THE PULSATORY LIPOSOMES RELEASING OF NEUROTRANSMITTERS INSIDE THE INTERNEURONAL SYNAPTIC CLEFT MAY BE A POSSIBLE DEVICE FOR THE DEPRESSION TREATMENT

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Abstract. In this paper, we have considered the problem of the pulsatory lipid vesicle which may be regarded as a two stroke engine using the osmotic solute as a fuel. The consumed fuel is usefulness material for neuromedical applications. 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. So, a very interesting application of pulsatory liposomes may be the compensation of neurotransmitter deficiency into synaptic cleft. The pulsatory liposomes may be used as a device available for two from the four ways to compensate the neurotransmitter depletion by drug action: 1. to increase the release of neurotransmitters from the presynaptic terminal; 2. to delay the reuptake of neurotransmitters in the presynaptic neurons.

Key words: pulsatory liposome, depression, neurotransmitters deficiency compensation.

INTRODUCTION

Liposomes were initially used as an artificial model of cell membrane in biophysical membrane studies. Being a closed lipid bilayer, this membrane has the quality to separate a certain volume from the rest of the medium. More often, liposomes are used as drug carriers [17, 18]. Many studies have been made until now in order to incorporate, carry and free therapeutic substances, enzymes, antigens, antibodies, genetic material; these substances may by incorporated either

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inside the lipid bilayer (lipophilic substances) or inside the aqueous compartment (hydrophilic substances) [2, 16].

Also, for release active pharmacological substances at the place of action, the lipid bilayer of the liposome fuses with the lipid bilayer of the target cell membrane, and releases at once all the content.

In order to release this content different physical and chemical properties are used: liposomes positive charged, pH-sensitive liposomes, liposome interacting with macrophage, etc.

In this paper, the author proposes another mechanism of *specific action substances* releasing, which does not destroy the liposome: releasing in a well-controlled and accurate fashion, through transitory transmembranary pores.

The large interest for the exploration of pores by experimental, computational and theoretical approaches is dramatically growing, not only for a better understanding of molecular traffic across biomembranes, but also for potential applications in medical biotechnology [1, 4–10, 11, 12, 19]. In general, the transmembrane pores are either proteinaceous or lipidic. Our approach in this paper is devoted only to those pores which are transiently appearing in the lipid bilayer, and have their wall composed by lipid molecules.

THE PHENOMENOLOGICAL BASIS OF A PULSATORY LIPOSOME

Let us consider a unilamellar lipid vesicle filled with an aqueous solution of an osmotic solute. This vesicle is inserted into a hypotonic aqueous medium. There are two opposed concentration gradients across the vesicle bilayer [13]:

– *The water concentration gradient*. The water concentration from outside the liposome is greater than the water concentration from inside the liposome. From this reason, the influx of water molecules is greater than the outflow of water molecules, due to water diffusion process across the vesicle bilayer. So, the net flow comes inside the vesicle and is named osmotic flow.

– *The solute concentration gradient*. The inside solute concentration is greater than the outside solute concentration.

Due to this osmotic solute concentration gradient, the osmotic pressure appears directed from inside to outside of the vesicle.

The supplementary water entered inside the liposome determines two processes [13]:

