A Possible Resolution of the Gating Paradox

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ABSTRACT　We introduce a Markov model for the gating of membrane channels. The model features a possible solution to the so-called gating current paradox, namely that the bell-shaped curve that describes the voltage dependence of the kinetics is broader than expected from, and shifted relative to, the sigmoidal curve that describes the voltage dependence of the activation. The model also predicts some temperature dependence of this shift, but presence of the latter has not been tested experimentally so far.

INTRODUCTION

The gating of membrane channels is of vital importance for the electrophysiological activity of nerve, heart, and muscle. While some of these channels appear to have fractal-like gating (Liebovitch, 1995), most membrane channels do display activity that can be well approximated by a simple Markov process (Korn and Horn, 1988). However, Clay et al. (1995) revealed a gating current paradox that has been difficult to explain with a standard type (Hille, 1992) Markov model. The paradox is that the bell-shaped curve that describes the voltage dependence of the kinetics is shifted significantly relative to the sigmoidal curve that describes the voltage dependence of the activation. The standard type model (Hille, 1992) does not allow such a shift. Also, the former curve is broader than the one predicted by the standard model.

Here we introduce a new Markov model that extends and generalizes the standard one. Our generalization consists of introducing an alternative route between the open and the closed positions of the gate. With two routes, or two membrane protein folding pathways, we are able to obtain results consistent with the observed ones. Thus such a model presents a possible resolution of the above paradox. A more complete resolution requires investigation of the detailed physical mechanism present in real membrane channels to see how they compare with the model. The idea with two routes, a rapid one and a slow one, is that the probability of choosing one or the other also depends upon the voltage through a Boltzmann factor. This will affect the kinetics, but not the equilibrium distribution (stationary state), and a relative shift of curves can take place.

THE MODEL

We imagine that a membrane channel has one open and one closed state, as in the simplest standard (Hille, 1992)

Markov model for this problem. However, between these states we now assume that there exist two routes ($i = 1, 2$). This gives,

$$C \underset{\underset{\beta_2}{\overset{\alpha_2}{\rightleftharpoons}}}{\overset{\overset{\alpha_1}{\rightleftharpoons}}{\beta_1}} O, \tag{1}$$

where the rate constants α_1, α_2 and β_1, β_2, which are functions of voltage (but are constant at any given voltage), control the transitions between the closed (C) and the open (O) states of the gate. The α_i is the rate for a closed channel to open, and β_i the rate for an open channel to close. We introduce effective rate constants α and β,

$$\alpha = p_1\alpha_1 + p_2\alpha_2 \tag{2}$$

$$\beta = p_1\beta_1 + p_2\beta_2, \tag{3}$$

where the probabilities p_1 and p_2 are related in a standard way to the difference ΔG_b in energy barriers that must be overcome for each of the two routes,

$$p_1 = \frac{\exp\left(-\dfrac{\Delta G_b}{2kT}\right)}{\exp\left(\dfrac{\Delta G_b}{2kT}\right) + \exp\left(-\dfrac{\Delta G_b}{2kT}\right)} \tag{4}$$

$$p_2 = \frac{\exp\left(\dfrac{\Delta G_b}{2kT}\right)}{\exp\left(-\dfrac{\Delta G_b}{2kT}\right) + \exp\left(\dfrac{\Delta G_b}{2kT}\right)}. \tag{5}$$

Let x denote the average fraction of gates that are open or, equivalently, the probability that a given gate will be open, and let us imagine that a Markov (1906) model is suitable to describe the gating. One then has, as usual

$$\frac{dx}{dt} = \alpha(1 - x) - \beta x = \frac{x_\infty - x}{\tau}, \tag{6}$$

where

$$x_\infty = \frac{\alpha}{\alpha + \beta}, \qquad (7)$$

$$\tau = \frac{1}{\alpha + \beta}. \qquad (8)$$

Here x_∞ denotes the steady stationary state fraction of open gates and τ the relaxation time. At equilibrium, the probability for a channel to be in the open state is x_∞, and the probability to be in the closed state is $(1 - x_\infty)$. The ratio of these two probabilities is given by the Boltzmann distribution,

$$\frac{x_\infty}{1 - x_\infty} = \exp\left(\frac{\Delta G_x}{kT}\right), \qquad (9)$$

where T is the absolute temperature, k is Boltzmann's constant, and ΔG_x denotes the energy difference between the open and the closed positions. Thus,

$$x_\infty = \left(1 + \exp\left[-\frac{\Delta G_x}{kT}\right]\right)^{-1}. \qquad (10)$$

