DIFFERENTIATION OF CHANNEL MODELS BY NOISE ANALYSIS

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ABSTRACT Differentiation of membrane channel models based on fluctuation (or noise) analysis is discussed. The theory is particularly useful in distinguishing a single-conductance model (Hodgkin-Huxley formalism) from a multiconductance model. When applied to the frog node of Ranvier, it seems likely that the potassium channels of the membrane may have multiconductance states.

The original Hodgkin-Huxley (1952) formalism for a potassium channel (or gate) in the squid axon membrane can be reinterpreted as applying to a complex system composed of four independent subunits each of which can exist in one of two states, *i* and *ii* (Hill and Chen, 1971*a*, *b*). The channel is open to K^+ only when all four subunits are in state *ii*. Thus an HH K^+ channel is in fact a *multistate* linear kinetic system with a *single* conducting state. But it is somewhat unreasonable, physically, to assume that a K^+ channel is "open" only if all subunits are in state *ii*. Let us suppose instead that if one subunit of the complex is in state *i*, there is an additional steric or electrostatic energy barrier W to passage of a potassium ion, with the result that the conductance of an all-*ii* channel is reduced by a factor $\kappa = e^{-W/kT} < 1$. If two subunits are in state *i*, the simplest and most likely assumption is that the barrier is $2W$ and hence that the conductance reduction factor is κ^2, etc. Thus the single-conductance HH model is extended to a *multiconductance* model. Another simple alternative is that the reduction factors are κ, $\kappa/2$, $\kappa/3, \ldots$, etc. We shall hereafter refer to the former case as a κ-model and the latter a κ'-model. Note, that the original HH model is a special case of these models with $\kappa = 0$. As shown by Chen and Hill (1973), it is always possible to fit the potassium conductance kinetics of the Hodgkin-Huxley voltage-clamp experiment with a κ (or κ') model of arbitrary κ value, if the kinetic parameters of the subunits are adequately adjusted. That is, it is not possible to distinguish between κ values using kinetic data alone. However, since fluctuations are very sensitive to the alteration of kinetic parameters, different models which fit the conductance kinetic data may produce different current (or voltage) noise power spectra. Thus, model differentiation may be possible if the noise analysis is also taken into account. This has been discussed in a recent paper (Chen and Hill, 1973) in which three simple noise functions have been proposed for model testing: the corner frequency ω_c; the low-frequency flat part of the power spectrum G^o; and the total fluctuation G^T. When these functions were calculated using the HH K^+ conductance kinetic equations, they were found to be sensitive to κ values only in the hyperpolarized potential region. Thus, they may not be very useful in practice. In this communication, we introduce a function [$\gamma(V)$ in Eq. 6] that is very sensitive to models in the depolarized region. It is also directly related to a noise function [$E(V)$ in Eq. 7] that can be measured experimentally and is therefore more useful in model testing than the corner frequency, etc.

We shall confine our discussion exclusively to the potassium channel case. The analysis can be generalized easily to other linear kinetic systems in which the measured quantity may be other than the electrical current. We start with a rather general multiconductance model and consider the κ models as special cases.

Let us consider an ensemble of M independent and equivalent systems (one system equals one K^+ channel or gate in the nerve membrane case) each of which can exist in the discrete set of states $0, 1, 2, \ldots, x$ ($x = 4$ in the HH formalism). Let state x represent the only fully open state with a unit conductance $\bar{g}_K$ and let $a_j \bar{g}_K$ ($j = 0, 1, 2, \ldots, x - 1$) be the unit conductance of a system in state j, where $a_j < 1$. Then the total fluctuation of the current across the membrance at voltage V is equal to (see Chen and Hill, 1973)

$$\sigma_I^2 = M[\overline{a^2} - (\bar{a})^2]\bar{g}_K^2(V - V_K)^2, \tag{1}$$

where V_K is the equilibrium potential of the potassium channel,

$$\overline{a^2} = \sum_{j=0}^{x} a_j^2 p_j^s, \tag{2}$$

$$\bar{a} = \sum_{j=0}^{x} a_j p_j^s, \tag{3}$$

and p_j^s is the steady-state probability of a system in state j. Note that the values of the p_j^s depend on the model under consideration. In order to evaluate the p_j^s, one must fit the conductance kinetics of the model with the experimental voltage clamp data (see Chen and Hill, 1973). The mean steady-state potassium current at voltage V can be shown easily to be

