COMPENSATION OF NEUROTRANSMITTERS DEFICIENCY IN THE SYNAPTIC CLEFT

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Abstract. The increase of pressure inside a cell determines the stretch of its membrane. In such conditions either the cellular membrane may be ruptured and the cell dyes, or one pore may appear through its lipid matrix and the membrane becomes unstretched. It was found that only one pore can appear in a lipid vesicle at one time, but a succession of pores can form in the same vesicle. The membrane recovery happens only if a part of the intracellular material comes out of the cell through these transmembrane pores. In this paper we have analyzed the pore formation in a streatched vesicle after application of an osmotic stress. If we see the successive pores appearance as a periodic process, then the time interval between the appearance of two successive pores is a characteristic of the cyclic process, and, as usually, is named period. In the case analyzed here it is the sum of the swelling time of the vesicle and the lifetime of the pore formed on the vesicle. If the solvent (as water) has a low viscosity, the pore life is very short, the period is equal to swelling time. The swelling time between any two successive pores in a vesicle was calculated. At the beginning of the process the swelling time decreases with the cycle's rank, but later it reaches a constant value. For this reason the liposome may be regarded as a time controller of drug administration at the ill place were the liposomes had been placed.

Key words: Stretched vesicle, pores, swelling time, time biocontroller, drug release.

INTRODUCTION

The transport of molecules through transmembrane pores is widely used by cells. In the last time the interest, both experimental and theoretical, for pores is continuously growing because of their use in biotechnological applications. A large number of papers appeared in the last decade, which have referred both to pores formed by proteins, and to lipidic pores formed in the lipid matrix of the cell membrane. Here we will focus our attention only on the pores, which appear in the

Received June 2006;
in final form August 2006.

ROMANIAN J. BIOPHYS., Vol. 16, No. 3, P. 189–204, BUCHAREST, 2006

lipid bilayer with their wall formed by lipid molecules. Some pores can appear due to structural and dynamic properties of lipid bilayers [42–45]. Usually these pores are named stochastic pores. Particularly, the presence of thickness fluctuations in hydrophobic BLM was demonstrated both by theory [18, 19] and experiment [3]. For BLMs composed by a mixture of lipids, a selective association between phospholipids takes place, thus generating phospholipid domains and local changes in hydrophobic thickness [46, 47]. The thickness of the phospholipid domain is dependent on the length of the hydrocarbon chain of phospholipids [31–35, 41]. The thickness fluctuations caused by thermal motion of lipid molecules superpose on local variations of the bilayer thickness, which already exist, due to selective association of phospholipids [37, 48].

A stochastic transbilayer pore can form and grow, following random and biased thermal fluctuations of the bilayer thickness or superficial density [14, 15, 16, 25, 27, 36, 42, 53].

The appearance of stochastic pores in BLMs, due to thickness fluctuations, was first proposed by Popescu *et al.* [42]. The height of the energy barrier for membrane perforation following such a mechanism is large (~ 91 $k_B T$ [42], where k_B and T are the Boltzmann constant and absolute temperature, respectively). In this case, the geometrical profile of the pore, on a perpendicular plane to BLM, is of elliptical toroydal form [43, 45]. It was also shown that such a transmembrane pore could evolve to a stable state [37]. The results obtained with this model were pretty surprising, because of the short time of resealing of the stochastic pores formed in membranes. Two years later, Zhelev and Needham [60] created large and quasi-stable pores in lipid bilayer vesicles, thus keeping with the previous model prediction [42, 43, 44]. The membrane resistance to rupture [40, 52], expressed in terms of line tension for a large pore formed in a bilayer vesicle, was calculated by Moroz and Nelson [28, 29, 30]. Notably, they suggested a new procedure for an accurate estimate of the line tension starting from Zhelev and Needham's experiment.

Recently, in vesicles stretched by optical induced tension, a single pore only (of several micrometers in size) was observed at a time in a given vesicle. However, in the same vesicle, 30 – 40 pores can appear successively [20]. In this paper we show that a successive formation of pores in a vesicle has an interesting biotechnological application.

