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Review

Distribution and roles of aquaporins in salivary glands

Christine Delporte [a,*], Serge Steinfeld [b]

[a] *Department of Biochemistry and Nutrition, Faculty of Medicine, Université Libre de Bruxelles, Bat G/E, CP 611, 808 route de Lennik, B-1070 Brussels, Belgium*
[b] *Department of Rheumatology, Erasme University Hospital, Brussels, Belgium*

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Abstract

Salivary glands are involved in secretion of saliva, which is known to participate in the protection and hydratation of mucosal structures within the oral cavity, oropharynx and oesophagus, the initiation of digestion, some antimicrobial defence, and the protection from chemical and mechanical stress. Saliva secretion is a watery fluid containing electrolytes and a mixture of proteins and can be stimulated by muscarinic and adrenergic agonists. Since water movement is involved in saliva secretion, the expression, localization and function of aquaporins (AQPs) have been studied in salivary glands. This review will focus on the expression, localization and functional roles of the AQPs identified in salivary glands. The presence of AQP1, AQP5 and AQP8 has been generally accepted by many, while the presence of AQP3, AQP4, AQP6 and AQP7 still remains controversial. Functionally, AQP5 seems to be the only AQP thus far to be clearly playing a major role in the salivary secretion process. Modifications in AQPs expression and/or distribution have been reported in xerostomic conditions.

Keywords: Aquaporin; Salivary gland; Review

Contents

* Corresponding author. Tel.: +32 2 555 62 10; fax: +32 2 555 62 30.
E-mail address: cdelport@ulb.ac.be (C. Delporte).

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1. Introduction

For several decades, the existence of proteins forming water-specific channels was postulated based on membrane permeability measurements performed in cells and epithelia. In 1992, the first water-specific channel, formerly called CHIP28 and now aquaporin-1 (AQP1), was cloned and biophysically characterized [1,2]. Since then, other aquaporins (AQPs) have been cloned from mammals and the AQP family has grown to 13 members (AQP0 to AQP12). The AQPs are small hydrophobic integral membrane proteins of about 270 amino acids expressed widely in the animals and plants. AQPs exist as monomers of ±30 kDa [1] and their hydropathy analyses showed the presence of six transmembrane helices in each monomer, as well as three extracellular and two intracellular loops ([3]; Fig. 1). The signature amino acid sequence motifs of the AQPs, two repeating Asn–Pro–Ala (NPA) sequences, are present in the first intracellular and the third extracellular loop ([4]; Fig. 1). According to the "hourglass model" and crystallographic studies, these two loops connect in the centre of the lipid bilayer and form a hydrophilic pore for the water transfer through the lipid bilayer [5]. A single N-glycosylation site, present in the second extracellular loop, accounts for the detection of glycosylated and non-glycosylated forms of AQP1 by immunoblotting (Fig. 1). In the plasma membrane, AQP1 assembles in tetramers [6] and each monomer contains a water channel [5]. For AQP1, the water selectivity is due to both the size-exclusion effect of the channel [4,7], and the orientation of Asn in the NPA motifs providing necessary hydrogen-bonding interactions to separate water molecules and avoid proton flow through the channel [4,8].

On the basis of their permeability characteristics that coincide generally with their specific amino acid sequence motifs, the members of the AQP family can be divided into two groups: the aquaporins and the aquaglyceroporins. The aquaporins are primarily permeable to water (AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8) whereas the aquaglyceroporins (AQP3, AQP7, AQP9 and AQP10) also transport glycerol and small solutes (reviewed by [4,9,10]). The transport function of many AQPs can be inhibited by mercurial–sulfhydral-reactive compounds such as $HgCl_2$ [11].