$$\bar{I}_K = M\bar{a}\bar{g}_K(V - V_K). \tag{4}$$

Eliminating M from Eq. 1 and 4, we get

$$\bar{g}_K\gamma(V) = E(V), \tag{5}$$

where

$$\gamma(V) = [\overline{a^2} - (\bar{a})^2]/[\bar{a} - (\bar{a})^2], \tag{6}$$

and

$$E(V) = \sigma_I^2/\bar{I}_K(1 - \bar{a})(V - V_K). \tag{7}$$

The $\gamma(V)$ in Eq. 6 is the desired function. As will be shown below, the $E(V)$ in Eq. 7 can be measured experimentally and is therefore model-independent. On the other hand, $\gamma(V)$ is a completely model-dependent quantity. Since $\bar{g}_K$ is a constant, Eq. 5 implies that the functional form of $\gamma(V)$ must be identical to that of $E(V)$. Thus, if the measured $E(V)$ is available, the $\gamma(V)$ in Eq. 6 can be used to differentiate models. After the model is determined, the single-channel conductance $\bar{g}_K$ can be evaluated from Eq. 5 and the total number of channels in the membrane M can be obtained from Eq. 4.

Before studying the properties of $\gamma(V)$, let us first show that $E(V)$ in Eq. 7 can be obtained experimentally. Since the total current fluctuation of the membrane σ_I^2 can be obtained by integrating the current noise power spectrum and the mean potassium current $\bar{I}_K$ can be measured directly, $\bar{a}$ is the only quantity requiring further discussion. Suppose, at maximum deplorization ($V \to \infty$) a system can exist only in state x. Then, since $\bar{a} = 1$, the total steady-state conductance of the membrane, $g_K(\infty)$, is equal to $M\bar{g}_K$. At an arbitrary voltage, $g_K(V) = M\bar{a}\bar{g}_K$. Thus, we have $\bar{a}(V) = g_K(V)/g_K(\infty)$. That is, $\bar{a}$ is equal to the ratio of the steady-state membrane conductance at V to that at maximum depolarization and is thus a measurable quantity.

Now let us examine the properties of $\gamma(V)$. If the systems have just one conducting state (single conductance, or HH model), $a_j = 0$ for $j = 0, 1, 2, \ldots, x - 1$ and $a_x = 1$. Then $\overline{a^2} = \bar{a} = p_x^t = n_x$ [HH n; see Eqs. 2 and 3] and $\gamma(V) \equiv 1$. That is, $\gamma(V)$ is independent of the membrane potential (see Fig. 1). According to Eq. 5, the $E(V)$ function is also independent of V and $E(V) \equiv \bar{g}_K$. Thus, for the single-conductance model, the single-channel conductance ($\bar{g}_K$) can be estimated using Eq. 7 with measured values of σ_I^2, $\bar{I}_K$, and $\bar{a}$. This has been discussed recently by Begenisich and Stevens (1975). If the systems have more than one conducting state (multiconductance model), the $\gamma(V)$ in Eq. 6 will usually be a nonlinear function of V, because $\overline{a^2}$ is, in general, not equal to $\bar{a}$. In fact $\overline{a^2}$ is always less than $\bar{a}$ because $a_j < 1$ for all j except $j = x$ where $a_x = 1$. As a result, $\gamma(V)$ is always less than unity (See Eq. 6) except at maximum depolarization where all the systems are in state x and $\gamma(\infty) = 1$. Thus, the $\gamma(V)$ function seems very sensitive to the a_j values and may be used to differentiate models.

For the purpose of illustration, we chose the κ and κ' models mentioned above for the values of the a_j and calculated the $\gamma(V)$ function of the K$^+$ channels of both the squid axon and the frog node of Ranvier. As indicated before, the kinetic parameters of the model must be adjusted to fit the experimental kinetic data. For squid axon membranes, a simple fitting procedure using the Hodgkin-Huxley empirical formulas has been discussed recently (Chen and Hill, 1973). We have here simply adopted the "adjusted" p_j^t values from that paper and used them in Eqs. 2 and 3. Note, we are actually considering the modified models here, because the last reduction factors $a_0(=\kappa_x$, or $\kappa/x)$ were set to zero values (see Fig. 1 of Chen and Hill, 1973). How-