1. The swelling of liposome.
2. The dilution of the internal solution.

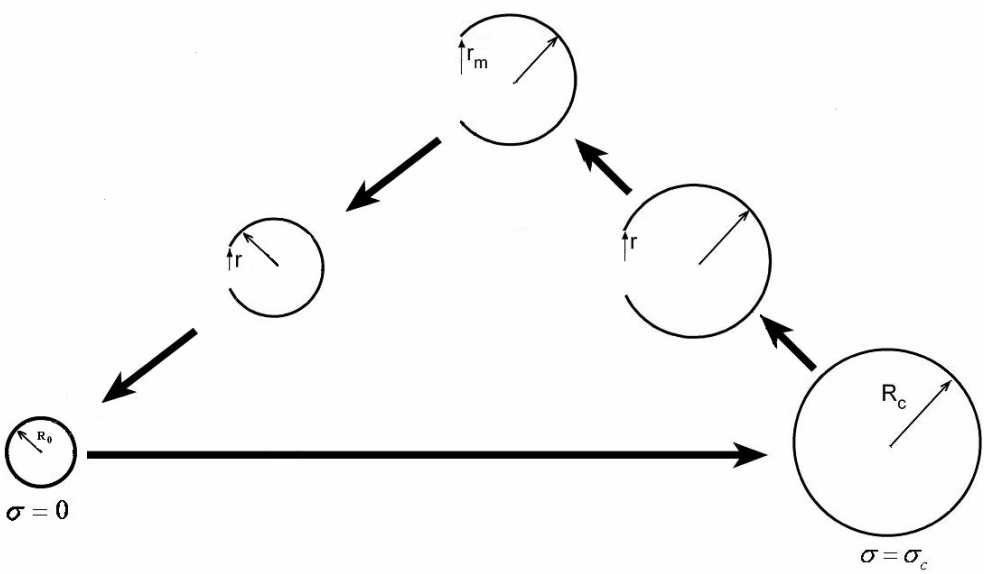

Fig. 1. A cycle from the evolution of a pulsatory liposome. In the first stage, the liposome swells from the initial state of radius R_0 to the critical state of radius R_c when o transbilayer pore appears (the top part of the picture). In the second stage, the pore radius increases to a maximum value r_m, after which it decreases to zero (equivalent to the pore disappearance). One must observe that, simultaneously with the pore evolution, the liposome relaxes until its radius equals to R_0 (the bottom part of the picture) [13, 16].

There are two direct and simultaneous consequences of the two above processes:

1. The vesicle surface tension grows at the same time with the liposome expansion and increases the Laplace pressure inside the vesicle.

2. The transmembrane solute concentration decreases, therefore the osmotic pressure decreases too.

Under these experimental conditions, at the end of a swelling stage, either the liposomal membrane may be ruptured and destroyed or one pore may appear through its lipid belayed [14].

Because the swelling process is slow enough, a transient transbilayer pore appears when the vesicle reaches the critical size. The pore appearance is an important event for the vesicle life, because it inverts the vesicle evolution sense. This event is followed by two simultaneous processes [13–16]:

1. The pore dynamics;

2. The leak out of the internal material of the vesicle, due to Laplace pressure.

The pore dynamics consists of two phases (Fig. 1). The pore radius increases up to the maximum value, r_m, in the first phase and decreases until the closure of the pore in the second one.

As a matter of fact, the pore dynamics is driven by the difference between the membrane tension and the edge (line) tension due to water exposure of the hydrophobic core membrane. In the first stage of the pore evolution, when it increases, the bilayer tension is greater than the edge tension.

Both phenomena, pore growth and internal liquid leakage, determine the membrane relaxation, due to the decrease of the membrane mechanical tension (the membrane tension decreases until it becomes equal to the tension of pore edge).

Do to Laplace pressure, the internal liquid continues to leak out the liposome even after the moment when the line tension equals the membrane tension, when in fact, the second part of dynamic pore starts, and therefore the pore radius decreases up to its disappearance.

After that, the vesicle dynamics described above can begin again (to swell itself) and a new cycle starts. The dynamics of a pulsatory liposome during a cycle is drawn in the picture from Fig. 1.

We can easily see that a cycle has two parts and one event: the vesicle swelling, the pore appearance and the vesicle relaxation, in the order of their succession.

In order to work a cycle, the following condition must be accomplished: the osmotic pressure must be greater than the Laplace pressure on the life span of the swelling stage. In other words, the working condition can be reformulated as: the osmotic pressure must be greater than the Laplace pressure if the vesicle would have reached its critical size.

When the osmotic pressure equals the Laplace pressure, the pulsatory liposome ceases its activity. This may happen at the last cycle.

Finally, we can imagine that such vesicle can work a number of cycles. During each cycle, the vesicle leaks out a certain amount of internal solution, which contains a number of solute molecules [12, 15, 16].

THE DEPRESSION MECHANISM. POSSIBLE APPLICATION OF PULSATORY LIPOSOMES

A very interesting application of pulsatory liposomes may be the compensation of neurotransmitter deficiency into synaptic cleft.

