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Opinion Article

OPINION ARTICLE

Pre-and Postfusion Tuning of Regulated Exocytosis by Cell Metabolites

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Physiological responses appear to rely on two main mechanisms, contracting a muscle, or secreting a substance; both being controlled by Ca^{2+}. The key events of stimulus–contraction, addressed at the end of 19th century by studying how the ionic composition of solutions support myocardial contraction,[1] are now understood to critically depend on an increase in free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$). Fundamental role of Ca^{2+} in stimulus–secretion–coupling was highlighted in 1957 by Jenkinson in experiments showing that increasing extracellular $[Ca^{2+}]$ nonlinearly augments transmitter release and that the injection of Ca^{2+} into the nerve terminal by Miledi in 1973 elicits neurotransmitter release. This paved the way towards the "Ca^{2+} hypothesis" of stimulus–secretion coupling, positing that during depolarization Ca^{2+} enters the cytoplasm and "becomes involved in a reaction between specific molecules on the inner surface of the axon membrane and molecules on the surface of synaptic vesicles colliding with the membrane, thereby leading to the release of quantal packets of transmitter substance". [2]

The notion "vesicles colliding" implicates that events instigated by Ca^{2+} entry evolve swiftly to minimize the delay between stimulus and secretion, which can be as short as 150 μs.[3] Synaptotagmin 1 (Syt1), a Ca^{2+} sensor of the release machinery responds sufficiently rapidly to Ca^{2+} to mediate the subsequent bringing together of the SNARE protein complex.[4] SNARE proteins exist in vesicular and plasmalemmal versions (v-SNAREs and t-SNAREs), which assemble into a thermally stable ternary SNARE complex,[5] an association of the three types of SNARE molecules, an essential step toward membrane fusion. Atomic force spectroscopy, however, showed that the SNARE complex is not as stable as previously thought.[6]

An alternative view of how to minimize the stimulus–secretion coupling delay suggests that Ca^{2+} not only sets in motion rapid assembly of the SNARE complex,[2] but activates an already preassembled release machinery. In such a case, a vesicle is already docked and even fused with the plasma membrane (i.e., Ca^{2+} trigger operates at the vesicle postfusion stage, see Figure 1).[7]

Neurotransmitter release from vesicles is mediated by exocytosis, a universal process existing in eukaryotic cells, which involves a membrane merger between the vesicle and the plasma membranes, leading to the formation of a fusion pore, which is essentially a channel through which vesicle content exits into the extracellular space. Hormones and enzymes are released in a similar manner, but typically with a slower dynamics compared to the synaptic events. The slowness of exocytosis in (neuro)endocrine and exocrine cells allowed us to discern the stages through which a vesicle has to pass to allow the content discharge (Figure 1). One of these stages also occurs in the absence of stimulation; it represents a formation of extremely narrow, subnanometer, fusion pores described in

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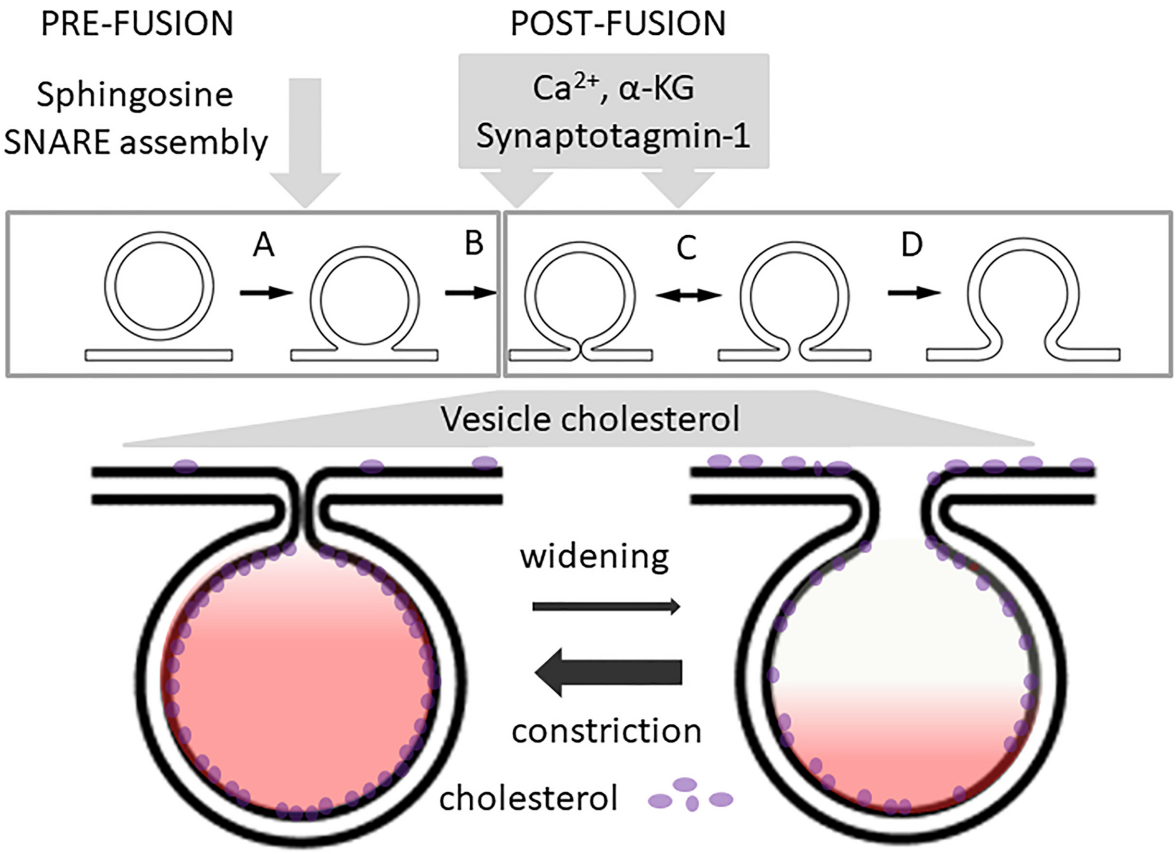

