Application of the one- and two-dimensional Ising models to studies of cooperativity between ion channels

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ABSTRACT The Ising model of statistical physics provides a framework for studying systems of protomers in which nearest neighbors interact with each other. In this article, the Ising model is applied to the study of cooperative phenomena between ligand-gated ion channels. Expressions for the mean open channel probability, p_o, and the variance, σ^2, are derived from the grand partition function. In the one-dimensional Ising model, interactions between neighboring open channels give rise to a sigmoidal p_o versus concentration curve and a nonquadratic relationship between σ^2 and p_o. Positive cooperativity increases the slope at the midpoint of the p_o versus concentration curve, shifts the apparent binding affinity to lower concentrations, and increases the variance for a given p_o. Negative cooperativity has the opposite effects. Strong negative cooperativity results in a bimodal σ^2 versus p_o curve. The slope of the p_o versus concentration curve increases linearly with the number of binding sites on a protomer, but the σ^2 versus p_o relationship is independent of the number of ligand binding sites. Thus, the σ^2 versus p_o curve provides unambiguous information about channel interactions. In the two-dimensional Ising model, p_o and σ^2 are calculated numerically from a series expansion of the grand partition function appropriate for weak interactions. Virtually all of the features exhibited by the one-dimensional model are qualitatively present in the two-dimensional model. These models are also applicable to voltage-gated ion channels.

INTRODUCTION

Cooperative behavior in biological systems has been the focus of many theoretical and experimental investigations. Cooperativity arises from direct, energetic interactions between components of a system. Such interactions give rise to a sigmoidal dependence between the state of the system (e.g., ligand binding to a receptor) and an extrinsic thermodynamic variable (e.g., ligand concentration). Two levels of molecular interaction can give rise to cooperativity in biological systems: (*a*) interactions between subunits of a macromolecule (protomer) and (*b*) interactions between individual protomers themselves. In the classic example of hemoglobin, the first type of interaction is apparent; the four oxygen binding sites on each hemoglobin molecule are coupled such that the binding of one oxygen molecule enhances binding affinity of hemoglobin for additional oxygen molecules (Pauling, 1935; Monod et al., 1965; Koshland et al., 1966; Thompson, 1968).

Transmembrane ion channels, enzymes that catalyze the transport of ions across cell membranes, may, in principle, have one or both levels of cooperativity. Several ligand-gated ion channels have more than one agonist binding site, and occupancy of all sites may either be required for channel opening or greatly stabilize the open conformation of the channel (Magleby and Pallotta, 1983; Sine and Steinbach, 1986*a*; Colquhoun and Ogden, 1988; Jackson, 1988). In the most thoroughly studied example, the nicotinic acetylcholine (ACh) receptor channel, however, the two agonist binding sites are thought to be either essentially equivalent or have a preexisting and constant difference in affinity (Sine and Taylor, 1980; Sine and Steinbach, 1986*b*; Colquhoun and Ogden, 1988; Jackson, 1988; Liu and Dilger, 1991). Many voltage-activated channels exhibit a sigmoidal dependence of activation parameters on voltage (Hodgkin and Huxley, 1952). When the details of this voltage dependence were examined for one particular type of potassium channel, there was no evidence for energetic interactions between voltage-gated subunits (Hill and Chen, 1971*a*, *b*).

Does cooperativity exist between individual ion channels? Most studies of ion channel behavior have relied on the assumption of channel gating independence (Katz and Miledi, 1972; Anderson and Stevens, 1973), which states that the opening or closing of one channel does not alter the probability of opening or closing of neighboring channels. When it became possible to measure the properties of single ion channels (Neher and Sakmann, 1976; Hamill et al., 1981), this assumption became testable. The first such test was made by Neher et al. (1978) on ACh receptor channels. They found that the distribution of open channels was consistent with a stationary Poisson process, suggesting channel independence. Evidence supporting channel independence also has been obtained from analysis of experiments on voltage-gated sodium channels (Sigworth, 1980) and potassium channels (Hill and Chen, 1971*a*, *b*). However, a number of investigations have suggested that neighboring ion channels may indeed interact with each other (Neumcke and Stampfli 1981, 1986; Kiss and Nagy, 1985; Iwasa et al., 1986; Yeramian et al., 1986). The issue of channel independence remains unresolved, and there is a need for some systematic way of assessing interactions between ion channels.

In this article, we use the one- and two-dimensional Ising models to describe nearest-neighbor energetic in-

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teractions between ion channels. Expressions for the mean open channel probability and the fluctuations around the mean (variance) are derived from the grand partition function. The conventional relationships that examine the dependence of mean open probability on ligand concentration, voltage, or pressure cannot always be used to distinguish cooperativity between channels from the molecular details (stoichiometry) of channel gating. In contrast, the relationship between the variance and the mean provides a sensitive and quantitative measure of the strength of interaction between channels. The model is applicable to many channel systems.

THEORY

The Ising model was first introduced to describe interactions between fermions (elementary particles such as electrons having half-integer spin or magnetic moment), which are arranged in an array (of 1, 2, or 3 dimensions) and are placed in an external magnetic field (Ising, 1925). The model can be applied directly to the study of interactions between ligand-gated ion channel proteins in a biological membrane. We assume that there are m ligand binding sites on the protein but there are only two distinct states of the protein: state A, the unliganded state, and state B, the fully liganded state. Furthermore, we associate A with the closed (nonconducting) state of the channel and B with the open (conducting) state. The open channel probability will be a function of the ligand concentration; this enters into the expression for the chemical potential of the system. The Ising model provides a means for considering the contribution of an additional source of energy—an interaction energy between nearest neighbor channels—in the overall open channel probability.