There are two very interesting biotechnological applications, which request the increase of membrane permeability: gene therapy and targeted drug delivery. In the first one, the transport of DNA fragments through cellular and nuclear membranes is requested [58]. The second application uses drug molecules encapsulated in vesicles, which have to be transported to a target place [23, 24, 58, 59]. There, the vesicle has to release the drug molecules, in a well-controlled fashion. The appearance of transbilayer pores may be stimulated using chemical and physical methods [1, 2, 4, 17, 49]. The chemical methods are based on the addition of a suitable agent [7–11]. Using physical methods, such as electroporation [60], osmotic shock [12], temperature jump, adhesion on porous or

decorated substrate and intense illumination one can produce a stretch of the vesicle membrane, which relaxes forming transient pores. These pores may reach diameters up to 10 μm [51].

The appearance of pores through the cellular membrane, caused by mechanical tension is a possibility for the intracellular material to be transported outside the cell.

In this paper, we have focused our attention on the successively formed transient pores induced in a vesicle by osmotic stress and on the time interval between two successive pore formations. In the first part of this paper we will describe the transient pore dynamics. Then, the solute concentration inside the vesicle, depending on the time elapsed, is calculated. The time period between two successive pores was also calculated. An interesting application in medicine is discussed: transient pores in liposomes could be used for compensation of neurotransmitter deficiency in the synaptic cleft.

THEORY OF PORE DYNAMICS

The surface of a closed membrane can be in a crumpled state, characterized by negative surface tension (σ), dynamic undulations due to thermal fluctuations and a positive excess area. The excess area is equal to the difference between projected (A_p) and real area (A_0) [6], or mathematically $\Delta A = A_p - A_0$.

In common circumstances the membrane tension of a unilamellar vesicle is equal to zero. Using one of the methods named above, the vesicle may reach a stretched state, characterized by surface tension and small or non-existent shape fluctuations.

The most important states of the vesicle dynamics are given in the Figure 1.

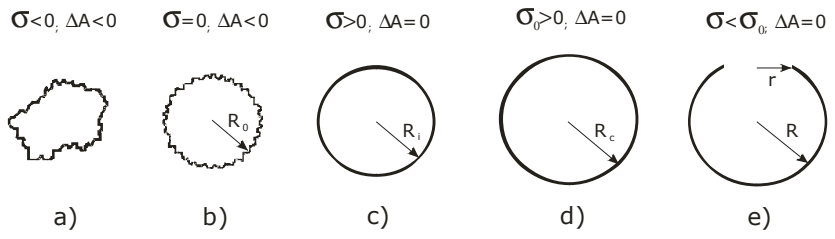

Fig. 1. The most important states of a vesicle in its dynamic cycle. a) The crumpled vesicle characterized by negative surface tension and excess area ($\sigma < 0$; $\Delta A < 0$); b) The spherical vesicle ($\sigma = 0$; $\Delta A < 0$); c) the perfect smooth vesicle ($\sigma > 0$; $\Delta A = 0$); d) The stage just before the opening of the pore, named the initial stage, characterized by surface tension $\sigma_0 > 0$); R_c is the vesicle mean radius just before the pore opening. e) The vesicle wearing an open evolving pore. The surface tension is $\sigma < \sigma_0$.

LIPIDIC MASS CONSERVATION

The stretched closed membrane can relax by opening a pore. Considering a spherical vesicle, which evolves according to the cycle from Fig. 1 and supposing that the vesicle does not lose lipid molecules we can write the following equalities:

$$A_0 = 4\pi R_0^2 \tag{1}$$

$$A = 4\pi R_i^2 = 4\pi R_0^2\left(1+\frac{\sigma_0}{E}\right) = 4\pi R_0^2\left(1+\frac{\sigma}{E}\right) + \pi r_p^2 = 4\pi R_0^2 + \pi r_c^2 \tag{2}$$

where R_0 is the vesicle mean radius in the state b) of Fig.1 ($\sigma = 0$); r_c is the maximal radius of the pore. The pore can take a maximal radius r_c, when the liposome is completely relaxed and no leakage of internal liquid occurs. This is the case of a viscous internal liquid. If the closed spherical membrane is stretched by a surface tension σ, its radius changes as:

$$R(\sigma) = R_0\sqrt{1+\frac{\sigma}{E}} \tag{3}$$

E is the elastic modulus for surface stretching, or compression, and is equal to: $E = \frac{48\pi K_H^2}{R_0^2 kT}$ [5, 6]. In this formula K_H is the Helfrich bending constant and kT is the thermal energy.