At equilibrium, each of the forward reactions must occur just as frequently as each of the reverse reactions, giving

$$\frac{\alpha_i}{\beta_i} = \exp\left(\frac{\Delta G_x}{kT}\right). \qquad (11)$$

This is the principle of detailed balance, which is present in dynamical systems (reversible mechanics). As in the standard model the rates are then assumed to be

$$\alpha_i = \lambda_i \exp\left(\frac{\Delta G_x}{2kT}\right) \qquad (12)$$

$$\beta_i = \lambda_i \exp\left(-\frac{\Delta G_x}{2kT}\right), \qquad (13)$$

where λ_i is assumed to be independent of ΔG_x. Thus the relaxation time (Eq. 8) can then be written as

$$\tau = \frac{1}{\alpha + \beta} = \frac{1}{p_1\alpha_1 + p_2\alpha_2 + p_1\beta_1 + p_2\beta_2}. \qquad (14)$$

Using Eqs. 4, 5, 12, and 13, we obtain

$$\tau = \frac{2\cosh\left(\frac{\Delta G_b}{2kT}\right)}{(\alpha_1 + \beta_1)\exp\left(\frac{-\Delta G_b}{2kT}\right) + (\alpha_2 + \beta_2)\exp\left(\frac{\Delta G_b}{2kT}\right)} \qquad (15)$$

$$= \frac{\cosh\left(\frac{\Delta G_b}{2kT}\right)}{\cosh\left(\frac{\Delta G_x}{2kT}\right)\left[\lambda_1\exp\left(-\frac{\Delta G_b}{2kT}\right) + \lambda_2\exp\left(\frac{\Delta G_b}{2kT}\right)\right]} \qquad (16)$$

$$= \frac{\cosh\left(\frac{\Delta G_b}{2kT}\right)}{\lambda \cosh\left(\frac{\Delta G_x}{2kT}\right)\left[\exp\left(-\frac{\Delta G_b}{2kT} - \gamma\right) + \exp\left(\frac{\Delta G_b}{2kT} + \gamma\right)\right]} \qquad (17)$$

$$= \frac{\cosh\left(\frac{\Delta G_b}{2kT}\right)}{2\lambda \cosh\left(\frac{\Delta G_x}{2kT}\right)\cosh\left(\frac{\Delta G_b}{2kT} + \gamma\right)}, \qquad (18)$$

where

$$\gamma = \frac{1}{2}\log\left(\frac{\lambda_2}{\lambda_1}\right) \qquad (19)$$

$$\lambda = \sqrt{\lambda_1\lambda_2}. \qquad (20)$$

To be more specific, the voltage dependences of ΔG_x and ΔG_b are needed. For the energy difference between the open state and the closed state we assume as usual,

$$\Delta G_x = G_{closed} - G_{open} \equiv q_x(v - v_x) - s_x T, \qquad (21)$$

where the term $q_x v_x$ is due to the difference in mechanical conformation energy between the two states; $q_x v$ represents the electrical potential energy change associated with the redistribution of charge during the transition, and s_x is due to the difference in entropy between the two states. A similar expression can be assumed for the energy difference between the two barriers in routes 1 and 2,

$$\Delta G_b = G_1 - G_2 \equiv q_b(v - v_b) - s_b T. \qquad (22)$$

Here v is voltage, while q_x, v_x, s_x, q_b, v_b, and s_b are constants. However, the assumed voltage dependence in Eq. 22 is in no way obvious, but we find it reasonable in the sense that the choice between the two routes may possibly depend upon the voltage in a way similar to the fraction x_∞ of open and closed gates.

One notes that the curve for the relaxation time τ has a shift in position due to the term γ. Inserted for the special case $\Delta G_b = \Delta G_x$ the above yields

$$x_\infty = \left(1 + \exp\left[\frac{-q_x(v - v_x) + s_x T}{kT}\right]\right)^{-1} \qquad (23)$$

$$\tau = \left(2\lambda \cosh\left[\frac{-q_x(v - v_x) + T(s_x - 2\gamma k)}{2kT}\right]\right)^{-1}. \qquad (24)$$

Here we find that the voltage dependence of the curve for the relaxation time (Eq. 24) is shifted by an amount $2\gamma kT/q_x$ relative to the steady-state activation curve (Eq. 23), which means that the magnitude of the shift depends upon temperature. With $\Delta G_b \neq \Delta G_x$, Eq. 24 becomes more complex, as follows from Eq. 18, and the shape of the former curve is modified. This, however, is dealt with in the next section.

