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Self-focusing of ion channels in cell adhesion

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Accumulation of ion channels in a cell membrane may be triggered by the flow of current through the channels if the membrane is closely attached to a surface and if the channels are electrophoretically mobile. Using the Smoluchowski-Kelvin equations to describe the channel density and the voltage in the cleft between membrane and surface, it is shown that this process may occur for parameters which may well be realized in a tissue or in cell culture.

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INTRODUCTION

We consider a cell (e.g., neuron, glial cell, granulocyte, amoeba, fibroblast) which is in close contact to a surface or to another cell (Fig. 1). We assume that there are ion channels in the cell membrane of the adhesion site and that current is flowing through them from the bath into the cell. This current has to pass the cleft between surface and membrane such that a drop of the voltage is built up there, i.e., a lateral electrical field is created which points towards the center of the adhesion site. If the channels are electrophoretically mobile, the radial field drives the channel molecules towards the center of the region of adhesion. This redistribution gives rise to a change of current flow and of the electrical field. The feedback of channel redistribution and of voltage relaxation leads to an accumulation of the channels in the center. In order to judge whether this simple process of cellular morphogenesis is feasible under realistic conditions of cell adhesion, we compute the coupled dynamics of the channel density and of the voltage profile in the cleft between membrane and surface on the basis of the Smoluchowski-Kelvin equations [1].

SMOLUCHOWSKI-KELVIN EQUATIONS

The density of channel molecules $n(x,y,t)$ in the membrane (Cartesian coordinates x,y, time t) changes by diffusion and by electrophoresis in the gradient of extracellular voltage $V(x,y,t)$. With the diffusion coefficient D, the mobility D/k_BT (k_BT thermal energy) and the electrophoretic charge ze_0 (e_0 elementary charge) we obtain

$$\partial_t n = D\nabla\left(\nabla n + \frac{ze_0}{k_BT} n\nabla V\right). \tag{1}$$

Kirchhoff's law of current conservation for every area element of the cleft determines the extracellular voltage. It leads to the two-dimensional analog of Kelvin's cable equation [2] [Eq. (2)] with the distance d between membrane and surface assumed to be constant, with the specific resistance ρ of the extracellular medium and with the channel conductance Λ,

$$\nabla^2 V = \frac{\rho\Lambda n}{d}(V - V_I + V_0). \tag{2}$$

The intracellular voltage V_I is held more negative than the reversal voltage V_0 of the channels such that current flows into the cell. The Kelvin equation is used in its quasistationary form because the relaxation of the voltage $V(x,y,t)$ is by orders of magnitude faster than the redistribution of the channel density $n(x,y,t)$ [1]. We study a circular site of adhesion with a radius r_0. We restrict the motion of the channels to this region and keep the voltage at the periphery at bath potential with $V(r_0)=0$.

The Smoluchowski and the Kelvin equations together describe a coupled system of nonlinear dynamics. For a numerical treatment we refer the channel density to the average density $\bar{n}$—introducing the normalized variable $N=n/\bar{n}$—and we refer the voltage to the thermal energy—introducing the normalized variable $U=Vze_0/k_BT$. We scale space and time by the length constant $\lambda=\sqrt{d/\rho\Lambda\bar{n}}$ and by the time constant $\tau=\lambda^2/D$, respectively, using the coor-

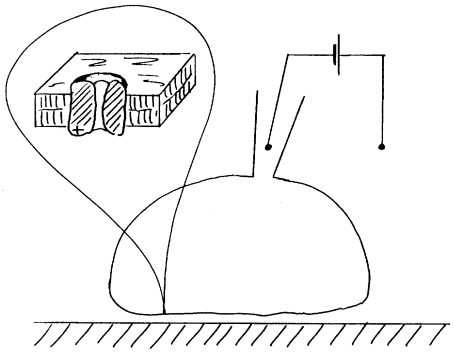

FIG. 1. Cell adhesion on a planar surface. The distance between the cell membrane and the surface is d with a specific resistance ρ of the extracellular medium. The intracellular voltage V_I may be controlled by a patch-clamp electrode. In the membrane of the adhesion region (inset) there are ion channels at an average density $\bar{n}$. These protein molecules are characterized by their ionic conductance Λ, by their reversal voltage V_0 of Ohmic current, by their diffusion coefficient D of lateral motion and by their effective charge number z of lateral electrophoresis.

