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Topical Review

Kiss and Run Mechanism in Exocytosis

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Introduction

Secretion of cellular content, stored in vesicles, is a fundamental process which requires assembly of distinct proteins as well as merging of two lipid bilayers. Exocytosis can be induced by specific secretagogues (regulated exocytosis) or can occur constitutively. Our knowledge about proteins involved in vesicle fusion has expanded dramatically during the last 5 years. Nonetheless, little is known about the morphological aspects of vesicle fusion. The classical model of exocytosis, also known as "total fusion", predicts the complete incorporation of the vesicle membrane into the plasma membrane. An alternative model suggests that fused vesicles can detach from the cell membrane and be retracted into the cytoplasm. Vesicles preserve their integrity during this process, which is also known as the "transient fusion" or "kiss and run" model. New morphological approaches, such as fluorescent microscopy or atomic force microscopy, provide new evidence for transient vesicle fusion. Exocytosis of neurotransmitters, amylase, or surfactant are examples of regulated secretion by a "prolonged kiss" between vesicle and plasma membrane. This review will focus on new fluorescent optical approaches and on atomic force microscopy to study vesicle fusion with the plasma membrane. These techniques provide data in support of the kiss and run model of exocytosis.

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Key words: Exocytosis — Secretion — Fluorescence — AFM — Transient fusion — Total fusion

Exocytosis and Vesicle Cycling

Membrane fusion involves the merging of two phospholipid bilayers and is a regular occurrence in eukaryotic cells. Intracellular vesicle targeting and subsequent membrane fusion regulate protein processing and sorting, intracellular digestion, integrity and dynamics of cellular compartments as well as cell growth and proliferation. Fusion of vesicles with the plasma membrane has three aims: the first is to incorporate integral membrane proteins, such as ion transporters or ion channels, etc., into the cell membrane. The second is to incorporate membrane material into the plasma membrane. The third is to release intravesicular contents into the extracellular space by exocytosis.

During regulated exocytosis, vesicles storing proteins that are prepackaged for secretion, actively move to specific sites at the plasma membrane (active zone) and fuse after stimulation by specific secretagogues. Vesicles fuse fully (total fusion) with the plasma membrane and after a delay, components of the vesicle membrane are recaptured by endocytosis. Endocytosis is found at sites distant from the active zones. Endocytosed vesicles fuse with the endosomal compartment for repair and resorting. Total fusion has been described in neurons and endocrine cells (*see* Fig. 1) [27, 28, 34].

In addition, a transient state of vesicle fusion with the plasma membrane, referred to as "transient fusion" or "kiss and run", can be documented [3, 4, 10, 11, 15, 35, 45, 50, 87] (*see* Fig. 1). This process has been the subject of controversy in recent years. These vesicles fuse with the plasma membrane by forming a transient fusion pore while preserving vesicle integrity. After detachment, vesicles can fuse again without any preceding endosomal fusion. Therefore, the advantage of transient