Mammals possess three major pairs of salivary glands: parotid, submandibular and sublingual as well as numerous minor salivary glands scattered among the oral cavity. Salivary glands are composed of acinar cells either serous or mucous, and ductal cells of several varieties. Saliva is a watery fluid containing electrolytes and a mixture of proteins. The Na^+/K^+ ATPase, located at the basolateral membrane of acinar cells, produces an inward-directed Na^+ chemical gradient (Fig. 2). The $Na^+/K^+/2Cl^-$ cotransporter, located at the basolateral membrane, uses the Na^+ gradient to elevate the intracellular Cl^- concentration. Intracellular Cl^- level also increases through the paired basolateral Cl^-/HCO_3^- and Na^+/H^+ exchangers. The Na^+-dependent Cl^- uptake mechanism allows concentration of intracellular Cl^- above its electrochemical gradient. Agonist-stimulated saliva secretion is initiated by the opening of K^+ and Cl^- channels located in the basolateral and apical membranes, respectively. HCO_3^- is secreted across the apical membrane via an ion channel, possibly via the same channel involved in Cl^- secretion. The opening of K^+ and Cl^- channels leads to a negative electrical potential difference allowing the passive movement of cations across the acinar cell tight junctions. The accumulation of ions in the lumen generates a transepithelial osmotic gradient driving water movement through the apical AQP5 channels and paracellular pathways. This leads to the secretion of an isotonic plasma-like primary fluid ([12]; Fig. 2). Although little is known about ion transport pathways in salivary ducts, while saliva passes through the ducts, NaCl is resorbed via Na^+ channels, Cl^- channels, and Na^+/H^+ exchangers, while K^+ and HCO_3^- are excreted via Cl^-/HCO_3^- and K^+/H^+ exchangers (Fig. 2). The resulting hypotonic secreted saliva is due to the relative impermeability of the ducts to water [13–17]. The final saliva composition depends on both the origin of the stimulation (sympathetic or parasympathetic) and the type of salivary gland stimulated.

Typically, humans secrete about 1 l of saliva per day. The functions of saliva include the protection and hydration of mucosal structures within the oral cavity, oropharynx and oesophagus, the initiation of digestion, some antimicrobial defence, and the protection from chemical and mechanical stress.

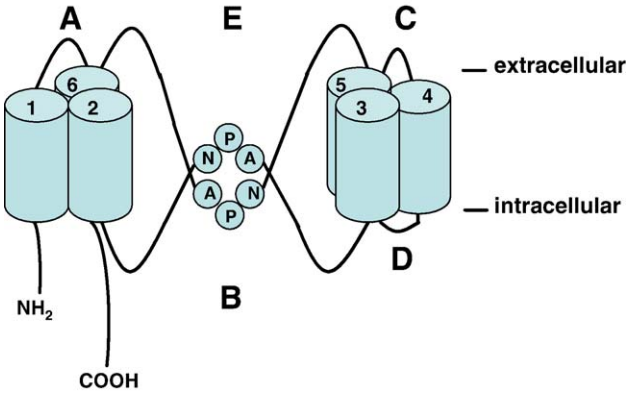

Fig. 1. Hourglass model of AQP1. AQP1 contains six transmembrane domains, three extracellular and two intracellular loops. The loops B and E, folding in the lipid bilayer, form a single aqueous pore.

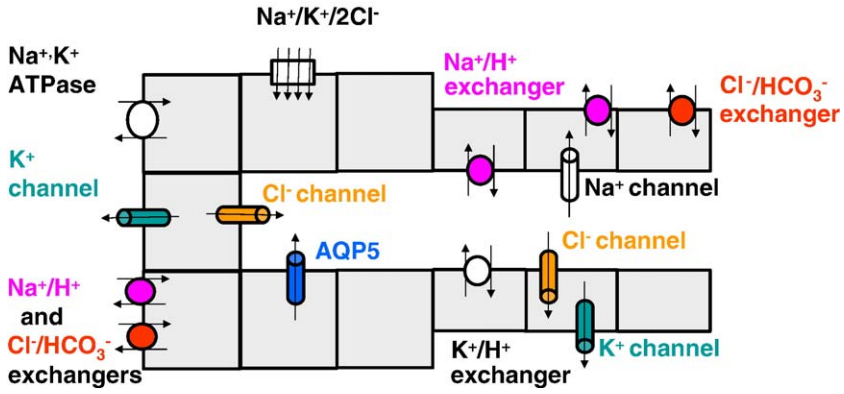

Fig. 2. Salivary secretion. The acinar cell secretes a primary isotonic plasma-like fluid based on the entry of Cl^- across the basolateral membrane and its exit across the apical membrane. During the passage of saliva through the ducts, NaCl is resorbed while K^+ and HCO_3^- secreted. All the known ion transporters involved in these processes are indicated in the figure.

Salivary gland dysfunction manifests clinically as dysphagia, oral pain, increased dental carries, and infections by opportunistic microorganisms [18].

The expression, localization and functional roles of the AQPs detected in salivary glands (AQP1, AQP3, AQP4, AQP5, AQP6, AQP7 and AQP8; see Table 1) are discussed in the present review.

