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Special Issue Histochemistry of Salivary Glands

Review

Function of the Membrane Water Channel Aquaporin-5 in the Salivary Gland

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The process of saliva production in the salivary glands requires transepithelial water transfer from the interstitium to the acinar lumen. There are two transepithelial pathways: the transcellular and paracellular. In the transcellular pathway, the aquaporin water channels induce passive water diffusion across the membrane lipid bilayer. It is well known that aquaporin-5 (AQP5) is expressed in the salivary glands, in which it is mainly localized at the apical membrane of the acinar cells. This suggests the physiological importance of AQP5 in transcellular water transfer. Reduced saliva secretion under pilocarpine stimulation in AQP5-null mice compared with normal mice further indicates the importance of AQP5 in this process, at least in stimulated saliva secretion. Questions remain therefore regarding the role and importance of AQP5 in basal saliva secretion. It has been speculated that there would be some short-term regulation of AQP5 such as a trafficking mechanism to regulate saliva secretion. However, no histochemical evidence of AQP5-trafficking has been found, although some of biochemical analyses suggested that it may occur. There are no reports of human disease caused by AQP5 mutations, but some studies have revealed an abnormal subcellular distribution of AQP5 in patients or animals with xerostomia caused by Sjögren's syndrome and X-irradiation. These findings suggest the possible pathophysiological importance of AQP5 in the salivary glands.

Key words: water channel, aquaporin-5, salivary gland, xerostomia

I. Introduction

The aquaporins (AQPs) are membrane water channel proteins that enable passive water transfer across the lipid bilayer. As water transfer is a ubiquitous phenomenon, the aquaporins are widely distributed among bacterial, plant, and animal species [7]. To date, 13 AQP isoforms (AQP0–AQP12) have been identified in mammals [7]. In this review, we focus on the role of AQP5 in the salivary gland of mammals and discuss its physiological functions in saliva secretion as well as its relationship to human disease.

II. Membrane Water Channel Aquaporins and Transepithelial Water Transfer

Cells are enclosed by a membrane lipid bilayer which restricts water permeability. If a high level of water transfer is required, water channels such as the AQPs provide a system for enabling this. These proteins contain six transmembrane domains and maintain their NH_2- and COOH-termini within the cytoplasm. Characteristic sequences for the AQP family members are two highly conserved short stretches of asparagine-proline-alanine (NPA), which form the pore of the channel [7].

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The 13 AQP isoforms that have been identified in mammals [7, 33] are usually subdivided into three groups based on the primary sequences. These are: 1) the classical aquaporins (AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8), which mainly permeate water; 2) the aquaglyceroporins (AQP3, AQP7, AQP9, and AQP10), which are permeable to small solutes such as glycerol and urea as well as water; and 3) unorthodox aquaporins (AQP11 and AQP12). The AQPs are widely distributed among water-handling organs such as the kidney and exocrine glands (Table 1) [15, 17, 19, 20, 33]. At least seven AQP isoforms are expressed in the kidney, in which a large amount of water is reabsorbed along the uriniferous tubules [35]. Among these isoforms it is well known that AQP2 plays a key role in water reabsorption in the collecting duct cells and a functional defect in this protein causes diabetes insipidus [4, 28]. AQP2 is largely localized to the intracellular storage vesicles when the plasma vasopressin level is low and when it is elevated AQP2 accumulates on the apical membrane through vesicular trafficking (Fig. 1) [2, 34, 36]. Transcellular water reabsorption is accelerated by AQP2 on the apical membrane and by both AQP3 and AQP4 on the basolateral membrane in accordance with the osmotic gradient between the luminar and basolateral sides [15, 35]. This is one example of a well-established transepithelial water transport mechanism. Transepithelial water transport also occurs in the salivary glands which in humans secrete 1000–1500 mL of saliva per day.