Consider an ordered one- or two-dimensional square lattice consisting of a number (N) of identical receptor proteins. We assume that the ligand solution of concentration c is in thermodynamic equilibrium with the receptors, that only the nearest-neighbor receptors interact with each other, and that binding of one ligand molecule reduces the energy of the system by E ($E > 0$, $\exp(-E/kT) \propto K_m$, the equilibrium binding constant. The energy of the unoccupied state is taken as zero). Let n be the number of liganded receptors. The canonical ensemble partition function can then be written as

$$Q(n, N, T) = \sum_{i=1}^{\Omega} \exp(-E_i/kT), \tag{1}$$

where k is Boltzmann's constant, T is absolute temperature, E_i the sum of nearest-neighbor pair energies for the ith configuration of the lattice system plus the energy due to binding of $n \times m$ ligand molecules, and Ω is the number of possible configurations

$$\Omega = \frac{N!}{n!(N-n)!}. \tag{2}$$

Let ϵ_{AA}, ϵ_{BB}, and ϵ_{AB} be the potential energies of interaction (in units of kT) between the nearest-neighbor AA, BB, and AB pairs, respectively, and N^i_{AA}, N^i_{BB}, and N^i_{AB} be the numbers of nearest-neighbor AA, BB, and AB pairs in the ith configuration. It then follows that

$$E_i = kT(\epsilon_{AA}N^i_{AA} + \epsilon_{AB}N^i_{AB} + \epsilon_{BB}N^i_{BB}) - mnE. \tag{3}$$

The grand partition function is

$$\begin{aligned} Z(\mu, N, T) &= \sum_{n=0}^{N} \exp(mn\mu/kT)Q(n, N, T) \\ &= \sum_{n=0}^{N} \exp[mn(E+\mu)/kT] \\ &\quad \times \sum_{i=1}^{\Omega} \exp[-(\epsilon_{AA}N^i_{AA} \\ &\quad + \epsilon_{AB}N^i_{AB} + \epsilon_{BB}N^i_{BB})], \end{aligned} \tag{4}$$

where μ is the chemical potential of the ligand in the solution:

$$\mu = \mu_0 + kT \ln (c), \tag{5}$$

μ_0 is the standard chemical potential of the ligand and c is the ligand concentration.

The measurable quantities of interest to electrophysiologists are the mean current $\langle I \rangle$ and the current variance, σ^2, due to the random opening and closing of channels. (Electrophysiologists measure the mean occupancy and variance of a state [open channel] as opposed to the mean number of bound ligand molecules. Thus, some of these equations differ by a factor of m from those relevant to binding isotherms.) The mean current is proportional to the open channel probability, p_o,

$$\langle I \rangle = Nip_o, \tag{6}$$

where i is the current that flows through a single channel. Np_o is simply the expectation value of n, so that:

$$\begin{aligned} p_o &= \frac{1}{NZ} \sum_{n=0}^{N} n \exp[mn(E+\mu)/kT] \\ &\quad \times \sum_{i=1}^{\Omega} \exp[-(\epsilon_{AA}N^i_{AA} + \epsilon_{AB}N^i_{AB} + \epsilon_{BB}N^i_{BB})] \\ &= \frac{kT}{mN}\left(\frac{\partial \ln Z}{\partial \mu}\right)_{N,T}. \end{aligned} \tag{7}$$

The current variance is defined as:

$$\sigma^2 = \langle (I - \langle I \rangle)^2 \rangle = \langle I^2 \rangle - \langle I \rangle^2. \tag{8}$$

Statistical mechanics provides us with an expression for fluctuations in the number of bound ligand molecules, nm, (e.g., Hill, 1956, p. 105)

$$\langle (nm)^2 \rangle - \langle nm \rangle^2 = kT \frac{\partial \langle nm \rangle}{\partial \mu}. \quad (9)$$

Combining this with Eqs. 6–8, we get

$$\sigma^2 = \frac{i^2 kTN}{m} \left(\frac{\partial p_o}{\partial \mu} \right)_{N,T}. \quad (10)$$

In the case of the one-dimensional Ising model, an analytical expression for the partition function exists. The resulting expressions for p_o and σ^2 are given in the Appendix as Eqs. A-17 and A-18. They are expressed in terms of an energy, J, that is a linear combination of the three interaction energies:

$$J = \epsilon_{AB}/2 - \epsilon_{AA}/4 - \epsilon_{BB}/4. \quad (11)$$

There is no general analytical solution of the two-dimensional Ising model, but series expansion approximations have been found. The Domb expansion used here for the square lattice is appropriate for weak interactions. In the Appendix, we use the nine known terms of the expansion (Domb, 1949; Brooks and Domb, 1951). We found that the series appears to converge for $-0.125 < J < 0.325$. When $-0.10 < J < 0.20$, inclusion of the ninth term changes the calculated value of σ^2 by <1%.

RESULTS AND DISCUSSION

Initially, we will illustrate the results of the Ising model calculations with a particular example: the case where there is only one ligand binding site on the receptor ($m = 1$) and where the only type of interaction is between neighboring open channels ($\epsilon_{AA} = \epsilon_{AB} = 0$, $J = -\epsilon_{BB}/4$). Thus, the opening of one channel will either stabilize ($\epsilon_{BB} < 0$) or destabilize ($\epsilon_{BB} > 0$) the open state of neighboring channels. More general cases will be considered later.

Qualitative expectations

Before presenting quantitative results of our calculations, we will consider how interactions between channels might be expected to affect open channel probability and variance. In the absence of interactions, the concentration-response relationship (p_o versus c) takes the form of a Langmuir isotherm. This relationship is characterized by a half-maximal activation concentration, $c_{0.5}$, that is equal to the ligand binding equilibrium constant, K_m, and a Hill coefficient, n_H, of unity (see Eqs. A-25 and A-26). We expect that the effect of positive cooperativity between open channels is to make the open probability more sensitive to concentration: p_o versus c will have a steeper concentration dependence ($n_H > 1$), and the $c_{0.5}$ will shift to lower concentrations ($c_{0.5} < K_m$). The opposite effects are expected for negative cooperativity.