In the second part of the dynamic cycle, after the pore formation, an equality of areas appears:

$$A = 4\pi R_i^2 = 4\pi R_0^2\left(1+\frac{\sigma}{E}\right) + \pi r^2 \tag{4}$$

where r is the pore radius when the membrane has the surface tension σ.

It is useful to define the critical radius pore as:

$$4\pi R_i^2 - 4\pi R_0^2 = \pi r_c^2 \tag{5}$$

The critical pore exists when the vesicle is completely relaxed ($\sigma = 0$) and assuming zero leakage. The last supposition signifies that $R_i = R$. The relation (5) is obtained from relation (4) if $\sigma = 0$.

Writing areas relations, we suppose that the vesicle dynamics is perfectly described by median vesicles, drawn with dashed line in Fig. 1. They have a surface tension equal to the bilayer tension.

ENERGETICAL CONDITIONS

Most of the models describing pore formation in membranes are based on a simple hypothesis proposed three decades ago by Litster [24, 56, 57]. According to this hypothesis, the membrane free energy change due to pore appearance is given by Litster relation:

$$\Delta E_p = 2\pi r\,\gamma - \pi r^2 \sigma \tag{6}$$

A stochastic pore may tend to open or close, depending on the forces acting on its boundary (Fig. 2). The appearance of a circular pore of radius r, in a membrane with surface tension coefficient σ, is determined by the presence of two competing energetic terms: a reduction in free energy by a surface tension component ($-\pi r^2\sigma$), and an increase in free energy by a line tension component ($+2\pi r\gamma$).

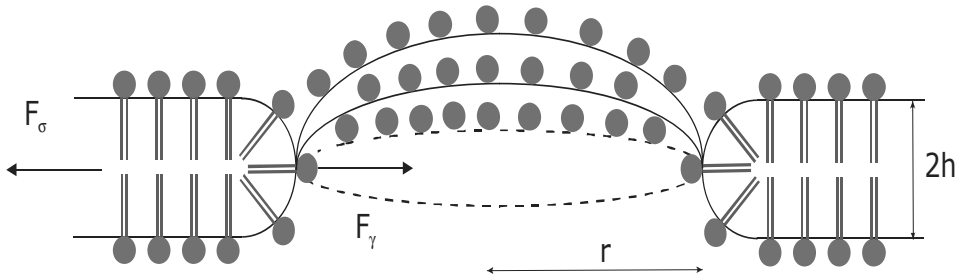

Fig. 2. A cross section through a bilayer with a pore. Its evolution is determined by the balance of two opposing forces. The opening of the pore is driven by the force F_σ based on the membrane tension and the closure is driven by F_γ based on the line tension.

The height of the energy barrier is equal to: $\Delta E_{\max} = \pi \frac{\gamma^2}{\sigma}$ and is realized for a pore radius, $r = \frac{\gamma}{\sigma}$.

The line tension, γ, is caused by the hydrophobic property of phospholipids, and contributes to the energy barrier which hinders pore formation. The surface tension coefficient, σ, reduces the barrier height for pore formation.

The free energy change due to the bilayer deformation following the pore appearance is lost due to the internal viscosity of the lipid bilayer. The energy change due to internal viscosity of the bilayer is:

$$\Delta E_v = 2\pi r \eta_m d \frac{\partial r}{\partial t} \tag{7}$$

Equating the two energy changes for the lipid bilayer, one obtains a differential equation for the pore radius:

$$\pi r^2 \sigma - 2\pi r \gamma = 2\pi r \eta_m d \frac{\partial r}{\partial t}$$
$$r\sigma - 2\gamma = 2\eta_m d \frac{\partial r}{\partial t} \tag{8}$$

LEAK OUT THROUGHT THE PORE

After pore appearance the internal liquid comes out and the vesicle decreases its size.