Table 1. *Tissue distributions of mammalian aquaporins*

Isoform	Tissue distribution
AQP0	eye (lens)
AQP1	capillary, erythrocyte, kidney (proximal tubule, descending thin limb)
AQP2	kidney (collecting duct)
AQP3	kidney (collecting duct), urinary epithelium, skin, digestive epithelium, respiratory epithelium, eye
AQP4	kidney (collecting duct), stomach, respiratory epithelium, brain
AQP5	salivary gland, respiratory epithelium, lacrimal gland, sweat gland
AQP6	kidney (collecting duct)
AQP7	kidney, testis, skeletal muscle
AQP8	unclear
AQP9	liver, brain
AQP10	unclear
AQP11	kidney, liver, testis, thymus, brain
AQP12	pancreas

III. Histology of the Salivary Glands

The major salivary glands consist of the parotid, submandibular, and sublingual glands. The basic secretory unit of each salivary gland consists of the acinus and duct system. Acinar cells are basically classified into two types depending on the components of secretion: 1) the serous cells which secret proteins and 2) the mucous cells which secret mucin. As it is sometimes difficult to distinguish between these cell types, the term “seromucous cells” has been used to describe the acinar cells which have both characteristics. The duct system consists of the intercalated, striated, and excretory ducts.

Histological structure and the words describing each membrane domain of salivary acinar cells are confusing because of the presence of the intercellular canaliculus. Acinar cells have the apical and basolateral membrane domains, since they are polarized epithelial cells. Apical

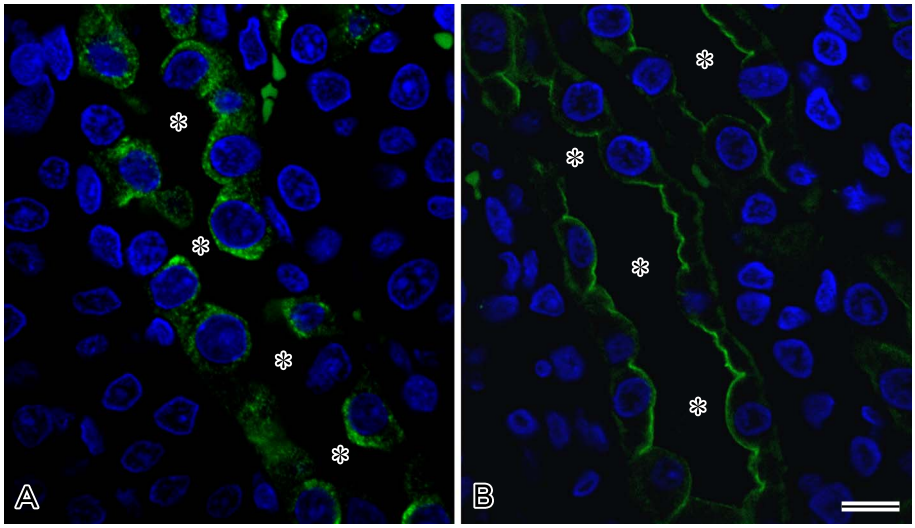


Fig. 1. Immunofluorescent localization of AQP2 in water-loaded and vasopressin-treated rat kidney collecting ducts. Paraffin sections were immunolabeled for AQP2 (green signals). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI) and are indicated by the blue signals. Confocal images are shown. **A:** Water-loaded rat kidney. AQP2 is largely distributed in the cytoplasm. **B:** Vasopressin-treated rat kidney. AQP2 is highly accumulated in the apical membrane. Asterisks indicate the lumen of the collecting ducts. Bar=10 μm.

