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A model of the gating of ion channels

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The gating of ion channels in biological membranes has usually been described in terms of Markov transitions between a few discrete open or closed states. Such models predict that the distributions of open and closed durations decay as a sum of exponential terms. Recent experimental data have indicated that certain channels are not easily described by these models. We show that distributions of open and closed times similar to those seen experimentally are predicted by a model that involves only one open and closed state but that assumes the activation energy of the gating process to be stochastic. This model involves only a few parameters and these have direct physical interpretations. Measurements of the correlation between the durations of successive open or closed events is shown to provide an experimental method for distinguishing between this and other models.

Introduction

Early studies of the gating of ion channels in biological membranes by spectral analysis of fluctuations in macroscopic membrane current fostered Markov models of transitions between a few discrete states [1]. More revealing direct studies of ion channel gating were made possible by the development of patch clamp recording [2]. This technique allows measurement of the gating of a single channel and tabulation of the lengths of time between gating transitions. The distributions of open and closed durations can then be used to calculate the rate constants of gating within an assumed model [3].

Several ion channels have been found recently that are not easily described by simple Markov models [4,5,6,7]. The distributions of open or closed durations for these channels require a sum of many exponential terms for an adequate fit but resemble a simple power law. We propose a model to explain such data that is based on gating between only two states. The energy barrier for the gating transitions is assumed to vary slowly in time and to have a Gaussian distribution. This model is shown to approximate both simple exponential and power law forms of the event duration histograms of ion channels.

Description of the model

In this discussion we will give expressions only for channel open times. Analogous expressions for closed intervals can be obtained by interchanging the subscripts o and c in any of the following equations. In the simplest Markov model, the probability that the channel first closes between times t and $t + \mathrm{d}t$, given that it opened at time zero, is

$$f_o(t)\,\mathrm{d}t = k_c \exp(-k_c t)\,\mathrm{d}t \quad \text{(two-state Markov)} \tag{1}$$

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where k_c is the closing rate constant and $dt \rightarrow 0$. We consider an extension of the two-state Markov model defined by the following assumptions: (1) that rate constants are of the form

$$k_c = A_c \exp(-g(t)) \qquad (2)$$

where A_c is a constant and $g(t)$ is a stochastic variable; (2) that $g(t)$ has a Gaussian distribution with mean zero; and (3) that changes in $g(t)$ are very slow in comparison to the time between gating transitions.

The first of these assumptions is suggested by an Arrhenius interpretation of the gating rate constants, namely $k_c = C_c \exp(-\Delta G_c/RT)$ where C_c is a constant, ΔG_c is the activation energy of the closing reaction, R is the ideal gas constant, and T is the absolute temperature. Formally separating constant and stochastic contributions to ΔG_c with the definition $\Delta G_c/RT = \Delta G_c^\circ/RT + g(t)$ and substituting $A_c = C_c \exp(-\Delta G_c^\circ/RT)$ we obtain Eqn. 2. The use of a single stochastic variable, $g(t)$, for both k_c and k_o makes the implicit assumption that the energies of the closed and open states are fixed and only the energy of a transient transition state is variable. A Gaussian form for $g(t)$ would be expected if variations in activation energy arise from the additive contributions of a large number of independent factors. This second assumption is expressed mathematically by

$$P(g) = (\pi\gamma^2)^{-1/2} \exp(-g^2/\gamma^2) \qquad (3)$$

where $P(g)$ is the probability density function for $g(t)$ and $\gamma^2/2$ is the variance of this distribution. The third assumption of our model implies that the value of $g(t)$ is essentially constant throughout any particular open interval. Although this assumption is introduced for mathematical expedience, it is consistent with the notion that changes in the conformation of proteins can occur on a time scale of seconds or minutes [8,9].