In the case of noninteracting channels, there is a quadratic relationship between the variance and the open channel probability:

$$\sigma^2 = Ni^2 p_o(1 - p_o) = i\langle I \rangle - \langle I \rangle^2/N. \quad (12)$$

To assess the effects of cooperativity on variance, consider a model in which each pair of channels act as completely coupled dimers. (This is not actually an example of an Ising model; we use it only to illustrate a point.) In this case, the single channel current would appear to be twice as large as it would in the absence of interactions and there would appear to be only half as many channels. The variance, then, would be $\sigma^2 = N/2(2i)^2 p_o(1 - p_o) = 2Ni^2 p_o(1 - p_o)$, which is twice as large as the variance without interactions. This suggests that the effect of positive cooperativity will be to increase the total measured variance. Conversely, negative cooperativity should decrease σ^2. (See Hill [1985] for other examples of the effects of cooperativity on variance.)

One-dimensional Ising model

Fig. 1 *A* shows the predictions of Eq. A-17 for the dependence of open channel probability on ligand concentration for $\epsilon_{BB} = 0, \pm 0.5$ and ± 1.0. The Ising model predicts that both the position and shape of the curves are different when there are interactions between channels; e.g., when open channels exhibit positive cooperativity ($\epsilon_{BB} < 0$), the apparent binding affinity is shifted to lower concentrations and the slope becomes steeper. When $\epsilon_{BB} > 0$, the apparent binding affinity is shifted to higher concentrations and the slope becomes less steep.

The magnitude of these changes can be calculated explicitly. The half-maximal activation concentration is given by Eq. A-25

$$c_{0.5} = K_m \exp(\epsilon_{BB}), \quad (13)$$

and the Hill slope of the curve at $c_{0.5}$ is obtained from Eqs. A-18 and A-26

$$n_H = \exp(-\epsilon_{BB}/2). \quad (14)$$

Thus, for example, when $\epsilon_{BB} = -0.5$, $c_{0.5} = 0.61 \cdot K_m$ and $n_H = 1.28$. For $\epsilon_{BB} = 0.5$, $c_{0.5} = 1.65 \cdot K_m$ and $n_H = 0.78$.

Fig. 1 *B* illustrates the effects of interactions on the variance versus open channel probability curve. The variance at $p_o = 0.5$, $\sigma^2_{0.5}$, remains the maximum variance, but there is no longer a quadratic relationship between variance and p_o. Positive cooperativity increases $\sigma^2_{0.5}$, whereas negative cooperativity decreases it. The value of $\sigma^2_{0.5}$ has the same dependence on ϵ_{BB} as does n_H:

$$\sigma^2_{0.5} = \frac{i^2 N}{4} \exp(-\epsilon_{BB}/2). \quad (15)$$

It can be shown that the variance versus mean current curve has an initial slope of i (the single channel

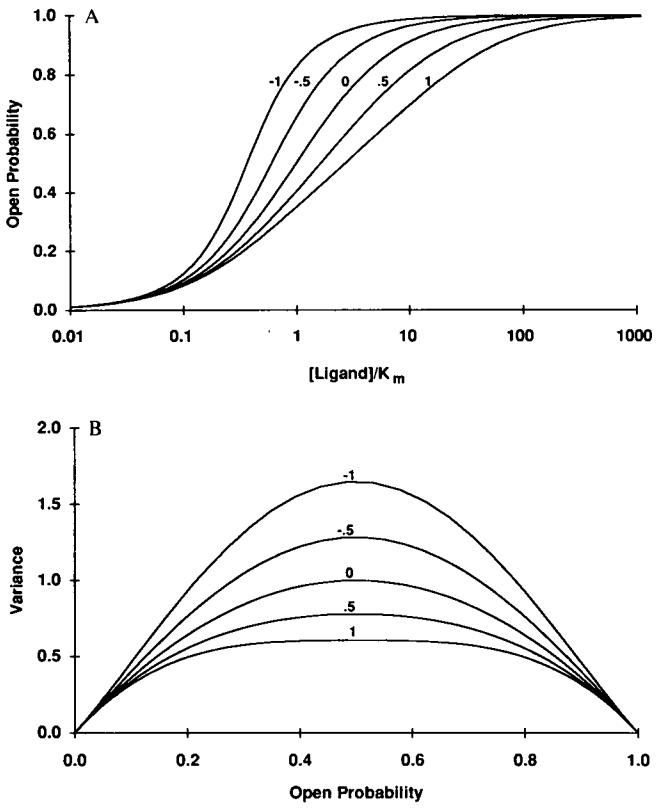

FIGURE 1 Predictions of the one-dimensional Ising model applied to interacting, two-state ion channels for different values of the energy of interaction between adjacent open channels, ϵ_{BB}. The curves are labeled with the interaction energy in units of kT. (*A*) The concentration dependence of the open probability. (*B*) The relationship between variance and open probability. The variance is normalized to its value at $p_o = 0.5$ without interactions ($\epsilon_{BB} = 0$).

current), independent of ϵ_{BB}, as suggested by the curves in Fig. 1 *B*.

In the case of strong negative cooperativity, the Ising model predicts a different sort of concentration dependence for the mean current and variance. The $\langle I \rangle$ versus log (c) curve flattens near $p_o = 0.5$ (Fig. 2 *A*) and the σ^2 versus $\langle I \rangle$ curve becomes bimodal with a local minimum at $p_o = 0.5$ (Fig. 2 *B*). These changes occur for

$$\left.\frac{\partial^2\sigma^2}{\partial p_o^2}\right|_{p_o=0.5} > 0,$$

that is, for values of $\epsilon_{BB} > \ln(3) = 1.099$. The origin of this phenomenon is that negative cooperativity increases the stability of the state in which adjacent channels are alternately open and closed.

