Eur Biophys J (2012) 41:73–78
DOI 10.1007/s00249-011-0759-3

ORIGINAL PAPER

EBSA
Biophysics in Europe

Hydrodynamic flow in a synaptic cleft during exocytosis

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Received: 21 March 2011 / Revised: 14 September 2011 / Accepted: 8 October 2011 / Published online: 1 November 2011

Abstract It is shown that exocytosis in a chemical synapse may be accompanied by "microjet" formation due to the overpressure that exists in the vesicles. This mechanism may take place either at complete fusion of a vesicle with the presynaptic membrane or in the so-called kiss-and-run mode of neurotransmitter release. A simple hydrodynamic model of the viscous incompressible flow arising in the synaptic cleft is suggested. The occurrence of hydrodynamic flow (microjet) leads to more efficient transport of neurotransmitter than in the case of classical diffusive transport.

Keywords Exocytosis · Chemical synapse

Introduction

In recent years, there has been considerable progress in studying the process of exocytosis, which is a universal mechanism of secretion in neuronal and endocrine cells. At present, the proteins (SNARE proteins) involved in this process have been identified and the main stages through which the vesicles undergo exocytosis are known (Südhof 2008; McMahon et al. 2010). The relatively short rising phase of postsynaptic responses in cholinergic and some other synapses of vertebrates implies that the secretion of neurotransmitter (acetylcholine) must be sufficiently fast (Edmonds et al. 1995; Stiles et al. 1996). A number of mathematical models have been developed so far to describe the processes leading to the generation of postsynaptic currents in both neuromuscular (Land et al. 1984; Bartol et al. 1991; Stiles et al. 1996; Khanin et al. 1994, 1997; Smart and McCammon 1998; Naka 1999; Naka and Sakamoto 2002) and central synapses (Faber et al. 1985; Wahl et al. 1996; Kruk et al. 1997; Nielsen et al. 2004). Some of these assume that neurotransmitter appears in the synaptic cleft instantaneously at the onset of simulation, while other researchers attempted to reproduce the process of exocytosis in more detail (Stiles et al. 1996; Wahl et al. 1996; Khanin et al. 1994, 1997; Naka 1999; Naka and Sakamoto 2002; Fan and Fedorov 2004). The main conclusion of these studies is that passive diffusion through a rapidly opening pore (rate of expansion should be at least 10 nm/ms) can account for the experimentally estimated amplitude and time course of endplate currents (Stiles et al. 1996; Wahl et al. 1996; Smart and McCammon 1998; Naka 1999; Fan and Fedorov 2004) in both central and peripheral synapses. However, release through a small fusion pore leads to a neurotransmitter concentration on the postsynaptic membrane below obtained estimates, and some acceleration of mediator diffusion is required to compensate for slow pore expansion. One of the mechanisms to provide sufficiently rapid discharge of mediator was proposed by Khanin et al. (1994, 1997). They speculated that the rapid pore expansion (full fusion) suggested by quick-freezing micrographs (Torri-Tarelli et al. 1985) may take place already after transmitter discharge is completed through a narrow pore formed at the early stages of exocytosis. As a possible mechanism of fast discharge of

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charged mediator through a small pore, the authors suggest influx of cocharged ions or coefflux of countercharged particles (ion interchange). Kharakoz (2001) suggested that neutralization by Ca^{2+} of the negative charge at the vesicle outer surface may result in a lateral constringent force that could flatten the vesicular membrane, thus contributing to the force ejecting the vesicle content. Unfortunately, at the moment, there are no decisive experimental tests to distinguish between different mechanisms of mediator secretion—simple passive diffusion in case of fast pore extension or accelerated release thorough a narrow pore.

In this paper, we show for the first time that both release modes (fast pore opening and release through the narrow pore) may be accompanied by "microjet" formation due to the overpressure that exists in the vesicles. At the same time, the role of the SNARE protein remains unchanged.

The overpressure, δp, between the inner and outer regions of the vesicles may be the result of several factors (Tanford 1979; White 1980; Amatore et al. 2003; Kelly et al. 2004). The surface tension of the vesicle membrane creates a pressure jump across the membrane defined by the Laplace equation, $\delta p_{\mathrm{L}} = 2\sigma/R_{\mathrm{v}}$, where σ and R_{v} are the surface tension and the radius of curvature of the surface. The membrane of the vesicles is stretched and undergoes mechanical stress because of the gradient of osmotic pressure, and the concentrations of osmotically active substances (acetylcholine and comediator) are significantly higher inside than outside the vesicle membrane. Finally, at the merging of the membranes, a force tending to straighten the membrane vesicles appears. This force can serve as an additional factor in accelerating the expulsion of the synaptic vesicle contents into the synaptic cleft. When the vesicle merges with the cytoplasmic membrane, the overpressure in the vesicle may drive a hydrodynamic flow of fluid. Mediators suspended in the fluid are carried with this flow. This additional form of transport can significantly contribute to diffusion transport in the synaptic gap.

