Functional Organization of the Porosome Complex and Associated Structures Facilitating Cellular Secretion

Porosomes, the universal secretory machinery at the cell plasma membrane, are cup-shaped supramolecular lipoprotein structures, where membrane-bound vesicles transiently dock and fuse to release intravesicular contents during cell secretion. In this review, the discovery of the porosome and its structure, dynamics, composition, and functional reconstitution are outlined. Furthermore, the architecture of porosome-like structures such as the "canaliculi system" in human platelets and various associated structures such as the T bars at the Drosophila synapse or the "beams," "ribs," and "pags" at the frog neuromuscular junction, each organized to facilitate a certain specialized secretory activity, are briefly discussed.

Cells synthesize and release products such as hormones, growth factors, neurotransmitters, or digestive enzymes by the process of secretion and do so in a highly regulated manner. Cellular cargo destined for secretion are packaged and stored in membranous sacs or vesicles that, when required by cells to be released to the outside, dock and establish continuity at the base of specialized plasma membrane structures called porosomes (FIGURE 1) (13, 15, 16, 18, 20–22, 51, 53, 56, 57, 60, 75, 97). In the past decade, the isolation of the "porosome complex" and determination of its composition, structure, and its dynamics at nm resolution and in real time, and its functional reconstitution into artificial lipid membrane (13, 15, 16, 18, 20–22, 51, 53, 56, 57, 60, 75, 97) has greatly advanced our understanding of the secretory process in cells (1, 3–5, 23, 27, 30, 46–48, 50, 62, 71, 74, 84, 91, 92, 101, 113, 114, 119, 120). Interestingly, in nature, even single-cell organisms have developed specialized and sophisticated secretory machinery, such as the secretion apparatus of Euplamosa gondii (FIGURE 2) (63), the contractile vacuoles in paramecium (43), or the various types of secretory structures in bacteria (70). Therefore, it is not surprising that mammalian cells too have evolved such highly sophisticated and specialized cup-shaped supramolecular lipoprotein structures—the porosome complexes and porosome-like "canaliculi system" in human platelets (FIGURE 2) (115, 116), for the precise and regulated docking, fusion, and release of intravesicular contents from cells. In addition to the presence of porosome as the universal secretory machinery at the cell plasma membrane, various forms of specialized structures, such as the T bars at the Drosophila synapse (FIGURE 2) (82) or the "beams," "ribs," and "pags" at the frog neuromuscular junction (FIGURE 2) (42), have evolved, each organized to facilitate a certain specialized secretory activity in different cell types.

Porosomes were first discovered in the mid 1990s (97) in acinar cells of the exocrine pancreas and subsequently in growth hormone (GH)-secreting cells of the pituitary gland (13), in chromaffin cells (16), and β-cells of the endocrine pancreas (53), in neurons (21), and in astrocytes (75). Porosome size ranges from ~100 to 180 nm in pancreatic acinar cells to ~12 to 17 nm in neurons and astrocytes. Several proteins constitute the porosome complex (51), including structural proteins such as actin, ion channels such as the chloride and calcium channels (51, 18), and the t-SNARE membrane fusion proteins located at its base (60), where secretory vesicles transiently dock and fuse to expel their contents. During cell secretion, the porosome opening dilates to allow release of secretory products, returning to its resting size following completion of the process (13, 15, 97). Accordingly, secretory vesicles can undergo multiple rounds of docking-fusion-release cycles, generating partially empty and empty vesicles as observed in electron micrographs. The empty vesicles can then be recycled via endosomes and the Golgi complex. In contrast to transient fusion, if secretory vesicles were to completely merge at the porosome base, it would result in a total obliteration of the structure, since the surface area of secretory vesicles is several-fold larger than that of the porosome complex. In human platelets, where a one-time massive and complete degranulation need to occur following cell activation, the porosome has become invaginated into the cell giving rise to the "canaliculi system" (115, 116) and, as a consequence, greatly increased the surface area for a large population of secretory vesicles to simultaneously dock and fuse for release. Similarly, in a variety of specialized nerve endings, electron-dense structures like the "beams," "ribs," and "pags" at the frog neuromuscular junction (42), the T bars at the Drosophila neuromuscular junction (82), the long projections in skate
In the past five decades, our understanding of the secretory process has greatly helped manage to predict the cell plasma membrane (ZG head) (60). Porosomes were to transience “kiss-and-run” during secretion, a process greatly helped by the neuronal packet may acquire vesicle content nonneuronal to strate the accretion cell secretory vesicle were to transition to become free vesicle membrane (ZG head) (60). In the past five decades, our understanding of the secretory process has greatly helped manage to predict the cell plasma membrane (ZG head) (60).