Effects of the number of channels

Up to this point, we have assumed that the number of interacting channels, N, is large. Expressions for the partition function and occupancy of a one-dimensional Ising model with a small number of channels are given, along with an expression for the variance, in the Appendix (Eqs. A-14 and A-15). The variance corresponding to half activation is:

$$\sigma^2_{0.5} = \frac{Ni^2}{4}\exp(-\epsilon_{BB}/2) \times \frac{\cosh^N(-\epsilon_{BB}/4) - \sinh^N(-\epsilon_{BB}/4)}{\cosh^N(-\epsilon_{BB}/4) + \sinh^N(-\epsilon_{BB}/4)}. \quad (16)$$

In Fig. 3 *A* we show the dependence of $\sigma^2_{0.5}$ (normalized to its value for large N, Eq. 15) on the number of channels from different amounts of positive cooperativity. The normalized variance is smaller when there are fewer channels. A similar situation exists for negative cooperativity, except when the number of channels is an odd number. In this case, the "unpaired" channel decreases the stability of the configuration. These figures also indicate that N does not have to be extraordinarily large for the large-N form of the Ising model to be valid.

Two-dimensional Ising model

Nearly all of the features exhibited by the one-dimensional model are also present in the two-dimensional

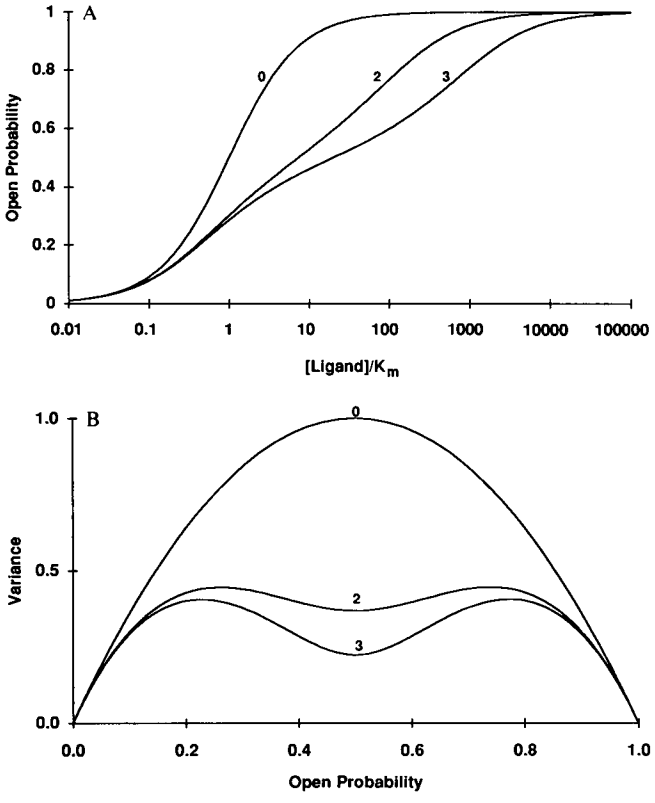

FIGURE 2 Predictions of the one-dimensional Ising model in the case of strong negative cooperativity between adjacent open channels, ϵ_{BB}. The curves are labeled with the interaction energy in units of kT. Note the differences in the concentration and variance scales compared with Fig. 1. (*A*) The concentration dependence of the open probability. (*B*) The relationship between variance and open probability.

model, but the effects are more pronounced for a given interaction strength. This arises because each channel has four instead of two nearest neighbors. Using Eq. A-25, we find that the effect of interaction energy on shifting the p_o versus c curve is directly related to the number of nearest neighbors:

$$c_{0.5} = K_m \exp(2\epsilon_{BB}). \quad (17)$$

Thus, a given interaction strength in the two-dimensional model is as effective in changing the apparent binding affinity as twice that strength in the one-dimensional model. The effect of interaction strength on variance (and, according to Eq. A-26, on n_H) is illustrated in Fig. 4.

The lack of analytical expressions for the two-dimensional model makes it difficult to prove additional relationships between the variance and the mean. However, we can make a few empirical observations based on our numerical calculations in the range of interaction energies over which the series appears to converge. Just as in the one-dimensional case, the initial slope of σ^2 versus $\langle I \rangle$ appears to be i. A bimodal relationship between σ^2 versus $\langle I \rangle$ may exist for $\epsilon_{BB} > 0.45$. Moreover, for strong negative cooperativity, there may be additional stable configurations near $p_o = 1/3$ and $p_o = 2/3$. For example, $p_o = 1/3$ can be achieved with a square lattice of channels in which the open channels are aligned diagonally (1 diagonal containing open channels followed by 2 diagonals with closed channels). In this arrangement, each open channel has four closed neighbors and each closed channel has two open and two closed neighbors.

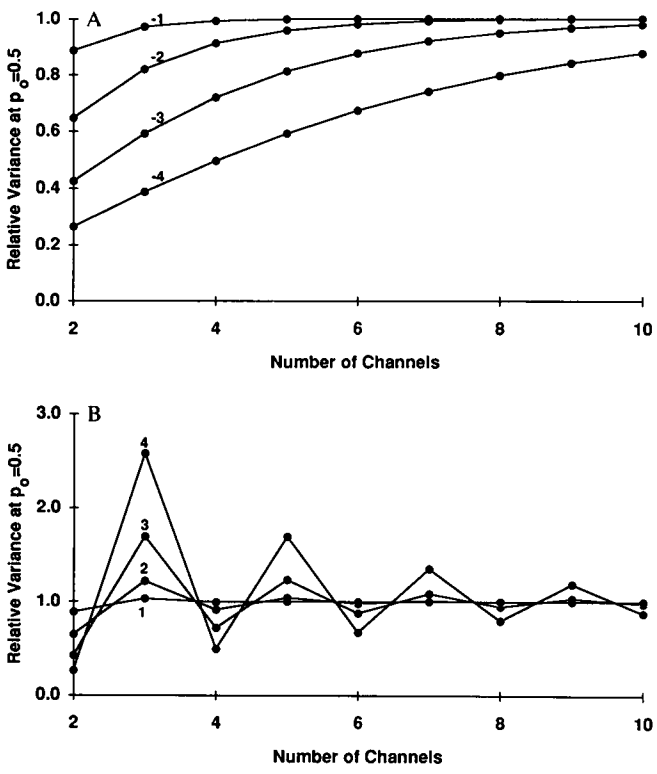

FIGURE 3 The effect of the number of channels on the variance at p_o = 0.5 for different open-open channel interaction energies (ϵ_{BB}). The curves are labeled with the interaction energy in units of kT. The variance is normalized to the variance that would be obtained for a large number of channels, thus, relative variance = $[\cosh^N(-\epsilon_{BB}/4) - \sinh^N(-\epsilon_{BB}/4)]/[\cosh^N(-\epsilon_{BB}/4) + \sinh^N(-\epsilon_{BB}/4)]$. (*A*) Positive cooperativity ($\epsilon_{BB} < 0$). (*B*) Negative cooperativity ($\epsilon_{BB} > 0$). Note that for even N, the relative variance does not depend on the sign of ϵ_{BB}.