This model predicts (Appendix A) the probability density function of open times given by

$$f_o(t) = A_c \pi^{-1/2} \exp(3\gamma^2/4) \times \int_{-\infty}^{\infty} dx \exp(-x^2 - A_c t \exp(\gamma(\gamma - x))) \qquad (4)$$

It is easily verified that this distribution is normalized, is finite for $t = 0$ with value $A_c \exp(3\gamma^2/4)$, and reduces to Eqn. 1 with $k_c = A_c$ in the limit $\gamma \rightarrow 0$. The nth moment of $f_o(t)$, calculated as the integral from 0 to ∞ of $t^n f_o(t)\, dt$, is $n!\, A_c^{-n} \exp\{n(n-2)\gamma^2/4\}$. In particular, the first moment or mean open time of the channel is $A_c^{-1} \exp(-\gamma^2/4)$.

It can be shown that for $A_c t > 1$ the integrand in Eqn. 4 is a maximum value at x^* where $\gamma - 1/\gamma < x^* < \gamma + \ln(A_c t)/\gamma$ and that the integrand is less than 0.001-times this maximum outside the interval $\gamma - 3/\gamma < x < \gamma + [5 + \ln(A_c t)]/\gamma$. Thus, for large γ the integrand is peaked sharply near γ and can be approximated by $\exp\{\gamma^2 - 2\gamma x - A_c t \exp(\gamma(\gamma - x))\}$. In this approximation the argument of the exponent is correct to first order in $(x - \gamma)$ and the integral of Eqn. 4 can be evaluated analytically to obtain $f_o(t) = [\pi^{1/2} A_c \gamma \exp(\gamma^2/4)]^{-1}\, t^{-2}$. Thus, for $t \geq A_c^{-1}$ and large γ, this model predicts a power law form for $f_o(t)$ with exponent -2. Power law behavior is not followed for small t, however, since $f_o(t)$ approaches a finite value in that limit.

Fig. 1 is a log–log plot of $f_o(t)$ vs. t for a channel with $A_c = 0.01$ and various values of γ. Changes in A_c shift these curves equally relative to the axes but do not change their shapes. In-

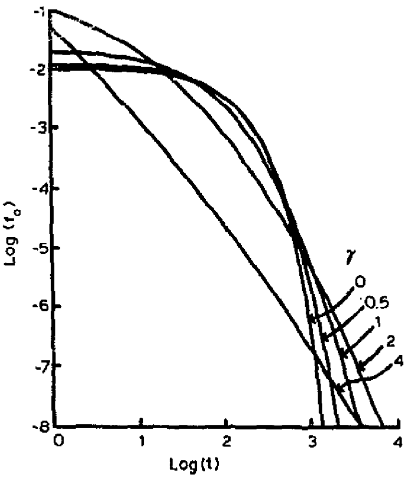

Fig. 1. Log(f_o) vs. log(t) for $A_c = 0.01$ and various values of γ. The trapezoid rule with $\Delta x = 0.01$ was used to evaluate the integral in Eqn. 4 over the interval $-20 \leq x \leq 30$.

creasing linearity of the log–log plot with larger γ indicates a greater number of both very brief and very long event durations relative to the exponential decay of the two-state Markov model.

A useful device for characterizing complex kinetic behavior of channel gating is the effective rate constant on time-scale t defined by Liebovitch et al. [6] as

$$k_c^{eff}(t) = -\frac{d}{d\tau} \ln F_0(\tau) |_{\tau=t} \tag{5}$$

where $F_o(\tau) = \int_\tau^\infty dt' f_o(t')$. For our model it is straightforward to show that

$$k_c^{eff}(t) = A_c \frac{\int_{-\infty}^{\infty} dx \exp\{-x^2 - A_c t \exp(\gamma(\gamma - x))\}}{\int_{-\infty}^{\infty} dx \exp\{-x^2 - \gamma(\gamma - x) - A_c t \exp(\gamma(\gamma - x))\}} \tag{6}$$

Fig. 2 shows the dependence of k_c^{eff} on t and γ. For $\gamma = 0$, k_c^{eff} is independent of t, demonstrating appropriate reduction to the two-state Markov result. For $\gamma > 0$, k_c^{eff} declines with increasing t.