**FIGURE 1.** Po

A: A high-resolution image of the plasma membrane (ZG head) (60). B: an exocrine pancreatic cell shown at zero time point of the nonstimulated; C: a synaptic micrograph of the neuronal p order of magnitude of magnified ridges with some protein complex attached to the rim of the structure (118). D: some complex added to the cell increase in capacitance determined using.

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**Porosome Dynamics**

**Reconstitution**

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Porosome: the secretory portal in mammalian cells

Porosomes were first discovered in acinar cells of the exocrine pancreas (97), which are polarized secretory cells having an apical and a basal end. Pancreatic acinar cells synthesize digestive enzymes and stored them within 0.2–1.2 μm in diameter membranous sacs or vesicles, called zymogen granules (ZGs), located apically. Following a secretory stimulus, ZGs dock and fuse with the apical plasma membrane to release their contents to the outside. In contrast to neurons, where secretion of neurotransmitters occurs in milliseconds, pancreatic acinar cells secrete digestive enzymes over minutes following a secretory stimulus and therefore were ideal for investigating the molecular steps involved in cell secretion. In the mid 1990s, atomic force microscopy (AFM) studies on live pancreatic acinar cells were performed to evaluate, at high resolution, the surface topology of the apical plasma membrane in both resting and stimulated cells. Circular “pits” measuring 0.4–1.2 μm, and typically 3–4 “depressions” or porosomes (FIGURE 1, A–C) measuring 100–180 nm in diameter within each pit, are found at the apical end of the cell (97). The basolateral membrane in acinar cells is devoid of such pit or porosome structures. High-resolution AFM images of porosomes in live acinar cells further reveal a cone-shaped morphological identity with the depth of each cone measuring 15–35 nm. Similarly, examination of resting GLUT-containing cells of the pituitary gland (13), neurons (21), astrocytes (75), β-cells of the endocrine pancreas, mast cells, and chromaffin cells of the adrenal medulla (53) all have demonstrated the presence of porosomes as permanent structures at the cell plasma membrane.

Exposure of pancreatic acinar cells to the secretagogue mastoparan (Mast 7) results in a time-dependent increase (25–45%) in both the diameter and relative depth of porosomes (FIGURE 1D). Studies demonstrate that porosomes return to their resting size on completion of cell secretion (FIGURE 1D) with no demonstrable change in pit size (15, 97). Enlargement of porosome diameter and relative depth follows exposure to the secretagogue and correlates with cell secretion (FIGURE 1D). Exposure of acinar