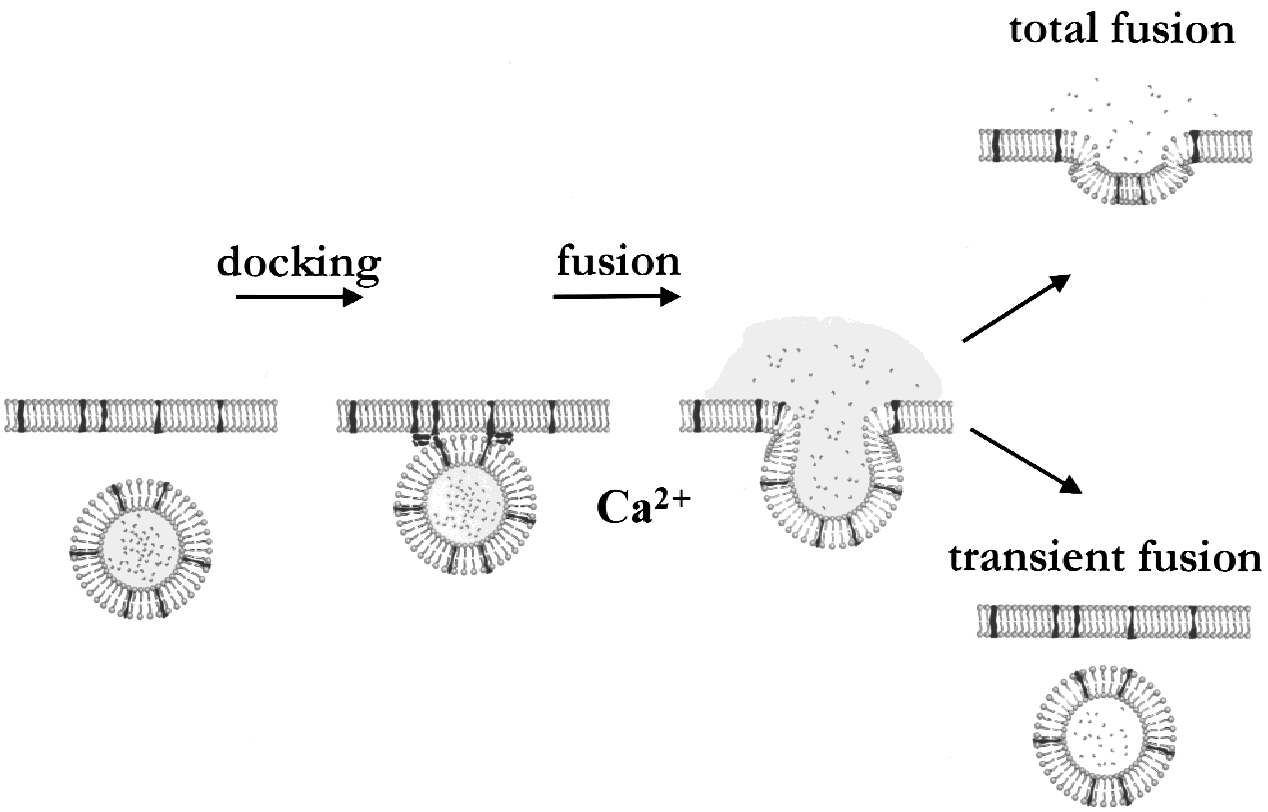

Fig. 1. A schematic drawing of vesicle cycling. After vesicle movement to the cell periphery, the vesicle is primed and becomes docked to the plasma membrane. After an intracellular calcium increase the vesicle fuses with the plasma membrane (rarely is a cAMP increase necessary for fusion). Priming, docking and fusion of vesicles require several cytosolic as well as membrane-bound proteins. The illustration displays the two discussed morphological concepts for vesicle fusion. First, the vesicle gets integrated into the plasma membrane, and subsequent endocytosis happens at a distinct membrane area with subsequent endosomal fusion ("total fusion"). Second, the vesicle fuses and detaches from the plasma membrane in a reversible manner. Thereby the vesicle integrity is maintained and repeated fusion events are possible ("transient fusion").

fusion is a rapid cycling between a fusion state and a nonfusion state. It seems that some cells, such as chromaffin cells, can switch between total and transient fusion by varying extracellular calcium [1]. Data supporting the idea of transient fusion states were first established by patch-clamp techniques [3, 10, 15]. In recent years, morphological methods, such as fluorescent microscopy or atomic force microscopy (AFM) have provided evidence for the kiss and run model. Both morphological techniques showed that vesicles release their contents within seconds [38] to minutes [69]. After the fusion event, the vesicles detach and return into the cytosol while maintaining their integrity and morphology.

This review summarizes data that support the kiss and run model in different cell types. Transient fusion events have been shown in pancreatic, pulmonary, neuroendocrine and neuronal cells. The review is organized according to the different cell types and the technical approaches used in studies of exocytosis.

A New Cell Imaging Tool: Atomic Force Microscopy

The AFM is a tactile instrument that was invented in 1986 by Binnig, Gerber and Quate [8]. In the AFM, a fine silicon or silicon nitride tip scans the surface of the sample. Any deflection of the tip due to surface topography is recorded. The AFM provides three-dimensional data of biological samples with a resolution in the nanometer range. The lateral or x, y resolution of the AFM is in the range of 50–100 nm, whereas the height or z-resolution is better (~1 nm). Resolution is limited mainly due to the softness of living cells. An increasing loading force by the tip leads to an indentation of the plasma membrane followed by an increase in contact area between the tip and the membrane.

The benefit of the AFM is that it makes possible the study of the morphology of living cells in their physiological environment in a time-dependent manner. About 6 years after the invention of AFM the first studies

on living cells were published [25, 30, 61]. The authors predicted that the main potential of AFM was to follow membrane dynamics, such as exo- or endocytosis [32, 37], on living cells. One year later, Oberleithner and collaborators demonstrated vesicle transport and endocytosis on living cells imaged by AFM [51]. Scanning the lamellipodium of migrating cells they visualized endocytosis in this area. Endocytotic pores were measured and they showed a mean diameter of ~90 nm and a lifetime of 1 min. The authors found ~10 dimples per μm^2, enough vesicles to contribute to membrane expansion after reinsertion at the leading edge to pass ahead with migration [51]. Similar studies were done by Fritz and coworkers [20] and by Braunstein and Spudich [9]. Other examples of how AFM can be used to address specific questions in cell physiology are studies on exocytosis, cell migration, cell volume regulation, cell division or secretion of ATP by lung epithelial cells [25, 41, 52, 61, 64, 67, 68, 70–73].