Although the cause of depression is not fully known, it is accepted that the biogenic amine neurotransmitters (norepinephrine, dopamine, serotonin) and acetylcholine may be all implicated in depression, especially their depletion 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 (Fig. 2). The tricyclic amines (desipramine, imipramine and amitriptyline) which block the reuptake of noradrenaline and serotonin into the presynaptic neuron are powerful antidepressant drugs. 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.

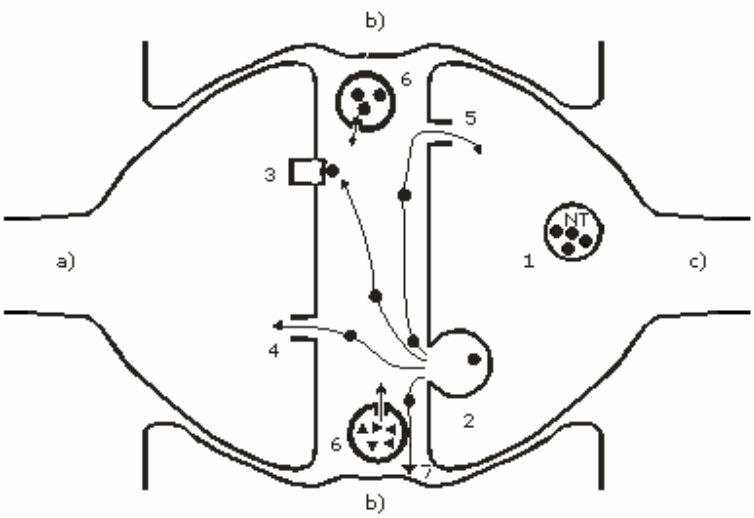

Fig. 2. The biocontroller liposome introduced in a synaptic cleft. a) the presynaptic neuron; b) the glial cell; c) the postsynaptic neuron. 1. The natural vesicles which encapsulate the neurotransmitter synthesized by the neuron. 2. The delivery of natural neurotransmitter by exocytosis in the synaptic 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.

MATERIALS AND METHODS

THE AMOUNT OF NEUROTRANSMITTER RELEASED DURING A CYCLE

The amount of neurotransmitter delivered during a cycle is given by efflux through the open pore and is equal to:

$$\Delta m_i = C_i^f V_f - C_{i+1}^0 V_0, \tag{1}$$

where C_i^f, V^f is the neurotransmitter concentration and the liposome volume, respectively, at the end of the swelling stage of liposome in the cycle of "*i*" rank; V^f is the liposome volume at its critical size; C_{i+1}^0, V_0 is the neurotransmitter concentration and the liposome volume, respectively, at the beginning of the swelling stage of liposome in the following cycle, that is the cycle of rank "*i*+1"; V_0 is the liposome volume at its initial state.

Taking into account that the solute amount is conserved during the swelling stage of each cycle ($C_i^f V_f = C_i^0 V_0$), the equation (1) becomes:

$$\Delta m_i = 4\pi R_0^3 (C_{i-1}^0 - C_i^0). \tag{2}$$

THE CHANGE IN COMPOSITION OF THE INTERNAL LIQUID

The amount of solute inside the liposome is modified by solute efflux through the open pore according to the equation:

$$\frac{\mathrm{d}(C_i V)}{\mathrm{d}t} = -\pi r^2 C_i v, \tag{3}$$

which is equivalent with:

$$\frac{\mathrm{d}\ln(C_i V)}{\mathrm{d}t} = -\frac{3r^2 v}{4R^3}, \tag{4}$$

where $C_i(t)$ is the neurotransmitter concentration inside the liposome, $r(t)$ is the pore radius, $R(t)$ is the liposome radius and v is the mean transport velocity of internal fluid through the pore.

But the outward flow velocity of the internal liquid is given by the formula:

$$v = \frac{\sigma_c}{6\eta_l\left(R_c^2 - R_0^2\right)} \frac{r}{R}\left[4\left(R^2 - R_0^2\right) - r^2\right]. \tag{5}$$

Taking into account the (5) the final form of equation (4) is:

$$\frac{\mathrm{d}\ln(C_i V)}{dt} = \frac{-2\sigma_c}{\eta_l\left(R_c^2 - R_0^2\right)} \frac{r^3}{R^4}\left[4\left(R^2 - R_0^2\right) - r^2\right]. \tag{6}$$

The equation (6) may be integrated, only if we know the time dependence of liposome vesicle size, $R(t)$, and of pore radius, $r(t)$, during the second stage of the life cycle of a pulsatory liposome.

