Lyotropic Ion Channel Current Model Compared with Ising Model

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Abstract. Trans-membrane currents in ligand-gated ion channels are calculated in a non-equilibrium, chemically open whole cell system. The model is lyotropic in the sense that dynamics and parameters such as ligand concentration for half-maximal response (scale of response), and threshold for firing in neurons, are nonlinear functions of the reactant concentrations. The derived total current fits recorded data significantly better than those derived from mass action, Ising, and other equilibrium type models, in which the derived response can be displaced from the assessed response by several orders in the ligand concentration. A comparison of the model obtained with an Ising-like model provides a methodology to obtain the non-equilibrium scaling dependence of Ising-like models on the reactant concentrations.

Key words: ion-channel currents, non-local coopeartivity, non-equilibrium neuron firing, drug potency, patch clamp, Lyotropic ion-channel system, Ising model

Ion channels produce and transduce electric signals in living cells and are responsible for a large number of cell regulatory functions and network interactions such as in the immune system and central nervous system. Ion channels are therefore major targets for drug therapy. Their structure and function, for instance, various gating mechanisms and different forms of cooperativity between ion channels, are hence central issues in biological physics. Usually, ligand-gated ion channel currents are described in equilibrium models such as the Hill [1] and Langmuir [2] equations, and thermotropic lattice type theories such as the Ising model [3]. In the present work a ligand-gated ion channel system on a cell membrane is studied in a non-equilibrium model that has been previously proposed for the control of DNA replication and the cell cycle [4, 5].

At least two different levels of interaction can take place in a system of ion-channels: local cooperation between different receptors on the same channel protein, and non-local cooperation between different channel proteins. Local cooperativity is here illustrated by the Hill equation. Let n be the number of receptors

per ion channel protein, r and ρ the concentrations of receptors and ligands. The rate for binding of ligands to receptors is then given by $\partial\psi/\partial t = kr\rho^n - k'\psi$, k' and k being the dissociation and association constants, and $K = k'/k$. The binding is also restricted by the initial constraints, $\rho_0 = \rho + \psi$ and $r_0 = r + \psi$, ρ_0 and r_0 being the initial reactant concentrations. Assuming that dynamical equilibrium prevails, $\partial\psi/\partial t = 0$ hence $kr\rho^n = k'\psi$, the ion channel current is then given by

$$I = I_{\max} \frac{\psi}{r_0} = I_{\max} \frac{\rho^n}{\rho^n + K}, \quad (1)$$

where the slope exponent n is called the Hill coefficient henceforth denoted by n_H . The scale derived in the Langmuir ($n_H = 1$) and Hill ($n_H > 1$) equations, K and $K^{1/n}$, often deviate by several orders from the assessed EC_{50} value [6, 7]. This should not come as a surprise because a response theory derived in an equilibrium system could not be expected to describe non-equilibrium biological systems. As will be shown in this work, the Ising model has essentially the same type of scaling problems, obscuring an interpretation of the macroscopic properties of the system in terms of the behavior of the constituent molecules.

For simplicity it is assumed that there is only one receptor on each ion channel protein, $n_H = 1$, and no local cooperativity between ion channels. In this model, the ion channels are assumed to be opened when a threshold number N of receptors have been liganded. The model is derived in one spatial dimension, assuming that all ion channels are distributed along the x -axis within a finite interval L . The non-equilibrium binding of ligands to receptors then reads

$$\begin{aligned} \frac{\partial\psi}{\partial t} &= k\rho r - k'\psi = k((a - \psi)^2 - (a^2 - b^2)) \\ &= k(1 - \psi/a)^2(a^2 - (a^2 - b^2)/(1 - \psi/a)^2) \end{aligned} \quad (2)$$

where $a^2 = (\rho_0 + r_0 + K)^2/4$ and $b^2 = \rho_0 r_0$ are obtained by inserting the initial constraints. If $k' \ll k$ the solution to Eq. (2) becomes $\ln\{(\rho/r)(r_0/\rho_0) = k(\rho_0 - r_0)t\}$. This relates the time parameter to the reactant concentrations as if all reactants and complexes were homogeneously distributed in space. However, in reality the rate of binding also depends on the spatial distribution of the receptors.