2. AQP1

2.1. Normal salivary glands

In rat parotid glands, both AQP1 mRNA and protein were detected [19]. Immunolocalization studies using thin and ultrathin cryosections and light and electron microscopy revealed no labelling of the glandular tissue. In contrast, labelling was detected on both apical and basolateral membranes from non-fenestrated endothelial cells of capillaries and venules [19–22]. In rat submandibular gland, AQP1 expression was shown to be constitutive and distributed to microvasculature during embryonic and postnatal development [23,24].

In human salivary glands including parotid, submandibular, sublingual and labial, AQP1 mRNA was detected by RT-PCR [25–27]. AQP1 immunoreactivity was not confined to the capillaries, but also associated with myoepithelial cells. These are contractile cells with polygonal cell body, central nucleus, and long tapering processes that form a basketlike framework around the acini and smaller ducts [25,26]. Normal tissue microarray using parotid salivary gland revealed some AQP1 expression. Additional immunohistochemistry and semiquantitative histomorphometric analyses indicated that AQP1 expression was localized to endothelial barriers and myoepithelial cells [28].

In transgenic mice lacking AQP1, pilocarpine-induced of salivary secretion showed no defect in volume or composition of saliva [37,38]. These data suggested that AQP1 does not play a major role in saliva secretion.

2.2. Salivary glands from Sjögren's syndrome patients

Sjögren's syndrome is a common inflammatory disease characterized by lymphocytic infiltration of salivary and lacrimal glands leading to glandular hypofunction and dry mouth and eyes [29]. Several factors may contribute to impaired salivary flow, including proinflammatory cytokines, such as interleukin-1 and tumour necrosis factor-α (TNF-α), that can inhibit both basal and stimulated secretion [30,31]. In Sjögren's syndrome patients, the presence of autoantibodies against M3R could prevent the normal salivary secretion in response to neuronal stimulation [32]. In biopsies of labial salivary gland from Sjögren's syndrome patients, compared to control subjects, the AQP1 distribution was decreased by 38% in myoepithelial cells. No change was observed in endothelial cells of nonfenestrated capillaries [33].

Decreased AQP1 expression in myoepithelial cells from the labial salivary glands of Sjögren's syndrome patients has led to

Table 1
AQPs localization in salivary gland cell types

AQP	Cell type	Subcellular localization			Remarks
			Rat	Human	
AQP1	Endothelial Myoepithelial	A+B	[19–24]	[25,26,28,33]	
AQP3	Acinar	B		[26,33]	Controversial data: see [20,23,24]
AQP4	Ductal	B	[46]		Controversial data: see [23,24,26,27,47]
AQP5	Acinar	A	[21,47,50,61]	[26,55,79]	
		B	[51]		Not confirmed by others
		SG	[51]		Not confirmed by others
	Ductal	A	[47,52,54]		Controversial data: see [21,50,53]
AQP6	Unknown				Controversial data
AQP7	Unknown				Controversial data
AQP8	Myoepithelial		[88,89]		

References are indicated between brackets. A: apical; B: basolateral; SG: secretory granules.

the hypothesis that myoepithelial cell dysfunction plays a role in its pathogenesis [33]. Myoepithelial cell contraction [39] can be induced by the type 3 muscarinic receptor (M3R) activation in response to acetylcholine [40]. Also, cell volume variation mediated by AQP1 might contribute to vascular smooth muscle contraction [41]. If AQP1, similarly to AQP5 [42], translocates upon M3R stimulation, AQP1 might contribute to rapid myoepithelial cell volume modifications and contraction [41]. Since myoepithelial cells embrace the acini, their contraction would constrict the lumen of the acini and facilitate saliva flow. Nevertheless, this hypothesis is not supported by the data obtained with transgenic mice lacking AQP1 [37,38].

2.3. Salivary gland cell lines

Well-studied salivary epithelial cell lines have been shown to be useful tools to study the cell biology of salivary glands. The HSG cell line derived from an irradiated human submandibular gland and thought to originate from pluripotential intercalated duct region [34] did not express AQP1 [35]. The SMIE cell line, derived from rat submandibular epithelial duct cells immortalized with adenovirus 12S E1A gene product is also devoid of AQP1 expression [36]. However, the A5 cell line, derived from rat submandibular epithelial cells transformed with 3-methylcholanthrene and sharing immunological characteristics with intra- and extra-lobular salivary ducts cells [36], was shown to express both AQP1 mRNA and protein as well as a high water-permeability coefficient inhibited by $HgCl_2$ [35]. The presence of AQP1 in A5 cells, exhibiting ductal immunological characteristics, seems rather inappropriate considering the water impermeability of intra- and extra-lobular ducts [13–17].

