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Extrusion of transmitter, water and ions generates forces to close fusion pore

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ABSTRACT

During exocytosis the fusion pore opens rapidly, then dilates gradually, and may subsequently close completely, but what controls its dynamics is not well understood. In this study we focus our attention on forces acting on the pore wall, and which are generated solely by the passage of transmitter, ions and water through the open fusion pore. The transport through the charged cylindrical nano-size pore is simulated using a coupled system of Poisson–Nernst–Planck and Navier–Stokes equations and the forces that act radially on the wall of the fusion pore are then estimated. Four forces are considered: a) inertial force, b) pressure, c) viscotic force, and d) electrostatic force. The inertial and viscotic forces are small, but the electrostatic force and the pressure are typically significant. High vesicular pressure tends to open the fusion pore, but the pressure induced by the transport of charged particles (glutamate, ions), which is predominant when the pore wall charge density is high tends to close the pore. The electrostatic force, which also depends on the charge density on the pore wall, is weakly repulsive before the pore dilates, but becomes attractive and pronounced as the pore dilates. Given that the vesicular concentration of free transmitter can change rapidly due to the release, or owing to the dissociation from the gel matrix, we evaluated how much and how rapidly a change of the vesicular K^+–glutamate$^-$ concentration affects the concentration of glutamate$^-$ and ions in the pore and how such changes alter the radial force on the wall of the fusion pore. A step-like rise of the vesicular K^+–glutamate$^-$ concentration leads to a chain of events. Pore concentration (and efflux) of both K^+ and glutamate$^-$ rise reaching their new steady-state values in less than 100 ns. Interestingly within a similar time interval the pore concentration of Na^+ also rises, whereas that of Cl^- diminishes, although their extra-cellular concentration does not change. Finally such changes affect also the water movement. Water efflux changes bi-phasically, first increasing before decreasing to a new, but lower steady-state value. Nevertheless, even under such conditions an overall approximate neutrality of the pore is maintained remarkably well, and the electrostatic, but also inertial, viscotic and pressure forces acting on the pore wall remain constant. In conclusion the extrusion of the vesicular content generates forces, primarily the force due to the electro-kinetically induced pressure and electrostatic force (both influenced by the pore radius and even more by the charge density on the pore wall), which tend to close the fusion pore.

1. Introduction

Hormones, transmitters and peptides are released from secretory cells by exocytosis of vesicles (synaptic, large dense-core vesicles and other types of vesicles) or granules. During exocytosis the first aqueous connection that forms between the lumen of a secretory vesicle and the cell exterior is provided by the formation of a fusion pore [1]. Following its opening the fusion pore expands, but this expansion may stop and be reversed, and the pore may close completely [2]. Dynamics of the pore opening and closing may critically influence how rapidly hormones, transmitters or peptides are released into the extracellular space, and may thus be involved in regulating the quantal size [3–5].

It is now generally accepted that the presynaptic quantal size can change rapidly and very significantly as a result of stimulation or various pharmacological treatments [6–8]. Moreover the nature of secretory mechanism is such that the quantal size plasticity should generally be expected. First, the quantal size will change if the homotypic fusion changes [9]. Second, the quantal size should change because vesicles of different size are not equally efficient as barriers to diffusion of Ca^{2+} [10]. In all secretory systems, but especially in neuroendocrine cells with large vesicles and highly variable diameters, large vesicles will be released preferentially, but this is more pronounced when opening of Ca^{2+} channels becomes synchronous, as is the case during evoked release. Third, the fused vesicles may release only a fraction of their content ('kiss-and-run'; [11–13]. In such a case the quantal size should decrease at high release levels, due to incomplete re-filling of the vesicle. The importance of kiss-and-run mechanism however is presently hotly debated [14]. Finally, any

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change of the fusion pore dynamics may alter the postsynaptic quantal size irrespective of whether the release of the quantal content is complete or not [15].

Understanding mechanisms controlling the dynamics of exocytotic fusion pore dilatation and contraction is thus fundamental for understanding of quantal size plasticity. The cytoskeletal proteins may be considered as possible candidates controlling its dynamics [16]. They are known to play an important role in vesicular trafficking to the release sites. As a result of stimulation and calcium entry a depolymerization of the cortical actin network occurs facilitating exocytosis [17–19]. In this scenario the vesicular transport occurs by the action of molecular motors (such as myosin II) acting on F-actin trails. However, the molecular motors may also exert a tensional pressure on the F-actin network, which will alter the membrane tension, fusion pore expansion, and extrusion of vesicular contents [20]. Myosin II appears to act as a molecular motor on the fusion pore expansion by hindering its dilation when it lacks the phosphorylation sites, but the differences of the fusion pore dynamics and of the catecholamine release between control and transfected chromaffin cells with the unphosphorylatable form of myosin II are very limited [16]. Although their contribution may be greater in other secretory systems, it is necessary to consider the importance of other forces in regulating the dynamics of fusion pore dilatation.

