REVISTA COLOMBIANA DE FÍSICA, VOL. 37, No. 1. 2005

DEFORMATION OF A SPHERICAL SYNAPTIC VESICLE INDUCED BY AN APPLIED ELECTRIC FIELD

Xaira Cortés Sañudo y Ramón Fayad Naffah
Facultad de Ciencias. Departamento de Física. Universidad Nacional de Colombia.

SUMMARY

In the present work a theoretical model of a synaptic vesicle is developed. The vesicle is considered a spherical organelle, neglecting the contribution of neurotransmitters. In addition, its lumen, its membrane and the neuronal cytoplasm behave like linear, homogenous and isotropic media characterized by specific conductivities and permittivities. The theoretical approach considers the application of an electric field (invariant in time) over this vesicle, which induces, through its membrane, an electric potential difference. The value of transmembrane potential under natural physiological conditions agrees with the reported in the existing literature for the aforesaid organelles. A polarization of the vesicular membrane is produced by the action of the applied electric field eliciting an electromechanical force that stretches the vesicle's membrane. As a result, the vesicle is extended in the direction of the electric field indicating a transition from its spherical morphology to a prolate vesicle.

RESUMEN

En el presente trabajo se desarrolla un modelo teórico de una vesícula sináptica esférica. Esta se considera como un organelo esferoidal, despojada de neurotransmisores en la que su lumen, su membrana y el citoplasma neuronal se comportan como medios lineales, homogéneos e isotrópicos con permitividades y conductividades específicas. El método utilizado será el análisis del sistema bajo la acción de un campo eléctrico invariante en el tiempo, lo que induce, a través de su membrana, una diferencia de potencial. El valor que toma dicho potencial transmembranal bajo condiciones fisiológicas naturales coincide con el reportado en la literatura para los susodichos organelos. Una polarización de la membrana vesicular es producida por la acción del campo eléctrico aplicado, lo que a su vez origina una fuerza electromecánica que actúa sobre dicha membrana. Como resultado, la vesícula es elongada en la dirección del campo eléctrico evidenciando una variación desde su forma esférica hacía una vesícula proloide.

INTRODUCTION

The biological system of this study is the synaptic vesicle: A small membrane-bounded organelle. Following to Lee et al. [1], such a vesicle is assumed as a spherical organelle neglecting the neurotransmitters contribution; in addition, its lumen, its membrane and the neuronal cytoplasm are considered linear, homogeneous and isotropic media, characterized by both specific conductivities and permittivities. Such approach is consistent with the physiological state in which a spherical synaptic vesicle is being recycled or acidified [2]. The physical phenomenon to study will be the transmembrane potential induction by a time-invariant electric field applied over the vesicle. As a result, there will be a polarization of the vesicular membrane turning into an electromechanical force over the membrane whose final effect is the vesicle's deformation.

METHOD

In this study, the mathematical representation of the intended biological system is obtained from Maxwell's equations stated as follows: The homogenous electric field *E* is deformed into an electric field *E* due to the vesicle presence. The electric field *E* can be described in terms of the gradient of a scalar potential field Φ; where *r* represents general coordinates (spherical coordinates):

$$E = -\nabla\Phi$$

Where Φ satisfies the Laplace's equation and the following conditions:

(1) The electric field far from the vesicle must be homogeneous:

$$\lim_{r\to\infty}(-\nabla\Phi) = E_{\text{o}}$$

(2) The scalar potential field Φ(r) within the vesicle must be finite:

$$\lim_{r\to 0}\Phi(r) < \infty$$

(3) The continuity conditions take the following form:

$$\left(\Phi_i - \Phi_m\right)\Big|_A = 0$$
$$n\cdot\left(\sigma_i\nabla\Phi_i - \sigma_m\nabla\Phi_m\right)\Big|_A = 0$$
$$\left(\Phi_m - \Phi_e\right)\Big|_B = 0$$
$$n\cdot\left(\sigma_m\nabla\Phi_m - \sigma_e\nabla\Phi_e\right)\Big|_B = 0$$

Where *A* stands for the vesicle-membrane internal surface and *B* refers to the vesicle-membrane external surface; Φ_i , Φ_m and Φ_e denote the scalar potential field function $\Phi(r)$ in the following order: inside the vesicle (the so-called *lumen*), within the vesicle membrane and outside the vesicle (the so-called neuronal *cytoplasm*); σ_i , σ_m and σ_e are the conductivities associated with the media related above; ε_i , ε_m and ε_e are the corresponding permittivities and n is the unitary vector normal to the boundary. The system parameters thus stated are depicted on Fig 1.