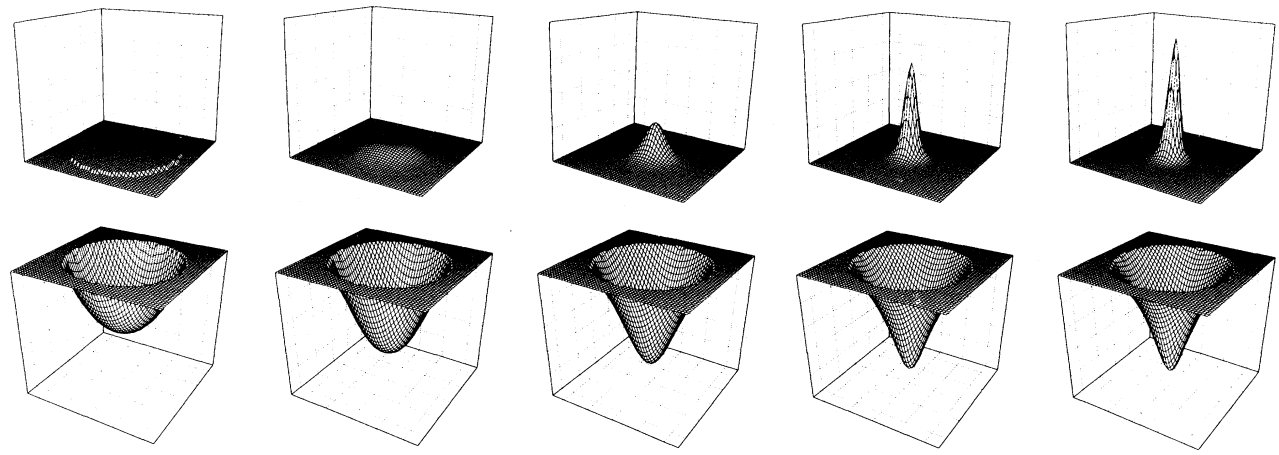

FIG. 2. Self-focusing. The density of ion channels in the membrance (top row) and the voltage in the cleft between membrane and surface (bottom row) is shown at five stages from left to right. The values of the scaled time are $T=0$, 0.1, 0.2, 0.4, and 0.8. The scaled radius of the circular region of adhesion is $R_0=2$. The ordinates of the normalized channel density and of the normalized voltage are drawn from $N=0$ to 40 and from $U=0$ to -10, respectively. The initial normalized channel density is $N=1$. The motion of the channels is restricted to the attachment site (Neumann condition). The voltage in the surround is kept at $U=0$ (Dirichelet condition). The drive parameter (scaled difference of intracellular voltage and reversal voltage) is $\hat{U}=-10$.

dinates $X=x/\lambda$ and $Y=y/\lambda$ and the normalized time $T=t/\tau$. We obtain Eqs. (3) and (4) which are controlled by a "drive parameter" $\hat{U}=(V_I-V_0)ze_0/k_BT$ of the scaled difference of intracellular voltage and reversal voltage,

$$\partial_T N=\nabla(\nabla N+N\nabla U), \tag{3}$$

$$\nabla^2 U=N(U-\hat{U}). \tag{4}$$

The constraints at the periphery with scaled radius $R_0=r_0/\lambda$, are $U(R_0)=0$ (Dirichelet condition) and $\vec{R}\cdot\nabla_{XY}N=0$ (Neumann condition), respectively. We integrate the Smoluchowski equation by a Euler-forward algorithm. After each time step the stationary Kelvin equation is solved by an algorithm of simultaneous overrelaxation [3].

SELF-FOCUSING

As an example we consider an adhesion site with a radius $r_0=10\ \mu$m with a distance $d=10$ nm between membrane and interface and a specific resistance of the electrolyte $\rho=100\ \Omega$ cm. The extracellular voltage at the periphery is $V(r_0)=0$ mV. The intracellular voltage is $V_I=-100$ mV. We assume that there exist open cation selective channels in the region of adhesion with a reversal voltage $V_0=0$ mV and a conductance $\Lambda=20$ pS [4] at an average density $\bar{n}=20\ \mu\text{m}^{-2}$ [4]. (Specific membrane conductance $\bar{g}=40$ mS/cm^2.) The length constant is then $\lambda=5\,\mu$m and the scaled radius is $R_0=2$. We assume that the channels are mobile with a diffusion coefficient $D=0.1\ \mu\text{m}^2/\text{s}$ [5]. The time constant is then $\tau=250$ s. We assume a number of electrophoretic charges $z=2.5$ [4] such that the drive parameter at room temperature is $\hat{U}=-10$.

The result of the numerical integration is shown in Fig. 2 for a time interval $T=0.8$. We start with a homogeneous distribution of channels. Due to current flow the initial voltage profile has a typical shape of a hanging rope. The channels move in the primary electrical field towards the center. This accumulation leads to a sharpening of the voltage profile. Mutual feedback of the dynamics of channels density and of voltage leads to a focusing of the channel in the center of the attachment site. In the stationary state the density of the channels is by a factor 35 higher than in the original situation. The average distance of the channel molecules is reduced to about 40 nm.

For the sake of simplicity the computation disregards the diffusion of channels from the surround into the adhesion site and also the current through other membrane conductances. The first effect would lead to further enhancement of channel density on a longer time scale [6] and the second effect could give rise to periodic patterns [1,6].

The computation of self-focusing in cell adhesion as shown in Fig. 2 may be applied to two other situations: (i) To a postsynaptic membrane separated by the narrow synaptic cleft from the presynaptic terminal [6,7], and (ii) to an artificial membrane spanned over a shallow well in a solid support [8]. (The shallow well models the extracellular cleft). In the case of a synapse with its small diameter we would need a higher primary density of open channels to attain a sufficiently short length constant. In the case of the large artificial system the process would take a long time.

Self-focusing could be visualized by fluorescence using labeled proteins or voltage sensitive dyes [9]. The voltage profile could also be detected directly by a set of transistors beneath the membrane [10]. The process can be controlled by imposing an appropriate intracellular voltage by a patch-clamp electrode (Fig. 1).

CONCLUSION

Self-focusing of ion channels is a process which may occur in a situation of cell adhesion for reasonable values of the pertinent parameters, provided that there exist open and mobile ion channels, of course. It is the intention of this paper to

point out that this morphogenetic process may well be effective in a biological situation. Whether it is really of biological relevance—or whether it is slowed down or avoided in real situations by immobilization of the membrane proteins—has to be investigated. On the other hand it is suggestive to consider controlled self-focusing as an experimental tool to trigger aggregation and 2D crystallization of ion channels in a membrane.

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