In this study we focus our attention on the fluidic, electro-static and electro-kinetic forces acting on the fusion pore, and which are present simply because K^+ and glutamate$^-$ are extruded through the fusion pore. We simulate the fusion pore as a nano-sized cylinder with positively charged surface connected to two compartments equal in size using the computational methods of continuous nanofluidics [21–26]. The transmitter considered is glutamate, and it is negatively charged. K^+–glutamate$^-$ concentration in the vesicular compartment is either constant (stationary simulations) or changes in a step-like manner (time-dependent simulations). The extra-cellular compartment contains Na^+Cl^-, whose concentration does not vary during simulation. In most simulations the pressure and potential difference between two compartments are zero, whereas the surface charge density varies from one simulation to another over a wide range. Coupled system of Poisson–Nernst–Planck and Navier–Stokes equations is used to estimate the potential, electric field, pressure, fluid and ionic fluxes in the nanofluidic pore and to assess the forces acting on the fusion pore. Four forces are considered: a) inertial force, b) pressure, c) viscous force, and d) electrostatic force. The inertial and viscous forces are found to be very small, but the electrostatic force and force due to pressure may be significant. Counter-intuitively, the direction of both forces is often such to close the pore. The extrusion of the vesicular content thus by itself generates forces, which not only may affect the fusion pore dynamics, but more unexpectedly tend to close the pore. Moreover, the force generated by the extrusion of vesicular content can be sufficiently large to overcome forces caused by the tension difference between vesicular and plasma membrane.

2. Methods

2.1. Mathematical model

Poisson–Nernst–Planck (PNP) equations are used to calculate ionic current through a pore for all ionic species. PNP equations are composed of the Poisson (1) and Nernst–Planck (2) equations. The electrostatic potential (Φ) is calculated using Poisson equation:

$$-\nabla.\varepsilon_0\varepsilon_r\nabla\Phi = \rho_e \tag{1}$$

where ε_0 is the permittivity of vacuum, ε_r is the relative dielectric constant of solution. Note that the same equation applies to the membrane, but in the membrane the relative dielectric constant is ε_m, and the charge density ρ is zero. In solution the charge density ρ_e is given by:

$$\rho_e = F\sum z_a c_a \left(= e\sum z_a n_a\right) \tag{2}$$

where c_a is the molar concentration of ion a [mol/m^3], F is Faraday constant ($9.648*10^4$ C/mol), z_a is the valence of ion a, n_a is the number density of ion a. The following factors also influence the potential in the fusion pore: a) the fixed charges on the pore wall, b) the mobile charges inside the pore, and c) the charges in the solution and on control edges outside the pore.

The movement (by convection–diffusion–migration) of ionic species in the electrolytic fluid/solution is given by the Nernst–Planck equation:

$$\mathbf{J}_a = \mathbf{u}c_a - D_a\nabla c_a - m_a z_a F c_a \nabla\Phi \tag{3}$$

where $\mathbf{J}_a$ is molar flux [mol/m^2 s], D_a and m_a are diffusivity and mobility of ion a ($m_a = D_a/RT$), respectively; $\mathbf{u}$ is fluid velocity and F, R and T are Faraday constant, gas constant [8.315 Jl/(Kmol) and temperature (in Kelvin), respectively. Finally, the conservation of ionic mass of a dynamic problem is given by:

$$\frac{\partial c_a}{\partial t} + \nabla.\mathbf{J}_a = 0. \tag{4}$$

Note that the divergence operator is defined as:

$$\nabla.\mathbf{J} = \frac{1}{r}\frac{\partial}{\partial r}(rJ_r) + \frac{\partial J_z}{\partial z} \tag{5}$$

where J_r and J_z are the r- and z-components of vector $\mathbf{J}$.

The electrolytic fluid velocity $\mathbf{u}$ that is responsible for the convective transport of ions can be computed from the time dependent Navier–Stokes (NS) equations:

$$\rho\left(\frac{\partial\mathbf{u}}{\partial t} + \mathbf{u}.\nabla\mathbf{u}\right) = -\nabla p + \nabla.\left[\mu\left[\nabla\mathbf{u} + (\nabla\mathbf{u})^T\right]\right] + \mathbf{F}_e \tag{6}$$

$$\nabla.\mathbf{u} = 0. \tag{7}$$

Eq. (6) describes the conservation of momentum, while Eq. (7) accounts for the conservation of mass. In these equations ρ_f, μ, and p are respectively the density, viscosity and pressure of the fluid, while $\mathbf{F}_e$ is the electric force per unit volume ($\mathbf{F}_e = \rho_e\nabla\Phi$).

2.2. Geometry, parameters and boundary conditions

The nano-size fusion pore considered in this study has a cylindrical geometry. The computational domain describing this system consists of the fusion pore, a piece of the membrane wall as well as portions of the vesicular interior and extra-cellular spaces (Fig. 1A). The length of the pore L and of each of the compartments representing the vesicular and extra-cellular spaces was 10 nm resulting in total length of the computational domain of 30.0 nm. The fusion pore radius R ranged from 1.0 to 4.0 nm, whereas the radius W of the compartments representing the vesicular and extracellular spaces was 11 nm.

Axial symmetry condition has been applied on all variables along the axis of the fusion pore (boundary 4; Fig. 1B). The boundary conditions for the Nernst–Planck equation are concentrations of K^+–glutamate$^-$ and Na^+–Cl^- on two external controlling edges of the upper or vesicular compartment (boundaries 1) and lower or extra-cellular compartment (boundaries 2). On the edges of the upper compartment the concentrations of K^+–glutamate$^-$ ranged from 30 to 75 mM (mol/m^3), whilst the concentration of Na^+–Cl^- was 0 mM. On the edges of the lower compartment the concentration of K^+–glutamate$^-$ was 0 mM, while the concentration of Na^+–Cl^- was 150 mM. Note that we assume that: a) glutamate is negatively charged