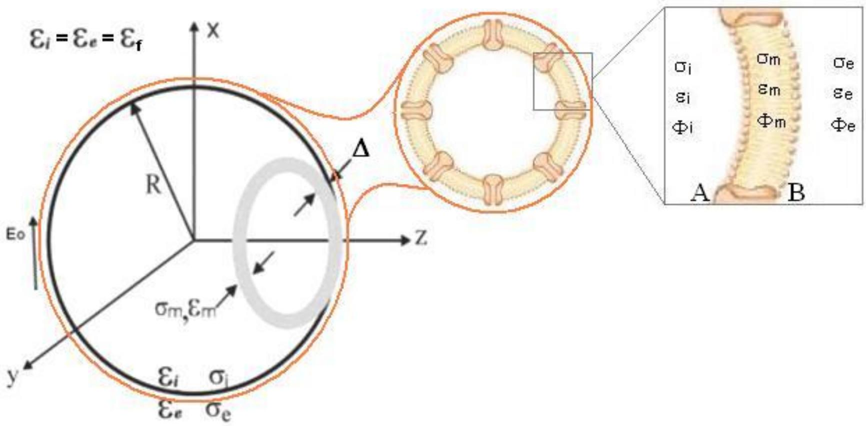

Figure 1. Assignment of notation used for boundary conditions.

The general solution for the electric potential on spherical coordinates obtained from Laplace's equation is described next.

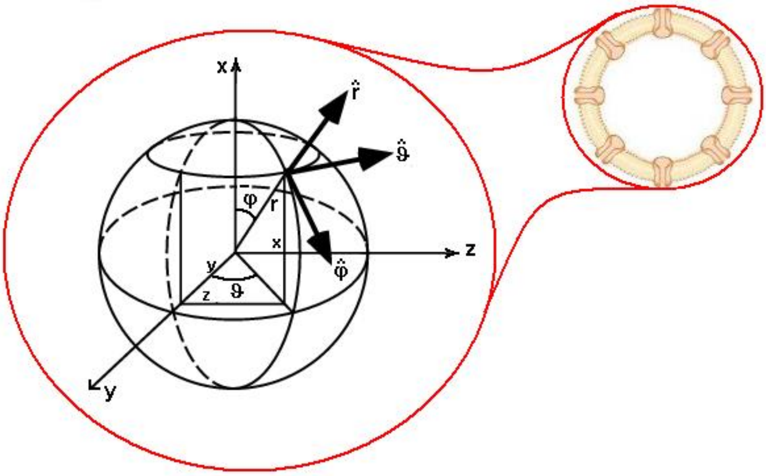

Figure 2. Coordinate system for a spherical vesicle

The coordinate system related to the spherical synaptic vesicle illustrated on Fig. 2 is stated as follows:

$$x = r\cos\varphi, \quad y = r\sin\varphi\cos\vartheta, \quad z = r\sin\varphi\sin\vartheta$$

where $\left\{(r,\vartheta,\varphi)\in\Re^3 : r\geq 0,\ \ 0\leq\varphi\leq\pi,\ \ 0\leq\vartheta\leq 2\pi\right\}$. The general solution of Laplace's equation on spherical coordinates is given by:

$$\Phi = \begin{cases} ar\cos\varphi & \text{para} \quad r < R \\ br\cos\varphi + \dfrac{c}{r^2}\cos\varphi & \text{para} \quad R < r < R + \Delta \\ -E_o r\cos\varphi + \dfrac{d}{r^2}\cos\varphi & \text{para} \quad r > R + \Delta \end{cases}$$

Being Δ, the vesicle-membrane thickness: Much less than the vesicle radius *R*; and *c, b, a* and *d* must be found from the above-mentioned set of boundary conditions.

The *transmembrane potential* across the vesicular membrane induced by the applied electric field is mathematically stated as $\Delta\Phi = \Phi_m\big|_A - \Phi_e\big|_B$. Therefore, based on the Laplace's equation solution and with the stated above boundary conditions is possible to find a mathematical equation for the scalar potential field $\Phi(r)$ on three regions: inside the vesicle, within the vesicle membrane and outside the vesicle. As a result, an equation for the induced transmembrane potential is also attainable.