From this reason the equation (6) must be coupled with the following two differential equations [13–16]:

– The differential equation for pore radius:

$$4\eta_b h\frac{dr}{dt}=\frac{Er}{R_0^2}\left(R^2-R_0^2-\frac{r^2}{4}\right)-2\gamma, \tag{7}$$

where h is the half thickness of lipid bilayer, η_b is the lipid bilayer viscosity, and E represents the elastic modulus for surface stretching or compressing.

– The differential equation for vesicle radius:

$$\frac{dR}{dt}=-\frac{Er^3}{6R_0^2\eta_l}\left(\frac{R^2}{R_0^2}-1-\frac{r^2}{4R_0^2}\right)+P_w V_{\mu w}\left(1-\frac{r^2}{4R^2}\right)\left[\frac{Q}{4\pi R^3}-\frac{2\beta E}{R_0^2}\left(\frac{R^2}{R_0^2}-1-\frac{r^2}{4R_0^2}\right)\right], \tag{8}$$

where η_l is the internal fluid viscosity, P_w (measured in m/s) is the water permeability through liposome membrane, $V_{\mu w}$ is the water molar volume (in m^3/mol), $\beta = 1/(N_A k_B T)$ N_A is the Avogadro number, k_B is Boltzmann constant, T is the absolute temperature and Q is the solute content.

The differential equations (6), (7) and (8) can be solved numerically using Euler's method to obtain the time dependence of vesicle radius, pore radius, and internal solute concentration ($R(t)$, $r(t)$ and $C_{in}(t)$). In the following chapter we have given the plots of these three parameters, characterizing the pulsatory liposome, for the relaxing stage in the first cycle (Figs. 3–5).

RESULTS

In order to calculate the neurotransmitter amount delivered during the pulsatory liposome running, we must know the concentration at the beginning to each cycle. We solved the system of three differential equations using Euler's method with a step size $\delta t = 1$ ms in order to see the time dependence of $r(t)$, $R(t)$, and $C(t)$. Before numerical integration all three equations were prepared by scaling the variables and parameters. The initial conditions were: $r(0) = 1.576$ μm; $R(0) = 20.6$ μm, and $C(0) = 0.01$M.

The differential equations were integrated for a liposome size used in experimental studies. The vesicle radius in relaxed state ($\sigma_0 = 0$) was $R_0 = 19.7$ μm, and the critical radius was $R_c = 20.6$ μm [1, 3, 19]. The membrane critical surface tension was $\sigma_c = 1.7\times10^{-5}$N/m [3]. The aqueous solution viscosity was $\eta_l = 3.2\times\times10^{-2}$ N.s/m^2 [19].