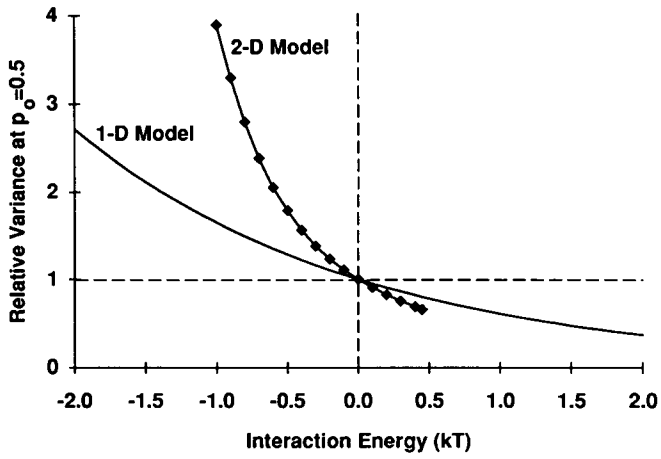

FIGURE 4 A comparison of the predictions of the one- and two-dimensional Ising models for the variance at p_o = 0.5 as a function of open-open channel interaction energy (ϵ_{BB}). The line for the one-dimensional model is drawn according to Eq. 15 and normalized to the variance when ϵ_{BB} = 0. The symbols represent the variance calculated from the series expansion of the two-dimensional model.

Effects of closed channel interactions

In generalizing from the special case of open-open channel interactions only, we can make two observations. (*a*) If there are both open-open and closed-closed channel interactions, such that $\epsilon_{AA} = \epsilon_{BB} \neq 0$, but no open-closed channel interactions ($\epsilon_{AB} = 0$), the Hill slope of the p_o versus c curve will change but the concentration at which half of the channels are open will not change (see Eq. A-6). (*b*) The variance versus open channel probability curve depends only on a linear combination of the interaction energies (J, see Eq. 11). Thus, this relationship cannot be used to determine the individual interaction energies.

Effects of additional ligand binding sites

When there is more than one ligand binding site ($m > 1$), the expression for the Hill slope at $c_{0.5}$ in the one-dimensional Ising model becomes (from Eqs. A-18 and A-26)

$$n_H = m \exp(2J), \quad (18)$$

which is m times larger than the slope for $m = 1$ (Eq. 14). However, the variance versus open channel probability

curve depends only on the interaction energies (J) and is independent of the number of binding sites (from Eqs. A-17 and A-18):

$$\sigma^2 = Ni^2 p_o(1 - p_o)\exp(2J) \times \{1 + (2p_o - 1)^2[\exp(-4J) - 1]\}^{1/2}. \quad (19)$$

This important result means that although the number of binding sites affects the concentration dependence of both the open channel probability and variance separately, it does not affect the relationship between the variance and the open channel probability. Because the expressions for p_o and σ^2 are parametric in H (Eqs. A-10 and A-11) and H is the only parameter that depends on m, it can be shown that in the two-dimensional model, n_H, is still proportional to m, whereas the relationship between σ^2 and p_o remains independent of m.

Application to other types of channels

We can easily extend this theory for use with channels that are not ligand gated. For example, with voltage-gated channels, the following substitutions should be made in Eq. 4: $m \rightarrow f$, $n \rightarrow 2n - N$ and $E + \mu \rightarrow e(V - V_o)$, where f is the voltage sensitivity of the channel (the number of gating charges), $2n - N$ is the difference between the number of open channels and the number of closed channels, V is the applied potential, and V_o is the potential at which $p_o = 0.5$. The rest of the derivation follows the outline presented in this article. In particular, the relationship between the variance and the open probability is the same as for ligand-gated channels (Equation 19).

Experimental investigations of ion channel cooperativity

Let us review the assumptions used in the Ising model: (a) the system consists of a number of identical protomers, (b) each protomer can assume only two conformations, and (c) nearest-neighbor promoters interact with a constant, conformation-dependent energy. Are there any channel systems that meet these criteria? The requirement of identical channels has, until recently, been difficult to achieve. In some cases, the experimental preparation contains hundreds or thousands of channels. (Examples of small electrophysiological preparations with large numbers of channels are [a] a micron-sized patch of membrane containing ACh receptor channels from muscle tissue where the channel density may be $>10^4/\mu m^2$ [Salpeter, 1987], [b] a patch from a tissue culture cell line—patches from BC3H-1 cells may contain several hundred ACh receptor channels [Dilger and Brett, 1990], and [c] a sodium-channel-containing node of Ranvier where the density is on the order of $10^4/\mu m^2$ [Sigworth, 1980].) Even when a patch contains only a few channels, it is hard to demonstrate their identity (Iwasa et al., 1986). However, the use of techniques to express identical clones of ion channels in a system suitable for electrophysiological study (Miller, 1989) should eliminate this problem. The second requirement may be mitigated by extending the theory to include multistate protomers (e.g., by using Monte Carlo simulations of the Ising model [Hill and Chen, 1981], by using the more general q-state Potts model [Wu, 1982], or by considering other models of protomer interactions [Changeux et al., 1967]). Many channels, however, have effectively only two states. The conditions for this are that there are only two conductance levels (closed and open; no subconductance levels) and, for ligand-gated channels, that the equilibrium occupancy of liganded-closed states and unliganded-open states is low. For the nicotinic ACh receptor, the fractional occupancy of both liganded-closed states (AR and A_2R) is never more than about 0.12 (based on the equilibrium constants used in Dilger and Brett, 1990) and spontaneous openings in the absence of agonist occur with an extremely low probability (Jackson, 1986). (Patch-clamp recordings from ACh receptor channels often reveal an additional open state [Sine and Steinbach, 1986*a*; Colquhoun and Sakmann, 1985]. However, its low rate of occurrence (<20%) and short open time (~10 times smaller than the longer duration open state) combine to give it a fractional occupancy of <0.02.) Although there are at least two open states detected in single channel experiments, these appear to arise from a single population of receptors (Sine and Steinbach, 1986*a*; 1987).