RESULTS AND DISCUSSION

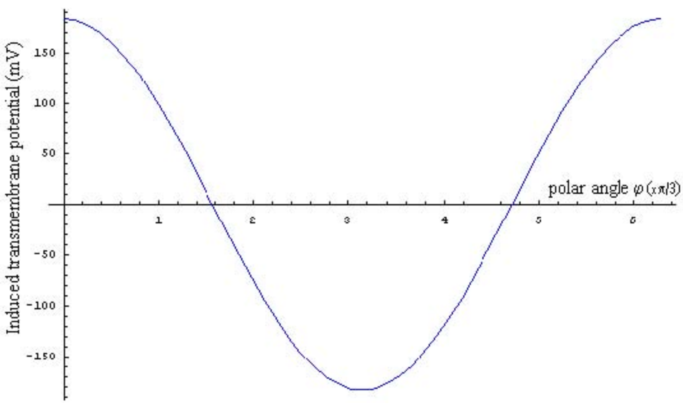

Figure 5. Induced transmembrane potential as a function of the polar angle in a spherical vesicle.

The transmembrane potential as a function of the polar angle is represented on figure 5. To analyze the behavior of the vesicle under normal physiological conditions, the applied electric field has been assumed as one with the same magnitude of the cognate electric field generated by the ionic gradient through the vesicular membrane. The effect of the electric stimulation of the vesicle is the change in the electric potential difference across the lumen and the neuronal cytoplasm; it is the so-called induced transmembrane potential and is shown on figure 5. Typical values (Table I) have been assigned to the electrical parameters on the electric potential equation.

TABLE I. VALUES USED IN THE ELECTRIC POTENTIAL EVALUATION

Parameter	Symbol	Value	Reference
Conductivity of the cytoplasm	$\sigma_C = \sigma_e$	2.83S/m	♠
Conductivity of the membrane	σ_m	$1.2\times10^{-6}\ S/m$	[6]
Conductivity of the lumen	$\sigma_L = \sigma_i$	1.82S/m	♠
Permittivity of the cytoplasm	$\varepsilon_C = \varepsilon_e$	$6.4\times10^{-10}\ Aseg/Vm$	[7]
Permittivity of the membrane	ε_m	$4.4\times10^{-11}\ Aseg/Vm$	[6]
Permittivity of the lumen	$\varepsilon_L = \varepsilon_i$	$6.4\times10^{-10}\ Aseg/Vm$	[7]
Membrane thickness	Δ	$2\ nm$	[8]
Vesicle radius	R	$120\ nm$	[1], [9]

♠ Evaluated in Cortés [3] from experimental values reported in both Weiss (1996) [4] and Grabe and Oster [5] (2001)

The numerical value of $\Delta\Phi$ depends on the location of the space in which this one is calculated. The values obtained here for the transmembrane potential induced by an electric field that is generated under natural physiological conditions agrees with those reported by Grabe and Oster [4] for this type of organelles. As it can be seen from figure 5, $\Delta\Phi$ takes positive or negative values for different angles supporting the fact that an electric polarization is produced over the spherical vesicle membrane under the action of the applied electric field. This electric polarization produces an electromechanical force that acts on this membrane and, as a result, this one is stretched in the direction of the electric field. Thus, if the spherical vesicle is deformed by the action of the electric field, the radial displacement must be accompanied by a tangential displacement. The magnitude of the vector displacement is found considering that the vesicular area is locally conserved before and after deformation. The area is conserved locally if:

$$\sqrt{g}\,d\varphi\, d\vartheta = \sqrt{g'}\,d\varphi\, d\vartheta \tag{1}$$

Where g and g' are the determinants of the metric tensor before and after the vesicular deformation:

$$\sqrt{g} = R^2 \sin\varphi \tag{2}$$

And, in the linear approach:

$$\sqrt{g'} = R^2 \sin\varphi\left(1 + 2\frac{1}{R}u_r + \frac{\cot\varphi}{R}u_\varphi + \frac{1}{R}\frac{du_\varphi}{d\varphi}\right) \tag{3}$$

When taking (3) and (2) into (1), the following equations must be satisfied:

$$u_r = \frac{1}{2}s_2\left(3\cos^2\varphi - 1\right)$$
$$u_\varphi = -s_2\cos\varphi\sin\varphi$$

Therefore, the vector displacement turns out to be $u = u_r\hat{e}_r + u_\varphi\hat{e}_\varphi$, being $\hat{e}_r = (\sin\varphi\cos\vartheta,\ \sin\varphi\cos\vartheta, \cos\varphi)$ the radial unitary vector and $\hat{e}_\varphi = (\cos\varphi\cos\varphi,\ \sin\varphi\cos\vartheta, -\sin\varphi)$ the tangential unitary vector. The equilibrium form of the vesicle is calculated minimizing the total free energy. The two contributions to this energy are: First, the total bending energy of the membrane [10] and, second, the energy required by the elements of the membrane to be displaced in the electric field; the total bending energy [11] is obtained from the equation found by Helfrich [11], [12] while the second one is attained from the electrical Maxwell stress tensor. Helfrich [11], [12] demonstrated that the deformation of a spherical vesicle gives rise to an increase in the total bending energy of the membrane given by:

$$G_{BENDING} = \frac{48\pi}{5}\left(1 - \frac{c_o r_o}{6}\right)k_o\left(\frac{s_2}{r_o}\right)^2$$