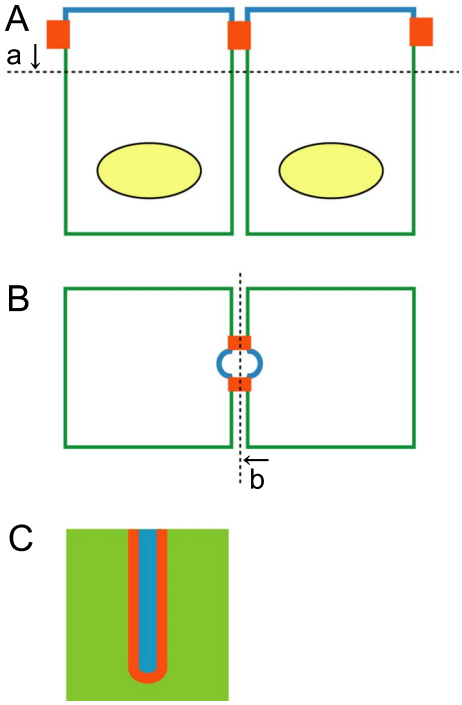


Fig. 2. Schematic diagram illustrating membrane domains of salivary acinar cells. **A:** Neighboring acinar cells are sealed with the tight junction (orange), which is a boundary between the apical (blue) and basolateral (green) membrane domains. **B:** Cells are cut along a dotted line (a) and viewed from the top (arrow). An intercellular canaliculus, which is part of lumen extending from the apical side, is seen between neighboring cells. Canalicular membrane is part of apical membrane (blue). **C:** Cells are separated along a dotted line (b) and a lateral surface view (arrow) is shown. Tight junction (orange) is seen along a boundary between canalicular membrane (blue); that is, apical membrane, and basolateral membrane (green).

membranes of each acinar cell surround the lumen of the acinus; on the other, basolateral membranes are bathed in the interstitial fluid. Apparent tight junctions are seen between the apical and basolateral membrane domains. Intercellular canaliculus is a specialized form of the lumen extending from the apical side to the basal side between acinar cells [24, 26]; therefore intercellular canalicular membrane is part of apical membrane domain (Fig. 2). Immunolabeling on the intercellular canalicular membrane between acinar cells are sometimes misunderstood as if the labeling was on the basolateral membrane domain. To clarify the membrane domain of acinar cells, we often show the immunolabeling of tight junction proteins such as occludin, which is always seen along the boundaries between the apical and basolateral membrane domain (Fig. 3) [14].

IV. Mechanism of Salivary Secretion

Saliva is a watery fluid containing electrolytes and a mixture of proteins and is secreted in two main stages:

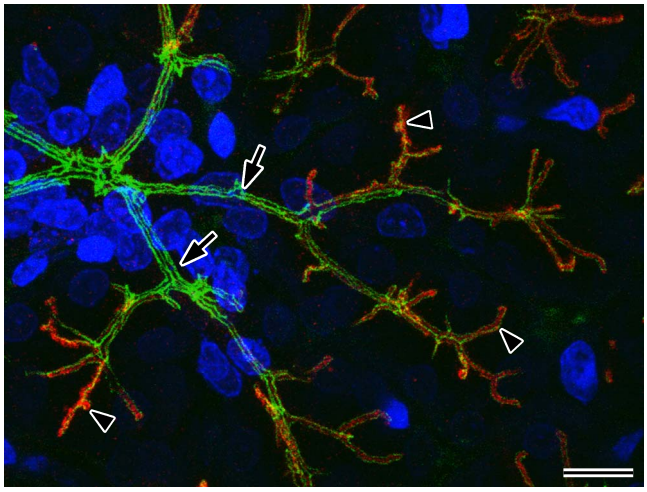


Fig. 3. Immunofluorescent localization of AQP5 in the rat parotid gland. A cryostat section was immunolabeled for AQP5 (red) and occludin (green). Nuclei were counterstained with TO-PRO-3 (blue). A projection image of consecutive confocal images is shown. AQP5 is localized to the apical membrane of the acinar cells, including the intercellular secretory canaliculi along the region of occludin labeling (arrowheads). Arrows indicate ducts. Bar=10 μm. Reproduced from Figure 4b of ref. 14 with the kind permission of Springer Science+Business Media.

through the production of primary saliva in the acini and a subsequent reabsorption process along the duct system [22]. In the process of primary saliva production, net movements of ions from the interstitium to the lumen via active processes cause near iso-osmolar transepithelial water transfer [22], i.e. in the absence of an osmotic gradient across the epithelial cell layer [29]. As a result, primary saliva is almost isotonic with plasma. Within the duct system, Na^+ and Cl^- are reabsorbed and K^+ and HCO_3^- are secreted. As the ducts are basically impermeable to water, the final saliva secreted to the oral cavity is hypotonic compared with the primary saliva [22].

