only one that is *chemically specific* and thus suggestive for independent experimental investigations.

Another characteristic of nerve excitation is a slower contribution to the ion currents, which is usually carried by K ions. An analysis of voltage clamp data of the K component in a manner similar to the method described here, together with a more detailed representation of the analysis of the rapid (Na ion) control system, will be submitted to the *Journal of Membrane Biology*.

We thank Prof. Manfred Eigen, Göttingen, for helpful discussions and Dr. v. d. Malsburg, Göttingen, for his critical comments. The Alexander-von-Humboldt fellowship to P.K.R. is gratefully acknowledged. We also thank the Stiftung Volkswagenwerk and the Sloan Foundation for financial support.

1. Hodgkin, A. L. & Huxley, A. F. (1952) *J. Physiol. (London)* **117**, 500–544.
2. Hodgkin, A. L. & Huxley A. F. (1952) *J. Physiol. (London)* **116**, 497–506.
3. Cole, K. S. (1968) *Membranes, Ions, and Impulses*, ed. Tobias, C. A. (University of California Press, Berkeley, Calif).
4. Goldman, L. & Schauf, C. L. (1973) *J. Gen. Physiol.* **61**, 361–384.
5. Abbott, B. C., Hill, A. V. & Howarth, J. V. (1958) *Proc. R. Soc. London Ser. B* **148**, 149–187.
6. Nachmansohn, D. (1955) in *Harvey Lectures 1953/54* (Academic Press, New York), pp. 57–99.
7. Nachmansohn, D. (1959) and Nachmansohn, D. & Neumann, E. (1975) *Chemical and Molecular Basis of Nerve Activity* (Revised) (Academic Press, New York).
8. Neumann, E. (1974) in *Biochemistry of Sensory Function*, ed. Jaenicke, L. (Springer-Verlag, Berlin), pp. 456–514.
9. Magleby, K. L. & Stevens, C. F. (1972) *J. Physiol. (London)* **223**, 151–197.
10. Goldman L. & Schauf, C. L. (1972) *J. Gen. Physiol.* **59**, 659–675.
11. Schauf, C. L. (1974) *Biophys. J.* **14**, 151–154.
12. Dubois, D. M. & Schoffeniels, E. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2858–2862.
13. Gillespie, C. J. (1973) *J. Theor. Biol.* **40**, 409–428.
14. Moore, J. W. & Cox, E. B. (1976) *Biophys. J.* **16**, 171–192.
15. Jakobsson, E. (1976) *Biophys. J.* **16**, 291–301.
16. Goldman, L. (1975) *Biophys. J.* **15**, 119–136.
17. Neumann, E. & Chang, H. W. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3994–3998.
18. Changeux, J.-P. (1975) in *Handbook of Psychopharmacology*, eds. Iverson L. L., Iverson, S. D. & Snyder, S. H. (Plenum Publishing Corp., New York), Vol. 6, pp. 235–301.