To illustrate how the analyses presented here can be used to assess cooperative effects between ion channels, consider the nicotinic ACh receptor channel. The open probability of this channel has a sigmoidal dependence on acetylcholine concentration (Colquhoun and Ogden, 1988; Dilger and Brett, 1990). There is good evidence that this is fully explained by a model in which there are two (similar, if not equivalent) ACh binding sites per protomer; only doubly liganded receptors can open and most of the doubly liganded receptors are open. Experimentally, the Hill slope at $p_o = 0.5$ is ~1.6 (Fig. 5 *A*, *symbols* and *solid curve*). Suppose that the number of ligand binding sites was unknown. Could the sigmoidal p_o versus concentration curve arise from a model containing single ACh binding site per protomer and interactions between nearest-neighbor protomers? The same Hill slope is obtained in the one-dimensional Ising model with positive cooperativity, $\epsilon_{BB} = -0.9\ kT$ (Equation 14, Fig. 5 *A*, *dashed curve*). The two curves are virtually indistinguishable. These two interpretations of the p_o versus concentration curve do make very different predictions about the relationship between variance and mean current. For noninteracting channels, the variance versus mean current curve is a parabola with an initial slope equal to the single channel current (Fig. 5 *B*, *solid curve*). For interacting channels ($\epsilon_{BB} = -0.9\ kT$), the

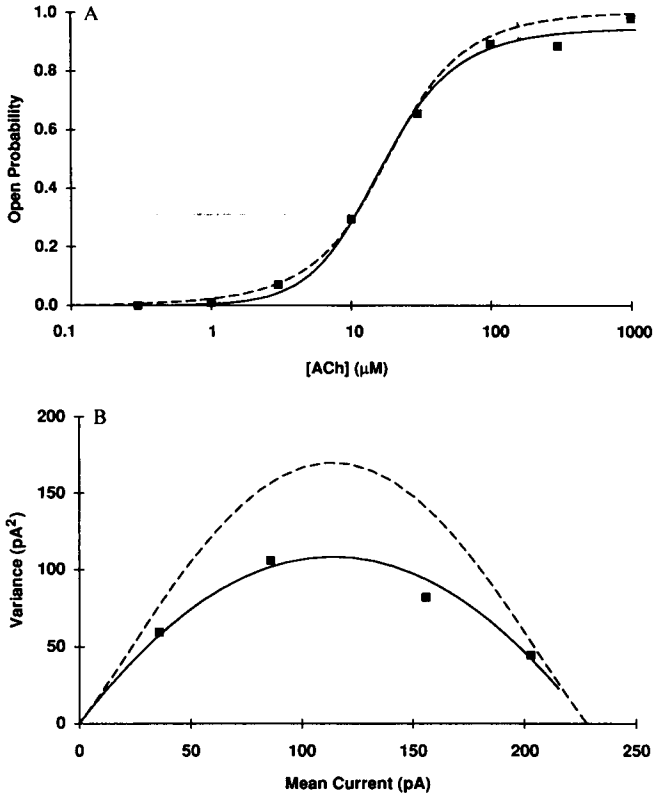

FIGURE 5 An examination of data obtained from nicotinic ACh receptor channels for evidence of interaction between channels. The data (Dilger and Brett, 1990) were obtained by rapidly perfusing outside-out patches (from BC3H-1 cells, holding potential = −50 mV) with ACh while measuring the peak current (before desensitization). (*A*) The p_o versus concentration curve. The symbols (■) represent the mean results obtained on eight patches. The solid line is the fit of the data to the kinetic scheme in which there are two identical ACh binding sites and only doubly liganded channels open ($p_o = c^2\beta/\alpha/[K^2 + 2Kc + c^2(1 + \beta/\alpha)]$, with $\beta/\alpha = 17$, $K = 60$ μM). The dashed line is the prediction of the one-dimensional Ising model with $\epsilon_{BB} = -0.9$ kT. The two interpretations of the data cannot be distinguished easily. (*B*) The variance versus mean current curve for one particular patch (Fig. 5 in Dilger and Brett, 1990). The symbols (■) represent the absolute value of the peak current and the average variance obtained while rapidly perfusing the patch with 3, 10, 30, and 100 μM ACh. The solid line is the best fit of Eq. 12 to the data: $N = 120$ and $|i| = 1.9$ pA. This value of the single channel current agrees with the value obtained from single channels directly, 2.0 pA. The dashed line corresponds to one-dimensional Ising model with $\epsilon_{BB} = -0.9$ kT (as in *A*). The two models make vastly different predictions and are easily distinguished. The data suggest that there is no measurable interaction between channels.

curve is nonparabolic and measurably larger (Fig. 5 *B*, *dashed curve*). The experimental variance versus mean current data (Fig. 5 *B*, *symbols*) are consistent with the noninteracting channel model. If there are interactions between these ACh receptor channels, then the interaction energy is quite small ($|\epsilon_{BB}| < 0.2\ kT$). This example demonstrates the power of single channel recording in determining the degree of cooperativity in ion channels. The scale of the variance versus mean current curve is determined by only one parameter, the number of channels in the patch, because the single channel current can be measured directly.