V. Distribution of AQP5 in the Salivary Gland

It was expected that water channels would operate in the salivary acinar cells and in 1995 Raina *et al.* [25] isolated AQP5 from the rat submandibular gland. In our laboratory, we raised anti-AQP5 antibodies and performed a detailed examination of AQP5 localization in the rat tissue [14]. Double-labeling of AQP5 and the tight junction protein occludin clearly showed that AQP5 is localized at the apical membrane (Fig. 3) [14]. AQP5-positive lateral aspects between neighboring acinar cells are intercellular canaliculi as mentioned in HISTOLOGY OF THE SALIVARY GLANDS.

An important question arising from these findings was whether any AQPs are present in the basolateral membrane that enable water uptake from the basolateral side for transcellular water transfer. No clear evidence of this had

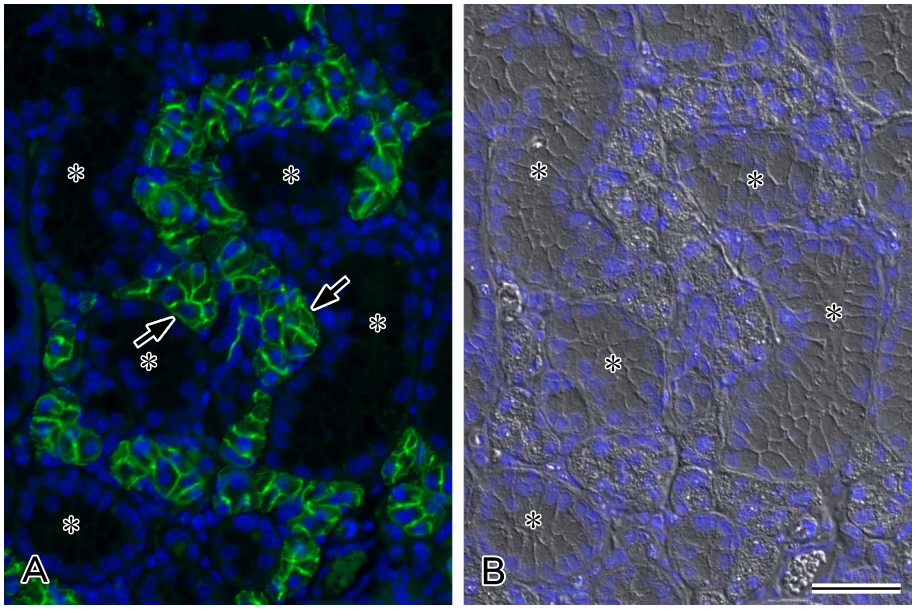
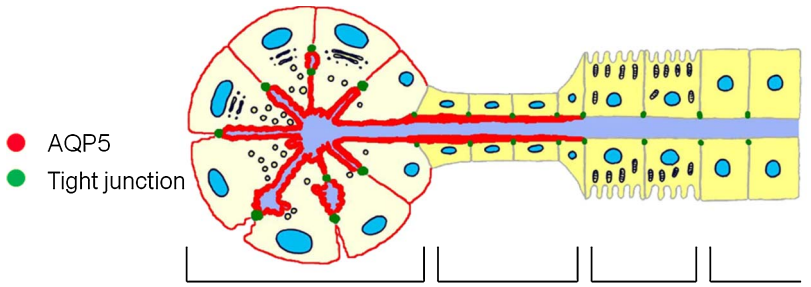


Fig. 4. Immunofluorescent localization of AQP5 in the mouse submandibular gland. A paraffin section was immunolabeled for AQP5 (green). Nuclei were counterstained with DAPI (blue). A merged fluorescence image for AQP5 and DAPI (**A**) and a corresponding Nomarski differential interference-contrast image merged with DAPI fluorescence image (**B**) is shown. AQP5 is highly localized at the apical membrane and marginally at the basolateral membrane (arrows). Asterisks indicate ducts, in which no labeling of AQP5 is seen. Bar=50 μm.