Following mitogenic stimulation of quiescent 3T3 mouse fibroblastic cells, DER-2 (the murine homolog of AQP1) was among the early-delayed response genes activated in response to 20% foetal bovine serum [43]. This observation suggested that AQP1 could be involved in cellular growth control. In A5 cells, the presence of AQP1 [35] might reflect the loss of growth control associated with transformation. AQP1 expression was first shown to vary during the cell cycle in A5 cells. The highest protein levels were observed when most cells were in G_0/G_1 followed by a 40–60% decrease when cells moved into S and G_2/M phase [44]. Since changes in cell size occur during cell division, AQP1 level modification, and consequently water-permeability fluctuation, might be necessary during that process.

3. AQP3 and AQP4

3.1. Normal salivary glands

During prenatal, but not postnatal, development of rat submandibular gland, AQP3 mRNA was detected by RT-PCR but not by Northern blot analysis or in situ hybridization [24]. Western blot analysis [23] as well as immunohistochemical analysis [20] did not reveal the AQP3 protein in rat submandibular gland. Thus, the expression of AQP3 in rat salivary glands is not clear at present.

In human parotid, submandibular, sublingual and labial salivary glands, AQP3 mRNA was detected by RT-PCR and Northern blot analysis [26,27]. Moreover, immunoreactive AQP3 was localized at basal and lateral, but not apical, membranes of both serous and mucous acinar cells, but not in ductal cells [26,33]. However, these observations have not been confirmed by other studies.

Contradictory data also exist for the assessment of AQP4 expression in rat salivary glands. Indeed, AQP4 mRNA was detected [45] or not [24] by RT-PCR in adult rat submandibular gland. During prenatal development of rat submandibular gland, AQP4 mRNA was detected by RT-PCR but not by Northern blot analysis [24]. Although AQP4 protein was localized by immunohistochemistry to epithelial cells of excretory ducts in rat submandibular gland, primarily at the basolateral membrane [46], both Western blot analysis of gland membranes [23] and other immunohistochemical studies [47] were unable to detect the AQP4 protein in rat submandibular gland.

In human parotid, submandibular, sublingual and labial salivary glands, AQP4 mRNA was detected by RT-PCR but not by Northern blot analysis [26,27]. Moreover, AQP4 protein was not detected by immunohistochemistry on salivary gland sections [26].

In both rat and human salivary glands, the AQP4 expression still remains controversial. If present, AQP4 might be expressed either at low levels or only in a small fraction of the cell population of the salivary gland.

No data are currently available concerning possible functional role of AQP3 in the salivary secretion process. In transgenic mice lacking AQP4 pilocarpine-stimulation of salivary secretion showed no defect in volume or composition of saliva [37,38]. These data suggested that AQP4 does not play a major role in saliva secretion.

3.2. Salivary glands from Sjögren's syndrome patients

Compared to control subjects, in Sjögren's syndrome patients labial salivary gland biopsies, the AQP3 distribution (basolateral membrane) was not altered in acinar cells [33]. Those data suggested that AQP3 was not involved in the pathogenesis of Sjögren's syndrome.

Since AQP3 distribution was not modified in acinar cells from labial salivary gland biopsies from Sjögren's syndrome patients, compared to control subjects [33], it is unlikely that AQP3 might be involved in the pathogenesis of Sjögren's syndrome.

4. AQP5

4.1. Expression and localization

4.1.1. Normal salivary glands

AQP5, initially isolated from rat submandibular gland [48], was also detected in rat [23,48] and mouse [49] parotid, submandibular and sublingual glands. Strong AQP5 labelling was located in the apical membrane, including the

intercellular canaliculi, of serous-type acinar cells from rat submandibular [21,47,50] and from rat minor salivary glands [51]. A weak AQP5 labelling located at the apical membrane of mucous-type acinar cells was observed in rat minor salivary gland [51], but not in rat submandibular gland [50]. Very weak AQP5 labelling was observed in basal and lateral membranes of acinar cells in rat minor salivary gland [51], but not in rat submandibular gland [50]. At high magnification, confocal microscopy revealed that the apical acinar AQP5 labelling observed in rat minor salivary gland did not form a straight line, but rather dispersed omega-shape indentations, suggesting AQP5 might be present in secretory granules [51].