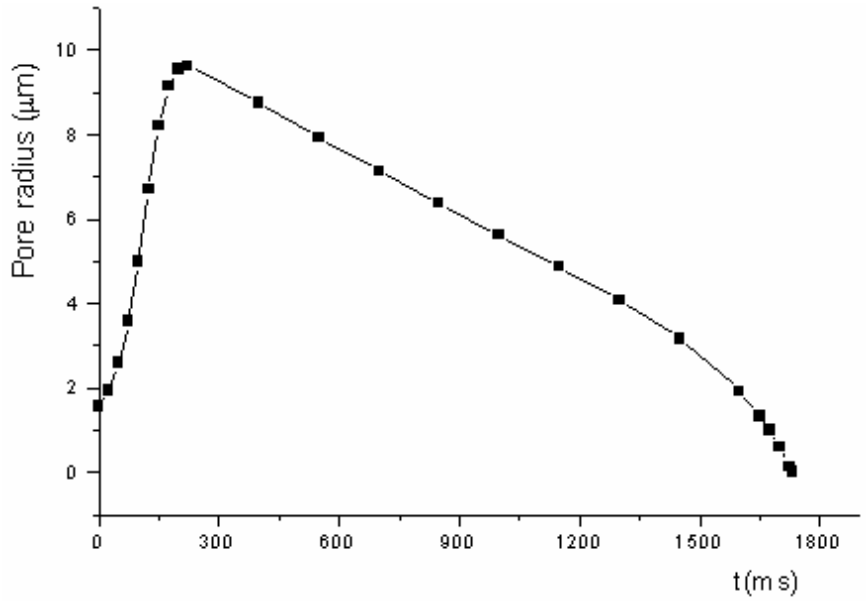

Fig. 3. The plot of the pore radius as a function of time starting from pore nucleation up to its disappearance. This is a solution of differential equation (7).

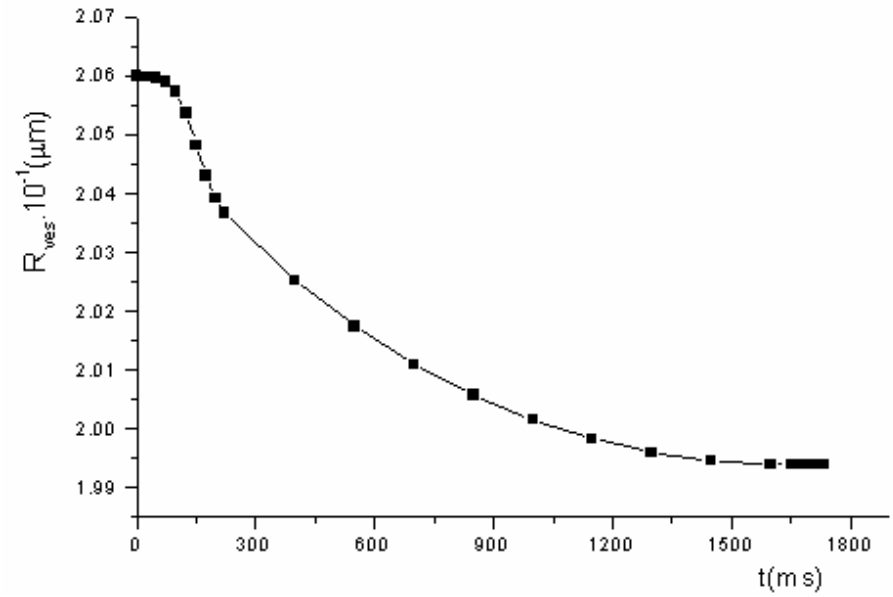

Fig. 4. The plot of the vesicle radius as a function of time during the relaxing stage of a cycle of a pulsatory liposome. This is the solution of differential equation (8).

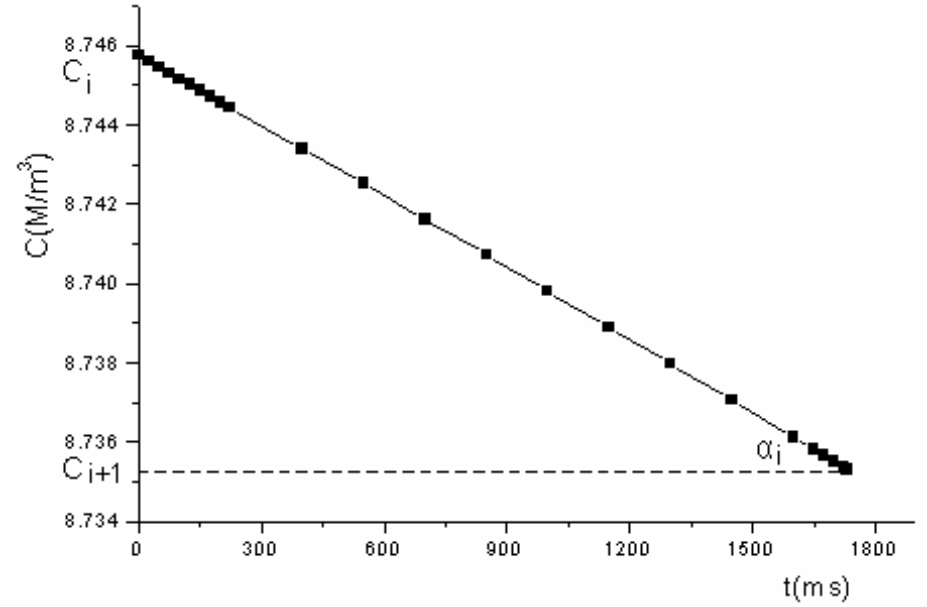

Fig. 5. The plot of the solute concentration inside a liposome as a function of time, during the relaxing stage of the liposome. It is the solution of differential equation (6).