The flow of expelled liquid in time unit is: $Q = \pi r^2 v$, where r is the pore radius and v is the mean leak-out velocity of internal liquid. The flow on time unit has to be equal to the decrease rate of the vesicle volume:

$$Q = \frac{\partial V_{\text{vez}}}{\partial R} \tag{9}$$

The internal liquid is pushed out through the pore by Laplace pressure ΔP:

$$\Delta P = \frac{2\sigma}{R} \tag{10}$$

The pushing out force is:

$$F_{\text{p}} = \Delta P \cdot \pi r^2 \tag{11}$$

This force may be equal to the shear viscosity force involved in the outward flow:

$$F_{\text{v}} = 3\pi\eta_{\text{l}} r v \tag{12}$$

Taking into account the above relations (10)–(12) the outward flow velocity of the internal liquid is:

$$v = \frac{2\sigma r}{3R\eta_{\text{l}}} \tag{13}$$

Introducing the formula (13) in the relation (9) one obtains an equation for the vesicle radius:

$$\frac{2\pi\sigma r^3}{3R\eta_{\text{l}}} = 4\pi R^2 \frac{\partial R}{\partial t} \tag{14}$$

DYNAMICS OF CLOSED MEMBRANES UNDER OSMOTIC STRESS

Let us analyze a vesicle, which incorporates a solution of concentration C_{s0}. The solvent is water, and the solute may be a drug molecule. It is more useful here, to define the concentration as:

$$C = \frac{\text{number of molecules of a solute}}{\text{solution volume}}$$

VARIATION OF INTERNAL SUBSTANCES AFTER THE N-TH DYNAMIC CYCLE OF A VESICLE

Before the beginning of the first cycle, the solute concentration C_{s0}, and the solvent (water) concentration C_{w0} are considered in a vesicle of volume equal to $V_0 = 4\pi R_{i0}^3/3$.

Each dynamic cycle has two parts. In the first part the vesicle swells due to the osmotic flow. Its internal radius increases from R_{i0} (vesicle is smooth and $\sigma = 0$) to R_{ic}. R_{ic} is the internal vesicle radius just before the pore formation. In the second part of the cycle, the vesicle size decreases because of the leakage out of internal solution. Its radius decreases from R_{ic} to R_{i0}. The index "*i*" denote "inside".

The number of water molecules, N^+, that enters inside the vesicle during the swelling stage is:

$$N^+ = \frac{V - V_0}{\bar{v}} = \frac{4\pi\left(R_{ic}^3 - R_{i0}^3\right)}{3\bar{v}} = \frac{4\pi R_{ic}^3}{3\bar{v}}\left(1 - \frac{R_{i0}^3}{R_{ic}^3}\right) = N\left(1 - f\right) \qquad (15)$$

In the above formula we have introduced the following notations:

$\bar{v} = \frac{\mu}{\rho N_A}$ is the volume of a water molecule in the liquid state and in the working condition. N_A is Avogadro's number, μ is the molar mass, ρ is the water density.

$N = \frac{V_c}{\bar{v}} = \frac{4\pi R_{ic}^3}{3\bar{v}}$ is the number of water molecules which would fill the stretched vesicle just before the pore appearance if only water would be present.

$f = \frac{V_0}{V_c} = \frac{R_{i0}^3}{R_{ic}^3}$ is the ratio between the vesicle volumes in the complete relaxed state and in the stretched state just before pore formation.

The first cycle

a) Swelling stage.

Initially, in a complete relaxation state the vesicle contains:

$N_{s0} = C_{s0}V_0$ solute molecules and $N_{w0} = C_{w0}V_0$ molecules of water.

At the end of the sweeling stage, just before the pore opening, the same number of solute molecules is present in the vesicle, but this contains a larger number of water molecules N_{w1};

$$N_{w1} = N_{w0} + N^+ = C_{w0}V_0 + N(1-f) \tag{16}$$

The new concentrations are:

$$\begin{aligned} C_{s1} &= \frac{N_{s0}}{V_c} = \frac{C_{s0}V_0}{V_c} = fC_{s0} \\ C_{w1} &= \frac{N_{w1}}{V_c} = \frac{C_{w0}V_0 + N(1-f)}{V_c} = fC_{w0} + \frac{1-f}{\bar{v}} \end{aligned} \tag{17}$$

b) Relaxation stage.

After the pore opening, the pore radius increases up to a maximal value, then it decreases and finally the pore closes. During the pore evolution an amount of internal liquid leaks out. At the end of the first cycle and the beginning of the second, the vesicle is in a complete relaxation state and contains N_{s1} molecules of solute and N_{w1} molecules of water.

$$\begin{aligned} N_{s1} &= V_0C_{s1} = fV_0C_{s0} \\ N_{w1} &= V_0C_{w1} = fV_0C_{w0} + \frac{V_0(1-f)}{\bar{v}} \end{aligned} \tag{18}$$

The second cycle

a) Swelling stage.