Where C_0 it is the spontaneous curvature of the membrane, r_o refers to the middle of the vesicular membrane given by $\left(2R - \Delta\right)/2$ [13], s_2 is the deformation amplitude and is a proportionality constant known as elastic module of curvature. Table II shows typical values associated to these parameters in a spherical vesicle.

Table II: Typical curvature values to spherical synaptic vesicle.

Parameter	Symbol	Value	Reference
Spontaneous curvature	C_o	0•	[14]
Elastic module of curvature	k_o	4×10^{-9}	[15]

• That is satisfied in the case in which the phospholipids monolayers of the vesicular membrane are considered symmetrical in their composition.

On the other hand, the second contribution to the free energy of the vesicle is obtained from the work done by the electrical forces on the membrane to deform it. Considering that the electric field in every vesicular region (lumen, membrane and neuronal cytoplasm) has been found above through the solution of the Laplace's equation, it is clearly possible to obtain the associated electric field to these regions, and, when taking them to the electrical Maxwell stress tensor, the force exerted by the electric field on the vesicle will be thus found. In fact, the surface force densities acting on the interfaces membrane - lumen and cytoplasm - membrane are:

$$f_{\text{int}} = \left(T^{(membrana)} - T^{(lumen)}\right)\hat{e}_r$$

$$f_{ext} = \left(T^{(citoplasma)} - T^{(membrana)}\right)\hat{e}_r$$

Where T is the electrical Maxwell stress tensor corresponding to j = *lumen, membrana, citoplasma*:

$$T_{kl}{}^{(j)} = \varepsilon^{(j)}\left(E_k{}^{(j)}E_l{}^{(j)} - \frac{1}{2}\left(E^{(j)}\right)^2 \delta_{kl}\right)$$

Thus, the membrane deformation due to the electric field requires an energy given by:

$$G_{ELECTRIC\ \ FIELD} = -\int\left(f_{ext}\cdot u\right) dA_{ext} - \int\left(f_{\text{int}}\cdot u\right) dA_{\text{int}}$$

With the integral extended over the sphere:

$$dA_{ext} = R_{ext}^2 \sin\varphi d\varphi d\vartheta = (R+d)^2 \sin\varphi d\varphi d\vartheta$$

and

$$dA_{\text{int}} = R_{\text{int}}^2 \sin\varphi d\varphi d\vartheta = R^2 \sin\varphi d\varphi d\vartheta$$

Hence, the energy required by the elements of the membrane to be displaced in the electric field is:

$$G_{ELECTRICFIELD} = -\frac{12\pi E_o{}^2 (R+\Delta)^2 s_2}{5}\frac{K_1}{K_2{}^2}$$

Where

$$\begin{aligned} K_1 = {} & 9R^2(R+\Delta)^4 \sigma_e{}^2(\varepsilon_m(\sigma_i+\sigma_m)^2 - 4\varepsilon_i\sigma_m{}^2) \\ & - \varepsilon_m\sigma_e{}^2(3R^3(\sigma_i+\sigma_m) + 2(\sigma_i+2\sigma_m)(\Delta^3+3\Delta^2R+3\Delta R^2))^2 \\ & + (3R^3\sigma_m(\sigma_e+\sigma_i) + (\sigma_e+\sigma_m)(\sigma_i+2\sigma_m)(\Delta^3+3\Delta^2R+3\Delta R^2))^2\varepsilon_e \end{aligned}$$

and

$$K_2 = 3R^3\sigma_m(2\sigma_e+\sigma_i) + (\Delta^3+3\Delta^2R+3\Delta R^2)(2\sigma_e+\sigma_m)(\sigma_i+2\sigma_m)$$

Therefore, the equilibrium of the vesicle under the action of an applied electric field is calculated minimizing the total free energy:

$$G_{TOTAL} = G_{BENDING} + G_{ELECTRIC\ FIELD}$$

Obtaining finally:

$$s_2 = \frac{3E_o^{\,2}(R+d)^2(2R+d)^2}{8k_o\left(12-(2R+d)c_o\right)}\frac{K_1}{K_2}$$