FIGURE 1 Porosome: the secretory portal in mammalian cells

A. A high-resolution AFM micrograph shows a single pit with four 100- to 180-nm porosomes within (blue arrowhead) at the apical plasma membrane in a live pancreatic acinar cell (97). B: an electron micrograph depicting a porosome (blue arrowhead) close to a microvilli (MV) at the apical plasma membrane (PM) of a pancreatic acinar cell. Note the association of the porosome membrane (yellow arrowhead) and the zymogen granule membrane (ZGM) (red arrow head) of a docked ZG (inset). Cross section of a circular complex at the mouth of the porosome is seen (blue arrowhead–60). C: schematic diagram of pits (yellow arrow) and porosomes (blue arrow) at the cell plasma membrane. ZGs, the secretory vesicles in exocrine pancreas, called dock and transiently fuse at the porosome base to exapitate intracellular contents (70). D: several porosomes within a pit are shown at zero time and 5 min and 30 min following stimulation of secretion. Section analysis across three porosomes is shown at top, with the blue arrowhead pointing at a porosome (97). Note the dilatation of the porosome at the 5-min time point and its return to near resting size after 30 min following stimulation of secretion. E: % total cellular amylase release in the presence (yellow bars) and absence (blue bars) of the secretagogue Mas7. Note the increase in porosome diameter, correlating with an increase in total cellular amylase release at 5 min following stimulation of secretion. At 30 min following a secretory stimulus, there is a decrease in porosome diameter and no further increase in amylase secretion beyond the 5- min time point. No significant changes in amylase secretion or porosome diameter were observed in control cells in either the presence or absence of the nonstimulatory masotaran analog Mas17 (97). F: electron micrograph of a porosome (blue arrowhead) at the nerve terminal, in association with a synaptic vesicle (SV) at the presynaptic membrane (Pre-SM). Notice the central plug-like structure at the neuronal porosome opening (21). G: an electron micrograph of a neuronal porosome in physiological buffer, also showing the central plug (blue arrowhead) at its opening. The central plug in the neuronal porosome complex may regulate its rapid close-open conformation during neurotransmitter release. The neuronal porosome is an order of magnitude smaller (10–15 nm) compared with porosome in the exocrine pancreas (100–180 nm). Note the central plug and eight interconnected ridges within the porosome complex (20). H: electron density maps of negatively stained electron micrographs of isolated neuronal porosome protein complex. Note the ~12-nm complex exhibiting a circular profile and having a central plug, with eight interconnected protein densities at the rim of the complex (22). I: atomic force micrograph of a pit and three porosomes within (one shown by the blue arrowhead) in pancreatic acinar cell and the specific immunolocalization of amylase-specific immunoglobin (yellow spots) demonstrating amylase secretion through the structure (10). J: electron micrograph of liposome-reconstituted porosome complex isolated from pancreatic acinar cell. Note the cup-shaped basket-like morphology of the porosome complex reconstituted in a 500-nm lipid vesicle. Bar = 150 nm (60). K: the lipid bilayer-reconstituted porosome complex is functional. Top shows a schematic drawing of the EPC9 setup for electrophysiological measurements. Isolated zymogen granules added to the cis compartment of the bilayer chamber dock and fuse with the reconstituted porosomes at the bilayer and are detected as an increase in capacitance and current activity and a concomitant time-dependent release of amylase to the trans compartment of the bilayer chamber determined using immunoblot assay (60).
cells to cytochalasin B, a fungal toxin that inhibits actin polymerization and cell secretion, results in a 15–20% decrease in porosome size and a consequent 50–60% loss in secretion (97). Results from these studies suggested porosomes to be the secretory portal in cells. These studies further demonstrated the involvement of actin in regulation of both the structure and function of porosomes. Similarly, following stimulation of secretion, porosomes in resting GH cells measuring 154 ± 4.5 nm (mean ± SE) in diameter result in a 40% increase in porosome diameter (215 ± 4.6 nm, P < 0.01), with no appreciable change in pit size (13). The direct determination that porosomes are the portals via which secretory proteins are discharged from cells finally came from immuno-AFM studies (13, 15). Localization at porosomes of gold-conjugated