Rather than imaging entire cells AFM can be used to study isolated cell membranes containing secretory vesicles [5] as well as to study isolated secretory vesicles [36] with both high temporal and high spatial resolution. In addition to morphological data physical parameters can be measured, such as cell elasticity or intermolecular binding forces [19, 24, 44, 59, 60, 64]. Forces that involve protein-protein interaction that occur in exo- or endocytosis, can be measured with picoNewton resolution [14, 18].

Distinct Areas of Transient Exocytosis in Pancreatic Epithelial Cells Imaged by AFM

Previous studies postulated "active zones" of exocytosis in neurons. Such active zones are characterized as distinct locations in the plasma membrane where vesicle fusion and exocytosis occur [27, 28, 39]. Steyer and coworkers suggested these active zones exist in chromaffin cells [78]. In our own studies we also proposed distinct areas in the plasma membrane of epithelial cells where exocytosis occurs. We studied pancreatic acinar cells in the rat using AFM (*see* Fig. 2; images were generated using BioScope from Digital Instruments). In this previous study we looked at single events of exocytosis in exocrine pancreatic cells [69]. By probing the outer surface of acinar cells we found large craterlike areas (diameter 0.5–2 μm) we called "pits". The plasma membrane in such an area has a lowered elasticity. Diminished elasticity of this area is most likely due to a reduced or missing cortical cytoskeletal network. Pits were only found at the apical surface where regulated secretory vesicle docking and fusion are hypothesized to take place. Inside the pits we found "depressions" (diameter ~150 nm) representing the docking and fusion sites for zymogen vesicles (vesicle diameter: 0.2–1.0 μm) (*see* Fig. 2, low resolution). After stimulating the cells the diameter of the depressions reversibly increased within 5 min from around 150 to 200 nm. The diameter of the depressions returned to normal after another 20 min (Fig. 2, high resolution). The opening and closing of the depressions correlated well with the amount and with the kinetics of secreted enzymes (amylase) stored in pancreatic vesicles. This confirmed that depressions are areas of secretion. The pits did not change their morphology during exocytosis. Depressions within the pits represent preselected areas of docked vesicles that are ready to fuse after stimulation. This prepared vesicle pool was described in neuronal cells and is called the "readily releasable pool" [11, 12, 56, 63]. After stimulation the readily releasable vesicle pool is able to fuse with the plasma membrane within seconds to release its vesicle content. In our own studies we found an immediate diameter increase of the depressions after stimulation consistent with an instant fusion process of a readily releasable vesicle pool with the plasma membrane. However, in contrast to neuronal cells, depressions remain enlarged for several minutes before their diameter decreases again. Thus, exocytotic secretion in epithelial cells seems to be slow (minutes) when compared with neuronal cells. In addition exocytotic pores cause significant morphological alterations of the plasma membrane. The "opening" and "closing" of the depressions suggest that the vesicle fuses with the plasma membrane, releases its content into the extracellular space and finally detaches from the plasma membrane. This model is called "kiss and run" or "transient fusion" (*see* Fig. 1). In case of a total collapse of the zymogen vesicles into the plasma membrane ("total fusion") we would expect a permanent increase of the depressions' diameter. The depressions' diameter should increase until the vesicle membrane and the plasma membrane are indistinguishable from each other. Such a complete incorporation of vesicles into the plasma membrane was shown in frog neuromuscular junctions after electrical stimulation [27, 28]. By using freeze-fracture electron microscopy the authors showed a permanent increase of the exocytotic pore until complete integration of the vesicle membrane into the plasma membrane.

The slow process of secretion involving large vesicles may be an explanation for the alternative pathway of transient vesicle fusion in pancreatic cells. In exocrine pancreatic cells it is not necessary to secrete amylase within milliseconds, as is the case in neuronal cells. A fusion pore limits the diffusion velocity of vesicle content into the extracellular space. Therefore a mechanism that involves a fusion pore increases the duration of release. A prolonged release of amylase avoids bursts of high amylase amounts in the intestine and ensures a more balanced enzyme concentration. Moreover by varying fusion pore size and fusion pore lifetime the cell is able to regulate the amount of amylase release