Evidence has been presented for interactions between ACh receptor channels in rat myotubes (Yeramian et al., 1986). From measurements of correlations between successive openings, these authors concluded that the opening rate for channels that have an open neighbor is 5–100 times higher than the normal opening rate. These experiments were performed with a concentration of ACh (200 nM), which produces very low open probability ($0.0004 < p_o < 0.0083$). We cannot assess cooperativity in these experiments with the Ising model, because the variance versus open probability curve is very insensitive to interactions at low p_o (see Figs. 1 *B* and 2 *B*).

There are several examples of variance versus mean current curves that suggest cooperativity between voltage-gated Na channels in the node of Ranvier (Neumcke and Stampfli, 1981, 1986). Neumcke and Stampfli (1981) present a plausible argument for apparent negative cooperativity arising from the depletion of permeant ions around the channel when the channel density is high. However, given the large number of channels present in the nodal membrane and the known heterogeneity of sodium channels, the conclusion that the other observations made in this system can be attributed to interacting channels should be considered tentative.

Perhaps the study that best approaches the ideal is that of Iwasa et al. (1986). They studied batrachotoxin-modified sodium channels from NG108-15 neuroblastoma cells. They reported on nine patches that contained only two channels each. These channels were indistinguishable on the basis of some conductance and kinetic criteria. In seven of the patches, the distribution of probabilities for zero, one, and two open channels differed from a binomial distribution. They showed that this data could not have arisen from two independent but nonidentical channels. They concluded that the channels were interacting with each other, but they could not determine whether or not the channels were identical. We used their published data on five patches to calculate the variance in open channel probability. The results are shown in Fig. 6. Clearly the variance versus open probability data are not in agreement with the prediction of a binomial distribution (curve labeled 0). An interaction energy of 0.25 kT, (negative cooperativity) fits most of the data, except for the points near $p_o = 0.5$. A stronger interaction energy (1.25 kT) fits the data near $p_o = 0.5$ and has a bimodal shape but does not fit most of the data. The data are also inconsistent with a model in which the channels have different voltage dependencies but do not interact (curves not shown). We are forced to conclude that the channels interact but one or more of the assumptions of the Ising model does not apply to this system. Thus, the channels may be nonidentical, may not be well described by a two-state model, or may have a variable interaction energy (the nearest-neighbor restric-

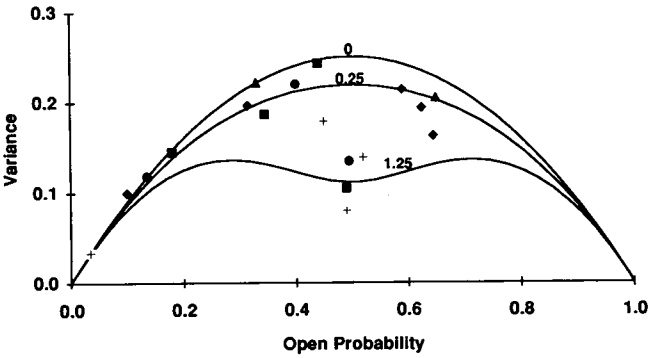

FIGURE 6 The variance (per channel) versus open probability curve for batrachotoxin-modified sodium channels calculated and replotted from Iwasa et al. (1986). The data from five patches are shown with different symbols. For each patch, measurements were made at two to five potentials ranging from −99 to −37 mV; these channels are open by depolarizing potentials. The three lines are drawn according to Eqs. A-14 and A-15 (one-dimensional model) with $N = 2$ and the indicated values of interaction energy, ϵ_{BB}. The curve for $\epsilon_{BB} = 0$ is a parabola—the prediction of a binomial analysis. See text for discussion.

tion of the Ising model is moot for a two channel system). This last possibility is particularly intriguing: the interaction energy may be voltage dependent such that the interaction is stronger when $p_o \approx 0.5$, that is, for voltages close to −60 mV. Patch-to-patch variability might arise from differences in either the distance between channels or the coupling mechanism itself.

Looking forward to future studies in which a known number of identical cloned channels can be made to sit at the tip of a patch clamper's pipette, we can suggest ways in which interactions between channels can be detected. First, comparison of the p_o versus concentration (or voltage) curve for a single channel with that of an ensemble of channels may provide evidence for cooperativity. This analysis would test for cooperativity whether or not the kinetic reaction scheme of the channel were known. Differences in both the steepness and $c_{0.5}$ may be seen. Second, variance versus mean current curves can be examined. Cooperativity may be detected as a deviation from a parabolic curve and as a discrepancy between the maximum variance measured and the variance calculated from $Ni^2/4$. Finally, an estimate of the interaction strength may be made by assuming nearest-neighbor interactions in a one- or two-dimensional lattice, using the Ising model calculations. The ability to measure single channel (protomer) properties gives these studies a distinct advantage over studies of cooperativity in other biological systems.

APPENDIX

Here we outline the solutions of the Ising models in our application to interactions between ion channels. For given values of n and N, there is only one independent value among N^i_{AA}, N^i_{AB}, and N^i_{BB}. If the number of nearest neighbors is b ($b = 2$ for the linear lattice of the one-dimensional Ising model and $b = 4$ for the square lattice of the two-dimensional Ising model):

$$2N^i_{AA} + N^i_{AB} = b(N - n), \tag{A-1}$$

$$2N^i_{BB} + N^i_{AB} = bn. \tag{A-2}$$

Eq. 3 can then be rewritten as (using Eq. 11):

$$E_i = [2JN^i_{AB} + bn\epsilon_{BB}/2 + b(N - n)\epsilon_{AA}/2]kT - mnE. \tag{A-3}$$

The grand partition function (Eq. 4) becomes:

$$Z(\mu, N, T) = \exp(-bN\epsilon_{AA}/2) \sum_{n=0}^{N} \exp[(mn\mu + mnE)/kT + bn\epsilon_{AA}/2 - bn\epsilon_{BB}/2] \times \sum_{i=1}^{\Omega} \exp(-2JN^i_{AB}). \tag{A-4}$$

