

Fluctuation-dissipation theorem imposes high-voltage fluctuations in biological ionic channels

Joaquim Procopio

Instituto de Ciências Biomédicas, Universidade de São Paulo, Caixa Postal 66208, 05508-970 São Paulo, SP, Brazil

José A. Fornés*

Instituto de Matemática e Física, Universidade Federal de Goiás, Caixa Postal 131, 74001-970 Goiânia, GO, Brazil

(Received 20 June 1994)

In order to determine the voltage fluctuations across cell membranes, the theory of generalized susceptibility was applied to a system consisting of a capacitor and a resistor in parallel, resembling a fragment of biological cell membrane harboring an ionic channel. We found, in accordance with others, that the mean square voltage fluctuation is given by $\langle V^2 \rangle = kT/C$, where C is the electrical capacitance of the membrane. When the above equation is applied to different combinations of membrane patch areas and corresponding channels, it is possible to identify a range of membrane areas and channel conductivities where voltage fluctuations may influence the behavior of a putative gate. It appears that for lower channel conductivities high amplitude voltage fluctuations fall in the range of typical gating response times. It is proposed that voltage fluctuations may be included among the many mechanisms influencing gating behavior.

PACS number(s): 87.10.+e, 87.22.Bt

I. INTRODUCTION

Biological ionic channels are microscopic entities capable of transferring ions at a great rate. Most ion channels exist in one of two basic configurations, namely, open or closed. Transitions between channel configurations are mediated by a specialized region of the channel, the gate. Gates can be modulated by chemicals, by voltage, or just be insensitive. In either case, transitions between the various configurations (open, closed, intermediate states) display a stochastic component whose nature is poorly understood [1–3].

Cell membranes, on the other hand, are the substratum upon which channels insert. Since the membrane matrix is basically electrically insulating and separates two electrolytical media, it acts as an electrical capacitor. Channels, inserted alongside the matrix, can then be viewed as leaks upon the membrane dielectric, what leads to the parallel RC equivalent for the channel-membrane system [4,2].

Living cell membranes are usually subject to high electrical fields. A series of processes contribute to maintain a voltage difference of about 60–90 mV across the membrane. This is a time-averaged voltage, easily measurable with special electrodes. At a smaller time scale it is expected, from thermodynamics, that the voltage across the membrane fluctuates. Also, when the dimension is stepped down to molecular sizes, it is not possible to probe into the very vicinity of the membrane. Voltage fluctuations at a molecular scale cannot be measured due both to the unavailability of microscopic probes and to

the response limitation of measuring electronics. Measurement of these fluctuating voltages is also inherently elusive due to the thermal noise of electronic apparatuses. Ionic channels and respective gates, on the other hand, are sufficiently small and fast as to both sense and respond to local fluctuating electrical fields [3,1].

In addition, voltage fluctuations are predicted to exist across the cell membrane. Values of the fluctuating voltages across cell membranes are, however, conflicting in the literature, due mostly to the different theoretical methods employed. Weaver and Astumian [5] have presented a recent calculation of the effects of weak fields upon cells. Oosawa [6] has calculated the magnitude of fluctuating voltages across different points in electrolytical solutions. Such studies point to average fluctuating voltages reaching, in some conditions, the 100 mV mark.

An analysis of the effects of fluctuating electric fields on membrane components has to take into account two main aspects: first, the factors that influence the magnitudes and spectral distribution of voltage fluctuations, and second, the mechanisms permitting the cell to react to such fluctuating fields. In the present work we confine ourselves specifically to the effects of fluctuating membrane voltages on the functioning of an idealized channel and its respective gate.

II. THEORY

We model the ionic channel and the corresponding membrane segment as an RC circuit in parallel (R is the channel electrical resistance and C is the membrane patch capacitance) [4]. In this circuit the relation between the Fourier components of the spontaneous fluctuational current I_ω and voltage, V_ω is given by

*Corresponding author.

$$V_\omega = Z(\omega)I_\omega, \quad (1)$$

where $Z(\omega)$ is the complex generalized impedance of the system as a function of the radial frequency ω . Equation (1) can be written as

$$q_\omega = \alpha(\omega)V_\omega, \quad (2)$$

where $\alpha(\omega)$ is the complex generalized susceptibility ($\alpha(\omega) = -1/[i\omega Z(\omega)]$), q_ω is the Fourier component of the fluctuational charge in the capacitor, and i is the imaginary unit.