antibody to secretory proteins provided direct evidence that secretion occurs through them (FIGURE 1I) (13, 15). ZGs contain the starch digesting enzyme amylace. AFM micrographs of the localization of gold-tagged amylace-specific antibodies at the porosome opening following stimulation of cell secretion (15, 66) demonstrate porosomes to be the cells secretory portals. Similarly, gold-tagged growth hormone-specific antibody bound to selectively localize at porosomes following stimulation of secretion (13) establishes them to be the secretory portals in GH cells. The morphology of the porosome complex facing the cytosolic compartment in exocrine pancreas (51), and in neurons (21), has also been determined at near-nm resolution in subcellular fractions. AFM studies on isolated plasma membrane preparations in near physiological conditions reveal scattered circular disks measuring 8.5–1 μm in diameter, with inverted cup-shaped structures (51). The inverted cups at the cytosolic compartment of isolated pancreatic plasma membrane preparations range in height from 10 to 15 nm, and on several occasions ZGs ranging in size from 0.4–1 μm in diameter are observed in association with one or more of these inverted cups, suggesting them to be porosomes. To further confirm that the cup-shaped structures are indeed porosomes, immuno-AFM studies were carried out. Target membrane proteins SNAP-23/-25 (90) and syntxin (t-SNARE) (7) and secretory vesicle-associated membrane protein v-SNARE complex (20, 21, 51, 60). Additionally, chloride ion channel (22, 60) have also been reported (88). In agreement with these earlier findings, our studies (20, 21, 51, 60) demonstrate the association of SNAP-23, syntxin 2, cytoskeletal proteins actin, α-fodrin, vimentin, and calcium channels β3 and α1c, together with the SNARE regulatory protein NSE at the porosome complex (20, 21, 51, 60). Additionally, chloride ion channel proteins CIC2 and CIC3 were also identified as part of the porosome complex. Isoforms of a number of other proteins identified as components of the porosome complex have also been reported using 2D-BAC gels electrophoresis (51). For example, three isoforms each of the calcium ion channel and vimentin have been identified in pancreatic porosomes. Using yeast two-hybrid analysis, we confirmed the presence of SNAP-23/-25 (t-SNARE) in the porosome complex with SNARE regulatory protein NSF, at the porosome complex (20-22). Furthermore, actin- and microtubule-based cytoskeleton have been implicated in intracellular vesicle traffic. Fodrin, which was previously implicated in exocytosis, has also been shown to directly interact with SNAP-23 (87). Studies demonstrate α-fodrin to regulate exocytosis via its interaction with the t-SNARE syntxin family of proteins, perhaps through interaction with the COOH-terminal region of syntxin, which is known to interact with α-fodrin. Similarly, vimentin filaments interact with SNAP23/25 and hence are able to control the availability of free SNAP23/25 for assembly of the t-v-SNARE complex (41). Collectively, these findings suggested that vimentin, α-fodrin, actin, and SNAP23/25 may be part of the porosome complex. Additional proteins such as α-fodrin, synaptophysin, and myosin may associate when the porosome establishes continuity with the secretory vesicle membrane. The globular tail domain of myosin V containing a binding site for VAMP, which is abundant in a calcium-dependent manner (88). Further interaction of myosin V with syntxin had previously been shown to require both calcium and calmodulin. It has also been suggested that VAMP acts as a myosin V receptor on secretory vesicles and may regulate formation of the SNARE complex (87). Interaction of VAMP with synaptophysin and myosin V had also been reported (88). In agreement with these earlier findings, our studies (20, 21, 51, 60) demonstrated the association of SNAP-23, syntxin 2, cytoskeletal proteins actin, α-fodrin, vimentin, and calcium channels β3 and α1c, together with the SNAP23/25 for assembly of the t-v-SNARE complex (20, 21, 51, 60). Additionally, chloride ion channels CIC2 and CIC3 were also identified as part of the porosome complex. Isoforms of a number of other proteins identified as components of the porosome complex have also been reported using 2D-BAC gels electrophoresis (51). For example, three isoforms each of the calcium ion channel and vimentin have been
vesicle docking, fusion, and the transfer of intravesicular contents from the cis to the trans compartments of the bilayer chamber is monitored as an increase in capacitance (vesicle fusion and concomitant increase in membrane surface) and current (conductance).