Due to the osmotic flow the vesicles swells again up to the state in which a new pore may form. N^+ water molecules flow inside the vesicle. Before the pore opens, we have inside the vesicle:

$$\begin{aligned} N_{s2} &= N_{s1} \\ N_{w2} &= N_{w1} + N^+ = fV_0C_{w0} + \frac{V_0(1-f)}{\bar{v}} + N(1-f) \end{aligned} \tag{19}$$

The new concentrations at the end of the swelling stage are:

$$\begin{aligned} C_{s2} &= \frac{N_{s2}}{V_c} = \frac{fV_0C_{s0}}{V_c} = f^2C_{s0} \\ C_{w2} &= \frac{N_{w2}}{V_c} = \frac{fV_0C_{w0}}{V_c} + \frac{V_0(1-f)}{V_c\bar{v}} + \frac{N(1-f)}{V_c} = f^2C_{w0} + \frac{f(1-f)}{\bar{v}} + \frac{(1-f)}{\bar{v}} = f^2C_{w0} + \frac{1-f^2}{\bar{v}} \end{aligned} \tag{20}$$

b) Relaxation stage

At the end of the complete relaxation stage, the vesicle has the radius equal to R_0 and σ = 0. The number of solute and water molecules is:

$$N_{s3} = V_0 C_{s2} = fV_0 C_{s0}$$
$$N_{w3} = V_0 C_{w2} = f^2 V_0 C_{w0} + \frac{V_0\left(1-f^2\right)}{\bar{v}} \tag{21}$$

The nth cycle

At the end of the nth cycle, the inside of the liposome is characterized by:

$$C_{sn} = \frac{N_{s(n-1)}}{V_c} = \frac{f^{n-1}V_0 C_{s0}}{V_c} = f^n C_{s0}$$
$$C_{wn} = \frac{N_{w(n-1)}}{V_c} = \frac{f^{n-1}V_0 C_{w0}}{V_c} + \frac{V_0\left(1-f^{n-1}\right)}{V_c\bar{v}} + \frac{N\left(1-f^{n-1}\right)}{V_c} = f^n C_{w0} + \frac{1-f^n}{\bar{v}} \tag{22}$$
$$N_{sn} = V_0 C_{sn} = f^n V_0 C_{s0}$$
$$N_{wn} = V_0 C_{wn} = f^n V_0 C_{w0} + \frac{V_0\left(1-f^n\right)}{\bar{v}}$$

CALCULATION OF THE SWELLING TIME OF THE VESICLE

Suppose that the inside of the vesicle is occupied by a mixture of water and pharmacological molecules. Additionally, we suppose that the vesicle charged with drug molecules has reached a closed region around the ill “place”, which is filled with water. Due to the tonicity difference between the two adjacent media separated by the membrane, water will diffuse through the lipid bilayer into the vesicles, which are swelling up to a critical diameter. The size increase of the vesicle in a *dt* time interval is determined by the water molecules which entered. So:

$$dV_i = J_w A_i \bar{v}\, v dt \tag{23}$$

where V_i is the internal volume of the vesicle, J_w is the water flow through the internal surface of the lipid bilayer, A_i, $\bar{v}$ is the volume of a water molecule and v is the transport velocity of water molecules through the lipid bilayer. The relation (23) may be written:

$$4\pi R_i^2\, dR_i = J_w\, 4\pi R_i^2 \bar{v} dt \tag{24}$$

$$dR_i = J_w \bar{v}\, \mathrm{d}t \tag{25}$$

Integrating from R_0 (when the lipid bilayer is not stretched, $\sigma_0 = 0$) to R_c (when the liposome is just before pore forming) we obtain:

$$R_c - R_0 = J_w \bar{v} \tau \tag{26}$$

We noted with τ the swelling time of the liposome which is the time needed by the liposome to reach its critical state starting from the initial completely relaxed state. We introduce a mean concentration of water molecules in the lipid bilayer as:

$$\overline{C}_w = \frac{\kappa\left(C_{we} + C_{wi}\right)}{2} \tag{27}$$

where C_{we} and C_{wi} are the water concentration outside and inside the vesicle, respectively. The constant κ is the partition coefficient of water in the lipid region of the vesicle.

