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Ionic Currents Generated by Voltage-Dependent Channels.

B. DENET and P. PELCE

Université de Provence-St Jérome, Biophysique, Case 252
13397 Marseille Cedex 20, France

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Abstract. – Early stages of growth of many cells are associated with the development of ionic currents flowing through the cells. Previous works suggest that the lateral mobility of pumps or channels in the plasma membrane can lead to such amplification. We show that the voltage dependence of the influx of these pumps and channels is sufficient to amplify them. We perform a numerical integration of the electrodiffusion equations for the ions and the Poisson equation for the electric potential in and outside a circular cell, with boundary conditions at the plasma membrane determined by the *I-V* curves of the relevant pumps and channels. When the ions considered are potassium and calcium ions, the cell is shown to amplify initial disturbances when its size exceeds a critical value (1 to 100 μm). Then, on the diffusive time scale, disturbances organize in a loop, with current intensity of the order of 100 pA.

Early stages of growth of many animal and vegetal cells are associated with the development of ionic currents flowing through the cells [1-4]. These currents can play an important role in the further differentiation or morphogenesis of the cells since the polarity of the growing cells often follows the initial polarity of the currents. These transcellular currents, which are largely controlled by calcium ions, have a magnitude of the order of 100 pA. Their origin and role in establishing and maintaining cell polarity are still unclear. The purpose of this paper is to analyse a mechanism which, in a given cell, spontaneously generates non-uniform membrane potential and ionic concentrations. This mechanism involves the sensitivity of the pumps and channels which maintain the membrane potential to concentration and membrane potential variations. Because of the sensitivity of pumps and channels, an asymmetric perception of a signal (light, electric field) causes membrane potential and ionic concentration modulations. These modulations generate an asymmetric transmembrane transport of the various ionic species, and currents begin to flow. These currents generate electric fields and concentration fluxes which can reinforce the initial modulations and thus maintain steadily flowing currents. In a previous study [5] we show that, following this hypothesis, and under some conditions involving the derivatives of the pumps and channel currents with membrane potential, cells can maintain non-uniform

membrane potential and thus generate ionic currents. We analyse here the characteristics of these currents.

For simplicity, we assume the cell plasma membrane to be a circle which limits intra and extracellular media. We expect that the cell geometry (a sphere for the fucus egg) influences the dynamics analysed here only qualitatively. In and outside the cell, we integrate the electrodiffusion equations for the ions (potassium of concentration c_1 and calcium of concentration c_2) and the Poisson equation for the electric potential which govern the dynamics of the ionic disturbances. At the cell boundary, concentration and potential gradients are determined as a function of the transmembrane currents [5, 6]. The dependence of these currents on membrane potential and ionic concentrations can be obtained from the *I-V* curves of the corresponding pumps and channels. As suggested by the composition of the currents flowing through the focus egg [7], we consider the case where potassium and calcium ions are relevant for the dynamics. For technical reasons associated to the numerical scheme, we assume the same coefficients of diffusion D for the ions in and outside the cell. This is not the case in experiments since diffusion coefficients are expected to be smaller in the cytoplasm than in a usual liquid. However, we expect that this approximation does not weaken the qualitative predictions described in the following. As the Debye length is much smaller than the cell size, electroneutrality of the intra and extracellular media is satisfied ($z_1 c_1 + z_2 c_2 = \text{const}$). It follows that the dynamics is driven by the diffusion equation for one ion of concentration $c = c_1$, for instance:

$$\frac{\partial c}{\partial t} = D \Delta c , \tag{1}$$

coupled to the Laplace equation for the electric potential,

$$\Delta \Phi = 0 , \tag{2}$$

by the conditions applied at the cell boundary ($r = R$):

$$J_{1,2} = -D \left(\frac{\partial c_{1,2}}{\partial r} + \frac{z_{1,2} e}{kT} c_{10,20} \frac{\partial \Phi}{\partial r} \right). \tag{3}$$

