The Steady State Stability Criterion of a Simple Model of Calcium Channels

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Abstract. A simple known model of calcium inactivation is described and qualitatively analysed. Stability conditions at the level of a stationary state with respect to some small perturbations in the concentration of Ca^{2+} ions are analysed from the point of view of the Prigogine non-equilibrium thermodynamics. Possible internal fluctuations in Ca^{2+} ion concentration are discussed as connected with fluctuations of the potential energy of interaction between calcium ions and the binding sites.

Key words: Calcium channel — Ca binding sites — Non-equilibrium thermodynamics analysis — Potential energy fluctuations

Calcium is now recognized as an important intracellular messenger and fluctuations in its concentration have been implicated in the regulation of a large number of molecular events (Hagiwara and Byerly 1981). The understanding of the origin of this regulation mechanism is one of the basic problems in biophysical chemistry. To recapitulate, Ca channels play a crucial role in coupling membrane excitation to cellular responses such as secretion or contraction. Unlike Na channels, Ca channels are often modulated by hormones and neurotransmitters. It is known that Ca channels are pores capable of transferring millions of permeant ions per second; their voltage-dependent properties clearly distinguish them from pumps or exchange mechanisms. This unique role of Ca ions as activators and regulators of biological processes is related to their physico-chemical properties which are optimal for interactions with high molecular weight bioorganic substances (Carafoli and Crompton 1978).

Recently Poledna (1989) has developed a simple model of a calcium channel inactivation and has analysed it qualitatively. Here we repeat briefly the principal assumptions of this model which is based on the Brehm and Eckert hypothesis (1978) of an inactivation mechanism depending on calcium concentration at the inner mouth of the calcium channel.

First, surface concentration of calcium, C_i, at the inner side of membrane depends on the diffusion, flow from the outer side of the membrane inside the cell. This transmembrane transport process can be formally described by the kinetic equation

$$w_1 = \frac{dC_i}{dt} = kC_t(C_o - C_i) - C_i \tag{1}$$

where C_i is the number of non-inactivated channels per unit surface of the membrane at time t, C_o is the total extracellular surface concentration of Ca^{2+} ions which is related with intermembrane potential by Boltzmann relationship. Membrane channels are present at rather low densities in membranes, usually no more than 50 or 100 per cell. This implies that, on the average, channels are about 3 μm apart and that they can be treated as a dilute two-dimensional solution.

For simplicity it is supposed that the Boltzmann equilibrium relationship holds at the condition on non-zero fluxes and at existing non-zero transmembrane potential. We introduce the formal specific rate constants k and $\varkappa$ that control the activity of working channels (C — closed channel), described simply as

$$C + \text{Ca (outer)} \underset{\varkappa}{\overset{k}{\rightleftharpoons}} \text{Ca (inner)} \tag{2}$$

Since calcium ions are divalent, they rapidly bind to negative charges within the cell and only a relatively small amount of them is actually free inside the channel.

Second, a depolarizing voltage step opens the channels in the surface unit of a membrane. Calcium can be bond on the channel at the inner side of the membrane and block it. The inactivation of the Ca^{2+} channels by Ca^{2+} can be represented as

$$C + \text{Ca (inner)} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} C^* \tag{3}$$

The system is assumed to be in a stationary state, the total surface concentration of channels is C_{tot}, C^* stands for the surface concentration of inactivated channels, k_f is the specific rate constant for the forward reaction of Ca^{2+} binding to the channel, and k_r is the specific rate constant of the reverse reaction, release of Ca^{2+} from the receptor. (The asterisk denotes inactivated state.) The rate equation corresponding to the Ca^{2+} inactivation kinetics scheme (3) can be written as

$$w_2 = \frac{dC_i}{dt} = k_r(C_{tot} - C_i) - k_f C_i C_i \tag{4}$$

assuming Eq. (5) to hold for C_{tot}

$$C_{tot} = C_t + C^* \tag{5}$$

The same symbols are used for the chemicals and the individual channels as well as their concentrations (C_{tot}, C_o, C_t, C_i).