The spectral densities of charge fluctuations are given by (see, for instance, Landau and Lifshitz [7]):

$$\langle q^2 \rangle_\omega = |\alpha(\omega)|^2 (V^2)_\omega \quad (3)$$

and the spectral density of the mean square of the fluctuational force (in our case, voltage) is

$$(V^2)_\omega = \frac{\hbar \alpha''(\omega)}{|\alpha(\omega)|^2} \coth \frac{\hbar \omega}{2kT}, \quad (4)$$

where $\alpha''(\omega)$ is the imaginary part of $\alpha(\omega)$, \hbar is Planck's constant divided by 2π , k is the Boltzmann constant, and T is the absolute temperature. Then the mean square of the fluctuating potential is given by the integral

$$\langle V^2 \rangle = \frac{1}{\pi} \int_0^\infty (V^2)_\omega d\omega. \quad (5)$$

For a RC circuit, we have

$$\frac{1}{Z(\omega)} = \frac{1}{R} + i\omega C, \quad (6)$$

$R = R_{ch}R_m/(R_{ch} + R_m)$, where R_{ch} and R_m are, respectively, the electrical resistances of the channel and of the corresponding membrane patch. Consequently,

$$\alpha(\omega) = -C + i \frac{1}{\omega R}. \quad (7)$$

Replacing $\alpha(\omega)$ given by Eq. (7) into Eq. (4) we have

$$(V^2)_\omega = \frac{\hbar \omega R}{1 + (\omega RC)^2} \coth \frac{\hbar \omega}{2kT}. \quad (8)$$

The characteristic frequency $\omega_c = (RC)^{-1}$ (frequency response) of biological channels is less than 10^{11} s^{-1} which permits one to leave out quantum effects and make the approximation $\coth \frac{\hbar \omega}{2kT} \approx \frac{2kT}{\hbar \omega}$ in Eq. 8, namely,

$$(V^2)_\omega = \frac{2RkT}{1 + (\omega RC)^2}. \quad (9)$$

Replacing $(V^2)_\omega$ given by Eq. (9) in Eq. (5) and performing the integration we obtain

$$\langle V^2 \rangle = \frac{kT}{C}, \quad (10)$$

in accordance with Weaver and Astumian [5] and DeFelle [2].

III. RESULTS AND DISCUSSION

Equation (10) was applied to a system consisting of an ion channel and the corresponding patch of lipid bilayer membrane. We consider a typical channel density of 600 channels/ $\mu\text{m}^2 = 600 \text{ channels}/10^8 \text{ \AA}^2$, which is equivalent to $1.66 \times 10^5 \text{ \AA}^2/\text{channel}$ (see the table in Hille [1], for example). This means that a given channel is separated from its neighbors by a distance s of about 400 \AA in the membrane plane and the system consists of a channel embedded in a membrane patch of about 400 \AA wide. We also analyze smaller patches of membrane.

We consider the channel as existing in two basic states, open and closed. The transition between the two configurations is the result of a voltage sensitive gate. The gating "particle" is envisioned as bearing an electric charge and being fast enough to respond to a certain range of fluctuating voltages. No further speculation on mechanisms are made up to this point. We assume the resistance of the open channel to be $R_{open} = 5 \times 10^{10} \Omega$ (for an idealized channel). The system resistance when the channel is fully closed tends to approach the resistance of the bare matrix making the RC time approach the seconds time scale. In order to avoid this extreme we chose $R_{closed} = 5 \times 10^{13} \Omega$, representing a state not fully closed. This values of course can be varied but the choice allows for a preliminary analysis. For each channel configuration (open or closed) we applied Eq. (10) to obtain the mean square voltage fluctuation $\langle V^2 \rangle$ as a function of the membrane patch area A given as s^2 . For each value of the membrane patch area, a relaxation time $\tau = RC$ of the fluctuation is defined. The membrane capacitance associated with the patch harboring the channel is given by

$$C = \frac{\epsilon \epsilon_0 A}{d}, \quad (11)$$

where $A = s^2$ is the area of the small membrane patch associated with the channel, ϵ and ϵ_0 are, respectively, the relative dielectric constant of the membrane ($\epsilon = 2$) and permittivity of vacuum ($\epsilon_0 = 8.85 \times 10^{-12} \text{ c}^2\text{N}^{-1}\text{m}^{-2}$), and d is the membrane thickness. The MKS system of units was employed throughout.

Table I summarizes our results. The first column denotes the square root of the area of the capacitor equivalent to the membrane patch (in \AA). Columns 2 and 3 present the characteristic relaxation times for the open and closed channel, respectively. Column 4 shows the average voltage fluctuation in mV. Column 5 is the capacitance of the system.

The problem of size is of fundamental importance in the present context. Since we are reporting fluctuations across the cell membrane, we consider systems of roughly the same dimension as the membrane thickness, i.e., patches of membrane about 20 – 100 \AA wide, and extend the analysis to about 400 \AA . Since for each size considered there is an associated root mean square voltage fluctuation, it is expected that the channels "sense" fluctuations arising in membrane patches of all sizes.

From examination of Table I it is readily observed that

TABLE I. Relaxation times and amplitudes of voltage fluctuations as related to membrane patch area, $A = s^2$. Values in brackets are exponents of 10.