Here, the currents J_1 and J_2, determined by Patch-Clamp experiments, are functions of the membrane potential $\delta\Phi$ and the ionic concentrations c_1 and c_2, $z_{1,2}$ are the charge numbers ($z_1 = 1$, $z_2 = 2$) and c_{10}, c_{20} are the uniform steady ionic concentrations. The transmembrane currents are taken here as $J_1 = J_{K^+} = \delta\Psi(\gamma_e \exp[-\delta\Psi] - \gamma_i)/(\exp[-\delta\Psi] - 1)$ [8] for the potassium ion as is usual for a passive transport at constant field, and $J_2 = J_{Ca^{2+}} = -th(\delta\Psi + 1)$ for the calcium ion. The zero calcium current corresponds to an equilibrium potential $\delta\Psi = -1$. The last expression approximates the *I-V* curve shape in the domain of membrane potential where the influx increases when the membrane depolarises [7]. $\delta\Psi$ is the dimensionless membrane potential $(e/kT)\delta\Phi$, $\gamma_e = c_e/c_{e0}$ and $\gamma_i = c_i/c_{e0}$ the dimensionless external and inner concentrations. Thus the results obtained from the numerical simulation are essentially qualitative.

We work in polar coordinates (r, θ) and use finite-difference methods in the r-direction and Fourier spectral methods in the θ-direction. As it is not possible to have an infinite computational domain in the r-direction, we will limit ourselves to work in an interval $[0, r_{max}]$. The boundary conditions at $r = r_{max}$ will be that the mean value of the potential and concentrations will be forced to have their values at infinity, whereas the other Fourier modes will have a zero slope at $r = r_{max}$. These boundary conditions are applied at finite distance, however the results obtained are supposed to be in qualitative agreement with the

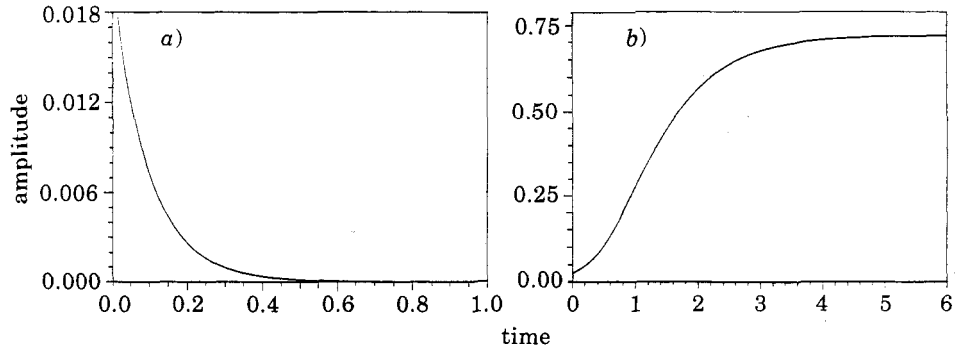

Fig. 1. – Time evolution of the amplitude of the first harmonic ($n = 1$) of the disturbance of the outer concentration at the cell boundary ($r = R$). *a*) $R < R_c$. The initial amplitude of the disturbance decays on the diffusive time scale R^2/D. *b*) $R > R_c$. The initial amplitude of the disturbance increases on the same diffusive time scale and finally saturates to a finite value.

behaviour of the equations in an infinite domain. The previous equations are linear partial differential equations in two domains, inside and outside the cell, connected by non-linear boundary conditions at the membrane. An inner iteration procedure is used to solve the non-linear system that arises at each time step.

The uniform steady state is first obtained. Concentration and potential jumps at the plasma membrane satisfy the condition that the transmembrane current of each ion vanishes. Then we consider the dynamical evolution of small disturbances of the uniform state.

Using the fact that, in the range of steady membrane potential measured in practice, the calcium influx increases when the membrane depolarizes [7], we observe two possible dynamics, depending on the cell size. We consider an initial disturbance where ionic concentration and electric potential vary proportionally to $\cos\theta$. For a cell size smaller than a critical value R_c, the initial disturbance decays on the diffusive time scale R^2/D (fig. 1*a*)) and the uniform steady state is finally obtained. For a cell size larger than R_c, the disturbance grows on the same time scale (fig. 1*b*)) and organizes in a well-defined loop where currents enter on one side of the cell and leave the opposite side (fig. 2).

The critical size

$$R_c = -\frac{Dc_0}{\frac{kT}{e}\frac{\partial J_{Ca^{2+}}}{\partial(\delta\Phi)}}, \tag{4}$$

where c_0 is a ionic concentration and $\delta\Phi$ the membrane potential, is determined by a linear stability analysis of the uniform state when the disturbance of the calcium current is assumed to be dominant. It corresponds a to a cell size for which disturbances neither grow nor decay [5]. It is remarkable that only a few quantities are involved in this formula: a derivative of a ionic transmembrane current with respect to the membrane potential (or, if sensitivity with ionic concentration is involved, a derivative with respect to the relevant concentration), a coefficient of diffusion and a ionic concentration.