It should be stressed that the above model is rather a combination of the diffusion transport kinetics represented by the quasichemical non-linear Eq. (1) and the non-linear kinetics Eq. (4). The presented model of the calcium channel kinetics (with the kinetic scheme introduced) represents a dissipative structure isomorphic to the Lotka — Voltera type of autocatalytic equations. The kinetic scheme of the functioning of the calcium channel, proposed by Poledna will be analysed elsewhere.

From the non-linear thermodynamics it is known that under certain conditions autocatalytic reactions or, more generally, reactions involving non-linear steps, tend to destabilize the system. The distance from equilibrium and the nonlinearity may both be the sources of order capable of driving the system to an ordered configuration. Naturally, the question arises whether the kinetics model of Ca channel (Poledna 1989) is stable with respect to some small perturbations in the concentration of intracellular Ca^{2+} ions. The purpose is to investigate this situation. We do this by the help of nonlinear thermodynamics of Prigogine (Nicolis and Prigogine 1971).

The principal variable which determines the stability of a thermodynamic system is the excess entropy production. In the case of chemical reactions occuring in an isothermal-isobaric system, the condition of stability of a nonequilibrium steady state would be as follows

$$\frac{\partial}{\partial t}(\delta^2 S(t)) > 0, \qquad \mathrm{d}T = \mathrm{d}P = 0 \tag{6}$$

i.e., the time derivative of the second differential of entropy ($\delta^2 S(t)$ which is $\delta^2 S(t) < 0$) corresponding to virtual change is greater than zero. It can be shown by thermodynamic arguments that

$$\frac{\partial}{\partial t}\frac{1}{2}(\delta^2 S(t)) = \int \mathrm{d}V \sum_k \delta w_k \, \delta(A_k/T) = \\ = \text{excess entropy production} = \delta P \tag{7}$$

Accordingly in the neighborhood of the steady state the excess entropy production per unit volume can be expressed as

$$\sum_k \delta w_k \delta(A_k/T) \geqslant 0 \tag{8}$$

Here, δw_k and $\delta(A_k/T)$ are the deviations of the rate w_k and affinity A_k/T from the reference state. In our case regard now the nonequilibrium steady state as

the reference state,

$$\begin{aligned} w_k &= w_{ok} + \delta w_k \\ A_k/T &= A_{ok}/T + \delta(A_k/T) \end{aligned} \tag{9}$$

where w_{ok} and (A_{ok}/T) respectively refer to the flux and force in this situation. If we perturb the steady state by perturbing only one of the forces A_m/T, the inequality is obtained

$$\delta w_m \delta(A_m/T) \geqslant 0 \tag{10}$$

which shows that the perturbation w_m and the perturbation (A_m/T) always have the same sign. The next step is to apply these ideas to the simple Poledna model of inactivation of calcium channel.

Stability criterion of excess entropy production

The system is taken to be isothermal and with no convective motion. We introduce the chemical affinities (11) and (12) of the process

$$A_1 = RT \ln \left\{ \frac{k}{\varkappa} \frac{C_t(C_o - C_i)}{C_i} \right\} \tag{11}$$

$$A_2 = RT \ln \left\{ \frac{k_r}{k_f} \frac{(C_{tot} - C_t)}{C_t C_i} \right\} \tag{12}$$

where R is the gas constant and T the temperature of the system. We consider that C_{tot}, C_o, C_t, T are maintained constant in time (and also k, $\varkappa$, k_f, k_r = const), so that only one independent variable, C_i, is left.

The corresponding perturbations in the chemical affinities and the reaction rates can be written as

$$\begin{aligned} \delta w_1 &= -(kC_t + \varkappa)\,\delta C_i \\ \delta A_1 &= -RT\left(\frac{1}{C_o - C_{oi}} + \frac{1}{C_{oi}}\right)\delta C_i \\ \delta w_2 &= -k_f C_t \delta C_i \\ \delta A_2 &= -RT\frac{1}{C_{oi}}\delta C_i \end{aligned} \tag{13}$$

where C_{oi} is the stationary nonequilibrium intracellular concentration of Ca^{2+} ions provided that the conditions allow simultaneous equilibrium of both reaction (2) and (3)

$$kC_t(C_o - C_{oi}) = C_{oi}$$
$$k_r(C_{tot} - C_t) = k_f C_t C_{oi} \tag{14}$$

It follows that reasonable value of C_{oi} is

$$C_{oi} = -\alpha/2 + (\alpha^2 + 4\beta)^{1/2}/2 \tag{15}$$

where

$$\alpha = \frac{(\varkappa - kC_{tot})k_r}{k_f \varkappa}, \quad \beta = \frac{kk_r C_o C_{tot}}{k_f \varkappa} \tag{15a}$$