	acinus	intercalated duct	striated duct	excretory duct
Rat				
Parotid	+ (AP)	–	–	–
Submandibular	+ (AP)	+++	–	–
Sublingual	+ (AP)	–	–	–
Mouse				
Parotid	+ + (AP,BL)	+	–	–
Submandibular	+ + (AP,BL)	+	–	–
Sublingual	+ (AP,BL)	+	–	–

AP: apical membrane, BL: basolateral membrane

Fig. 5. Summary of the distribution of AQP5 in the rat and mouse salivary glands. AQP5 is mainly distributed at the apical membrane and sometimes at low levels at the basolateral membrane of acinar cells, and at the apical membrane of some intercalated ducts. These expression levels are scored from – (negative) to +++ (highly positive).

been found previously in the rat or mouse. We found in our studies, however, that the total AQP5 expression levels are much higher in the mouse than in the rat for unknown reasons, and that AQP5 is present in the basolateral membrane as well as the apical membrane of acinar cells in the mouse salivary glands (Fig. 4) [18]. AQP5-labeling was still found to be much more abundant in the apical membrane than in the basolateral membrane in these experiments (Fig. 4). We further found some differences in the AQP5 expression profile in the intercalated duct among

each gland and between the mouse and rat; that is, in the rat, AQP5 is highly expressed only in the submandibular gland intercalated ducts, whereas is normally expressed in submandibular, parotid, and sublingual glands intercalated ducts in the mouse. We found no AQP5 expression in either the striated or excretory ducts (Fig. 4). The localization of AQP5 in the rat and mouse salivary glands is summarized in Figure 5.

In addition to the salivary gland, AQP5 was found to be distributed in many exocrine glands such as lacrimal glands, pyloric glands in the stomach, duodenal glands, and sweat glands [14, 16, 33].

VI. Physiological Roles of AQP5

The distribution of AQP5 on the apical membrane of acinar cells strongly suggested its physiological importance in water transfer during primary saliva production. To clarify this, Ma *et al.* [13] generated an AQP5-null mouse model in which they examined changes of the amount of saliva and its composition in a pilocarpine-induced state. These analyses revealed a more than 60% reduction in saliva, in which the concentrations of Na^+, K^+, and Cl^- were elevated, in AQP5-null mice. These data suggested the importance of the transcellular route of water secretion via AQP5, at least during pilocarpine-stimulated saliva secretion. Krane *et al.* [12] independently generated AQP5-deficient mice and examined water permeability in parotid and sublingual acinar cells isolated from these animals. They measured cell volume changes in response to hypertonicity-induced cell shrinkage and hypotonicity-induced cell swelling, and showed that water permeability decreased by over 65% in AQP5-deficient acinar cells. These results further indicated that AQP5 provides the major pathway for water transfer in acinar cells. However, the importance of AQP5 in basal salivary secretion remains unclear. Of note also, the AQP5-null mice were grossly normal in appearance except for mild growth retardation seen within the first weeks after weaning reported by Ma *et al.* [13]. A paracellular pathway in some epithelia could be also effective route for transepithelial water movement in addition to the transcellular pathway and is worthy of consideration (Fig. 6). It is well known that the intercellular spaces of the epithelial sheet, including the salivary acini, are sealed with a specialized barrier structure, tight junction, which is formed by several components such as occludin, the claudins, and ZO-1 [38]. However, tight junctions do not always provide a complete barrier and sometimes create a leaky pathway. The tightness of the junction seems to depend on the composition of its claudin isoform(s) [1]. For example, claudin-2 is a constituent of leaky and cation (Na^+)-selective paracellular channels in the renal proximal tubule cells [21]. Based on their analyses in the corneal endothelium, Fischbarg and colleagues assert that the paracellular pathway operating via tight junctions is much more important for fluid transport across leaky epithelia [5]. A previous report by Simson and Bank [27] has concluded using both ultrastructural and tracer-permeability criteria that tight junctions in the acinar cells appear to be leaky, even though the duct system is basically impermeable. The evidence to date thus suggests that the paracellular pathway may be more important than the transcellular pathway through AQP5 for near iso-osmolar fluid transport during basal saliva secretion. Further studies will be required to clarify this issue.