In Figs. 3–5 we have plotted the pore evolution along its lifetime and the evolution of the vesicle size during the second stage of a cycle that is during the relaxing of the vesicle [13, 15].

The change in solute concentration during the pore lifetime, when the aqueous solution leaks out the vesicle, was plotted in Fig. 5. It is very interesting that the solute concentration decreases linearly during vesicle relaxation.

The formula (2) is more adequate for the calculation of neurotransmitter amount delivered by the pulsatory liposome both in theoretical approach and for experimental measurements.

Also, in the experimental measurements, the amount of active substances delivered during a cycle may be calculated according to formula:

$$\Delta m_i = 4\pi R_0^3 \tau_i \operatorname{tg}(\alpha_i), \tag{9}$$

where τ_i and α_i are the pore lifetime, and the slope of the concentration graph as a time function during the liposome relaxation, respectively, corresponding to the i^{th} cycle.

For easy understanding of what follows, we introduce new notations: $\theta=2\beta\sigma_c = 13.63\ 10^{-9}$ mol/m^2; $V^f = V_c = 4\pi R_c^3$, the liposome volume at its final swelling stage; $s = \left(\frac{R_c}{R_0}\right)^3 = 1.1434$, the swelling ratio; $C_0^0 = C_0 = C(0)$.

Taking into account that $C_i^0 V_0 = C_i^f V_c$ and $C_{i+1}^0 = C_i^f$, then $C_{i-1}^0 = sC_i^0$.

We have considered that the neurotransmitter is dopamine having molar mass μ = 153.18 g/mol and density ρ = 1.26 g/cm^3. It results that its molar volume is 121.57 cm^3/mol.

If the initial dopamine concentration of C_0 = 0.01 M, then in the liposome one finds $19.287\cdot10^{10}$ dopamine molecules.

The number of cycles, n, performed by pulsatory liposome during its running is obtained using the formula [12]:

$$C_0 = \frac{\theta}{R_c} s^n . \tag{10}$$

Introducing the parameter values, it results n = 71 cycles.

The number of dopamine molecules delivered during each cycle is calculated according to the formula [12]:

$$\Delta N_{n,p} = \frac{V_0}{R_c}\theta(s-1)s^{n-p}, \tag{11}$$

where p is the rank of cycle ($p \le n$).

The number of dopamine molecules released by pulsatory liposome, filled with the initial concentration of dopamine C_0 = 0.01 M, calculated for the first ten cycles and the last one are given in Table 1.

In Figure 6 we have represented the molecules number, ΔN, delivered by pulsatory liposome, filled initially with dopamine with concentration C_0 = 0.01 M, as a function of cycle rank, p.

Table 1

The number of dopamine molecules delivered during some cycles of the pulsatory liposome selected in this paper. The initial concentration of dopamine is C_0 = 0.01 M

p	1	2	10	20	30	40	50	60	70	71
$\Delta N\times10^{-5}$	21693	18972	6494	1700	445	116.5	30.5	7.9	2.1	1.8