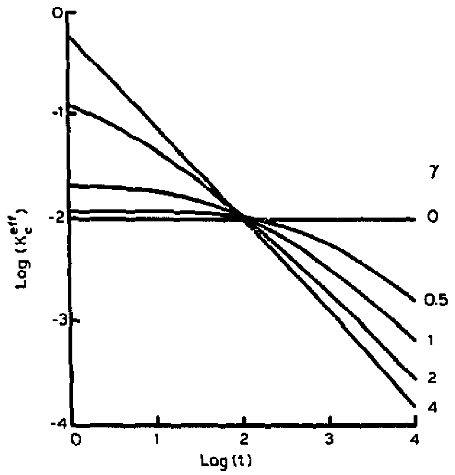

Fig. 2. Log(k_c^{eff}) vs. log(t) for $A_c = 0.01$ and various values of γ. The integrals in Eqn. 6 were evaluated as described in Fig. 1.

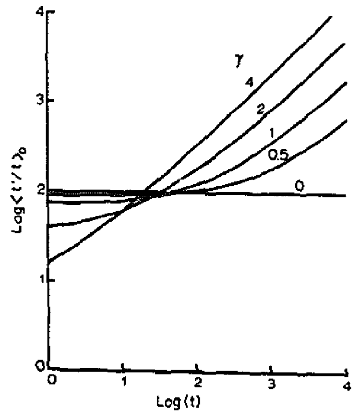

Fig. 3. Log $<t' | t>_0$ vs. log(t) for $A_c = 0.01$ and various values of γ. The integrals in Eqn. 7 were evaluated as described in Fig. 1.

There is a near-linear relationship between log k_c^{eff} and log t over several decades for larger γ.

Finally, our model predicts a specific form for the quantity $< t' | t >_0$, defined as the mean duration of open events, t', as a function of the duration of the preceding open event, t. This result is (Appendix B)

$$\langle t' | t \rangle_0 = A_c^{-1} \exp(-\gamma^2) \times \frac{\int_{-\infty}^{\infty} dx \exp\{-x^2 + 2\gamma x - A_c t \exp(\gamma(\gamma - x))\}}{\int_{-\infty}^{\infty} dx \exp\{-x^2 + \gamma x - A_c t \exp(\gamma(\gamma - x))\}} \tag{7}$$

Fig. 3 demonstrates the predicted dependence of event duration on the length of the preceding open interval. For $\gamma = 0$ the average open time is independent of the preceding open duration. Increasing γ leads to increasing correlation between the durations of successive open (or closed) intervals.

Results and Discussion

The ability of our model to describe certain experimental data is shown in Fig. 4. Using the total number of events, N, as an adjustable parameter, $N f_c(t)$ for our model was fitted by eye

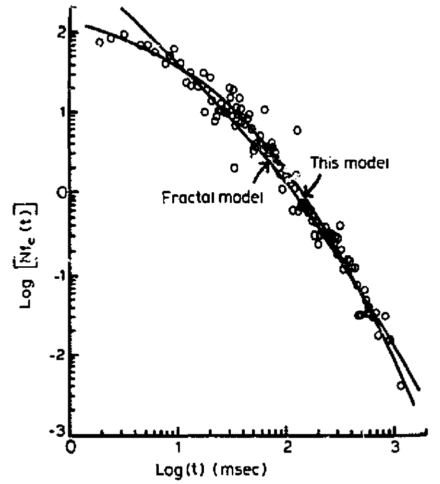

Fig. 4. Comparison of $N f_c(t)$ of this model to the experimental data and fractal model of Liebovitch et al. [6,8]. Parameters used for our model were $A_o = 0.01$ ms^{-1}, $\gamma = 1.8$, and $N = 1700$. Parameters of the fractal model were $A = 0.3493$ ms$^{-0.21}$, $D = 1.79$, and $N = 11000$. Data points were taken from Fig. 4 of Liebovitch et al. [6] using a computer digitizer pad.

to a histogram of 1465 closed durations obtained by Liebovitch et al. [6,10] from a channel in corneal epithelium. Values of $N = 1700$, $A_0 = 0.01$, and $\gamma = 1.8$ yielded an excellent fit to these data. At room temperature, $\gamma = 1.8$ corresponds to a standard deviation in the activation energy of the opening reaction of about 0.75 kcal/mol.