Model

In this paper, exocytosis in a chemical synapse is considered, namely the process when a vesicle within a nerve ending fuses to a plasma membrane and releases its content into the extracellular medium.

It is supposed that the synaptic cleft is filled with an incompressible viscous fluid (water). When the vesicle is opening, we assume that the overpressure

$$\delta p = \delta p_{\mathrm{L}} = (2\sigma/R_{\mathrm{v}}), \tag{1}$$

acts on water containing suspended neurotransmitter molecules.

A schematic of exocytosis and the geometry of the computational model in cylindrical geometry (r, z) are shown in Fig. 1. We consider the following two-dimensional nonstationary problem for a viscous incompressible fluid (ρ = const., where ρ is the fluid density) (Landau and Lifshitz 1987):

$$\begin{aligned} &\nabla \cdot \vec{u} = 0 \\ &\frac{\partial \vec{u}}{\partial t} + \left(\vec{u} \cdot \nabla\right)\vec{u} = -\nabla p/\rho + \nu \Delta \vec{u}. \end{aligned} \tag{2}$$

It is convenient to solve the problem in the stream function–vorticity variables in cylindrical coordinates:

$$\frac{\partial^2 \Psi}{\partial r^2} + \frac{\partial^2 \Psi}{\partial z^2} = -\xi r + \frac{1}{r}\frac{\partial \Psi}{\partial r}, \tag{3}$$

$$\frac{\partial \xi}{\partial t} + u_z \frac{\partial \xi}{\partial z} + u_r \frac{\partial \xi}{\partial r} - \frac{u_r}{r}\xi = \nu\left(\Delta\zeta - \frac{1}{r^2}\left(\varsigma + \frac{\partial u_z}{\partial r}\right)\right). \tag{4}$$

Here, Ψ is the stream function, which determines the velocity components as

$$u_r = -\frac{1}{r}\frac{\partial \Psi}{\partial z}, \quad u_z = \frac{1}{r}\frac{\partial \Psi}{\partial r}, \tag{5}$$

and $\vec{\xi} = \nabla \times \vec{u}$ is the vorticity,

$$\zeta = \frac{\partial u_r}{\partial z} - \frac{\partial u_z}{\partial r}. \tag{6}$$

The boundary conditions for system (2–6) are standard for the well-studied problem of a finite-radius jet directed toward an impermeable or partially permeable surface (a so-called impinging jet) in cylindrical geometry (Phares et al. 2000):

$$\begin{aligned} &\Psi(z,0) = 0 \\ &\Psi(h,r) = 0 \\ &\frac{\partial \Psi(z,R)}{\partial r} = 0 \\ &\Psi(0,r) = F(r). \end{aligned} \tag{7}$$

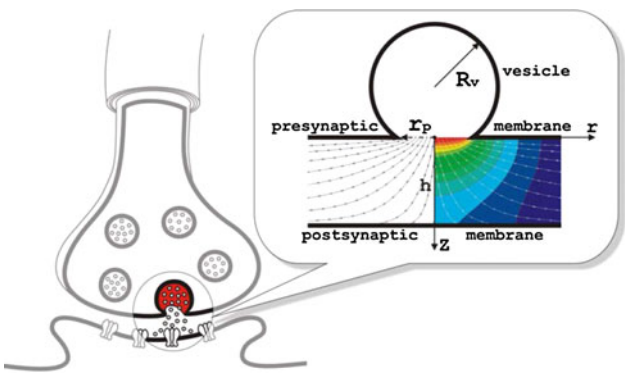

Fig. 1 Schematic of exocytosis in a chemical synapse. *Inset* geometry of the computational model