Applying the stability criterion (8), it follows that the stationary nonequilibrium state becomes (and stays) asymptotically stable as

$$\sum_k \delta w_k \delta(A_k/T) =$$
$$= \frac{R}{C_{oi}}\left[(k(C_{tot} - C^*) + \varkappa)\frac{C_o}{(C_o - C_{oi})} + k_f(C_{tot} - C^*)\right](\delta C_i)^2 > 0 \tag{16}$$

reference state for $t \geqslant t_0$. This condition warantees that the fluctuations cannot drive the calcium channel away from the steady state in the linear regime: i. e., the entropy production $P = dS_i/dt$ cannot increase with time so that the state of minimum entropy production is indeed stable under the above conditions of calcium channel operation.

Origin of fluctuations

The analysis outlined above has been based on a deterministic causal description provided by the equations of chemical kinetics. However there exist a number of instances in calcium channels where such a description may not be adequate. The main reason is that the very existence of many degrees of freedom in channels automatically implies the appearance of fluctuations in intracellular Ca^{2+} ion concentrations. Our task is now to understand how fluctuations in the calcium channels arise.

The introduced macroscopic description of the calcium channels has been based on the concentrations of free or bound binding sites, further on the concentrations of free and bound calcium ions and a restricted number of other variables, such as temperature and pressure. A given macroscopic state of calcium channel is always associated with rapid transitions between different Ca^{2+} ion states. For example, in the model proposed for the action of calcium in muscle it has been supposed that calcium is initially rapidly bound by an electrostatic mechanism with a binding constant of $10^3 - 10^4\,mol^{-1}.l$, followed by a relatively slow reaction with an equilibrium constant of $10^3 - 10^2$, giving

an overall binding constant of $10^6 - 10^7 \text{ mol}^{-1}.1$ (Ashley and Moisescu 1972). As a result, the macroscopic variables are subject of deviations around certain „reference" values correspond to the results obtained experimentally with macroscopic devices. These deviations appear to the observer as random molecular events and are precisely the fluctuations in binding sites and calcium concentrations as mentioned by Hagiwara and Byerly (1981).

R denotes the characteristic linear dimension of the calcium channel (i. e. the channel radius) and $\bar{r}$ denotes the average distance between Ca^{2+} ions in the channel. Then the number of calcium ions in the whole volume of a channel is $N \sim (R/\bar{r})^3$. Particularly interesting is the case when $\langle N \rangle = N \rangle\rangle 1$. The magnitude of the most probable fluctuations of potential energy V of Ca^{2+} ions are related by

$$V = \langle V \rangle_{eq} + \delta V, \quad \langle \delta V \rangle = 0 \tag{17}$$

where

$$\delta V = V - \langle V \rangle_{eq},$$

are fluctuations of the potential energy of calcium ions in calcium channel and we suppose that $\langle \delta V \rangle = 0$. Note that the validity of the central limit theorem automatically imposes an order of magnitude for the variance of the fluctuations relative to the mean value. Indeed in the case of stochastic potential energy variable V

$$\langle V \rangle = N \langle v \rangle \tag{18}$$

when N now is related to the size of the Ca channel and $\langle v \rangle$ is the potential energy of an individual Ca ion. According to the central limit theorem the variance $\langle \delta V^2 \rangle$ is bound to be of order $\sigma^2 N$, that is,

$$\langle \delta V^2 \rangle \propto \langle V \rangle \tag{19}$$

Alternatively, the order of magnitude of the most probable fluctuations V are related to $\langle V \rangle$ by

$$\delta V \propto \langle V \rangle^{1/2} \propto N^{1/2} \tag{20}$$

The relative importance of fluctuations in Ca^{2+} ion concentrations, therefore, diminishes as the size of the Ca channel increases:

$$\frac{\delta V}{\langle V \rangle} \sim \frac{\delta V}{N} \propto \frac{1}{N^{1/2}} \propto \xrightarrow[N \to \infty]{} 0 \tag{21}$$