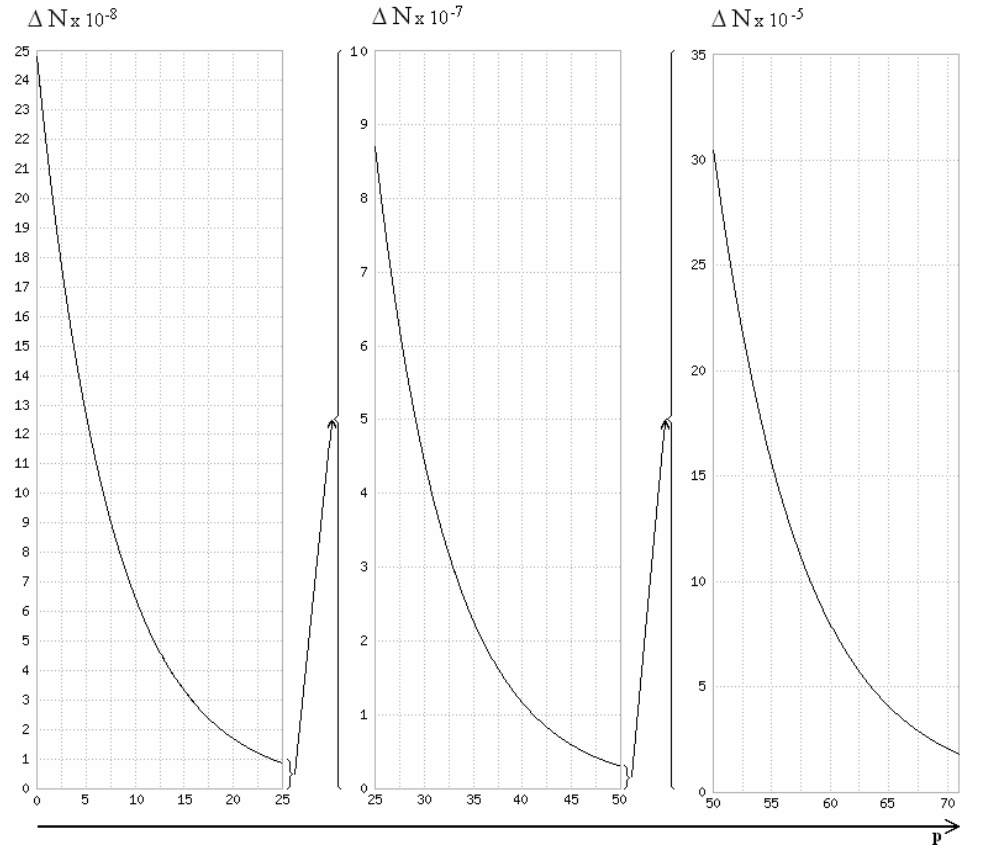

Fig. 6. The dopamine molecules number delivered by the pulsatory liposome along its running. The initial osmotic solute concentration was $C_0 = 0.001$ M. The pulsatory liposome works 71 cycles. The graph representing the dopamine amount released during each cycle was decomposed in three graphs of different degrees of amplification.

CONCLUSIONS

Pulsatory liposomes can be used for drug delivery in specified locations. In our opinion the amounts of drug should be sufficient to exert beneficial effects specifically in diseased regions, because there are no drug losses, as happens in other ways of drug delivery. The molecular mechanism of drug action should be known in detail in order to estimate the two parameters characterizing the pulsatory liposome: the time intervals between two successive pore openings, and the amount of drug released with the internal liquid leaked out during each cycle. Recently, an approximate solution for the working cycle of a pulsatory liposome was published [11, 12, 16].

Here, we made a theoretical simulation of the pulsatory liposomes working if they have been filled with 0.01 M initial concentration of dopamine. In its place could be any other neurotransmitter. The delivered amount of dopamine was given in molecules/cycle because we have considered that it is a more suggestive way to see the drug action. The validity of the theoretical results obtained in this paper could be verified by experiments in laboratory.

The preparation of pulsatory liposomes filled with one type of neurotransmitters, could not be a problem, it is possible. The external medium, where the liposomes will be introduced, could be a problem. It must have the same composition, or properties similar to those of the place where the liposome will work. The experimental tests may be accompanied by theoretical simulations.

From the practical point of view the liposomes transport at the site of action is a difficult problem which remains a biotechnology challenge. Some very interesting applications of pulsatory liposomes filled with drugs have been devised for targeting hepatic cells or the synaptic cleft. Endothelial pores (also known as fenestrae) control the exchange of fluids, solutes, and particles between the sinusoid blood capillaries and the space of Disse [9].

Pulsatory liposomes, free or included inside other vesicles, may reach hepatocytes due to hydrodynamic effects of blood circulation [9].

Finally, we consider that the pulsatory liposomes may be used as special devices for active substances controlled release, in the near future.

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