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AQPs and Control of Vesicle Volume in Secretory Cells

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Abstract. Aquaporins (AQPs) are a family of small, hydrophobic, integral membrane proteins. In mammals, they are expressed in many epithelia and endothelia and function as channels that permit water or small solutes to pass. Although the AQPs reside constitutively at the plasma membrane in most cell types, the presence of AQPs in intracellular organelles such as secretory granules and vesicles has currently been demonstrated. The secretory granules and vesicles contain secretory proteins, migrate to particular locations within the cell close to the plasma membrane and release their contents to the outside. During the process, including exocytosis, regulation of secretory granule or vesicle volume is important. This paper reviews the possible role of AQPs in secretory granules and vesicles.

Key words: Aquaporins — Secretory granules — Secretory cells — Volume Regulation

Introduction

In various exocrine and endocrine cells, cells release the contents of their secretory granules and vesicles to the outside. This involves the fusion of the granule or vesicle membrane with the plasma membrane at the secretory sites followed by the rupture of the fused membranes, which process is exocytosis. This process is continuous in most cells ('constitutive' exocytosis), but it can be greatly accelerated following an appropriate cellular signal such as neural stimulation and hormone ('regulatory' exocytosis). Secretory proteins are synthesized in the rough endoplasmic reticulum (RER), and exhibit vectorial transport from RER through a succession of membrane-bounded compartments including the Golgi complex, condensing vacuoles, and secretory granules and vesicles. The secretory granule or vesicle is formed from the condensing vacuole, which buds off from the *trans* face of the Golgi complex. In the condensing vacuole, secretory proteins exist in dilute form. In a subsequent packing process, the proteins are condensed. During this process, it is conceivable that transport of ions and water through the secretory granule membrane is necessary for the protein condensation, although there is not yet complete agreement on the basic principles involved (Arvan & Castle, 1998; Dannies, 2002). The secretory granules and vesicles migrate to particular locations within the cell close to the plasma membrane prior to the release of their contents to the outside. During the process, including exocytosis, regulation of secretory granule or vesicle volume is important, a regulation in which the contribution of various ion channels has been demonstrated (Trávenod, 2002).

Aquaporins (AQPs) are a family of small, hydrophobic, integral membrane proteins. At least 13 AQPs have so far been identified in mammals. In mammals, they are expressed in many epithelia and endothelia and function as channels that permit water or small solutes to pass (Borgnia et al., 1999; Verkman & Mitra, 2000). Although the AQPs reside constitutively at the plasma membrane in most cell types, they have recently been reported to be present in zymogen granules in rat pancreatic acinar cells (Cho, Cho & Jena, 2002), secretory granules in rat parotid acinar cells (Matsuki et al., 2005), the Brunner's gland of the rat duodenum (Parvin et al., 2005), intracellular vesicles in rat kidney (Yasui et al., 1999), the human parotid gland (Smith et al., 1999). mouse liver (Ferri et al., 2003) and synaptic vesicle from rat brain (Jeremic, Cho & Jena, 2005). It has been demonstrated that AQPs in secretory granules and vesicles contribute to their volume regulation.

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Topology

Matsuki et al. (2005) have demonstrated that AQP5 localizes on the membrane of secretory granules isolated from the rat salivary parotid gland, by immuno-blot analysis and immuno-electron microscopy. In the immuno-electron microscopy using anti-AQP5 antibody to the carboxyl terminus of AQP5, the immuno-gold particles were detected at the outside of the secretory granule membrane, indicating that the amino and carboxyl domains are oriented to the cytoplasmic side, because hydropathy analysis suggests that AQPs have six putative helical domains, and the studies utilizing epitope tagging, AQP-reporter chimeras and site-specific antibodies, confirmed this fundamental topological organization and indicated that the amino and carboxyl termini are cytoplasmically oriented (Verkman & Mitra, 2000). Cho et al. (2002) have demonstrated localization of AQP1 on rat exocrine pancreas zymogen granules, the membrane-bound secretory vesicles. Immuno-reacted AQP1 was detected by immuno-blot analysis when anti-AQP1 antibody to the carboxyl terminus of AQP1 was introduced into the zymogen granules permeabilized by streptolysin-O. In contrast, no immuno-reacted signal was detectable in the sample of intact zymogen granules pre-exposed to the anti-AQP1 antibody. Furthermore, immuno-gold labeling was observed at the inner side of the membrane of streptolysin-O-permeabilized zymogen granules by immuno-electron microscopy using the anti-AQP1 antibody. These observations indicate that amino and carboxyl domains of AQP1 localize at the luminal side of zymogen granule membranes in rat pancreas.