The Sprague–Dawley rat strain could be divided in two groups regarding the high or low level of AQP5 expression in rat submandibular gland. In animals expressing high level of AQP5, the AQP5 protein was localized at the apical, basal and lateral membrane of acinar cells as previously described [52], while in animals expressing low level of AQP5, the AQP5 protein was confined to the apical and/or lateral membrane of acinar cells [53].

In duct cells from rat submandibular glands, while most studies could not detect any AQP5 staining [21,50,53], few studies observed some apical staining in intercalated duct cells [47,52]. In rat parotid and sublingual salivary gland, no AQP5 staining was detected in the duct system [52]. In rat parotid tissue slices, some intracellular AQP5 labelling was detected in interlobular duct cells [54], but these findings still remain to be confirmed by others.

In human parotid, AQP5 mRNA was detected by RT-PCR [27]. In human parotid, submandibular, sublingual and labial glands, AQP5 labelling was confined to the apical membrane of acinar cells and not detected in duct cells [26,55].

4.1.2. Salivary gland cell lines

In A5 and SMIE rat submandibular salivary gland cell lines from ductal origin, the AQP5 expression was lacking [44].

An immortalized normal human salivary ductal (NS–SV–DC) cell line, lacking the expression of AQP5, acquires AQP5 gene expression in response to treatment with 5-aza-2′-deoxycitidine (5-Aza-CdR), a DNA demethylation agent [56]. The expressed AQP5 protein was mainly localized at the apical and lateral membrane of the cells, and was functionally active [56]. 5-Aza-CdR induces demethylation of two GC sites cooperatively in the AQP5 promoter to provoke AQP5 gene expression [56]. Since ductal cells are the main component of salivary glands following radiation therapy or Sjögren's syndrome, 5-Aza-CdR was suggested to provide efficient and useful means to impart facilitated water permeability to remaining ductal cells [56]. It should however be taken into account that agents inducing DNA demethylation are potential carcinogens.

4.2. Intracellular trafficking of AQP5

The translocation of AQP5 between intracellular and apical membranes from rat parotid gland (acini and ducts) occurs after M3R [42] or α1-adrenergic receptor [57,58] stimulation. This is thought to be due to an elevation in intracellular calcium concentration involving the stimulation of the nitric oxide/cyclic GMP pathway [59]. In rat minor salivary gland acinar cells, upon isoproterenol stimulation, the AQP5 labelling initially present in secretory granules became dispersed, suggesting that part of the apical membrane containing AQP5 are retrieved following exocytosis [51].

While only transient translocation of AQP5 to the apical membrane occurs following acetylcholine stimulation, long-lasting translocation of AQP5 could be obtained following SNI-2011 stimulation [60]. Since AQP5 trafficking studies were performed on tissue sections using biochemical methods, it was not clear if AQP5 could be subject to regulated trafficking in vivo. That issue was addressed by looking at AQP5 localization in rat parotid and submandibular gland after short in vivo stimulation (using muscarinic or adrenergic agonists) or inhibition (using muscarinic or adrenergic antagonist) of saliva secretion [61]. Under control conditions, most of the AQP5 labelling (> 90%) was associated with the apical membrane of acinar cells in both parotid and submandibular glands and intercalated duct cells in submandibular gland. While only rare AQP5 labelling was associated with intracellular membrane domains [61]. Short intervals (3 or 10 min) of stimulation or inhibition of salivary secretion did not reveal any significant translocation of AQP5 between intracellular vesicles or secretory granules and the apical plasma membrane [61]. One can, however, not exclude that shorter or longer stimulation or inhibition of salivary secretion might lead to AQP5 trafficking, or that the anaesthetic used in these experiments to tranquilize the animals may have interfered with protein trafficking.

AQP5 trafficking was observed in intracellular structures of interlobular duct cells following M3R agonist stimulation [54,62]. Also, AQP5 translocated with lipid rafts from intracellular structures to the apical membrane of interlobular duct cells, and then AQP5 moved from the lipid rafts to non-rafts within the apical membrane [54]. These data should be considered with care since the presence of AQP5 in interlobular duct cells still remains to be confirmed by others. Also, it should be determined if the AQP5 translocation occurs in all duct types, and if this mechanism is physiologically relevant regarding salivary secretion since duct cells have been considered water impermeable [13–17].