Results from these experiments demonstrate that the lipid membrane-reconstituted porosomes are indeed functional (21, 51, 60), demonstrating isolated secretory vesicle docking, fusion, and the transfer of intravesicular contents from the cis to the trans compartment of the bilayer chamber. ZGs fuse with the porosome-reconstituted bilayer as demonstrated by an increase in capacitance and conductance, and a time-dependent transport of the ZG enzyme amylase from cis to trans using immunoblot analysis (21, 51, 60). Chloride channel activity is also present in the reconstituted porosome complex, and the channel inhibitor 4,4'-diisothiocyanato-2,2'-stilbene-5,5'-disulfonic acid (DIDS) inhibits current activity through the porosome-reconstituted bilayer, demonstrating its requirement for porosome function. Similarly, the structure and biochemical composition of the neuronal porosome complex (FIGURE 1H) and the docking and fusion of synaptic vesicles at the structure have also been determined (20-22).

Regulation of Intravesicle Content Expulsion at the Porosome Complex

Studies demonstrate that secretory vesicle swelling is a requirement for the expulsion of intravesicular contents during cell secretion (65). Vesicle volume increases following stimulation of cell secretion had been proposed from earlier electrophysiological measurements in mast cells (2, 24, 29, 85) and studies on bovine adrenal chromaffin cells (89). However, a direct observation of secretory vesicle swelling in live cells following a secretory stimulus, at nanometer resolution and in real time, was first demonstrated in acinar cells of the exocrine pancreas (32, 65) and in synaptic vesicles (65) using AFM. Live pancreatic acinar cells, when imaged using the AFM, reveal at nanometer resolution the presence of ZGs lying immediately below the surface of the plasma membrane. Within 2.5 min of exposure to a secretory stimulus, a majority of ZGs within cells swell, followed by a decrease in ZG size and a concomitant discharge of secretory products. These studies directly demonstrated intracellular swelling of secretory vesicles following stimulation of cell secretion in live cells and vesicle deflation following partial discharge of vesicular contents. A similar mechanism of synaptic vesicle swelling for neurotransmitter release has also been demonstrated. This direct estimation of vesicle size dynamics at nanometer resolution under various experimental conditions has enabled determination of some of the molecular players in secretory vesicle swelling. To be able to determine the molecular mechanism of vesicle swelling, isolated secretory vesicles have frequently been utilized. Early studies using isolated ZG demonstrated the presence of Cl- and ATP-sensitive K- selective ion channels in the ZG membrane, whose activities were implicated in ZG swelling (35, 36, 38, 39, 94, 103, 105, 106). These studies further demonstrated that secretion of ZG contents from pancreatic acinar cells requires the presence of both K- and Cl- channels and ATP-sensitive K- selective ion channels in the ZG membrane, whose activities were implicated in ZG swelling (35, 36, 38, 39, 94, 103, 105, 106). These studies further demonstrated that secretion of ZG contents from pancreatic acinar cells requires the presence of both K- and Cl- ion channels in a number of tissues (49, 66, 98, 99). Analogous to the regulation of K- and Cl- ion channels at the cell plasma membrane, the regulation of these channels at the ZG membrane by a G-protein has also been demonstrated (52). Isolated ZGs from exocrine pancreas swell rapidly in response to ATP and NaF (52), suggesting the involvement of rapid water entry into ZGs following ATP exposure. As opposed to osmotic swelling, membrane-associated water channels called aquaporins (AQP) are involved in rapid water gating in cells (25, 67, 68, 80). The presence of AQP1 at the ZG membrane (11), and AQP3 and G-protein protein at the synaptic vesicle membrane (99), and their involvement in GTP-mediated vesicle water gating and swelling has been demonstrated (11, 59). The secretagog mastoparan (Mast 7) is an amphiphilic tetradecapeptide from wasp venom and is known to potentiate the GTPase activity of Gs/Cot proteins (45, 69, 111). Stimulation of G-protein receptors has been shown to lead to the phosphorylation of phospholipid membrane and formation of a highly structured alpha-helix that resembles the intracellular loops of G-protein-coupled receptors. Analogous to receptor activation, mastoparan is thought to interact with the COOH-terminal domain of the G protein α-subunit (112). Active

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mastoparan (Mas7) in the presence of [γ-32P]GTP demonstrates a significant increase in swelling and GTase activity in ZG over control inactive mastoparan (Mas17), confirming the presence and activity of Gi proteins at the ZG membrane (52). Similarly, Mas7 has also been demonstrated to stimulate synaptic vesicle swelling (59).

It has been reported that vH+-ATPase present at the ZG membrane (open arrowhead) participates in swelling (60, 65). Synaptic vesicles are formed by a release of synaptic vesicles (60, 65) and reconstituted by a release of synaptic vesicles (60, 65). Synaptic vesicles are formed by a release of synaptic vesicles (60, 65) and reconstituted by a release of synaptic vesicles (60, 65).