The presynaptic and postsynaptic membranes are impermeable to water within the considered time interval. Due to the hydrophilicity, no-slip boundary conditions are valid for the flow velocity; i.e., the normal and radial components of flow velocity are assumed to be zero at the membrane surface. In the pore, $r \le r_p$, a parabolic velocity profile is assumed, i.e., $u_z(0,r) = u_0(1-(r/r_p)^2)$. The influx stream function, $F(r)$, can be determined from the given influx velocity profile (Phares et al. 2000), $F(r) = \int_0^r u_z(0,r)r\mathrm{d}r$. In the calculations, the balance of mass flow rate is strictly obeyed:

$$\int_0^{r_p} u_z(r,0)r\mathrm{d}r = R\int_0^{z=h} u_r(R,z)\mathrm{d}z, \tag{8}$$

where R is the radial boundary for the accepted numerical domain. The pressure distribution was determined by solving the Poisson equation

$$\Delta p = -\rho\left[\left(\frac{\partial u_z}{\partial z}\right)^2 + 2\frac{\partial u_r}{\partial z}\frac{\partial u_z}{\partial r} + \left(\frac{\partial u_r}{\partial r}\right)^2 + \left(\frac{u_r}{r}\right)^2\right] \tag{9}$$

for each instant distribution of flow velocity and adopted boundary conditions. The pressure distribution on the boundary of the pores and synaptic cleft must satisfy the following condition, which follows from Bernoulli's equation:

$$p(0,r) = p_0 + \delta p - 0.5\rho u_z(0,r)^2, \quad r \le r_p. \tag{10}$$

Other boundary conditions for pressure are:

$$\begin{aligned} &p(z,R) = p_0 \\ &\frac{\partial p(z,0)}{\partial r} = 0 \\ &\frac{\partial p(0,r)}{\partial z} = \frac{\partial p(h,r)}{\partial z} = 0. \end{aligned} \tag{11}$$

Results and discussion

All partial differential equations relevant to the problem were solved in finite differences on a rectangular 2D mesh. The results obtained are grid independent. The time-dependent equation for vorticity (4) was solved using a McCormack second-order scheme (Anderson et al. 1984). The elliptic equations for the stream function (3) were solved with the boundary conditions (7) at each time step using the successive overrelaxation method (Anderson et al. 1984). New projections of velocity were then calculated using definition (5). Then, the equation for pressure (9) with boundary conditions (11) was also solved by the successive overrelaxation method (Anderson et al. 1984).

The results of calculations are shown in Figs. 2–4. These results correspond to quasistationary flow obtained for the typical parameters of chemical synapses listed in Table 1: width of the synaptic cleft, $h = 20$ nm; radius of the vesicles and pores, respectively, $R_v = 20$ and $r_p = 5$ nm. The density and kinematic viscosity of the liquid correspond to water at $T = 293$ K, i.e., $\rho = 998.2$ kg/m^3 and $\nu = 1.004 \times 10^{-6}$ m^2/s. As an example, it was assumed that the surface tension of the membrane vesicles is $\sigma = 0.1$ mN/m (Feller and Pastor 1996; Sandre et al. 1999). In this case, according to formula (1), the overpressure is $\delta p \approx 10^4$ Pa $= 0.1p_0$, where p_0 is the background unperturbed pressure, which is assumed to be equal to atmospheric pressure.

We consider the initial stage of exocytosis, when the radius of the vesicle is still close to its original value. In the continuous medium approximation, there are no ruptures in the liquid. Leakage of fluid from the vesicles is accompanied by a change in radius, which results from surface tension forces. The mass balance of fluid in the vesicles is determined by leakage through the pore: $\frac{\mathrm{d}M}{\mathrm{d}t} = 2\pi\rho\int_0^{r_p} u_z(0,r)r\mathrm{d}r$, where $M = \frac{4}{3}\pi R_v^3\rho$. Assuming that the hydrodynamic stage of fluid ejection from the vesicles takes time τ_h, and also that the exhaust velocity is constant, we can estimate the velocity of the fluid through the pore. If the earlier assumption of a parabolic velocity profile in the pore is taken, the listed assumptions give $u_0 \approx \frac{8}{3}\frac{R_v^3}{r_p^2\tau_h}$.

According to experimental measurements (Bruns and Jahn 1995) and computer modeling (Stiles et al. 1996; Smart and McCammon 1998), the release time of synaptic vesicle content is on the order of 100 μs. We assume that the duration of the hydrodynamic stage, τ_h, is only a part of the total release time; therefore, the choice of $\tau_h = 10$ μs seems to be reasonable. Note that the rising phase of transmitter release has a duration of approximately 40 μs (Skorinkin et al. 2008). This value was calculated using