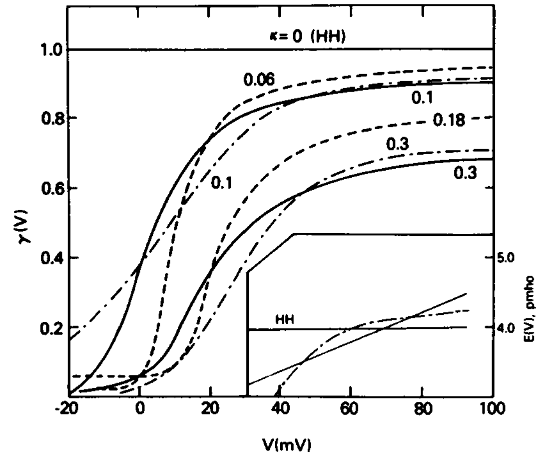

FIGURE 1 $\gamma(V)$ as a function of V for K^+ channels with $x = 4$. $V = 0$ at rest; $V > 0$ for depolarizations. The values of κ are shown in the figure. Solid lines (——): modified κ model for the squid axon. Dashed lines (- - -): modified κ' model for the squid axon. Broken lines (-·-): κ model for the frog node of Ranvier. *Inset:* $E(V)$ functions for the frog node of Ranvier. The vertical scale is indicated at the right edge of the figure. The linearly regressed experimental data of Begenisich and Stevens (1975) is shown by the tilted straight line. The horizontal line is the optimum $E(V)$ of the HH model ($\kappa = 0$) with a $\bar{g}_k$ value of 4 pmho. The optimum $E(V)$ for $\kappa = 0.3$ model is shown as a broken line. The corresponding $\bar{g}_k$ value is 6 pmho. See text for further details.

ever, since the κ values are small, these modified models are not very different from the original κ (or κ') models.

For the frog node of Ranvier, we used the empirical formulas of Dodge (1963) for the K^+ kinetic data and considered the κ model only. In this case, it is not necessary to use the fitting procedure mentioned above. Instead, we used the following equations in calculating the adjusted p_j^s (see Appendix III of Hill and Chen, 1972):

$$p_j^s = x!n^j(1 - n)^{x-j}/j!(x - j)!, \tag{8}$$

$$n = (n_D - \kappa)/(1 - \kappa), \tag{9}$$

where n_D is the probability of a subunit in state *ii* in Dodge's formulas.

Calculated $\gamma(V)$ curves for $x = 4$ are shown in Fig. 1. Note that the potential coordinate in the figure represents the displacement from the rest potential; $V = 0$ at rest; $V > 0$ for depolarizations therefrom. Incidentally, the rest potential is about -65 mV for the squid axon and about -75 mV for the frog node. As shown in Fig. 1, for all nonzero κ values considered, $\gamma(V)$ is very small near and below the rest potential and rises very rapidly upon depolarization. At high depolarizations, $\gamma(V)$ is almost constant and parallel to the horizontal line of the HH model. It is not unexpected that $\gamma(V)$ is very small at resting potential or below since the channels will stay mostly in states with small a-values so that $\overline{a^2}$ is very small compared with $\bar{a}$. The sharp increase of $\gamma(V)$ upon depolarization means that the equilibrium distribution of channel states changes in such a way that a^2 increases faster than $\bar{a}$ (see Eq. 6). At high depolarizations the main channel state is the state x with $a_x = 1$. Thus, the increase of $\overline{a^2}$ is roughly equal to that of $\bar{a}$, which is in turn roughly equal to the change of p_x. This accounts for the level-off behavior of $\gamma(V)$ at high depolarizations. Undoubtedly, the sigmoid shape of $\gamma(V)$ as found in Fig. 1 derives from the sigmoid nature of the potassium channel conductance at steady state. The fact that the $\gamma(V)$ curve for a nonzero κ model is very different from a horizontal line at moderate depolarizations ($V < 20$ mV, for example) suggests that this function can be used to distinguish an HH (single-conductance) model from a κ or κ' (multiconductance) model. Or, if the measured noise data are accurate enough, one may even be able to determine the κ values by comparing the measured $E(V)$ and the calculated $\gamma(V)$. Note, the crucial region for model differentiation is near the rest potential area. Therefore, for practical model testing, it is important to have noise measurements in that region.

We have also calculated the modified κ and κ' models with $x = 6$ for the squid axon case. With same κ values, the $\gamma(V)$ of $x = 6$ are found indistinguishable from those of $x = 4$ and are therefore not shown in Fig. 1. Thus, the present method can not be used to determine the variable x.