As mentioned above calcium ion bind by an electrostatic mechanism to the binding sites. However, except in high accumulation of calcium ions inside a channel the number of calcium binding sites generally exceeds the average

number of bound calcium ions, so that there are many possible configurations of the calcium ions, differing little in potential energy, among which fluctuations may occur as the result of thermal motion. Similar fluctuations may occur in the configurations of other ions bound to the binding sites, when the number of binding sites exceeds the average number such ions which are bound. Fluctuations in the number and configuration of the mobile ions impart fluctuating charges and fluctuating electric multipole moments to the system, which would not exist between static constellations of electric charge. In these conditions chaotic translational motion of free calcium ions generate a stochastic electromagnetic field, which gives rise to fluctuations of the calcium ions in the channel.

By physical means at the stationary nonequilibrium state the potential energy of interaction between the Ca^{2+} ion and the binding site (e. g. carboxylic group) V is kept constant on the average, leaving inevitable fluctuations around this fixed average value $\langle V\rangle$, adequately described by equation (17). Now, it is natural to anticipate that under the described conditions the mean square deviation of the potential energy V of calcium ions is

$$V \approx \langle V\rangle + \langle V\rangle \left(\frac{\bar{r}}{R}\right)^{3/2}, \left(\frac{F}{R}\right)^{3/2} \ll 1 \tag{22}$$

which is the heart of our considerations. From this relation it follows that if the distance $\bar{r}$ between calcium ions increases, e. g., if the concentration of calcium in the channel decreases, also fluctuations of V increase. Fluctuations of V, although measurable, might be expected to remain small compared to the macroscopic value $\langle V\rangle$. However as the volume of the calcium channel is limited fluctuations of δV in the calcium channel may be accompanied by long-range fluctuations.

Now we briefly make an estimate of the potential energy fluctuations of the calcium channel. Information about the total number of functional calcium channels in a membrane patch or whole cell comes along with estimates of the unitary current. Estimates of channel density range from (5—15) μm^{-2} (or (1/5—1/15) μm^2 per channel) in cromaffin cells, up to (30—60) μm^{-2} (or (1/30 − 1/60) μm^2 per channel) in snail neurons (Krishtal et al. 1981). From the introduced data we can estimate the probable value of the channel radius R ($(R \in (2.523 - 1.457).\ 10^{-7}$m for chromaffin cells and $R \in (1.030 - 0.728).\ 10^{-7}$m for snail neurons). If we consider the two Ca^{2+} -occupied sites rigidly separated by $r = 1.15$ nm, then the deviation $(V - \langle V\rangle)/\langle V\rangle$ corresponds to $(3.077 - 7.012).\ 10^{-4}$ for chromaffine cells and $(1.179 - 1.983).\ 10^{-3}$ for snail neurons. For the chosen value of $r = 5.00$ nm, the deviation $(V - \langle V\rangle)/\langle V\rangle$ acquires a value of $(2.789 - 6.359).\ 10^{-3}$ for chromaffine cells and $(1.069 - 1.7980).\ 10^{-2}$ for snail neurons. From the presented data it can be

deduced that the effect of fluctuations in charge configurations can influence ionic transport processes and the functioning of calcium channels (Kostyuk and Mironov 1982).

Let us return to the properties of the calcium channels. The passage of ionized calcium into the cell through selective, voltage gated, channels in the surface membrane is an important and widespread phenomenon in excitable tissues. The entry of the calcium is significant because this ion can act as an intracellular „messenger" or regulatory agent controlling a variety of cell functions that include exocytoses, motility, enzyme activity, membrane conductance, etc. The different cell functions are dependent of the stability of the stationary nonequilibrium state under which calcium channels work. The aim of this contribution was to point to the coherence of the ionic transport processes and functioning of calcium channels under the stability conditions.

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