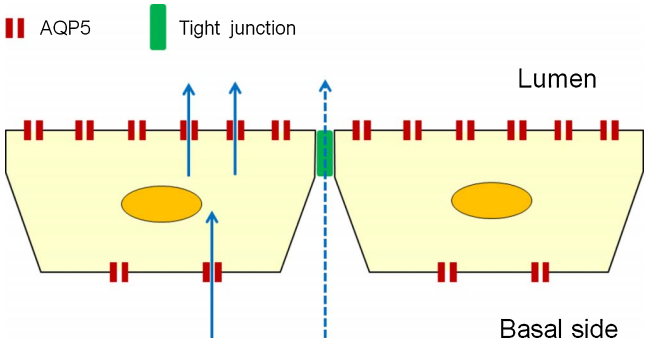


Fig. 6. Possible pathways underlying transepithelial water movement in salivary acini. Arrows indicate a transcellular pathway operating via AQP5. The dotted arrow indicates a paracellular pathway through the tight junction between cells.

VII. Short-term Regulation of AQP5

Saliva secretion is regulated by both sympathetic and parasympathetic nerve effects [40]. Hence, some short-term regulatory mechanisms for AQP5 and/or paracellular permeability have been predicted to be involved in the regulation of saliva secretion. One mechanistic possibility is AQP5-trafficking for a rapid localization change between the cell surface membrane and other intracellular membrane compartments. It is well known in this regard that the subcellular distribution of AQP2 is altered in renal collecting duct cells via its trafficking between intracellular vesicles and the plasma membrane, which in turn causes a rapid and enormous change in membrane water permeability (Fig. 1) [36].

The possibility of AQP5 trafficking as a short-term regulatory process for this protein has been predicted by its high homology to AQP2. Ishikawa *et al.* [9, 10] have reported changes in the AQP5 distribution from a biochemically isolated intracellular membrane fraction to the apical plasma membrane fraction using immunoblotting. We have carefully examined the immunohistochemical localization of AQP5 in rat and mouse salivary glands using AQP5 antibodies that we raised in our laboratory. Immunofluorescence microscopy has provided evidence of some dispersed immunolabeling at the luminar surfaces of acinar cells, suggesting the presence of AQP5 in the cytoplasm [14, 16, 18]. To clarify whether AQP5 is indeed localized intracellularly, we performed immunoelectron microscopy. However, no apparent labeling was detected in the cyto-

plasm in this analysis, indicating that the possibility of AQP5 trafficking is quite low. Gresz *et al.* [6] also examined whether AQP5 trafficking occurs by immunofluorescence and immunoelectron microscopy in the rat parotid and submandibular glands, in which the fluid secretion was stimulated or inhibited by drug administrations. Their results showed a predominant localization of AQP5 in the luminar membrane under all experimental conditions [6]. These results also suggested that AQP5 trafficking is unlikely to occur.

An unresolved question arising from these findings is the cause of the dispersed AQP5 immunolabeling observed by immunofluorescence microscopy. We have speculated that AQP5 localized at the apical membrane could transiently diffuse into the granule membrane that had fused to the apical membrane by exocytosis. This possibility is based on the fact that dispersed distribution of AQP5 in the parotid gland was emphasized when secretory granule release was induced by the administration of isoproterenol in rats and mice (Fig. 7A, B) [14, 18]. To further clarify this