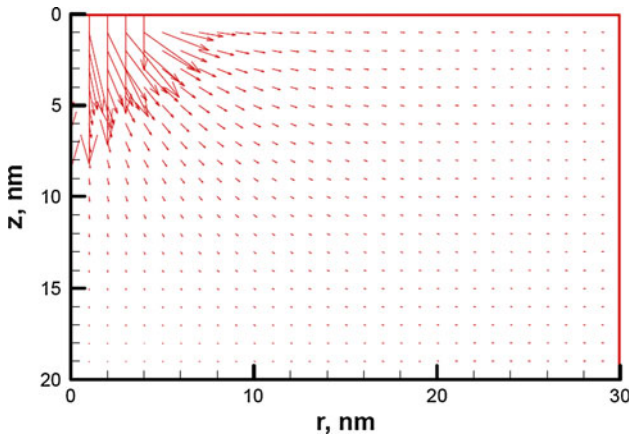

Fig. 2 Local flow velocity directions and relative amplitudes. The longest vector length corresponds to $u_z(z=0, r=0) \approx 8.5$ cm/s

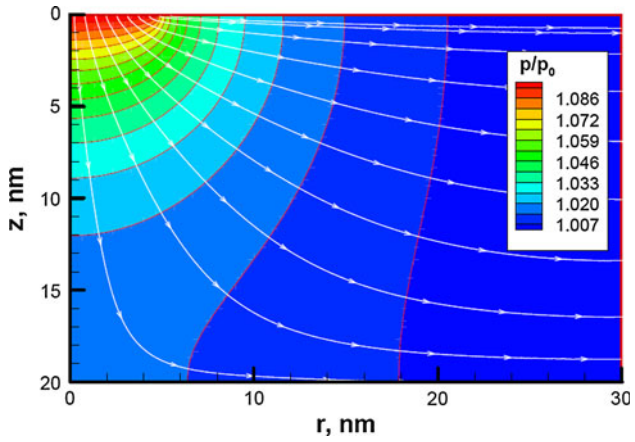

Fig. 3 Contours of the static pressure distribution, $p(z, r)/p_0$, and the flow streamlines

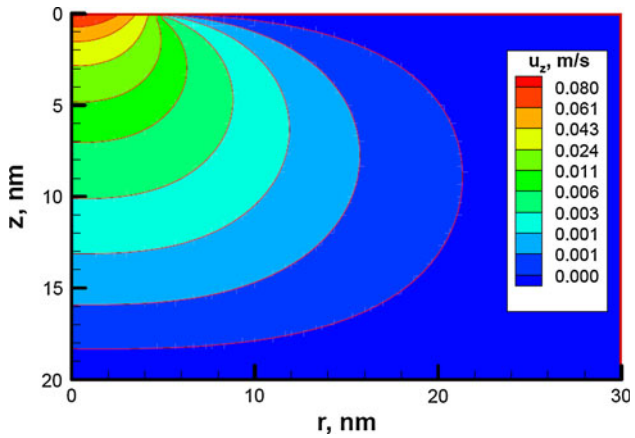

Fig. 4 Contours of the normal component of the flow velocity, u_z

experimental data and is close to our assumed value for τ_h. For the assumed sizes of the vesicles and pores, we obtain $u_0 \approx 8.5$ cm/s. All results presented in Figs. 2–4 correspond to this inflow velocity through the center of the pore. In the absence of viscosity, the maximum rate of outflow from the pores, estimated from Bernoulli's law, would be $u_{\max} = \sqrt{2\delta p/\rho} \approx 4.47$ m/s. In reality, not all the overpressure transfers into the flux kinetic energy; it is partially redistributed to ensure the flow of viscous incompressible fluid around the synaptic cleft (Fig. 3). In fact, the maximum velocity of the quasistationary flow, which corresponds to the outflow velocity from the pore, is significantly lower than the maximum speed, $u_{\max}$ (Figs. 2, 4).

In the model, the flow structure and pressure distribution are established in the synaptic cleft very quickly ($\ll 0.1$ μs $\ll \tau_h$), because the fluid is incompressible. During this time, the radius of the vesicles is approximately constant. Therefore, we can assume that the flow at any given moment in time is established and corresponds to the instantaneous value of the vesicle radius. Therefore, we can expect that the hydrodynamic transport mechanism works equally well for both modes of exocytosis—full fusion and "kiss and run", which lasts much shorter. The kiss-and-run mechanism is still a matter of debate for synaptic transmission, although it has been proved for some neurosecretory cells (Aravanis et al. 2003; Wightman and Haynes 2004).

The diffusion coefficient for a typical mediator (acetylcholine) molecule at $T = 293$ K is $D_M \approx 6 \times 10^{-10}$ m^2/s (Dionne 1976). The flux of mediators of density n_M from the pore into the cleft together with the microjet flow, $n_M u_z$, exceeds the diffusion flux, $-D_M \partial n_M/\partial z \sim D_M n_M/R_v$ at the axial flow velocity $u_0 = u_z(0,0) > D_M/R_v$. For the assumed vesicle radius, $R_v = 20$ nm, this happens at $u_0 > 3$ cm/s. As follows from our model for the parameters assumed, in the vicinity of the pore the flow velocity (and the hydrodynamic mediator molecule transport) is higher than the diffusive transport.