In an equilibrium system the conformational distribution of N liganded receptors could have been defined by $\varphi = \mu_c^{-N} \psi(x_1)\psi(x_2)\psi(x_3) \dots \psi(x_N)$, where ψ is the random distribution of one liganded receptor provided that c is chosen such that $c^{-1} \int \psi dx = 1$. Accordingly, φ also represents the probability to find N liganded receptors at x_i , ($1 \leq i \leq N$). However, at non-equilibrium conditions the concentrations of the reactants depend on a variable normalization. Therefore ψ may represent any finite number of liganded receptors. This problem can be partially solved by choosing c equal to $a \approx (\rho_0 + r_0)/2$, whereby ψ becomes normalized

($0 \leq c^{-1} \int \psi dx \leq 1$) such that it corresponds to one liganded receptor or less if $\rho_0 \geq r_0$.

The non-equilibrium (increasing) binding of ligands to receptors on ion channels therefore requires a variable (time-dependent) conformational distribution that can transit between the terms in the infinite series

$$\varphi = \mu \sum_{n=0}^{\infty} \left(\frac{1}{c}\right)^n \prod_{v=0}^n \psi(x_v) \approx \mu \sum_{n=0}^{\infty} \left(\frac{\psi}{c}\right)^n = \frac{\mu}{1 - \psi/c} \quad (3)$$

where $\psi(x_0) = 1$ accounts for the possibility that N ion channels may have been liganded already from the start. Thus apart from defining the probability to find an increasing number of liganded receptors, φ is also an order parameter. A truncation of the series in Eq. (3) at finite order could imply that less than N ion channels are liganded. The continuous form of φ is a result of L being divided into an infinite number of intervals, hence $\Delta x \approx 0$.

It is also assumed that ion channels are liganded, hence, opened in a given order from one end of the lattice to the other like a travelling wave at a velocity s along the x -axis. This does not affect the dynamics of the model system because the lattice spacing has already been neglected. In this way the system can comply both with the increasing number of liganded ion channels, which is controlled by Eq. (2), and the non-local correlations in Eq. (3). Dividing by s , introducing a non-linear coupling constant $g = (\rho_0 - r_0)/(\rho_0 + r_0)$ and assuming that $K \approx 0$, one can then rewrite the open-rate $\partial\varphi/\partial t = (\partial\varphi/\partial\psi)(\partial\psi/\partial t)$ as the first order equation of motion of a well-known non-linear potential theory [8]:

$$\frac{\partial\varphi}{\partial x} = \frac{1}{v} \frac{\partial\varphi}{\partial t} = \frac{\partial\varphi}{\partial\psi} \frac{1}{v} \frac{\partial\psi}{\partial t} = \frac{k(\rho_0 + r_0)}{2\mu v} (\mu^2 - g^2\varphi^2) \equiv \sqrt{2V(\varphi)}. \quad (4)$$

Since the time-dependent solution to Eq. (4), $\varphi = (\mu/g) \tanh(k(\rho_0 - r_0)t/2)$, is not normalized it cannot guarantee that N ion-channels are liganded/open at finite times. Therefore, in this approximate model N ion channels are assumed to be liganded/opened only after an infinite time, hence $N = \varphi(\infty) = \mu/g$. This also provides a topological type normalization of the order parameter. Since $k(\rho_0 + r_0)/2$ ion channels are liganded per unit time, it follows that $v = dk(\rho_0 + r_0)/2$ and $V(\varphi) = (g^2/2L^2)(\mu^2/g^2 - \varphi^2)^2$, $d = L/N$ being the lattice spacing in the finite order discrete lattice of ion channels.

The free energy density corresponding to the static field solution $\varphi = (\mu/g) \tanh(\mu x L)$ is $(1/2)(\partial\varphi/\partial x)^2 + V(\varphi)$. Unfortunately, $V \neq 0$ at $\varphi = 0$, before any receptors have been liganded. However, as is usually the case in Nature, the system goes to one of its lowest minima, $\varphi_{\text{old}} \rightarrow -N + \varphi_{\text{new}}$, thus breaking the symmetry of V . The asymmetric potential energy $V(\varphi) = (g^2/2L^2)\varphi^2(2N - \varphi)^2$ then

vanishes at $\varphi=0$ as expected. Inserting the relation $k(\rho_0 - r_0)t = \ln\{(\rho/r)(r_0/\rho_0)\}$ in the correspondingly displaced solution, the open probability becomes

$$\varphi(\rho) = \frac{\mu}{2} \frac{\rho_0 + r_0}{\rho_0 - r_0} \left[1 + \tanh \left\{ \frac{1}{2} \ln \left(\frac{\rho}{EC_{50}} \right) \right\} \right] \quad (5)$$