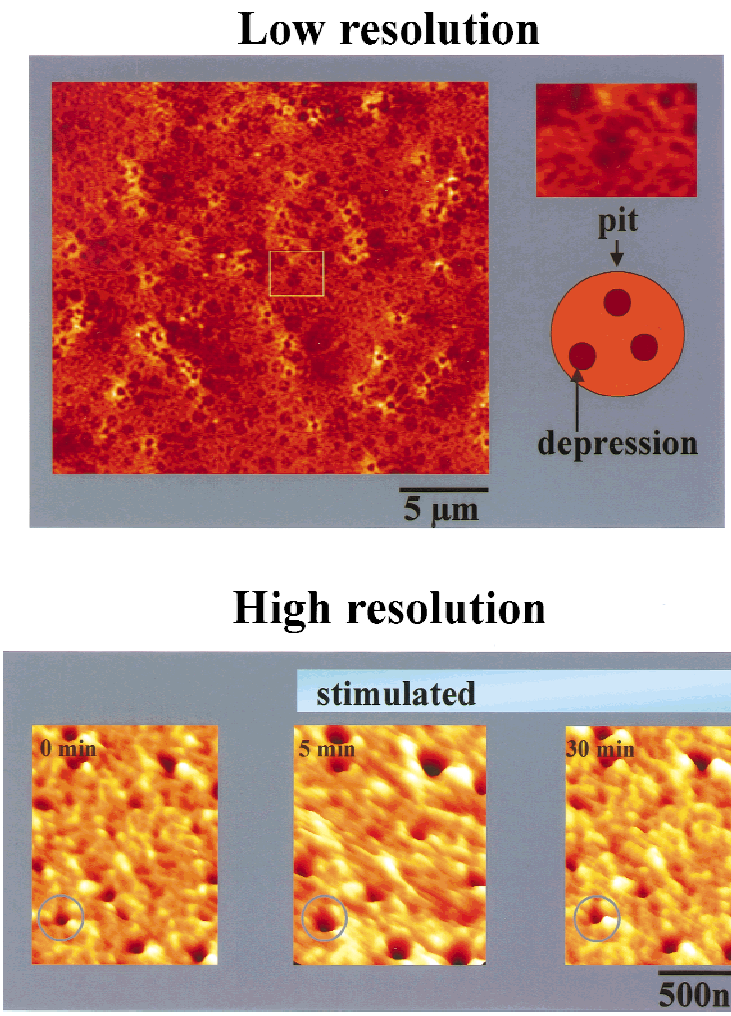

Fig. 2. Low resolution: Topography of the apical cell surface of living, isolated pancreatic acini observed by atomic force microscopy. Scattered, circlelike pits at the apical plasma membrane surface are seen. One pit (Insert) with 3 depressions is shown. High resolution: Dynamics of depression (there are no pits visible) following stimulation of secretion. Notice an increase in the depressions' diameter 5 min after stimulation, correlating with an increase in total cellular amylase release (*not shown*). At 30 min after stimulation there is a decrease in diameter of the depressions and no further increase in amylase release (*not shown*). Modified from [69].

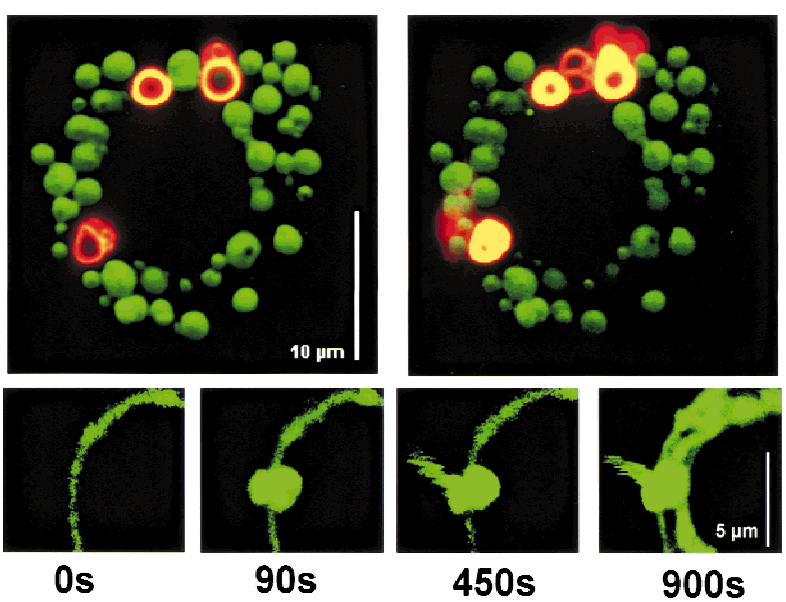

Fig. 3. Upper panel: Confocal laser scanning microscopy of images of a single pulmonary cell during stimulation with ATP (given 10 min before images were taken). Transition of 2 pre-exocytotic lamellar bodies (green; stained with LysoTracker Green, between the two yellow vesicles), to postexocytotic lamellar bodies (red; stained with FM1-43, between the two yellow vesicles) [43]. Lower panel: Confocal laser scanning microscopy of a single vesicle/plasma membrane fusion event using FM1-43. At time point 0 sec only plasma membrane staining is detectable. After stimulation with ATP additional staining of vesicle membrane and vesicle content is visible. Release continues over minutes. Image kindly provided by Thomas Haller, University Innsbruck, Austria.

according to demand. Previously it was shown that protein kinase C and extracellular calcium may be regulators of fusion pore kinetics and lifetime in chromaffin cells [1, 21]. In contrast, in neuronal cells it is necessary to secrete high and precise amounts of transmitters in a very short time. Therefore, a complete vesicle fusion with the plasma membrane with concomitant quantal transmitter release is useful. The amount of transmitter release is determined only by the numbers of vesicles that fuse with the plasma membrane.