Granule swelling

Isolated zymogen granules from rat exocrine pancreas have been reported to swell rapidly in response to GTP, mediated by Giα_3, a heterotrimeric GTP-binding protein (Jena et al., 1997). Cho et al. (2002) have demonstrated that AQP1 is localized in the granule membrane and contributes to the GTP-mediated swelling of zymogen granules. They have shown that GTP induces the increase in granule volume and water entry into granules, using atomic force microscopy and tritiated water, respectively. $HgCl_2$, an inhibitor of AQPs, and insertion of anti-AQP1 antibody into zymogen granules have also been reported to inhibit the GTP-mediated granule swelling and water permeability. Subsequently, Abu-Hamdah et al. (2004) have suggested that a Giα_3-phospholipase A_2-mediated pathway and K^+-channels are involved in the AQP1 regulation, using a channel blocker, an enzyme inhibitor and an agonist of GTP-binding protein.

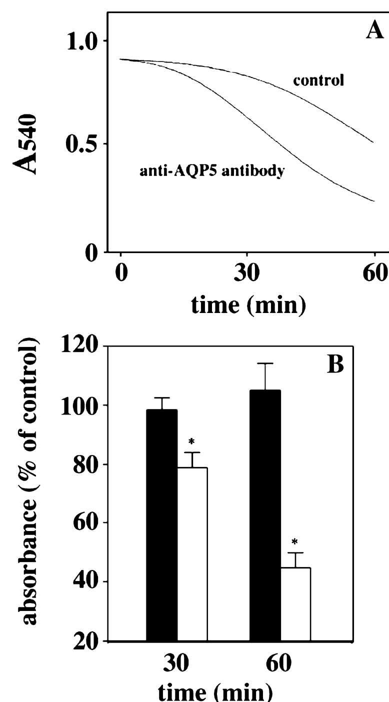

Fig. 1. Secretory granule lysis induced by anti-AQP5 antibody. (*A*) Parotid secretory granules were suspended in iso-osmotic KCl solution and incubated at 37°C for 1 h without or with anti-AQP5 antibody (5 μg/ml). (*B*) Secretory granules were incubated with anti-AQP1 (white columns) or anti-AQP5 antobodies (black columns) for 30 and 60 min. (Reproduced with permission by J. Membrane Biology from Matsuki et al., 2005).

To study the function of AQP5 in the parotid secretory granules, Matsuki et al. (2005) utilized a quantitative in vitro assay involving rapid osmotic swelling and end point measurements of granular osmotic lysis, which has been used for the investigation of ion conductance in secretory granules (De Lisle & Hopfer, 1986). In this assay system, it has been demonstrated that anti-AQP5 antibody induces secretory granule swelling and lysis in iso-osmotic KCl solution (Fig. 1), suggesting that inhibition of AQP5 function causes secretory granule swelling and lysis. The function of most AQPs is well known to be inhibited by mercurial agents which bind the -SH group of cysteines. The sequence of AQPs consists of six putative bilayer-spanning domains and five connecting loops containing two hydrophobic loops, loops B and E. In AQP5, a cysteine at residue 182 in loop E is considered to be a mercury-sensitive domain (Raina et al., 1995), and corresponds to the known mercurial-inhibitory site (Preston et al., 1993). Therefore, we used $HgCl_2$ instead of anti-AQP5 antibody. Interestingly, $HgCl_2$ clearly induced parotid secretory granule lysis as shown in Fig. 2 (Matsuki & Sugiya, *unpublished results*). The $HgCl_2$-induced granule lysis was also completely blocked in the presence of ß-mercaptoethanol, a protective agent for -SH groups, which has been demonstrated to restrain