Recently Conti et al. (1975), have suggested that the squid axon from *Loligo vulgaris* may have only one K$^+$ conductance state (HH model) based on the $G^p(V)$ data they measured. The present analysis would provide a sensitive check on their conclusion. To do this, one would simply plot the measured $E(V)$ in Eq. 7 as a function of the membrane potential. Any sign of a nonhorizontal line in the plot (particularly near the rest potential) would suggest a multiconductance model. Since the values of $\bar{g}_K$ and M do not enter into the fitting procedure, the present method is therefore more convenient.

In a recent report Begenisich and Stevens (1975) calculated the $E(V)$ of Eq. 7 for the potassium channels of the frog node of Ranvier. These authors stated that $E(V)$ in this case was not significantly dependent on membrane potential over the voltage range from -48 to $+16$ mV (corresponding to depolarizations from $+27$ to $+91$ mV in Fig. 1). Thus they suggested that the K$^+$ channels might possess only one conducting state with a unit conductance of 4 phmo. As shown in Fig. 1, the most sensitive test can be made only in the voltage region from rest to about 20 mV depolarization. It is therefore hard to draw any conclusion without the measured $E(V)$ in this region. In fact, their measured $E(V)$ is not really independent of the membrane potential. Rather, the linearly regressed $E(V)$ has a slope of 0.021 pmho/mV and a zero voltage intercept of 4.1 pmho. This linear line seems already to be adequate for model discrimination. As shown in Eq. 5, for a particular κ value a family of $E(V)$ functions can be calculated from the known $\gamma(V)$ (from kinetic data, see above) by varying the $\bar{g}_k$ value. However, there is only one $E(V)$ among this family that is *least* deviated from the experimental $E(V)$ function. Let us define this $E(V)$ as the *optimum* $E(V)$ of that particular κ value. Then, by comparing the set of optimum $E(V)$ functions with the experimental data, one can determine the value of κ. With Begenisich and Stevens's data the optimum $E(V)$ functions for $\kappa = 0$ (HH) and

$\kappa = 0.3$ were found by setting $\bar{g}_k$ equal to 4 and 6 pmho, respectively. The results are shown in the inset of Fig. 1, in which the experimental $E(V)$ of Begenisich and Stevens is also included. As is clearly shown in the figure, the optimum $E(V)$ of $\kappa = 0.3$ (broken line) fits experimental $E(V)$ better than the optimum HH $E(V)$ (horizontal line). Thus, it seems rather likely that the channels are not of Hodgkin-Huxley type and may have multiconducting states. An extension of $E(V)$ measurements down to the rest potential and a complete analysis of $\gamma(V)$ with actual kinetic data (instead of using Dodge's data) should readily resolve this question.

So far we have considered only the closed ensemble case. That is, the total number of systems in the ensemble is fixed. In some cases, the systems in the ensemble are not fixed because systems may be exchanged with one or more reservoirs (open ensemble). For example, the channels in an artificial lipid bilayer which is in contact with a solution containing channel-forming materials (such as EIM, etc.) form an open ensemble, because the channels (systems) in the membrane can be exchanged with those in the solution (reservoir) by adsorption and desorption processes. The channels in the post-synaptic membranes of neuromuscular junctions may also be considered as an open ensemble, if the channels are formed (activated) only through the adsorption of acetylcholine molecules. The fluctuations and noise of an open ensemble have been discussed in a recent paper (Chen, 1973). Using the analysis in that paper, one can easily show that Eq. 5 is also applicable to the open ensemble case, but with different $\gamma(V)$ and $E(V)$:

$$\gamma(V) = \overline{a^2}/\bar{a}, \tag{10}$$

$$E(V) = \frac{\sigma_I^2}{\bar{I}(V - V_E)} \tag{11}$$

where

$$\bar{a} = \sum_{j=0}^{x} a_j \bar{N}_j^s, \tag{12}$$

$$\overline{a^2} = \sum^{x} a_j^2 \bar{N}_j^s. \tag{13}$$

N_j^s is the steady-state mean number of systems in the ensemble in state j. Note that, as in a closed ensemble, $\gamma(V)$ is equal to unity for single-conductance models and is a nonlinear functional of the membrane potential for multiconductance models. Thus, Eqs. 5, 10, and 11 are useful in model determination and in evaluating the single-channel conductances. The same $E(V)$ as in Eq. 11 has been used to estimate the single-channel conductance of postsynaptic membranes by Colquhoun et al. (1975). As discussed before, this is valid only for single-conductance models. For multi-conductance models, $\bar{g}_K$ must be evaluated from Eq. 5 after the model is determined.

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