S (Å)	RC open (s)	RC closed (s)	Voltage fluctuation (mV)	Patch capacitance (F)
20	7.08[-10]	7.08[-7]	540.7	1.41[-20]
40	2.83[-9]	2.83[-6]	270.4	5.66[-20]
60	6.37[-9]	6.37[-6]	180.2	1.27[-19]
100	1.77[-8]	1.77[-5]	108.1	3.54[-19]
140	3.47[-8]	3.47[-5]	77.2	6.94[-19]
180	5.73[-8]	5.73[-5]	60.1	1.15[-18]
240	1.02[-7]	1.02[-4]	45.1	2.04[-18]
280	1.39[-7]	1.39[-4]	38.6	2.78[-18]
320	1.81[-7]	1.81[-4]	33.8	3.62[-18]
400	2.83[-7]	2.83[-4]	27.0	5.66[-18]

as the membrane patch area increases the characteristic time and the voltage fluctuation decrease, for both the open and closed channel configurations. From Table I it is also apparent that both channel states present the same value for the voltage fluctuation for each patch area. What differs in the open and closed states is the characteristic relaxation time. Also, for a given amplitude, voltage fluctuations in the closed state are slower and, as such, closer to the putative relaxation times of the biological gates (see [1], pp. 477 and 494, and [3], p. 1309).

Now, the relevant voltage fluctuations are, in principle, those capable of causing a conformational change in the channel-gate complex. For this to occur the fluctuation needs a minimum amplitude and duration. Since dc voltage changes as low as 20 mV are known to influence gating it is clear from Table I that at least, in what concerns amplitude, voltage fluctuations are capable of influencing the gate. Considering duration, it is expected that fluctuations occurring at frequencies equal to or smaller than the gate characteristic frequency can influence the gate conformation. On the other hand, fluctuations occurring at very high frequencies, despite being of great amplitudes (see Table I), probably do not last long enough to interact significantly with the gate. The range of frequencies is subject to speculation, since there is no data available concerning the mass or size of the

gated region of the channel. It is currently reported that the observed kinetic time constants describing gating are concentrated in the time range from 20 μ sec to 100 ms [1,3]. This means that, according to the data in Table I, there exists a range of membrane patch areas in which the corresponding relaxation times for the closed channel are of the order of the faster gating time constants. For these particular processes the hereby reported fluctuational changes in voltage are both sufficiently high and slow enough as to be important as inducers or modifiers of the gating process. We also observe from Table I that the relaxation times in the closed configuration are longer than those of the open one. One possible scenario in which such a mechanism could operate is in the transition from closed to open and vice versa: suppose that in a given channel the closed state is the one of lower energy. This means that the channel tends to go spontaneously to the closed state. In this state Table I shows that the high amplitude voltage fluctuations present characteristic frequencies well within the gate's own characteristic frequencies. Such fluctuations are in principle capable of interacting with the gate. In the open channel, on the other hand, Table I indicates that high amplitude voltage fluctuations are too fast to interact with the gate. Superimposed on the above fluctuations one also has to consider thermal fluctuations of the channel structure, including certainly the gated region [8]. If we consider the gate as a particle able to swing around some point of the channel superstructure, then the gate should present a constrained type of Brownian movement [9]. As such, we can describe a voltage-dependent gate as being influenced by the following actions: (1) deterministic behavior due to gross time averaged electric field across the membrane; (2) fluctuations in the electric field due to opening and closing of nearby channels [10,11]; (3) fluctuations in the local electric field due to RC behavior of the channel-membrane system (analyzed in this paper); (4) Brownian movement due to thermal interaction between the gating particle and the water molecules and ions.

ACKNOWLEDGMENT

This work was partially supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil).

-
- [1] B. Hille, *Ionic Channels of Excitable Membranes* (Sinauer Associates, Inc. Publishers, Sunderland, MA 1992).
 - [2] L. J. DeFelice, *Introduction to Membrane Noise* (Plenum Press, New York, 1981).
 - [3] P. Lauger, *Physiol. Rev.* **67**, 1296 (1987).
 - [4] A. Finkelstein and A. Mauro, in *Handbook of Physiology: Section 1, The Nervous System*, edited by E. R. Kandel (American Physiological Society, Washington, D.C., 1977), Vol. 1, Pt. 1.
 - [5] J. C. Weaver and R. D. Astumian, *Science* **247**, 459 (1990).
 - [6] F. Oosawa, *J. Theor. Biol.* **39**, 373 (1973).
 - [7] L. D. Landau and E. M. Lifshitz, *Statistical Physics* (Pergamon Press, Oxford, 1988), p. 387.
 - [8] G. R. Welch, B. Somogyi, and S. Damjanovich, *Prog. Biophys. Mol. Biol.* **39**, 109 (1982).
 - [9] R. K. Pathria, *Statistical Mechanics* (Pergamon Press, Oxford, 1988).
 - [10] R. D. Astumian, P. B. Chock, T. Y. Tsong, Y-der Chen, and H. V. Westerhoff, *Proc. Natl. Acad. Sci. USA* **84**, 434 (1987).
 - [11] T. Y. Tsong, *Annu. Rev. Biophys. Biophys. Chem.* **19**, 83 (1990).