FIGURE 2. Porosome-like structures and other specialized processes in cells. A: electron micrograph of a freeze-fractured discoid platelet. The organization and intercommunication of open canaliculi system (OCS) demonstrating docked and fused secretory vesicles. Several apertures (T) of the OCS open into a communicating web of fenestrated channels that stretch across the cytoplasm to openings (I) at the cell membrane. Elements of the dense tubular system (DTS) are interwoven with channels of the OCS in membrane complexes (MC) within the platelet (127,000). Reprinted from Ref. 115 with permission from the Rockefeller University Press. B: electron micrograph revealing the type III secretion system (needle complex) spanning the inner and outer membranes of Salmonella typhimurium. Note the depressions at the insertion point of the needle complex (open arrowhead). This system helps the bacteria inject proteins into host cell (70). Bar = 100 nm. C: electron micrograph of isolated needle complexes demonstrating the supramolecular structure of this type III protein secretion system of S. typhimurium. Bar = 100 nm (70). D: T. gondii parasite possesses a stable polarized secretory apparatus and a micropore. The secretory apparatus has three morphologically distinct organelles, namely micronemes, rhoptries (Rh), and dense granules. The conserved (Co) is present at the apical end of the parasite. Note the microtubule is continuous with the plasma membrane (PM) of the parasite. The parasite also possesses an inner membrane complex (IMC). Copyright, Joiner and Ross, 2002. Originally published in J Cell Biol 157: 557–563 (63). E–L: electron micrograph and schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). E and F: are diagrams of the active zone structure in the synaptic terminal electron micrographs shown at right: P, a neuromuscular junction (NMJ) terminal in Drosophila (B2) with a dense projection called a T bar (blue arrow). G: a tetrad synapse between photoreceptor and lamina monopolar cells in Drosophila. The T bar consists of a platform (double arrows) and a pedestal (single arrow). H: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). I: a tetrad synapse between photoreceptor and lamina monopolar cells in Drosophila. The T bar consists of a platform (double arrows) and a pedestal (single arrow). J: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). K: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). L: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). M: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). N: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). O: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). P: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). Q: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). R: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). S: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). T: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). U: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). V: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). W: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). X: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). Y: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). Z: electron micrograph schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118).
The synaptic vesicle membrane (44, 102) is responsible for the generation of the electrochemical H+ gradient (pH 5.2–5.5) within vesicles (34, 83), required for transport of neurotransmitters into the vesicle lumen. In addition to the established role of vH+-ATPase in neurotransmitter transport into synaptic vesicles, vH+-ATPase has also been suggested to participate in the secretion of the stored neurotransmitters (86, 93). Furthermore, guanine nucleotides have been reported to influence the glutamate-induced cellular response via diverse trophic, proliferative, and modulatory effects on neurons (95). Since synaptic vesicle swelling is G\(_\text{o}\)-mediated and is required for cell secretion (65), the involvement of vH+-ATPase at the synaptic vesicle membrane (44, 102), in G\(_\text{o}\)-mediated water gating through the AQP-6 channels resulting in vesicle swelling, was explored (100). In agreement, synaptic vesicle-associated vH+-ATPase is found to be required for GTP\(_\text{G}\_\text{o}\)-mediated swelling of synaptic vesicles. The sequence of events, however, involving vH+-ATPase and all associated players, remains to be determined. Since mastoparan behaves like an activated \(\beta\)-adrenergic receptor that stimulates vesicle swelling, it is possible that secretory vesicle swelling occurs via an internal agonist \(\beta\)-adrenergic receptor-PKA-G-protein-PLA\(_2\)-mediated pathway, resulting in the elevation of intravesicular [H\(^+\)]\(_\text{c}\) concentration, which then leads to active water entry via water channels at the synaptic vesicle membrane.

Measurements of intracellular ZG size, further reveals that different vesicles swell differently following a secretory stimulus (65). This differential swelling among ZGs within the same cell may explain why, following stimulation of cell secretion, some intracellular ZGs express less vesicular content than others and hence have discharged more of their contents. To determine precisely the role of swelling in vesicle-plasma membrane fusion and in the expulsion of intravesicular contents, electrophysiological ZG-reconstituted lipid bilayer fusion assays (68, 65) have been performed. Similar to what is observed in live acinar cells, each isolated ZG responds differently to the same swelling stimulus (11, 52, 65). These studies demonstrate that, following stimulation of cell secretion, ZGs within pancreatic acinar cells swell, followed by a release of intravesicular contents through porosomes (60, 65) and a consequent shrinkage of the vesicle. On the contrary, isolated ZGs stay swollen following GTP exposure since there is no release of the intravesicular contents (11, 52, 65). The role of vesicle swelling in membrane fusion and in the expulsion of intravesicular contents has been determined using electrophysiological bilayer fusion assays (60, 65). Reconstitution of the porosome into the lipid membrane results in a small increase in capacitance, possibly due to the increase in membrane surface area contributed by incorporation of porosome complexes, ranging in size from 100 to 180 nm in diameter (60, 65).