where $EC_{50} = r\rho_0/r_0$ is the ligand concentration for half-maximal response, i.e., the scale of response. Such a system, which depends on the reactant concentrations, is called lyotropic. Let $\varphi_1(\rho) = \varphi(\rho)/N$ be the open probability and i the current per ion channel, the total current becomes

$$I(\rho) = Ni\varphi_1(\rho) = Ni \frac{\rho}{\rho + EC_{50}}. \quad (6)$$

A direct comparison of Eq. (6) with the Langmuir equation, which is obtained from Eq. (1) for $n_H = 1$, reveals a major difference in the scaling parameter. The advantage of the proposed non-equilibrium model becomes apparent, for instance, by comparing Eq. (6) with whole cell currents assessed at different kainate concentrations in an AMPA receptor system expressed on *Xenopus* oocytes [9, 10]. With an initial kainate concentration $\rho_0 = 3.000 \mu\text{M}$ and a stationary spare receptor concentration $r/r_0 = 0.038$, both the derived scale $EC_{50} = 115 \mu\text{M}$ and derived total current (*solid line*) are in satisfactory agreement with the current assessed (*dots*) at various kainate concentrations (Figure 1) [9, 10].

In equilibrium-type models such as the Hill and Langmuir equations, on the contrary, the derived total currents are often displaced by several orders from the assessed values [4, 6], because the EC_{50} value is then essentially defined by the affinity constant K , such as in Eq. (1). For instance, in the case of AMPA receptors, for which $K = 9 \mu\text{M}$, the current derived in equilibrium-type models (*dashed line*) would be displaced by one order of magnitude (Figure 1).

Apart from solving these problems, the non-equilibrium model also changes the neuron's role as automaton. Although the firing still occurs at a critical transmembrane potential threshold, in the actual model this potential threshold is obtained at the definite threshold number N of receptor engagements. The two thresholds are related by $I = Ni = \Delta Q/\Delta T = C\Delta V/\Delta T$, ΔT being the open-time, ΔQ the transported charge, and C the capacitance, through the dependence of N on initial reactant concentrations. Thus, although the potential threshold is still a constant, the firing threshold can be attained at various receptor and ligand concentrations, which is not clear from the potential threshold itself. However this also changes the neuron's role as perceptron, because the output signal will then be high for certain combinations of the initial reactant concentrations and low for others. This appears to be more realistic because not only the number of vacant receptors expressed on the cell membrane may vary from organ to organ but also the initial concentration of ligands may vary from one time to another.

Figure 1. Ion channel currents (dots) induced by AMPA receptors expressed on *Xenopus oocytes*, at various kainate concentrations [8]. The solid line represents the current derived in the non-equilibrium model, and the dashed line is the current derived in equilibrium type models. The current I divided by its maximum value $I_{\max}$ is plotted as a function of the ligand concentration in Molars.

The derived non-equilibrium model illustrates the close connection between non-local cooperation between molecular complexes in a system and its scaling properties. Despite the inclusion of higher order powers of ψ in Eq. (3), the resulting Hill coefficient in Eq. (6) is still one, and thus there is no net contribution from local cooperativity between molecular complexes. The close connection between the Hill coefficient, $n_H > 1$, and local cooperativity, so-called allosteric effects, becomes particularly obvious in the binding of oxygen to hemoglobin. As demonstrated in the Ising model [3], however, the local cooperation between neighboring molecular complexes is more complex than a simple mass-action type power law as described here before.

In comparison with the Hill equation and from a statistical physics point of view the Ising model [3] is usually expected to provide a much more adequate platform to analyze the connection between local cooperativity and slope of response such as in ligand-gated ion channel systems. With N ion channel proteins, each with n binding sites for a ligand, the problem is simplified considerably by assuming that only two states are relevant for the biological function of an ion channel; state C, the

unliganded state, corresponding to closed channel, and state O, the fully liganded state, corresponding to open channel. Assuming that the binding of a ligand to a receptor lowers the energy of the system by $E > 0$, the equilibrium binding constant becomes $K \propto \exp(-E/kT)$. Let ε_{CO} , ε_{CC} , ε_{OO} be the local potential interaction energies between nearest neighbor CO, CC, OO pairs of ion channels in units of kT . The potential energy is then defined by $E_i = kT(\varepsilon_{CO}N_{CO}^i + \varepsilon_{CC}N_{CC}^i + \varepsilon_{OO}N_{OO}^i) - \nu n E$, where N_{ij}^i are the number of pairs of ion channels in the i th configuration and ν is the number of liganded receptors. The grand partition function is then given by