Hydrodynamic flow arising in the synaptic cleft under the influence of overpressure in the vesicles is similar to the well-studied problem of an impinging jet in macroscopic hydrodynamics (Phares et al. 2000). Therefore, we called the hydrodynamic flow arising in the synaptic cleft a "microjet."

Table 1 Input model parameters

Parameter	Symbol	Value(s)	Reported range	Sources
Diffusion constant	D_M	6×10^{-10} m^2/s	0.5×10^{-10}–1×10^{-9} m^2/s	Dionne (1976), Land et al. (1984), Naka (1999), Naka and Sakamoto (2002)
Cleft height	h	20 nm	10–50 nm	Khanin et al. (1994), Naka (1999), Naka and Sakamoto (2002), Savtchenko and Rusakov (2007)
Radius of vesicle	R_v	20 nm	18.5–20 nm	Heuser and Reese (1973), Fan and Fedorov (2004)
Radius of fusion pore	r_p	5 nm	≥1 nm	Spruce et al. (1990), Stiles et al. (1996), Fan and Fedorov (2004)
Surface tension	σ	0.1–1 mN/m	0.1–50 mN/m	Feller and Pastor (1996), Sandre et al. (1999)
Kinematic viscosity (water at $T = 293$ K)	ν	1.004×10^{-6} m^2/s		Grigoriev and Meilikhov (1997)

We understand that the model considered for the liquid ejection during exocytosis is significantly simplified; for example, we have neglected the effects associated with the actual thickness of the vesicles and presynaptic membranes. The real thickness of the membrane (5–9 nm) can lead to a significant, quantitative change in the results (White 1980). However, the qualitative picture of exocytosis, in our opinion, does not change. We took the surface tension of the membrane vesicles as $\sigma = 0.1$ mN/m and the liquid outflow time, $\tau_h = 10$ μs, only for a specific numerical example. At larger values of surface tension from the range listed in the Table 1, or shorter τ_h, the hydrodynamic transfer may be much stronger. Even for much longer exocytosis time, $\tau_h \sim 0.1$ ms, the hydrodynamic transport is greater than or comparable to the diffusion transport. The results obtained by solving the Navier–Stokes equations at small scales, typical for a synapse, can be regarded only as qualitative estimates. A quantitative analysis requires more appropriate methods of molecular dynamics. Also, we do not take into account the dynamics associated with the change in radii of vesicles and pores in the outflow of fluid into the synaptic gap. We hope to explore these important issues in the future.

Possible consequences of hydrodynamic flow (microjet) formation for chemical synapses are:

1. The hydrodynamic flow of neurotransmitters across the synaptic cleft with microjets is significantly greater than the transfer due to diffusion.
2. Formation of microjets allows more directed passage of mediator molecules through the basal membrane in the middle of the synaptic cleft, which leads to effective activation of postsynaptic receptors.
3. Formation of microjets creates directed motion of neurotransmitters through the cleft towards the postsynaptic cell, which decreases the probability of excessive activation of autoreceptors on the presynaptic membrane.

Conclusions

It is known that the properties of cellular and vesicular membranes are determined by several factors, such as ambient temperature, pH, concentration of divalent cations, and the phospholipid and protein composition of membranes, and can be affected by exposure to a number of surface-active substances (detergents, surfactants), neurotoxins, local anesthetics, etc. (Seeger et al. 2007; Roza and Fratangeli 2010). According to the model described above, it is reasonable to expect that some factors that modify the elastic and surface properties of plasma membrane should affect the parameters of the microjets. On the other hand, the hydrodynamic conditions in the synaptic gap affect the characteristics of the microjet and may be reflected in the amplitude and temporal characteristics of the parameters of synaptic responses (potentials and currents of the endplate), and hence in the efficiency of synaptic transmission. Electrophysiological recording of synaptic activity with modification of membrane properties through physical factors or pharmacological agents, as well as the direct pulsed field gradient–nuclear magnetic resonance (PFG-NMR) method (Kärger et al. 1998) to explore the transport properties of molecules transferred as a part of microjets, could contribute to model validation and definition of reasonable ranges for model parameters.

Acknowledgments We are grateful to L.G. Martinelli and A.V. Filippov for valuable comments and discussions. This work was partially supported by Russian Foundation for Basic Research and grant "Leading Scientific School".

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