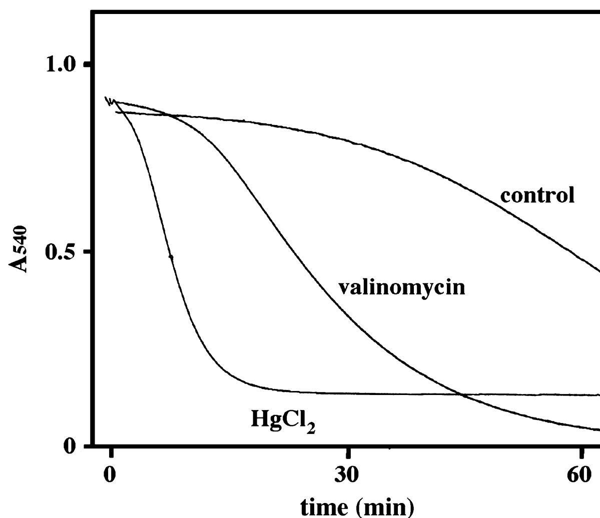

Fig. 2. Secretory granule lysis induced by $HgCl_2$. Parotid secretory granules were suspended in iso-osmotic KCl solution and incubated at 37°C for 1 h without or with $HgCl_2$ (1 μM) or valinomycin (10 μM). Although valinomycin, an electrogenic K^+-selective ionophore, induced osmotic lysis as previously described (Gasser & Hopfer, 1990), the effect of $HgCl_2$ was much stronger than it.

the effect of $HgCl_2$ on AQP5 (Frigeri et al., 1995). These observations support the view that secretory granule lysis is a result of the inhibition of AQP5 function, although further studies need to elucidate the mechanism of $HgCl_2$-induced secretory granule lysis, since $HgCl_2$ is not a specific inhibitor of AQPs.

Matsuki et al. (2005) have also demonstrated that the anti-AQP5 antibody-induced granule lysis is inhibited in the absence of Cl^- or in the presence of 4,4-diisothiocyanostilbene-2,2′-disulfonic acid, an anion channel blocker in the reaction mixture. There is no evidence that AQP5 acts as ion channel in AQP5-expressing oocytes (Agre et al., 1997; Anthony et al., 2000). The presence of Cl^- conductance in the secretory granule membrane of the rat parotid gland has been demonstrated (Gasser, Goldsmith & Hopfer, 1990; Gasser & Hopfer, 1990). On the other hand, in the secretory granules of pancreatic acinar cells, the expression of several ion channels, such as a ClC-2 Cl^- channel, a Ca^{2+}-activated and HCO_3^--permeable anion channel (CLCA) and a KCNQ1 K^+ channel, has been demonstrated and a specific set of ion channels has been considered to modulate the regulated granule swelling and the release of macromolecules (Thévenod, 2002). In airway secretory glands, the expression of cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-activated Cl^- channel (Jacquot et al., 1993), has been reported, and CFTR has been suggested to regulate the ion content in the secretory granules and granule expansion (Baconnais et al., 2005). As a consequence, we hypothesize that a balance of water permeation via AQP5 and Cl^- conductance is necessary for secretory granule volume regulation. Currently, the hypothesis that AQPs function as osmotic and turgor sensors rather than water channels, the sensor hypothesis, has been advocated (Shachar-Hill & Hill, 2002; Hill, Shachar-Hill & Shachar-Hill, 2004). Therefore, AQP5 appears to act as an osmotic sensor in the secretory granules of the parotid gland, although further studies with precise mechanisms of the relationship between AQP5 and the Cl^- channels are necessary. In secretory granules of the rat parotid gland, expression of the heterotrimeric GTP-binding protein Gsα and Gsα-regulated Cl^- conductance have been reported (Watson et al., 1992; 1997). However, AQP5 regulation by GTP-binding proteins in the secretory granule is still unknown.

Exocytosis and AQPs

It has been considered that the fluidity of the primary secretion is important for the discharge of granule contents from exocytosed secretory granules in pancreas (Gasser, DiDomenico & Hopfer, 1988). It is likely that components of the granule membrane, such as ion channels, might insert into the apical membrane during the fusion process, and subsequently salt and water would flush out the stored macromolecules into the acinar lumen and provide for an appropriate amount of fluid to be secreted with the proteins.