$$Z = \sum_{\nu=0}^N \exp(\nu n \theta / kT) \sum_i \exp(-E_i / kT) \quad (7)$$

where $\theta = \theta_0 + kT \ln(\rho)$ is the chemical potential of the ligand in solution, θ_0 the standard chemical potential of the ligand and ρ the ligand concentration. The open probability for a channel $\varphi_1(\rho)$ in this model is defined by

$$\varphi_1(\rho) = \frac{1}{NZ} \sum_{\nu=0}^N \nu \exp(\nu n \theta / kT) \sum_i \exp(-E_i / kT) = \frac{kT}{nN} \left(\frac{\partial \ln Z}{\partial \theta} \right)_{N,T}. \quad (8)$$

In the case of the one-dimensional Ising model, $\varphi_1(\rho)$ can be reduced to an analytic expression [3] and the total current then becomes

$$I(\rho) = N i \varphi_1(\rho) = \frac{N i}{2} \left[1 + \frac{\sinh(H)}{[\sinh^2(H) + \exp(-4J)]^{1/2}} \right]. \quad (9)$$

The Hill coefficient of this current is $n_H = n \exp(2J)$, $J = \varepsilon_{CO}/2 - \varepsilon_{CC}/4 - \varepsilon_{OO}/4$, $H = n(E + \theta)/(2kT) + \beta(\varepsilon_{CC} - \varepsilon_{OO})/4$ and β the number of nearest neighbours. In the one-dimensional Ising lattice $\beta = 2$. If there is only one receptor per ion channel and only neighboring open ion-channels interact the scale becomes $EC_{50} = K \exp(\varepsilon_{OO})$. Assuming that $n = 1$ and all short-range interaction energies ε_{ij} between neighboring pairs of ion-channels were equal, Eq. (8) would then be reduced to

$$I(\rho) = \frac{N i}{2} \left[1 + \tanh \left(\frac{1}{2} \frac{E + \theta_0 + kT \ln(\rho)}{kT} \right) \right] = N i \frac{\rho}{\rho + \exp[-(E + \theta_0)/kT]} \quad (10)$$

with a scale defined by $EC_{50} = \exp(-(E + \theta_0)/kT)$, which is essentially proportional to the affinity constant K .

When applied to non-equilibrium systems, in which the scale depends on the reactant concentrations, the Ising model will therefore meet the same type of scaling

problems as the Hill and Langmuir equations. The reason is that the actual Ising model, like most other models in condensed matter physics, are based on the assumption of dynamical equilibrium in the chemical sense, implying that the number of particles in the system is constant as assumed in the derivation of the Boltzmann distribution. Only small fluctuations in the particle number are permitted in these so called thermotropic systems. In the actual case Eq. (2) keeps the system far from dynamical equilibrium, making the dynamics of the system dependent on the reactant concentrations. Such a system is called lyotropic.

Therefore, a modification of the chemical potential from θ_0 to $\theta = \theta_0 + kT \ln(\rho)$ is not sufficient to accommodate the actual thermotropic type Ising model to the non-equilibrium, lyotropic conditions that prevail. For instance, as a consequence of the non-equilibrium driving equation, the scale $EC_{50} = r\rho_0/r_0$ in Eq. (6) depends on all reactant concentrations. In equilibrium-type models, this problem is usually overlooked and the derived scale is then simply replaced by the experimentally assessed one, thereby obscuring the interpretation of the macroscopic properties and biological functions in terms of the behaviour of the constituents.

Another problem occurring when the equilibrium-type Ising model is applied to a non-equilibrium system of ligand-gated ion channels is that all numbers N_{ij}^i of pairs of ion channels in the i th configuration, like the total number of liganded receptors in the above derived non-equilibrium model, $N = \mu/g = \mu(\rho_0 - r_0)/(\rho_0 + r_0)$, are continuous functions of the reactant concentrations. In other words, the configurations of the system are steadily changed by Eq. (2). A standard equilibrium-type statistical physics approach, making use of the usual Boltzmann distribution is therefore not directly applicable. Perhaps the method proposed here could provide a first clue to a better understanding of non-equilibrium and statistical properties of inanimate condensed systems, and of living matter and biological functions in particular.

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