In the case of vesicle cycling that is in line with a transient fusion model one question arises. How is it possible to maintain vesicle morphology during secretion? During amylase secretion vesicle diameter should decrease and therefore due to Laplace's law vesicle surface tension should increase. An increase of vesicle surface tension should further force enzyme release, which should further decrease vesicle dimensions until the vesicle collapses. To address this question we isolated pancreatic zymogen vesicles to follow single vesicle dynamics during stimulation. The 3-D imaging of isolated vesicles (diameter: 0.2–1.0 μm) from exocrine pancreas showed a vesicle swelling of about 20% upon stimulation [36]. Swelling of secretory vesicles has been suggested to be crucial for fusion with the plasma membrane measured by electrical techniques [17, 31]. Vesicle swelling during the fusion process prevents vesicle collapse, thus maintaining vesicle integrity and morphology—prerequisites for transient fusion, as mentioned above.

Slow Secretion Events and Transient Vesicle Fusion in Pulmonary Cells Imaged by Fluorescence Microscopy

Slow secretion occurring over a time constant of minutes can be also observed in alveolar type II cells [22]. Surfactant release by alveolar type II (AT II) cells via exocytosis of lamellar bodies is essential for normal lung function. Lamellar bodies are large vesicles with diameters of up to 3 μm. They exhibit an acidic intravesicular pH (pH ~6.1). Therefore they can be visualized with weak basic fluorescent markers such as LysoTracker Green or acridine orange, which accumulate in acidic compartments (*see* Fig. 3). Additionally, the phospholipid dye FM1-43 was used to stain the lipophilic vesicle content surfactant and the plasma membrane (red color in Fig. 3, upper panel and green color in the lower panel). Prestaining of the plasma membrane with styryl dyes, such as FM1-43, that are incorporated into the plasma membrane allows the visualization of vesicle fusion and recycling [13, 22, 38, 48, 65]. As soon as the unstained vesicle membrane fuses with the prestained plasma membrane the dye diffuses into the vesicle membrane. Therefore, fluorescence dye accumulation along the optical path can be visualized by bright fluorescence signals where vesicle fusion occurs [79]. Subsequent endocytosis of stained vesicles or plasma membrane is shown by the intracellular localization of fluorescence spots [48].

In the case of exocytosis of lamellar bodies, extracellular FM1-43 stains additionally the lipophilic vesicle content, surfactant. Stimulation of ATII cells with extracellular ATP increased intracellular calcium concentration, accompanied by exocytosis [22]. The calcium increase was transient (time period: 5 min). However, exocytosis of lamellar bodies outlasted the calcium signal (*see* Fig. 3, lower panel). The secretion of surfactant proceeded for more than 60 min. During exocytosis of lamellar bodies the dye FM1-43 got access to the vesicle content through the fusion pore. Therefore a bright fluorescent staining of intravesicular surfactant was observed (red color in Fig. 3, upper panel and green color in the lower panel). Simultaneously, the same fused vesicles lost their trapped dye (LysoTracker Green, green color in Fig. 3, upper panel) indicating loss of protons into the extracellular supernatant (extracellular pH 7.4). The fluorescent images have shown that lamellar bodies maintained their morphology during exocytosis and did not collapse into the plasma membrane. Moreover, some exocytosed vesicles were retrieved from the cell periphery to the perinuclear region, which is consistent with transient fusion rather than total fusion events [22]. In contrast, cell capacitance increased over 20 min after stimulation. The authors concluded that there is no endocytotic retrieval of vesicles within this time frame [43]. There are two possible explanations for this discrepancy. First, there was no intracellular ATP available during these measurements; ATP is required for endocytosis after stimulation, and ATP-free solutions were necessary to avoid additional stimulation of the cells. Second, endocytosis was too slow or too small to produce a measurable cell capacitance decrease within this time frame. In conclusion, the permanent cell capacitance increase over 20 min mainly confirmed the slow process of exocytosis of lamellar bodies.

The benefit of a slow process of lamellar body exocytosis is a more balanced secretion of surfactant. Moreover, a transient fusion of the large lamellar bodies with the plasma membrane preserves the vesicle membrane and avoids lipid mixing. It is likely that the lipid composition of vesicles and the plasma membrane is distinct [53]. A full fusion of the lamellar bodies with the plasma membrane during exocytosis would presumably be a rapid mixing of lipids and therefore result in a lipid sorting after endocytosis.