The water flow across the lipid bilayer is equal to:

$$J_w = \overline{C}_w v \tag{28}$$

From (26), (27) and (28) the swelling time is given by:

$$\tau = \frac{2\left(R_c - R_0\right)}{v \bar{v} \kappa \left(C_{we} + C_{wi}\right)} \tag{29}$$

Taking C_{we} and C_{wi} as C_{w0} and C_{wn} in formula (29), introducing the index n at τ and using the formula (22) for the internal concentration of water one obtains:

$$\tau_n = \frac{2\left(R_c - R_0\right)}{v \bar{v} \kappa \left[C_{we} + C_{w0} f^n + \frac{1 - f^n}{\bar{v}} \right]} = \frac{2\left(R_c - R_0\right)}{v \kappa \left[\bar{v} C_{we} + \bar{v} C_{w0} f^n + \left(1 - f^n\right) \right]} \tag{30}$$

LIMITING CONDITION FOR THE NUMBER OF SWELING CYCLES

The osmotic pressure is permanently equal to Laplace pressure due to elastic tension. So:

$$\sigma \left(\frac{1}{R - h} + \frac{1}{R + h} \right) = \Re T \left(C_s^{in} - C_s^{out} \right) \tag{31}$$

$$\frac{2\sigma R}{R^2 - h^2} = \Re T C_s^{in} \tag{32}$$

The formula (32) was obtained from (31) for $C_s^{out} = 0$, where R is the radius of the sphere between the two monolayers of the liposome bilayer, σ is the monolayer surface tension, and $2h$ is the hydrophobic core thickness, $\Re$ is the universal gas constant, T is the absolute temperature. The inward transmembrane diffusion of solvent will produce the pore appearance only if C_{sn} is greater than C_s^{in} determined from formula (32). With other words, the number of succesive pores, and consequently the time of drug delivery, is limited by both liposomes elastic and geometrical properties.

RESULTS

Let us consider a vesicle in a closed chamber, which contains water. The chamber may contain also other solute molecules for which the bilayer is impermeable. The radius of the relaxed liposome is $R_0 = 19.7$ μm. The value of the critical radius is $R_c = 20.6$ μm. The giant vesicles obtained experimentally in ref. [20] have such a value of critical radius.

The volume of a water molecule $\bar{v}$ is equal to 29.89 Å^3. The transport velocity through the lipid bilayer is $v = 10^5$ Å/s [50]. The partition coefficient of water in the lipid bilayer is taken equal to 1, because of lack of data. In fact κ must represent the partition coefficient of water in the hydrophobic core of the lipid bilayer.

We will consider that a quarter of the internal volume of the vesicle is occupied by water. So, $\bar{v}C_{w0} = 0.25$. For simplicity, we consider the chamber filled only with water. So, $\bar{v}C_{we} = 1$

Introducing these data in formula (30) one obtains the swelling time (τ_n) in the n-th cycle:

$$\tau_n = \frac{18}{200 - 75 f^n} \tag{33}$$

For the selected vesicle, $f = 0{,}9536$

Some values of τ_n are calculated in Table 1.

The dependence of τ_n on the pore order appearance for the described liposome is given in Fig. 3.

Tabel 1

Some value of τ_n calculated using the function (33) for $f = 0.9536$

n	1	2	3	4	5	10	15	20	∞
τ_n (ms)	140,32	136,97	133,92	131,12	128,56	118,01	111,36	106,31	90

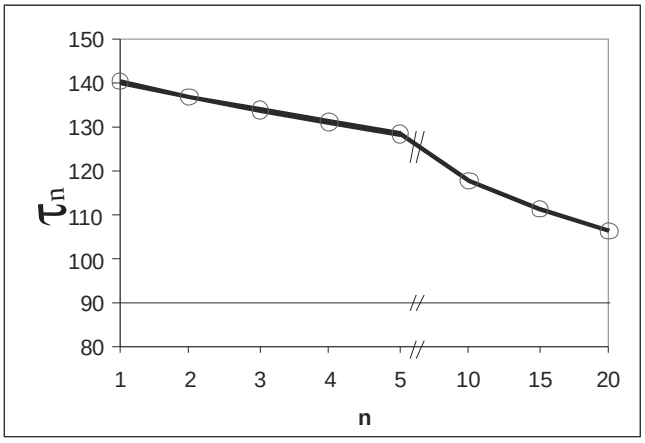

Fig. 3. Dependence of the swelling time on the order of the swelling cycle, if the ratio: radius of the vesicle in the relaxed state / radius in the stretched state just before pore formation is $f = 0.9536$.