Figure 1. Stages through which a vesicle interacts with the plasmalemma. Prefusion stage A denotes hemifusion, which proceeds to stage B, with a narrow fusion pore (postfusion stage), which can then reversibly open (stage C), before widening fully (stage D). Bottom: redistribution of cholesterol (depicted in pink on the surface) from the vesicle membrane to the outer leaflet of the plasmalemma controls fusion pore geometry (black arrows, modified from,[10] α-ketoglutarate, αKG).

2007 by Vardjan and colleagues, not permitting the release of vesicle content unless $[Ca^{2+}]_i$ is elevated.[7] Formation of these subnano-pores results in the "unproductive exocytosis." This stage has been suggested previously to potentially contribute to the minimization of the delay between the stimulus and secretion. The widening and persistence of the open fusion pore depends not only on $[Ca^{2+}]_i$, but also on the capacity of the dynamic assembly–disassembly of the SNARE complex,[7] indicating that this complex plays a role at postfusion stages as well (Figure 1).

In synapses, the stimulus–secretion coupling is tailored for the maximal speed, in analogy to a compressed spring, which rapidly relaxes upon being hit by a trigger Ca^{2+}. At the same time stimulus–secretion coupling is modulated by cell metabolites that are involved in energy provision (i.e., Krebs cycle) and those supporting the nanostructure of the exocytotic machinery (i.e., related to membrane constituents). An example of cell metabolites modulating exocytosis involving the Krebs cycle is the modulation of synptotagmin-1 (Syt-1) by α-ketoglutarate (αKG),[8] acting at the pre- and possibly also at the postfusion stages of exocytosis (Figure 1). Loss of the mitochondrial enzyme IDH3A (isocitrate dehydrogenase (NAD(+)) 3 catalytic subunit alpha) leads to a reduction in αKG, which impairs synaptic transmission through an ATP-independent pathway, where αKG was shown to promote membrane fusion by enhancing Syt1–lipid interaction,[8] thus prolonging synaptic activity. Cell metabolites may also affect exocytosis by targeting the nanostructure of release machinery. The availability of v-SNARE synaptobrevin-2 (also known as VAMP-2) to form a SNARE complex is critical to promote a merger between the vesicle and the plasma membranes. Activation of synaptopbrevin-2 depends on sphingosine, an essential releasable constituent of membrane sphingolipids, which enhances SNARE-mediated synaptic vesicle exocytosis[9] at the prefusion stage (Figure 1).

Cholesterol, the most common steroid in humans and a major constituent of the cell membrane, affects vesicles at postfusion stage, as revealed by analysis of the fusion pore conductance.[10] Previous studies indicated that cholesterol might regulate exocytosis by its ability to affect negatively curved lipid structures and to segregate membrane proteins into membrane rafts. As cholesterol also accumulates within vesicles, stimulation of exocytosis leads to the transfer and subsequent mixing of vesicle cholesterol with the plasmalemma. Cholesterol transfer reduces fusion pore conductance through a force due to surface tension, a new foundation for understanding exocytotic fusion pore regulation at the postfusion stage.[10]

In summary, while Ca^{2+} entry into the cytoplasm alone rapidly drives prefusion processes including the assembly of the apparatus required for the vesicular cargo release into extracellular space, this stage may well be tuned by cell metabolites including αKG and sphingosine to facilitate vesicle discharge. Moreover, stimulated vesicle cholesterol transfer to the plasma membrane may modulate the concomitant vesicle content release at the postfusion exocytotic stage, a process relevant to lysosomal storage diseases.

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Conflict of Interest Statement

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