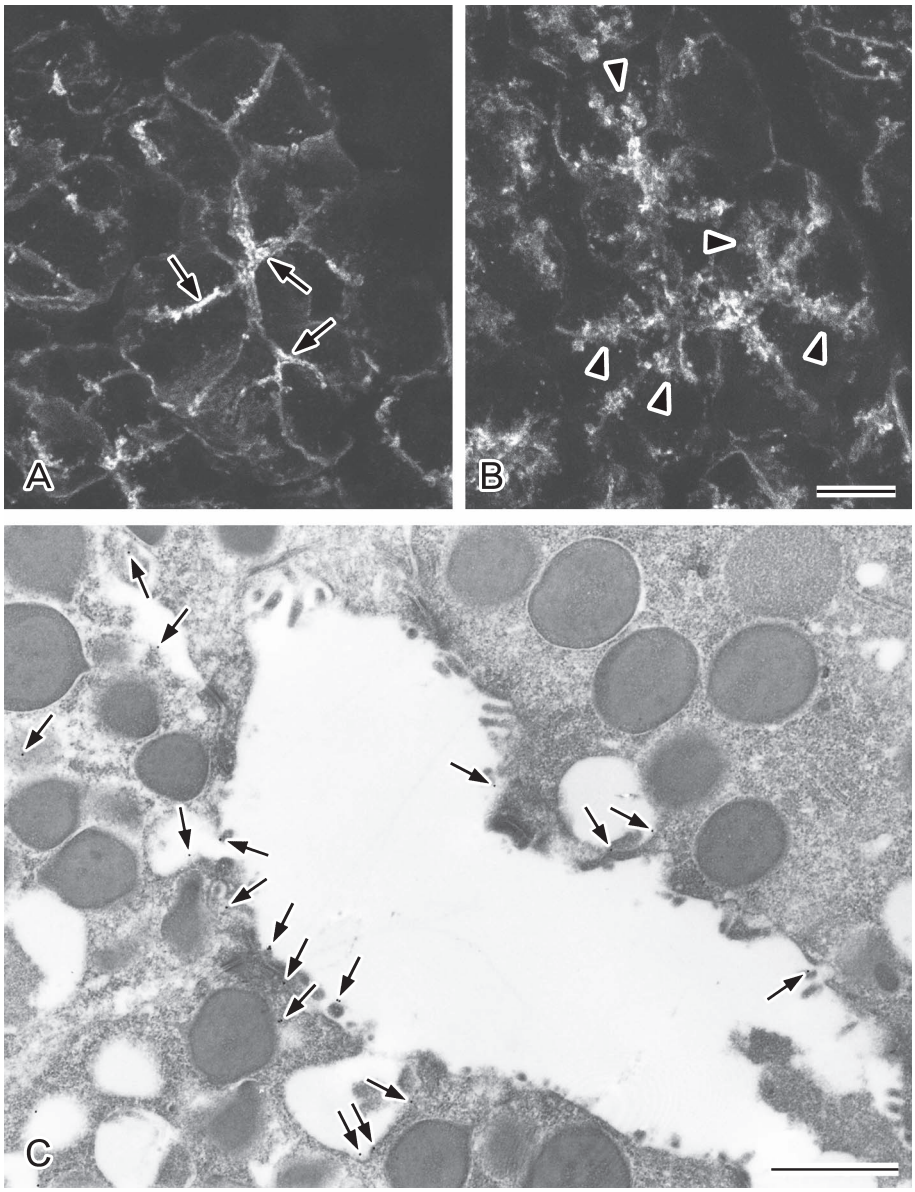


Fig. 7. Changes in AQP5 in the isoproterenol-treated mouse parotid gland. Mice were starved overnight as a control (**A**) and thereafter treated with isoproterenol (**B** and **C**; 5 min after the intraperitoneal injection of 0.8 mg/100 g body weight). Immunofluorescence microscopy (**A, B**) and immunoelectron microscopy via the post-embedding immunolabeling method (**C**) were performed. **A:** AQP5 is mainly localized at the apical membrane including the intercellular secretory canaliculi (arrows). **B:** Dispersed labeling of AQP5 is seen (arrowheads). **C:** AQP5 is detectable on the apical membrane and on some granule membranes fused to the apical membrane (arrows). Bars=50 μm (**A** and **B**), 1 μm (**C**). The images in **A** and **B** are reproduced from Figures 3a and 3b of ref. 18 with kind permission from the Japanese Society of Microscopy.