Fluorescent Proteins to Study Vesicle Fusion in Mast Cells

A promising tool in studying vesicle trafficking, fusion and endocytosis are fluorescent proteins, such as green fluorescent protein GFP [40, 83]. Fluorescent proteins fused with native proteins of interest, such as secretory

proteins, often colocalize with the endogenous protein. Vesicle or protein trafficking, for example of tissue plasminogen activator (tPA) and glucose transporter GLUT4, can be monitored in living cells [42, 58]. Miesenböck and coworkers designed two pH-sensitive GFPs, so-called "pHluorins". One is called ratiometric pHluorin and displays a reversible excitation ratio between pH 7.5 and 5.5 with a response time of less than 20 msec. The second pHluorin is called ecliptic pHluorin and gradually loses fluorescence as the pH is lowered [46]. Ecliptic pHluorins are invisible in an environment of pH <6.0 at 475 nm excitation wavelength, but can still be weakly seen at 410 nm. pH-sensitive fluorescent proteins allow the distinction between vesicles in nonfused and a fused state. In intact cells intravesicular pH is usually acidic (pH 5.0–6.0). When vesicles fuse, the pH change of the vesicle interior is followed by altered GFP fluorescence intensity. Miesenböck and coworkers used the ecliptic synapto-pHluorin (a fusion protein of synaptobrevin and the ecliptic pHluorin) to study vesicle fusion events in mast cells. Synaptobrevin or VAMP is a transmembrane protein of the vesicle and belongs to a group of structurally related and conserved proteins, known as SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) (for further detail *see* [6, 16, 34, 62]). These proteins are necessary for vesicle fusion. Synaptobrevin forms a stable complex with other SNARE proteins anchored at the plasma membrane. Complex formation bridges the opposing membranes and thereby mediates vesicle fusion [34]. Ecliptic synapto-pHluorin was exposed to the vesicle lumen (pH ~5.2) and therefore was only seen with 410- but not 470-nm excitation. After triggering exocytosis, vesicle content was released into the medium and locations harboring vesicles became fluorescent at 470 nm excitation. Beside the expected fusion events (incorporation of pHluorin-stained vesicles into the plasma membrane) fluorescence "flickering" can be monitored. This phenomenon of appearing and disappearing fluorescent signals at the same location was interpreted as a transient opening and closing of a fusion pore causing the internal pH of the vesicle to fluctuate. Flickering due to vesicle fusion events with the plasma membrane in mast cells was also described by using the patch-clamp technique and amperometry [10, 15, 49, 82]. The data suggest a transient fusion model that may occur in parallel with a total fusion of the vesicle with the plasma membrane.

Transient or Total Vesicle Fusion in Neuroendocrine and Neuronal Cells?

The classical model of exocytosis was first described at the frog neuromuscular junction by Heuser and Reese [27]. In their model, vesicles collapse completely (total fusion) into the plasma membrane at active zones. Subsequent endocytosis happens slowly (>20 sec) at sites distant from active zones. Endocytotic vesicles then recycle through the endosome. New experiments show evidence for a transient fusion of vesicles in neuroendocrine and neuronal hippocampal cells mainly through the use of fluorescence microscopy.

Kiss and Run in Chromaffin Cells

Prior to exocytosis vesicles must move to the plasma membrane. In chromaffin bovine cells Steyer and coworkers measured vesicle dynamics with evanescent-wave fluorescence microscopy (*see* Fig. 4) [77, 78]. By illuminating a thin layer of cytosol (~300 nm) where cells adhere to the glass coverslip, motion of single prestained vesicles can be tracked in three dimensions. Vesicles were prestained briefly by acridine orange. Images were recorded with a diffraction-limited resolution of approximately 30 nm and with a millisecond time resolution [77]. Steyer and coworkers found about 1,000 docked vesicles. After stimulation (producing depolarization) with high K^+ about 50% of docked vesicles immediately were lost in groups (*see* Fig. 4) [78]. This is consistent with the view of localized secretion at distinct areas as mentioned above for pancreatic cells. Docked vesicles that instantaneously fuse with the plasma membrane after stimulation, forming the first burst of secretion, represent the so-called "readily releasable pool" [11, 12, 56]. The existence of these docked vesicles is well known for neurons [12]. After stimulation Steyer and coworkers measured a partial replenishing of secretory vesicles with a time course of 6 min. However, using patch-clamp measurements, the pool of readily releasable vesicles in chromaffin cells is replenished with a time constant of about 10 sec, similar as in neurons [47, 76, 77, 81]. These data suggest that more vesicles are bound to the plasma membrane than are rapidly released (1,000 vesicles docked, of which ~50% are readily releasable vesicles). As mentioned above, it takes ~6 min to replenish vesicles after secretion, not 10 sec. It is more likely that replenishment of the readily releasable pool reflects stimulation of dormant vesicles that were already docked rather than movement of "new" vesicles towards the plasma membrane [54, 57, 77]. The authors showed that vesicles need minutes to evidently reach fusion competence after reaching the plasma membrane ('docking'). Another interpretation of the rapid replenishment of the readily releasable pool is that some vesicles cycle between a fused, secreting state and a docked, readily releasable state. After fusion, a rapid refilling of these vesicles is necessary. Since Steyer and coworkers used acridine orange to prestain the vesicles it was not possible to study the destiny of the vesicles after their fusion with the plasma membrane.