The concept of fluctuations in the activation energy of the gating of ion channels represents the intuitive fact that random motions in one part of a protein can affect motions in another part [11,12]. This idea was used previously by Sigworth [13] in a model that assumed the time-scale of fluctuations to be much faster than that of the event durations. Our model involves the opposite assumption and is consistent with the lack of coupling between gating transitions and fluctuations in channel current that he observed.

The representation of the experimental data in Fig. 4 by our model is excellent. Nevertheless, the choice between alternative models should be based not only on their accuracy in fitting experimental data but also on their capabilities of illuminating the physical basis of the observed phenomena and of predicting additional characteristics of the system. In these latter regards our model offers several advantages over other models that have been proposed.

The conventional use of Markov models involves both practical and philosophical problems because of the multiplicity of parameters involved in more complex gating schemes. Fitting an expression with many adjustable parameters to a set of data does little to validate the model upon which the expression is based, particularly if the data can be represented well by a much simpler empirical expression. Our model retains the concept of a few discrete states but is capable of fitting a variety of data, using a minimum of adjustable parameters.

The fractal model [6,7,10] is an elegant descriptive device, but it is not derived from defined physical assumptions. In contrast, our model is closely related to well-established chemical theories and its parameters have thermodynamic meanings. Furthermore, the property of the fractal model that $f_0(t) \to \infty$ as $t \to 0$ implies that a large number of brief events are 'missed' experimentally. The observed distribution of event durations would then be significantly different from the actual distribution and fitting of observed data with the theoretical $f_0(t)$ would be inconsistent. Our model is finite at $t = 0$ and does not require postulation of a large number of "missed" events.

A recent 'diffusion' model of channel gating [14] assumes a simple relationship for the Markov rate constants of transition between a near-infinite number of discrete states. Such models can probably describe a variety of experimental data with few parameters. This approach does provide a link between the gating behavior and the chemical nature of ion channels. However, the adjustable nature of the models themselves and the arbitrariness of their assumptions make it likely that very different choices will perform equally well. The model described in the present paper provides a well-defined and more concise description of channel gating by treating the large number of available protein conformations in a statistical manner.

Additional analytic approaches may be required to distinguish between alternative models that fit the duration histograms equally well. Evaluation of models should take into account the fact that event duration histograms do not contain all of the information about ion channel gating that is available in single channel records. One possible approach would to be calculate $<t' \mid t>_{\mathrm{o}}$ and compare those data to the prediction of our model (Fig. 3). Agreement would provide additional evidence that the physical basis of our model wes valid. Neither the fractal model of Liebovitch et al. [6,10] nor the linear diffusion model described by Millhauser et al. [14] appears to predict any correlation between the durations of successive events.

Finally, the model I have proposed is extendable to include a variety of additional factors. Use of separate but partially correlated stochastic variables for the opening and closing transitions, i.e., $g_{\mathrm{c}}(t) \neq g_{\mathrm{o}}(t)$, would relax our assumption that the energies of the closed and open states are fixed. The variation of $g(t)$ with time could be described by a Lorentzian process to examine the effects of stochastic changes that occur on a time-scale similar to that of the event durations. Additional closed and open states could be added to permit description of ligand-activated channels or of other channels with complex behavior such as the fast chloride channel [5]. The inclusion of stochastic rate constants in models of ion channels, as illustrated by the simple model in this report, should significantly reduce the number of states that must be postulated and may lead to a clearer understanding of the physical basis of channel gating.