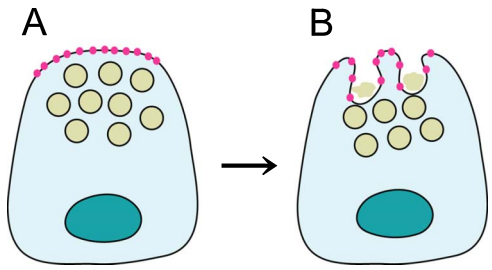


Fig. 8. Schematic diagram showing AQP5 localization in the salivary acinar cell. **A:** AQP5 (magenta) is localized on the apical membrane. **B:** Granule membrane fusion via exocytosis results in the lateral diffusion of AQP5 into the membrane derived from granules.

issue we performed immunoelectron microscopy and found that AQP5 labeling seemed to be restricted to the apical membrane and sometimes to granule membrane fused to the apical membrane (Fig. 7C) [18]. No labeling at any intracellular structure was apparent in this experiment. We therefore concluded that transient granule membrane fusion results in the lateral diffusion of AQP5 along the membrane, which is visualized as dispersed labeling by immunofluorescence microscopy (Fig. 8). These changes are not related to either the regulation of apical membrane water permeability or to AQP5-trafficking.

Another possible mechanism of short-term regulation of AQP5 is modification of the protein. Ishida *et al.* [8] have reported in the mouse lacrimal gland that an antibody against the AQP5 carboxyl terminus shows high immunoreactivity to the apical membrane in pilocarpine-treated lacrimal glands but not in saline-treated controls. An antibody to the extracellular domain of AQP5 showed similar immunolabeling patterns in both groups of animals. These results suggest that the AQP5 carboxyl terminus region may be modified by binding proteins or other factors that might regulate the functional properties of this aquaporin.

VIII. Long-term Regulation of AQP5

Changes in the AQP expression patterns in the kidney have been examined in many laboratories. For example, a 48-hour water restriction or a 5-day vasopressin infusion causes significant increases in AQP2 and AQP3 expression in the collecting duct cells [37]. The expression of AQP5 in the salivary glands may also be regulated by several long-term stimulations. This issue is under investigation in our laboratory.

IX. AQP5 and Human Disease

Many researchers are interested in the relationship between AQP5 and xerostomia, a disorder in which salivary secretion decreases and affected individuals suffer from difficulties in chewing, swallowing and speaking. The most common xerostomia-causing disease is Sjögren's syndrome, which is an autoimmune disorder. There are no reports that have associated Sjögren's syndrome with AQP5 mutations, but some studies have evaluated changes of the AQP5 distribution pattern in the salivary glands of Sjögren's syndrome patients [30] and in a mouse model of this disorder [11, 23]. Some of these has revealed the distribution of AQP5 in the basolateral membrane in addition to the apical membrane [11, 30], which is, however, not always abnormal since AQP5 is localized at the basolateral membrane as well as the apical membrane as discussed earlier. Nishimura *et al.* [23] have shown that AQP5 is localized not only to the apical membrane but also in the cytoplasm in Sjögren's syndrome model mice and that the administration of cevimeline, which is commonly used in Sjögren's syndrome patients to stimulate saliva and tear secretion, causes AQP5 accumulation on the apical membrane. Another major cause of xerostomia is radiation-induced salivary gland dysfunction. The expression of AQP5 and its distribution in the salivary glands is reported to be altered by irradiation [3, 31, 32]. It would be difficult, however, to elucidate whether these changes in AQP5 expression and distribution are directly related to the reduced salivary secretion in Sjögren's syndrome or radiation-induced xerostomia because the physiological importance of AQP5 in salivary secretion is still unclear. Muscarinic receptor agonists such as pilocarpine and cevimeline are clinically useful to treat patients suffering from xerostomia to increase saliva secretion [39]. The mechanism underlying the beneficial effect of these chemicals is enigmatic and it is uncertain whether it is related to AQP5. Further studies are required to elucidate these questions. It will be necessary in this regard to focus on the regulation of paracellular fluid transport as well as transcellular fluid transport that occurs via AQP5.

X. Perspectives

Despite the presence of the water channel protein AQP5 in salivary glands, its physiological roles during salivary secretion are unresolved. Further studies will be required to address this. To understand the general mechanism underlying transepithelial fluid transfer, combined analyses of transcellular and paracellular pathways will also be required.

XI. Acknowledgments

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