COMPENSATION OF NEUROTRANSMITTER DEFICIENCY

It is known that the process leading to depression is the depletion of neurotransmitters in the synaptic cleft. This is designated as the **biogenic amine theory of depression**. There are four ways to prevent the neurotransmitter depletion by drug action: a) to increase the release of neurotransmitters from the presynaptic terminal; b) to prolong the interaction time with the postsynaptic receptors; c) to inhibit the enzymes which inactivate or destroy the neurotransmitters; d) to delay the reuptake of neurotransmitters in the presynaptic neurons. The tricyclic amines (desipramine, imipramine, and amitriptyline), which block the reuptake of noradrenaline and serotonin into the presynaptic neuron are powerful antidepressants. However, the depletion of neurotransmitter in the synaptic space may be compensated regardless of its cause, by the existence in this space of some liposomes filled with the neurotransmitter molecules which are in deficit. It is possible to introduce liposomes filled with different types of neurotransmitters. The liposomes may deliver controlled quantities of neurotransmitter, periodically. The liposomes can contain drug molecules, such as tricyclic amines, to block the reuptake process in the presynaptic membrane.

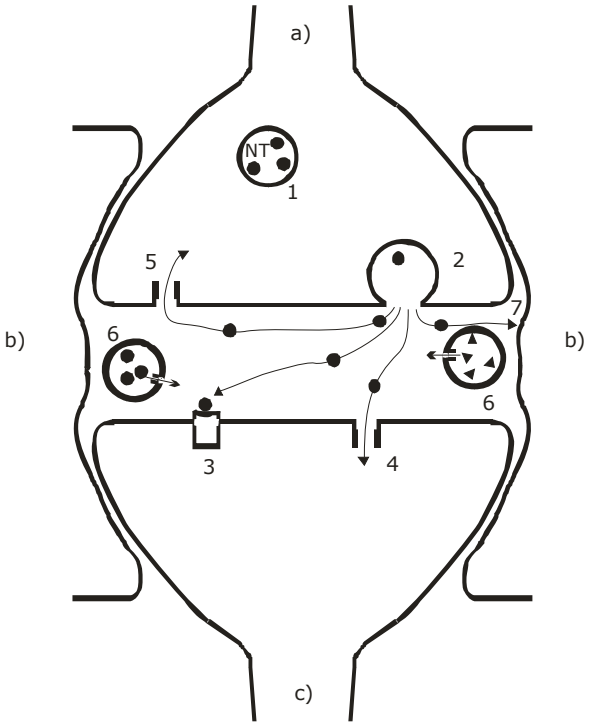

Fig. 4. The biocontroller liposome introduced in a synaptic cleft. a) The presynaptic neuron. b) The glial cell. c) The postsynaptic neuron. 1. The natural vesicles which incapsulate the neurotransmitter synthetised by the neuron. 2. The delivery of natural neurotransmitter by exocytose in the synaptic space. 3. The action on metabotropic receptors and G proteins, as second messenger. 4. The direct action on ionic channels. 5. The neurotransmitter loss due to reuptake in the presynaptic neuron. 6. Two liposomes are represented. Each of them releases only one type of neurotransmitter. 7. The leakage of neurotransmitters by transmembrane diffusion in the glial cells.

DISCUSSION

The use of liposomes as vehicle for drug transport to well defined places in the organism is a very ambitious aim of biotechnology. Here, we have endowed these liposome-vehicles with a supplementary property: the liposome can deliver some quantity of drug, or neurotransmitters in our case, from time to time. It acts as an automatic device. In the patent registered to OSIM we named it as “The biocontroller device for drug delivery”.

The length of the swelling time τ_n is dependent on the composition of both internal and external media of the liposome, its viscosity, and the partition coefficient of the solvent in the hydrophobic core. Some additives such as cholesterol or detergent, modifies the bilayer properties [13, 21, 22, 38, 39, 54, 55]. We have chosen for the coefficient κ a very unrealistic value: $\kappa = 1$. A more realistic value is $\kappa = 0.001$, which gives τ_n values in the range of minutes.

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