If values reported in tables I and II are applied to the previous equation, a deformation is obtained and taking it onto the vector displacement, a change of the spherical shape of the vesicle towards a prolate vesicle is thus shown. Therefore it is possible to figure out that the electrical polarization of the vesicular membrane (due to the action of the generated electric field under normal physiological conditions) gives place to the vesicle's morphological transition. The magnitude of deformation in the spherical vesicle turns out to be a direct function of the applied electric field magnitude. Therefore, when this one exceeds a breakdown intensity, the membrane will probably undergo a rupture in some region. In the same way, under the action of the applied electric field, there is an increase in the total energy of the membrane; this could be explained by the hydrophilic pores enlargement within the lipid bilayer taking into account that free polar molecule diffusion could take place across this membrane.

CONCLUSIONS

The effect of the stimulation of a vesicle by means of an electric field invariant in time is the change in the difference of the electric potential across its membrane known as induced transmembrane potential. Solving the Maxwell's equations for a spherical vesicle, the electric potential – inside the vesicle, outside the same one and over its membrane – is obtained. For a vesicle within physiological conditions (within the electrochemical proton gradient across the vesicular membrane) one explicitly obtains the induced transmembrane potential as a function of the polar angle. These values for the vesicle's transmembrane potential agree with those reported by Grabe and Oster for a spherical vesicle. The above-mentioned relationship shows an electrical polarization induced over the vesicular membrane due to the applied electric field. This polarization produces an electromechanical force over the membrane and, as a result, this one is stretched in the direction of the electric field. The equilibrium shape of the vesicle is evaluated minimizing its total free energy. For a spherical vesicle whose elastic module of curvature is 4×10^{-9} and with a radius of 120nm, the deformation amplitude of turns out to be 9.38nm which, into the displacement vector, shows a transition of the vesicle's spherical shape into a prolate one.

REFERENCES

[1] Lee SH, Valtschanoff JG, Kharazia VN, Weinberg R, Sheng M. Biochemical and morphological characterization of an intracellular membrane compartment containing AMPA receptors. 2001. Neuropharmacology. 41: 680-92.

[2] Cortés X. y Fayad R. Modelo eléctrico equivalente de una vesícula sináptica. Acta Biológica Colombiana. Vol. 8 No. 1, 2003.

[3] Cortés X. Modelo de polarización RC aplicado a vesículas sinápticas. Tesis de pregrado. Universidad Nacional de Colombia. Bogotá. 2003.

[4] Weiss T. F. *Cellular Biophysics* (Volumes 1 and 2). 1996. MIT Press, Cambridge, Massachusetts.

[5] M. Grabe and G. Oster. Regulation of organelle acidity. 2001. J. Gen. Physiol. 117:1-16.

[6] PRC Gascoyne, JPH Burt, FF Becker and R Pethig, *Membrane changes accompanying the induced differentiation of Friend erythroleukemia cells studied by dielectrophoresis*. 1993. Biochim. Biophys. Acta. 1149: 119-126.

[7] Buchner R, Hefter GT, and May PM. 1999. Dielectric relaxation of aqueous NaCl solutions. J. Phys. Chem. 103: 1–9.

[8] Loew LM. The electrical properties of biomembranes. *In* Biomembranes. Physical Aspects. 1993. M. Shinitzky, editor. VCH Publishers, Weinheim, Germany: 341-371.

[9] Henkel AW, Horstmann H, Henkel MK. Direct observation of membrane retrieval in chromaffin cells by capacitance measurements. 2001. FEBS Lett. 505:414-418.

[10] Sackmann E. Membrane bending energy concept of vesicle- and cell-shapes and shape-transitions. 1994. *FEBS Lett.* 346: 3-16.

[11] Helfrich W. Deformation of Lipid Bilayer Spheres by Electric Field. 1974. *Z.Naturforsch.* 29c: 182-183.

[12] Robledo A, Varea C, Fedeniuk RW,; Ramamurthi S, McCurdy AR. Can the Helfrich free energy for curved interfaces be derived from first principles. 1996. Physica A. 231: 178-190.

[13] Winterhalter M, Helfrich W. Deformation of Spherical Vesicles by Electric Field. 1988. *J.Coll.Int.Sci.* 122: 583-586.

[14] Lipowsky R. The Conformation of Membranes. 1991. *Nature* 349: 475-481.

[15] Seifert U and Lipowsky R. Morphology of vesicles, in *Structure and Dynamics of Membranes.* 1994. Edited by R. Lipowsky and E. Sackmann, Elsevier Science. Amsterdam.