Using the same cell type, Ales and coworkers

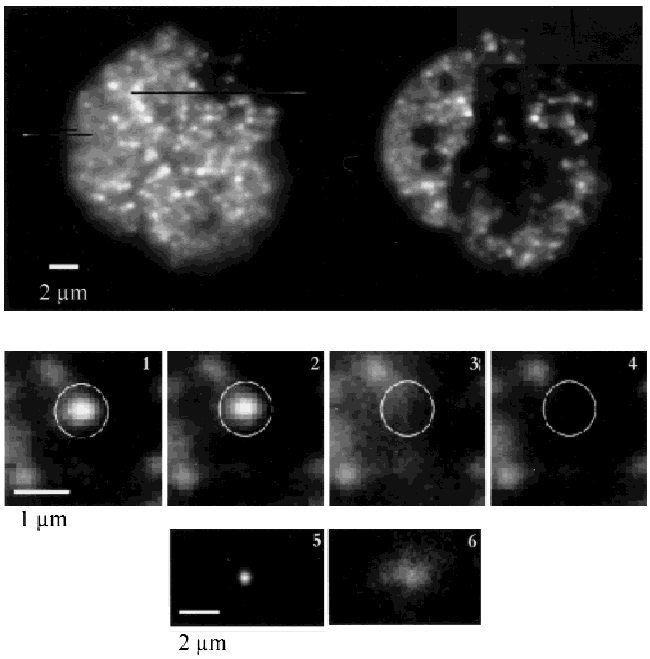

Fig. 4. Upper panel: Evanescent-wave fluorescence micrographs of a living chromaffin cell in normal buffer (left image). Many fluorescence-stained vesicles are visible. Two minutes after stimulation (right image) (high K^+) a selective loss of vesicles is visible, indicated by black patches within the cell. Lower panel: 1–4: Successive frames at 0.5 sec^{-1} show a vesicle (1,2) vanishing abruptly owing to exocytosis (2,3); the vesicle and its last location are indicated by circles. Images 5,6 are the same as 2,3 after subtracting image 4. Modified from [78].

showed by using the cell-attached patch-amperometry technique that in response to an increase of extracellular calcium chromaffin cells shift from the preferred model (total fusion) of exocytosis to the kiss and run mechanism [1]. A calcium increase from 5 to 90 mM increased the relative frequency of kiss and run events from 7 to 78%. During kiss and run events the fusion pore appeared to expand briefly to a large size, allowing for rapid and complete transmitter release. There was no size difference between vesicles that undergo permanent fusion and those that release transmitter by the kiss and run method (vesicle radius was always about 95 nm). Even though the authors used a high, and for chromaffin cells not physiological, calcium concentration, the concentration may be close to that attained in nerve terminals under physiological conditions. The authors propose that during phases of high electrical activity kiss and run is an excellent mechanism by which the pool of releasable vesicles can be rapidly replenished. This probably occurs through a direct pathway that bypasses the endosomal compartment. The advantages of the alternative cycling, involving total fusion and conventional endocytosis of the vesicle, are repair mechanisms that allow the elimination of defective membrane proteins and the reassembly of maximally refilled vesicles. The work by Ales and coworkers shows that vesicle cycling can be consistent with both total fusion and transient fusion.

Aside from calcium as an important regulator for vesicle fusion in chromaffin cells, Graham and coworkers showed that protein kinase C (PKC) modulates the kinetics of single vesicle release events [21]. Activation of PKC in chromaffin cells accelerated fusion pore expansion and subsequent pore closure and vesicle retrieval. The results suggest that PKC modulates the lifetime of vesicle fusion with the plasma membrane and therefore regulates transmitter release. Modulation of fusion pore expansion and closure to regulate transmitter release seems only to be important if vesicles undergo transient fusion events with the plasma membrane. Moreover, it is conceivable that PKC activation in addition to calcium (*see* the work by Ales and coauthors) shifts the preferred mode (total fusion) of exocytosis to the kiss and run mechanism. PKC activation promotes vesicle retrieval after fusion and should prevent vesicle collapse into the plasma membrane.

Transient Vesicle Fusion in Hippocampal Neuronal Cells

Evidence for kiss and run fusion events in hippocampal cells was shown by Klingauf and coworkers [38]. Presynaptic vesicles were loaded with FM1-43 in advance of stimulation. Cells were stimulated with a high-potassium solution and fluorescence destaining was measured. The authors showed an incomplete loss of prestained (FM1-43) vesicles during an initial round of exocytosis, allowing subsequent dye release to appear as a delayed burst. The time constant for endocytosis was ~6 sec, considerably faster than estimated for total fusion events (~20 sec) [66, 85]. Endocytosis was even faster (<2 sec) after increasing extracellular calcium (from 1 to 8 mM) or applying the nonselective kinase inhibitor staurosporin [38]. The fast endocytosis and the incomplete loss of dye reinforced the idea that a substantial fraction