Isolated ZGs, when added to the cis compartment of the bilayer chamber, fused at the porosome-reconstituted lipid membrane, and are detected as a step increase in membrane capacitance. However, even after 15 min of ZG addition to the cis compartment of the bilayer chamber, little or no release of the intravesicular enzyme \(\alpha\)-amylase is detected in the trans compartment. On the contrary, exposure of ZGs to 20 \(\mu\)M GTP induced swelling (11, 52) and results both in the potentiation of fusion as well as a robust expulsion of \(\alpha\)-amylase into the trans compartment of the bilayer chamber. These studies demonstrate that, following cell secretion, secretory vesicle swelling is required for the efficient discharge of intravesicular contents. Within minutes or even seconds following stimulation of secretion, empty and partially empty secretory vesicles accumulate within cells (12, 73). Following addition of ZGs to the cis compartment of the bilayer chamber, membrane capacitance continues to increase; however, little or no detectable secretion occurred even after 15 min, suggesting that either variable degrees of vesicle swelling or repetitive cycles of fusion and swelling of the same vesicle, or both, may operate during cell secretion. Under these circumstances, empty and partially empty vesicles could be generated within cells. To test this hypothesis, two key parameters were examined (65): 1) whether the extent of swelling is same for all ZGs exposed to a certain concentration of GTP and 2) whether there is a correlation between the extent of swelling and the quantity of intravesicular contents expelled. The answer to the first question was clearly yes; different ZGs respond to the same stimulus differently (65). Studies reveal that different ZGs within cells or in isolation undergo different degrees of swelling, even though they are exposed to the same stimuli (carbamylcholine for live pancreatic acinar cells or GTP for isolated ZGs). The requirement of ZG swelling for expulsion of vesicular contents was further confirmed when the GTP dose-dependent increase in ZG swelling (65) translated into increased secretion of \(\alpha\)-amylase. Although higher GTP concentrations elicited an increased ZG swelling, the extent of swelling between ZGs once again varied. Similar to ZGs, exposure of synaptic vesicles to 20 \(\mu\)M GTP also results in an increase in synaptic vesicle swelling (65) and, following exposure to Ca\(^{2+}\), results in the transient fusion of synaptic vesicles at the porosome, expulsion of intravesicular contents, and the consequent decrease in synaptic vesicle size (65). Additionally, as observed in ZG, not all synaptic vesicles respond to a swelling stimulus, and, if they do, they exhibit different swelling responses to the same stimuli. This differential response of synaptic vesicles within the same nerve ending may dictate and regulate the potency and efficacy of neurotransmitter release at the nerve terminal (65), explaining how partially empty vesicles are generated in cells (12, 73) following secretion. The generation of empty secretory
vesicles could result from multiple rounds of fusion-swelling-expulsion cycles during the secretory process. Another process termed "compound" secretion (78, 81) may also contribute to partial release of intravesicular contents and the generation of partially empty vesicles. In compound secretion, there is secretory vesicle-vesicle fusion, allowing large amounts of content release following such compound vesicle clusters to dock and fuse at the cell plasma membrane. Since secretory sites (porosomes) at the cell plasma membrane are limited, to overcome this limitation clusters to dock and fuse at the cell plasma membrane. However, even though this mechanism of compound secretion, compound vesicles (vesicles fused with each other) should transiently fuse membrane are limited, to overcome this limitation content release following such compound vesicle empty vesicles. In compound secretion, there is secretion (78, 81) may also contribute to partial release of process. Another process termed "compound" secretion-swelling-expulsion cycles during the secretory vesicles could result from multiple rounds of fusion-

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References


25. Curran MJ, Brodwick MS. Ionic control of the size of the vesicle fusion pore at the nerve terminal is an oversimplification of the secretory process in different cell types (FIGURE 2). The suggestion that these specialized structures merely serve as docking sites for the availability of synaptic vesicles for neurotransmitter release at the nerve terminal is an oversimplification of its function that remains to be fully resolved. In summary, porosomes are specialized plasma membrane structures universally present in secretory cells, from exocrine and endocrine cells to neuroendocrine cells and neurons. The fact that porosomes in exocrine and neuroendocrine cells measure 100–180 nm and that there is only a 20–35% increase in its diameter following the docking and fusion of secretory vesicles measuring 0.2–1.2 μm in diameter demonstrates that vesicles transiently dock and fuse at the base of the porosome complex to release their contents. The discovery of the porosome and an understanding of its structure and dynamics at nanometer resolution and in real time in live cells, its composition, and its functional reconstitution in artificial lipid membranes has greatly advanced our understanding of cell secretion. It is evident that the secretory process in cells is a well-coordinated, highly regulated, and finely tuned supramolecular orchestra. n
A force microscopy.

pores in live GH

Z. "Kiss-and-run": a multipotent; the universal

secretion pore in live
growth hormone cells of the pituitary.


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