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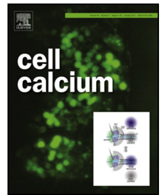

Exocytotic fusion pores as a target for therapy

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ABSTRACT

Regulated exocytosis can be split into a sequence of steps ending with the formation and the dilation of a fusion pore, a neck-like connection between the vesicle and the plasma membrane. Each of these steps is precisely controlled to achieve the optimal spatial and temporal profile of the release of signalling molecules. At the level of the fusion pore, tuning of the exocytosis can be achieved by preventing its formation, by stabilizing the unproductive narrow fusion pore, by altering the speed of fusion pore expansion and by completely closing the fusion pore. The molecular structure and dynamics of fusion pores have become a major focus of cell research, especially as a promising target for therapeutic strategies. Electrophysiological, optical and electrochemical methods have been used extensively to illuminate how cells regulate secretion at the level of a single fusion pore. Here, we describe recent advances in the structure and mechanisms of the initial fusion pore formation and the progress in therapeutic strategies with the focus on exocytosis.

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1. Introduction

Communication between cells is mediated by signalling molecules, including proteins, peptides, amino acids, bioamines and ions. The signalling process begins within cells, where signalling molecules have to be synthesized and delivered to designated places where they exit the cell. Small water-soluble signalling molecules can pass the plasma membrane via various channels and transporters; however, their reach is usually limited due to the relatively slow diffusion [1,2]. The most efficient mechanism for the transmission of signalling molecules to different parts of the cell involves various membrane-bound vesicles, which utilize the elaborate network of the cytoskeleton, to be transported to acceptor membranes [3–5]. Delivery of vesicles loaded with signalling molecules to the target membrane is the first step of the process termed regulated exocytosis. The following steps of regulated exocytosis include vesicle tethering, docking and priming. Finally, upon a stimulus, which is typically manifested by an increase in local cytosolic Ca^{2+} activity, vesicles fuse with the plasma membrane and release their cargo.

There are two proposed pathways of vesicle fusion; either the initially formed fusion pore (i.e. a low-conducting connection between the vesicle lumen and the extracellular space) swiftly expands, leading to complete merger of the vesicle into the plasma membrane (full-fusion exocytosis), or the fusion pore oscillates between relatively narrow states with different conductance (transient exocytosis) before it fully expands [6–9]. It is clear that by stabilizing the narrow fusion pore, by altering the speed of fusion pore expansion and by completely closing the fusion pore, tuning of exocytosis can be achieved [6]. The importance of such tuning for potential pharmacological interventions was demon-

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strated in a recent study by Collins et al. [10]. In this study, the authors have shown that the increased expression of the transcription factor Sox4 promotes transient versus full-fusion exocytosis, consequently inhibiting insulin secretion and increasing diabetes risk. How can a cell entail such tuning at the level of single-vesicle fusion? The answer is likely in the molecular components that participate in the formation of the fusion pore and in the components that make up the fusion pore, when the vesicle and the plasma membranes merge. However, the field has yet to reach an agreement on the interplay of the prominent candidates in the dynamic process of vesicle fusion [6]. The two main models, which predict fusion pores predominantly composed of either proteins [11] or lipids [12], diametrically oppose each other (Fig. 1). There is little doubt that vesicle priming and fusion are facilitated by three canonical soluble *N*-ethylmaleimide-sensitive-factor attachment protein receptor (SNARE) proteins [13]. In neuronal cells, the SNARE proteins involved in neuroexocytosis are synaptobrevin-2 (Syb2, also known as VAMP2), which is located on the vesicle membrane [14], plasma membrane anchored syntaxin-1 (Stx1) [15] and SNAP-25 [16]. In the model of a proteinaceous fusion pore, the transmembrane domains of these SNARE proteins line the initial fusion pore in a gap-junction-like manner (Fig. 1). This model is supported by observations of low-conducting (narrow) pores exhibiting fast reversals [8,17,18] and is consistent with the findings that tryptophan mutations of the syntaxin 1 transmembrane domain interfere with the flux through the fusion pore [11]. Nonetheless, the apparently small number of SNAREs required for fusion [19–22] suggests the presence of other fusion pore constituents, either proteins or lipids. In concordance with these results, it was proposed that the initial fusion pore is a hybrid structure composed of both lipids and proteins [19,23]. However, there is another possible model; i.e. the initial fusion pore is composed purely of lipids of both membranes [12]. According to this hypothesis, the sole purpose of the SNARE complex is to accelerate the formation of the fusion pore by bringing together the adjoining vesicular and the plasma membranes. In the next step, the outer leaflets of the membranes merge, initially forming a hemifusion stalk that proceeds into a hemifusion diaphragm and finally to the purely lipidic fusion pore [12,24,25] (Fig. 1). In line with this model, a hemifusion structure was detected by conical electron tomography [24], as well as in different in vitro fusion assays [12,26]. Nonetheless, it is still not clear if the hemifusion is not a dead end in Ca^{2+}-triggered exocytosis [27]. Unfortunately, most of the imaging techniques that allow live imaging suffer from a coarse spatial resolution, which prevents reliable measurements of structures in the range of a few nanometres (the initial fusion pores apparently have a conductance that corresponds to a diameter of less than a nanometre [7]). On the other hand, freeze-fracture electron microscopy, with sufficient resolution to resolve nanometre-sized objects, has not been able to confirm fusion pores smaller than 8 nm [6,28]. Structures, such as a hemifusion stalk and a narrow fusion pore, require the creation of highly curved membrane regions that are associated with large bending energies. These energy barriers may be in part overcome by membrane constituents, i.e. lipids, proteins or small membrane nanodomains, with negative spontaneous curvature [8,29–31]. Therefore, in the model predicting lipidic (or at least semi-lipidic) initial fusion pores, the shape and distribution of lipids is important for the formation and for the stability of a fusion pore.