of synaptic vesicles undergoes endocytosis quickly enough to retain the dye. After rapid endocytosis the vesicles maintain their fluorescence signal. However, a second stimulus (depolarization) produced a diminution in fluorescence immediately (as expected for a new round of exocytosis from internalized vesicles). The incompleteness of initial dye loss suggests a diffusion barrier that delays lateral diffusion of the dye into the plasma membrane. Such a barrier might arise from a fusion pore [86]. The results indicate a kiss and run mechanism for a large percentage of vesicles that undergo exocytosis. Moreover, internalized vesicles become fusion competent again without communication with the endosomal compartment. Vesicle cycling without intracellular fusion events after endocytosis was shown by Murthy and Stevens [48]. They showed that the amount of fluorescent dye uptake (styryl dye FM1-43) per vesicle in endocytosis matched the amount of vesicles lost after exocytosis. This means that during the vesicle recycling process there is no dye loss or leakage, for example by dilution due to fusion with the endosomal compartment. The vesicle maintains its integrity after fusion and internalization suggesting exo- and endocytosis without a total collapse of the vesicle into the plasma membrane.

The advantages of this model are a reduced membrane turnover by conservation of vesicle membrane and a rapid intracellular vesicle cycling. Reduced energy consumption is the consequence.

Conclusion

This review concentrates on optical approaches, especially on fluorescence microscopy and AFM, to study exocytosis. Quick-freeze electron microscopy revealed the fusion events in neuromuscular junctions already 20 years ago [27, 28]. Morphological data described in this review were mainly obtained from living cells and therefore give information on the dynamics of vesicle cycling. However, most of our knowledge about exocytosis was gained by electrical approaches, such as the patch-clamp technique and amperometry [2, 7, 26, 80, 84], or by biochemical analysis of proteins participating in vesicle fusion [6, 16, 23, 33, 34, 55, 62, 74, 75]. The knowledge from all these data suggests that neurons, epithelial and endocrine cells, and cells from different species share many common principles of secretion, such as SNARE proteins, a readily releasable pool of vesicles, Ca^{2+} dependence of secretion, etc. [16]. Nonetheless, there is no general model of exocytosis valid for all cell types. All cell types show typical characteristics concerning exocytosis, for example time course of secretion, total or transient fusion, vesicle size or vesicle geometry, which are likely to be due to individual specific cell function. Even intracellular calcium increase, commonly accepted as the major fusion stimulus, is not necessarily the final trigger for exocytosis. This was shown in kidney collecting duct for aquaporin insertion to increase water resorption or in parietal cells for H^+/K^+ ATPase insertion to increase HCl secretion [29]. In both examples an increase of intracellular cAMP concentration was the decisive trigger for membrane fusion.

The morphodynamics of the images described in this review support the kiss and run or transient fusion model as an alternative pathway for vesicle fusion and cycling. Kiss and run occurs in epithelial, chromaffin and even neuronal cells and was found to be in parallel with total vesicle fusion [1, 38, 39, 46]. Patch-clamp studies by Alvarez de Toledo and coworkers presented the first evidence for the transient fusion model of vesicle cycling in mast cells [3]. A flickering of membrane capacitance indicated a switch between a fused and nonfused vesicle with the plasma membrane. Vesicles maintain their integrity after secretion and therefore transient fusion prevents lipid mixing between the vesicle membrane and the plasma membrane. Advantages of this model are a reduced membrane turnover by conservation of vesicle membrane and a reduced intracellular vesicle cycling. Reduced energy consumption is the consequence. Moreover, kiss and run is an excellent mechanism for a rapid refilling of vesicles, probably through a direct pathway that bypasses the endosomal compartment [1, 53]. During periods of high activity the rapid recycling of vesicles between a fused and nonfused state is a useful mechanism to support cell function. Concerning epithelial or slowly secreting cells a further advantage is conceivable. A fusion pore that occurs during transient fusion events limits the diffusion velocity of vesicle content into the extracellular space. Therefore a mechanism that involves a fusion pore increases the duration of release. A prolonged release avoids bursts of high amounts of vesicle content in the extracellular space and ensures a more balanced concentration.

The present review shows that most of the morphological data are consistent with electrical data and provide new morphological aspects of exocytosis that complete our understanding of exocytosis. AFM or fluorescence microscopy (GFP or evanescent-field fluorescence microcopy), are novel approaches to study exo- and endocytosis in living cells to reveal complementary information about a fascinating cell function. A hypothesis that can be imagined and verified by these morphological approaches is that secretion may be regulated, not only by the number of fused vesicles, but also by the fusion pore size and fusion pore life time. Initial evidence for such a mechanism was produced from chromaffin cells [1, 21]. The authors of these studies showed that fusion pore opening and closing may be regulated by extracellular calcium and protein kinase C. Vesicle fusion that is consistent with the kiss and run model should benefit most from such a mechanism.

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