Several other definitions are required to get $Z(\mu, N, T)$ into the form used in standard texts:

$$E'_i = 2JN^i_{AB} - bJN/2, \tag{A-5}$$

$$H = m(E + \mu)/2kT + b(\epsilon_{AA} - \epsilon_{BB})/4, \tag{A-6}$$

and

$$M = N - n. \tag{A-7}$$

The term $m(E + \mu)/2$ is analogous to the external magnetic field in the original formulation of the Ising model. M is the total number of unliganded receptors. The constant terms of Eq. A-4 can be removed from the summation:

$$Z(\mu, N, T) = \exp(-bN\epsilon_{AA}/2) \exp[N(2H - bJ/2)] \times \sum_{M=0}^{N} \exp(-2HM) \sum_{i=1}^{\Omega} \exp(-E'_i). \tag{A-8}$$

Define

$$L = \sum_{M=0}^{N} \exp(-2HM) \sum_{i=1}^{\Omega} \exp(-E'_i). \tag{A-9}$$

Then, using the expressions for p_o and σ^2 in the text (Eqs. 7 and 10)

$$p_o = 1 + \frac{1}{2N} \frac{\partial \ln L}{\partial H}, \tag{A-10}$$

$$\sigma^2 = \frac{i^2 N}{2} \frac{\partial p_o}{\partial H}. \tag{A-11}$$

For the one-dimensional Ising model with finite N, L is given by (Hill, 1956, p. 313)

$$L = \lambda_1^N + \lambda_2^N, \tag{A-12}$$

where

$$\lambda_{1,2} = \exp(J - H) \times \{\cosh(H) \pm [\exp(-4J) + \sinh^2(H)]^{1/2}\}. \tag{A-13}$$

Using Eqs. A-10 and A-11, we find

$$p_o = 1 + \frac{1}{2(\lambda_1^N + \lambda_2^N)}\left[\lambda_1^{N-1}\frac{\partial\lambda_1}{\partial H} + \lambda_2^{N-1}\frac{\partial\lambda_2}{\partial H}\right], \quad \text{(A-14)}$$

$$\frac{4\sigma^2}{Ni^2} = \frac{1}{\lambda_1^N + \lambda_2^N}\left[\lambda_1^{N-1}\frac{\partial^2\lambda_1}{\partial H^2} + (N-1)\lambda_1^{N-2}\left(\frac{\partial\lambda_1}{\partial H}\right)^2 + \lambda_2^{N-1}\frac{\partial^2\lambda_2}{\partial H^2} + (N-1)\lambda_2^{N-2}\left(\frac{\partial\lambda_2}{\partial H}\right)^2\right] - \frac{N}{(\lambda_1^N + \lambda_2^N)^2}\left[\lambda_1^{N-1}\frac{\partial\lambda_1}{\partial H} + \lambda_2^{N-1}\frac{\partial\lambda_2}{\partial H}\right]^2, \quad \text{(A-15)}$$

which can be expressed in terms of H, J, and N after some tedious differentiation. When the number of channels is large, $\lambda_2^N \ll \lambda_1^N$ and L can be written as (Hill, 1956, p. 322)

$$L = \exp[N(J-H)] \times \{\cosh(H) + [\exp(-4J) + \sinh^2(H)]^{1/2}\}^N, \quad \text{(A-16)}$$

so that

$$p_o = \frac{1}{2}\left[1 + \frac{\sinh(H)}{[\sinh^2(H) + \exp(-4J)]^{1/2}}\right], \quad \text{(A-17)}$$

$$\sigma^2 = \frac{Ni^2}{4}\frac{\cosh(H)\exp(-4J)}{[\sinh^2(H) + \exp(-4J)]^{3/2}}. \quad \text{(A-18)}$$

For the two-dimensional Ising model, there is no known closed-form expression for the grand partition function except when $H = 0$ ($p_o = 0.5$). We shall consider the case of weak interactions between nearest-neighbor pairs and use the expansion method developed by Domb (1949). Let

$$y = \exp(-2H), \quad \text{(A-19)}$$

$$x = \exp(-2J), \quad \text{(A-20)}$$

$$\Lambda = L^{1/N}\exp(-2J) = L^{1/N}x. \quad \text{(A-21)}$$

Then,

$$p_o = 1 - y\frac{\partial \ln \Lambda}{\partial y}, \quad \text{(A-22)}$$

$$\sigma^2 = Ni^2 y\left[\frac{\partial \ln \Lambda}{\partial y} + y\frac{\partial^2 \ln \Lambda}{\partial y^2}\right]. \quad \text{(A-23)}$$

Specifically, if J is small, Domb assumed that the grand partition function can be expressed as an expansion about $t = 1 - x^2$.

$$\Lambda(y, x) = 1 + y + \sum_{r\geq 1}\frac{\phi_r(y)t^r}{(1+y)^{(2r-1)}}, \quad \text{(A-24)}$$

where $\phi_r(y)$ is a polynomial of y of degree not exceeding $2r$. The expressions for $\phi_r(y)$ are known through $r = 9$ (Domb, 1949; Brooks and Domb, 1951). Eq. A-24 can be substituted into Eqs. A-22 and A-23 to get series expansions for the open probability and variance.

There are two useful results that can be derived from the general Ising model. The first one is an expression for the half-maximal activation concentration, $c_{0.5}$. This is obtained from Eq. A-6 for $H = 0$.

$$c_{0.5} = K_m \exp\left[\frac{-b}{2m}(\epsilon_{AA} - \epsilon_{BB})\right], \quad \text{(A-25)}$$

where

$$K_m = \exp\left(-\frac{E + \mu_0}{kT}\right).$$

The other is an expression for the Hill slope of the p_o versus c curve in terms of the variance.

$$n_H = \frac{\partial}{\partial \log(c)}\left(\log\frac{p_o}{1-p_o}\right) = m\frac{\sigma^2}{Ni^2 p_o(1-p_o)}. \quad \text{(A-26)}$$

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