Regulated exocytosis is a fundamental signalling process, which contributes to neurophysiological performance and also, when impaired, to the development of pathological conditions. Vesicle fusion, and specifically the formation of the initial fusion pores, represents a key step in regulated exocytosis. In this review, we describe recent advances about the structures and mechanisms of the initial fusion pore formation and how these present a valid target for further research and medical applications.

2. Ensnaring the SNAREs

Regardless of the structure and of the exact mechanism leading to the initial fusion pore formation, SNARE proteins are considered to be essential for orderly execution of membrane fusion [32]. Hence, in the eons of evolution, they also became a target of toxins. When synaptobrevin, syntaxin and SNAP-25 are not assembled in a tight ternary complex, they are specific substrates for cleavage by *Clostridium botulinum* and tetanus neurotoxins (BoNT and TeNT, respectively) [33,34], which strongly inhibit synaptic release of neurotransmitters [35] (Fig. 2). The seven classic serotypes (recent molecular genetic analyses have confirmed novel BoNTs with different amino acid sequences that can be grouped within an existing serotype [36]) of BoNTs A–G are too large to pass the blood–brain barrier and therefore act at the periphery by inducing flaccid paralysis. On the other hand, TeNT has the ability to enter the spinal cord and results in spastic paralysis by blocking central motor neurons [34,37]. BoNTs and TeNT share the basic mechanism of blocking vesicle fusion; both are exquisitely specific metalloproteases that cleave different peptide bonds of the SNAREs. BoNTs A

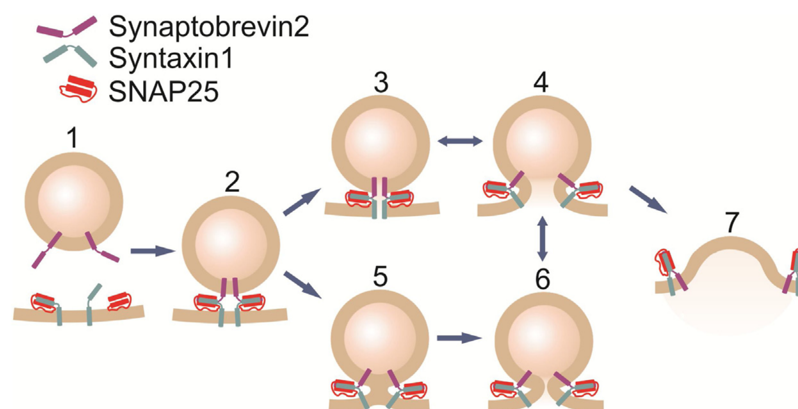

Fig. 1. Putative intermediates of fusion pore formation and expansion.
A vesicle is delivered (1) and appended (2) to the plasma membrane. Binding of plasmalemmal SNAREs (e.g. syntaxin-1 and SNAP-25) to secretory vesicle SNAREs (e.g. synaptobrevin-2), results in the formation of a four-helix bundle called the trans-SNARE complex, which brings membranes into close apposition. A vesicle then fuses with the plasma membrane, establishing an initial fusion pore. A model of a proteinaceous fusion pore predicts that transmembrane domains of SNAREs line the fusion pore (3), eventually leading to a lipidic fusion pore (4). An alternative mechanism implies the formation of a hemifusion stalk (5) that proceeds to a purely lipidic fusion pore (6). Either way, a fusion pore can fluctuate between different diameters (3–6, or 6–4), before complete merger of plasma and vesicle membranes (7).

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