Appendix A

Derivation of $f_o(t)$

Using the expression for k_{c} in Eqn. 2, the quantity given in Eqn. 1 can be interpreted as the probability that the channel first closes between times t and $t + \mathrm{d}t$ given that it opened at time zero *and* $g(t)$ had a specified value at time zero. This is written explicitly as

$$f_{\mathrm{o}}(t \mid g)\,\mathrm{d}t = A_{\mathrm{c}} \exp(-g) \exp\{-A_{\mathrm{c}} t \exp(-g)\}\,\mathrm{d}t \qquad \text{(A-1)}$$

The probability density function for channel openings in time, i.e., the frequency of openings, is k_{o} times the probability that the channel is closed at time t. Since the latter probability is given by $k_{\mathrm{c}}/(k_{\mathrm{c}} + k_{\mathrm{o}})$ we can write the conditional frequency of openings, $\nu_{\mathrm{o}}(t \mid g)$ as

$$\nu_{\mathrm{o}}(t|g) = k_o k_c/(k_c + k_o) = A_o A_c (A_c + A_o) \exp(-g) \qquad \text{(A-2)}$$

Using Bayes' theorem and Eqn. 3 we invert this frequency to obtain the probability density function for g, given a channel opening.

$$P(g \mid \text{opens at } t) = \nu_{\mathrm{o}}(t \mid g) P(g) \Big/ \left[\int_{-\infty}^{\infty} \mathrm{d}y\, \nu_{\mathrm{o}}(t \mid y) P(y) \right]$$

$$= \exp(-g) \exp(-g^2/\gamma^2)$$

$$\Big/ \left[\int_{-\infty}^{\infty} \mathrm{d}y \exp(-y) \exp(-y^2/\gamma^2) \right]$$

$$= (\pi\gamma^2)^{-1/2} \exp\{-(g/\gamma + \gamma/2)^2\} \qquad \text{(A-3)}$$

The net probability density function of open durations for our model, $f_{\mathrm{o}}(t)$, is obtained by integrating $f_{\mathrm{o}}(t \mid g)$ $P(g \mid \text{opens at } t = 0)$ over all g.

$$f_{\mathrm{o}}(t) = (\pi\gamma^2)^{-1/2} \int_{-\infty}^{\infty} \mathrm{d}g A_{\mathrm{c}} \exp(-g) \exp\{-A_{\mathrm{c}} t \exp(-g)\}$$

$$\times \exp\{-(g/\gamma + \gamma 2)^2\} \qquad \text{(A-4)}$$

This yields Eqn. 4 with the change of variable $x = g/\gamma + \gamma$.

Appendix B

Derivation of $<t' \mid t>_o$

Eqn. A-1 gives the conditional probability of open duration t, given a specified value of g. Bayes' theorem and Eqn. 3 can be used to invert this expression to obtain the conditional probability of g, given an event of duration t, denoted $P(g \mid t)$. This yields

$$P(g \mid t) = \frac{\exp\{-g^2/\gamma^2 - g - A_c t \exp(-g)\}}{\int_{-\infty}^{\infty} \mathrm{d}y \exp\{-y^2/\gamma^2 - y - A_c t \exp(-y)\}} \tag{B-1}$$

Assuming that g is essentially constant over two successive open intervals, we obtain the probability density function for open durations t', given that the previous open duration was t, by integrating the quantity $f_o(t' \mid g)\ P(g \mid t)\ \mathrm{d}g$ over all g. This yields

$$P(t' \mid t) = A_c \frac{\int_{-\infty}^{\infty} \mathrm{d}g \exp\{-g^2/\gamma^2 - 2g - A_c(t'+t)\exp(-g)\}}{\int_{-\infty}^{\infty} \mathrm{d}y \exp\{-y^2/\gamma^2 - y - A_c t \exp(-y)\}} \tag{B-2}$$

The expression for $< t' \mid t >_o$ given in Eqn. 7 is finally obtained by integrating $t'P(t' \mid t)\ \mathrm{d}t'$ over all t' and making the change of variable $x = g/\gamma + \gamma$ in the numerator and $x = y/\gamma + \gamma$ in the denominator.

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