In rat duodenal Brunner's gland, vasoactive intestinal polypeptide (VIP) has been reported to increase the flow rate as well as bicarbonate and protein output (Kirkegaard et al., 1981). Parvin et al. (2005) have currently demonstrated that AQP5 localizes in the granule membrane and the apical membrane in rat Brunner's gland by immuno-histochemistry and electron microscopic immuno-histochemistry, and that the AQP5 level in the apical membrane is increased by VIP stimulation, suggesting the translocation of AQP5 from the granule membrane to the apical membrane on exocytosis is provoked by VIP. In the rat parotid gland, it has been reported that immuno-stained AQP5 was scattered as clusters in the sub-membranous area of the acinar cells in the rat injected with isoproterenol (Matsuzaki et al., 1999). AQP5 has been reported to translocate from the intracellular vesicles to the apical membrane in vitro in response to stimulation of muscarinic receptors (Ishikawa et al., 1998), although such translocation of AQP5 has not been detected in vivo (Gresz et al., 2004). All of these reports imply redistribution of AQP5 from inside the cells to the apical membrane on stimulation with secretagogues, and support the hypothesis proposed by Gasser, DiDomenico & Hopfer (1988).

Swelling of secretory granules has been demonstrated to be a primary role in the expulsion of granule contents during secretion (Jena, 2004; 2005;

Kelly et al., 2004). An increase in secretory granule volume after stimulation of secretion has been determined in mast cells using electrophysiological measurements (Fernandez, Villalon & Verdugo, 1991). In pancreatic acinar cells, direct evidence of secretory granule swelling induced by stimulation of secretion has been demonstrated using atomic force microscopy (Cho, Cho & Jena, 2002). It has been considered that swelling of secretory granules results in a build-up of intra-granular pressure, which allows expulsion of the granular contents.

In the exocytotic process, release of granule contents follows fusion of secretory granules with the plasma membrane. In beige mouse mast cells, membrane fusion has been demonstrated to precede secretory granule swelling during exocytosis by studies with electrophysiological membrane capacitance measurements (Breckenridge & Alders, 1987; Zimmerberg et al., 1987). Granule swelling has been inferred to be necessary to stabilize and widen the exocytotic pore and to be caused by movement of small solutes through the exocytotic pore into the granule matrix. On the contrary, secretory granule swelling has been proposed to be prerequisite for secretory granule fusion with the plasma membrane (Stanley & Ehrenstein, 1985; Finkelstein, Zimmerberg & Cohen, 1986; Almers, 1990). In Ca^{2+}-induced exocytosis, it has been considered that activation of K^+ channels in granule membrane by Ca^{2+} causes an influx of K^+ and anions and subsequently an increase in the osmotic pressure in granule membrane induces swelling of granules situated close to the plasma membrane (Stanley & Ehrenstein, 1985). In rat pancreas, fusion of zymogen granules with the plasma membrane has been demonstrated to be facilitated by activation of the GTP-binding protein by $Gi\alpha_3$ in zymogen granule membrane by in vitro fusion assay (Nadin et al., 1989; Sattar et al., 2002). Furthermore, GTP-binding proteins have been demonstrated to contribute to secretory granule swelling using atomic force microscopy (Jena et al., 1997). These observations all suggest that vesicle swelling is an important prerequisite for secretory granule fusion with the plasma membrane. As described above, AQP1 in the pancreas zymogen granule membrane contributes to the GTP-mediated swelling of zymogen granules (Cho et al., 2002; Abu-Hamdah et al. 2004). Therefore, it is most likely that AQPs contribute to granule fusion with the plasma membrane and expulsion of the granule contents during secretion.

Perspective

There are many reports of the functions of AQPs in the plasma membrane, such as transcellular fluid transport and tissue swelling. On the other hand, AQPs have been suggested to be involved in cell migration and neural signal transduction (Verkman, 2005). The roles of AQPs in secretory granule and vesicle could provide a new insight for the elucidation of AQP function. AQPs in the granule membrane have been demonstrated to be involved in secretory granule and vesicle swelling, which appears to contribute to exocytotic release of the contents in secretory granules and vesicles. However, a specific set of ion channels, including AQP, is probably necessary for the regulation of granule and vesicle swelling, as demonstrated in the secretory granules of pancreatic acinar cells (Thévenod, 2002). Therefore, the relationship between AQPs and ion channels has to be elucidated. It is of interest that AQP6 resides exclusively in intracellular vesicles in renal epithelia (Yasui et al., 1999). Such observations imply the presence of granule- or vesicle-specific AQPs and their function. Because the involvement of AQPs in a variety of cellular functions has been confirmed by analysis of AQP-deficient mice, analysis of secretory and vesicle functions in these mice will allow us to elucidate a physiological role of AQPs in the secretory granule and vesicle.

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