For a quantitative evaluation of eq. (4), Dc_0 must be replaced by $D_{Ca^{2+}i}c_{0i}$ [5], where $D_{Ca^{2+}i}$ is the cytoplasmic coefficient of diffusion of the calcium ion and c_{0i} the cytoplasmic concentration of the potassium ion. It is well known that the calcium ion diffuses more slowly in the cytoplasma than in an ordinary liquid phase. One can expect that the cytoplasmic

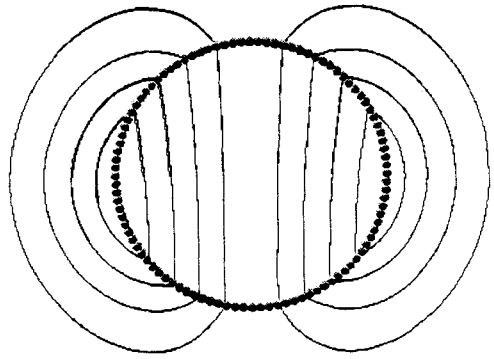

Fig. 2. – Steadily flowing currents corresponding to the final state of fig. 1*b*). The lines of force of electric fields are drawn. The currents enter the cell at the top and leave it at the bottom. Contrary to the patterns usually observed in experiments [1], lines slightly converge towards the bottom of the cell. We expect this effect to be associated to the particular dependence of the current on the membrane potential.

coefficient of diffusion of the calcium ion is 10^{-1} smaller than in an ordinary liquid phase so that $D_{Ca^{2+}i} \approx 10^{-7}\,cm^2/s$. Thus, to evaluate an order of magnitude of the critical wave number, consider $D = 10^{-7}\,cm^2/s$, $c_0 = 10^{-2}$ mol/l. A typical value for $(kT/e)\,\partial J_{Ca^{2+}}/\partial(\delta\Phi)$ can be obtained from the *I-V* curve of a calcium channel of *Fucus serratus L.* [7]. Such a channel is activated above -35 mV and disactivates slowly. From -35 to 0 mV, accessible range of membrane potential during the transient phase which follows the fertilization, the peak inward current density increases by $10\,\mu A/cm^2$. As $kT/e \approx 25$ mV, one evaluates $(kT/e)\,\partial J_{Ca^{2+}}/\partial(\delta\Phi) \approx 10^{-6}$ mole/m^2 s. Then, $R_c \approx 10\,\mu m$, which is of the order of the cell size. For a cell of 30 μm, the associated diffusion time is $R^2/D \approx 10^2$ s.

The proposed mechanism must be compared to a previous one proposed by Jaffe *et al.* [3] and calculated by Larter and Ortoleva [6] based on the mobility of channels. From the observation that Con*A* receptors can be displaced by an external electric field [9], they deduce that a similar effect could occur for mobile charged channels [10]. An amplifying effect can effectively occur on a diffusive time scale R^2/D_m, where D_m is the coefficient of diffusion of membrane bound particles when the channels are assumed to carry an external negative charge [6]. After this typical time, a steady state is reached [11]. As $D_m \approx 10^{-9}\,cm^2/s$, $R^2/D_m \approx 10^4$ s for a cell size of 30 μm.

The equations for the dynamics of the disturbances are linear. Thus the only effect which can saturate the growing disturbances is the non-linear dependence of the transmembrane currents with membrane potential or ionic concentrations. Furthermore, the final intensity of the corrents must be related to the scale of the currents given by the *I-V* curve of the calcium channel, *i.e.* 100 pA if one considers again the cell of *Fucus serratus L.* [7]. This is the order of magnitude of the currents measured in experiments.

In summary we have analysed a new mechanism for the generation of ionic currents flowing in growing cells. This mechanism is based on the voltage dependence or concentration dependence of the pumps and channels which sustain the membrane potential. When calcium and potassium ions are considered, this mechanism can generate currents on a time scale of some minutes when the cell size exceeds 100 μm as an order of magnitude. Amplification of the currents saturates when the intensity reaches the order of 100 pA. This particular example could be well adapted to the generation of currents in *Fucus serratus* fertilized zygotes [7].

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