FIGURE 1   The steady-state activation curve (Eq. 25) and the bell-shaped curve for the relaxation time (Eq. 26), with the parameters $v_x = 6.31$ mV, $k_x = 7.31$ mV, $\lambda = 0.31$ s^{-1}, $v_b = -1.79$ mV, $k_b = 7.99$ mV, and $\gamma = 1.89$. The error bars indicate the mean $\pm$ standard deviation from the six experiments of Clay et al. (1995).
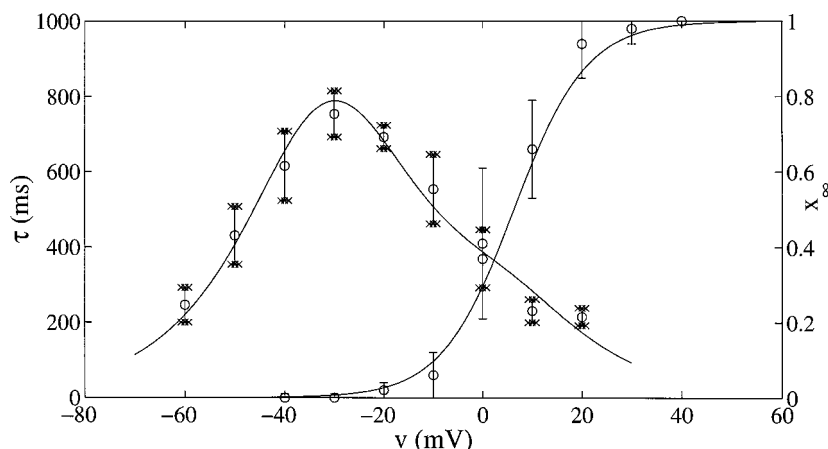
RESULTS

We will now compare the model with the experimental results of Clay et al. (1995) and show that it is consistent with the latter. Thus it presents a mechanism that represents a possible solution to the gating current paradox. The temperature dependence of the currents was not considered in those experiments, so here s_x and s_b can be incorporated into v_x and v_b. With the use of Eqs. 21 and 22, Eqs. 10 and 18 become

$$x_\infty = \frac{1}{1 + \exp\left(\dfrac{v_x - v}{k_x}\right)} \qquad (25)$$

$$\tau = \frac{\cosh\left(\dfrac{v - v_b}{2k_b}\right)}{2\lambda \cosh\left(\dfrac{v - v_x}{2k_x}\right)\cosh\left(\dfrac{v - v_b}{2k_b} + \gamma\right)}, \qquad (26)$$

where $k_x = kT/q_x$ and $k_b = kT/q_b$. These expressions were evaluated numerically, adjusting the parameters present to obtain a best possible fit to the experimental data. A least-squares fit weighting various points in accordance with experimental uncertainty was used. The results of this evaluation are shown in the figure below, where the data of Clay et al. (1995) are presented together with the curves given by Eqs. 25 and 26 using the parameters shown in the figure legend.

However, the curves are not very sensitive to the values of these parameters except γ, i.e., the other parameters can be varied quite a bit and still give essentially the same curves. From these curves we find that the model is fully consistent with the experimental results within the uncertainties in the latter. Since the results of our proposed model for the gating heavily rely upon the assumption in Eq. 22, one can ask oneself whether other known models will fit experimental data in a similar way by adjusting parameters. As far as we can see, this is not possible, e.g., Clay et al. (1995) tried to do so with the standard model, and as we find

too, the obvious shift in the two curves can in no way be accounted for even with some asymmetry between α and β. That is, asymmetry can only produce a minor shift before the bell-shaped form of the curve for τ is lost. In this respect we did a standard statistical test evaluating the expression

$$\frac{\chi^2}{n} = \frac{1}{N - M}\sum_{i=1}^{N}\left(\frac{t_i - e_i}{\sigma_i}\right)^2, \qquad (27)$$

where $n = N - M$ is the number of degrees of freedom, N is the total number of experimental points, M is the number of adjustable parameters, t_i are the various theoretical values, e_i the experimental averages, and σ_i the corresponding uncertainties of the latter. In our case with $M = 6$ we find $\chi^2/n = 0.91$ while the standard theory referred to above with $M = 4$ yields $\chi^2/n = 16.8$.

DISCUSSION

We have presented a Markov model that yields a possible solution to the gating current paradox announced by Clay et al. (1995). It gives a simple explanation of the voltage shift of the bell-shaped curve for the relaxation time relative to the steady-state activation curve. Also, the width and shape of the relaxation time curve can be modified in a way consistent with experiments. A novel feature of the present model is that the voltage shift is temperature-dependent. It is not clear whether such a temperature dependence can be observed experimentally.

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