In rat parotid gland, AQP5 has been shown to be involved in osmoregulation of the secretory granules [63]. In light of these recent data [63] and the hypothesis that AQPs can function as osmotic sensors, rather than water channels [64,65], AQP5 has been suggested to act as an osmosensor in the secretory granules from rat parotid gland. AQP5 was colocalized with the small GTP-binding proteins Rab4 from the apical endosome of acinar cells, suggesting that Rab4 participated in both endocytosis of the apical membrane and the transport of apical membranes components such as AQP5 [66].

In rat AQP5-transfected HSG cells, the AQP5 trafficking from intracellular vesicles to plasma membrane was shown to be triggered by intracellular calcium concentration increase and

involved the interaction of AQP5-containing vesicles with the cytoskeleton [67]. Polarized differentiated HSG cells expressing an acinar phenotype after culture on a basement membrane matrix [68] could have been a more appropriate model to study such protein trafficking.

4.3. Functional roles

Compared to wild-type mice, transgenic mice lacking AQP5 displayed reduced pilocarpine-stimulated saliva secretion (~60%), hypertonic (420 mosm) and more viscous saliva, while amylase and protein secretion were not modified [37]. This suggested that AQP5 played a key role in saliva fluid secretion and that high epithelial cell membrane water permeability is required for active near-isosmolar fluid transport. Further studies showed that hyposalivation was not caused by changes in whole body fluid homeostasis, but instead that water membrane permeability of salivary gland acinar cells was dramatically reduced in mice lacking AQP5 [69].

The abundant presence of AQP5 in intercalated ducts from rat submandibular gland suggests that AQP5 may play a role in the absorption and/or secretion of small solutes. The fact that AQP5 was not present in the duct system, with the exception of the intercalated ducts from rat submandibular gland, is in agreement with the water impermeability characteristics of the duct cells [13–17]. Further studies are required to investigate the functional role of AQP5 in the absorption and/or secretion of small solutes in intercalated ducts.

In Sprague–Dawley rats, the variation of AQP5 expression observed in submandibular glands seems to account for the variation of the saliva secretion rate observed between high and low AQP5 expressors (7-fold higher saliva rate in high expressors than low expressors) [53]. These data are in agreement with the key role of AQP5 in saliva secretion [37].

4.4. AQP5 and xerostomic conditions

Decreased salivary function (xerostomia) occurs for many reasons associated with increased age, drug use, radiation therapy of malignant tumours of the head and neck, and disease (such as Sjögren's syndrome) [70].

4.4.1. Senescence

In parotid glands (acini and ducts) from senescent rats, decreased AQP5 translocation to the apical membrane in response to acetylcholine, but not to epinephrine, was not due to decreased M3R and/or Gq protein expression, but rather to decreased nitric oxide synthase activity (NOS) [71]. NOS activity has been shown to be involved in AQP5 translocation [59]. In adult and senescent rats, the quinuclidine derivative SNI-2011 (a structurally rigid analogue of acetylcholine called cevimeline and known to act on M3R) was shown to induce persistent increase in the amount of AQP5 in the apical membrane of parotid gland, suggesting that this drug might be therapeutically beneficial for the treatment of age-related xerostomia [71].

4.4.2. Radiation therapy of malignant tumours of head and neck

For most of the patients diagnosed each year with head and neck cancer, ionizing radiation is a key component of therapy but has the adverse effect to cause severe damage to the acinar cells of the salivary glands lying in the radiation field [72]. In irradiated salivary glands from patients with head and neck cancer, the loss of AQP5 expression and possibly other proteins involved in the secretory process may participate to the mechanism that accounts for the loss of salivary flow observed in irradiated rat salivary glands [22,73]. In rat irradiated salivary gland, the AQP5 labelling intensity was markedly decreased [74], the number of AQP5-labelled intercalated ducts was decreased and the acinar cells were devoid of AQP5 labelling [Delporte, Redman and Baum, unpublished data]. Recombinant adenovirus encoding AQP1 has been shown to be of therapeutic benefit for the improvement of salivary secretion in irradiated rats, non-human primates and miniature pigs salivary glands ([22,75,76] and for review: [77]).

4.4.3. Sjögren's syndrome

Using *anti-human AQP5* antibodies, the immunohistochemical distribution of AQP5 in minor salivary glands biopsies from normal subjects, as well as from non-Sjögren's syndrome patients with dry mouth, revealed AQP5 primarily at the apical membranes of the acinar cells ([55]; Fig. 3). In contrast, in minor salivary glands, biopsies from Sjögren's syndrome

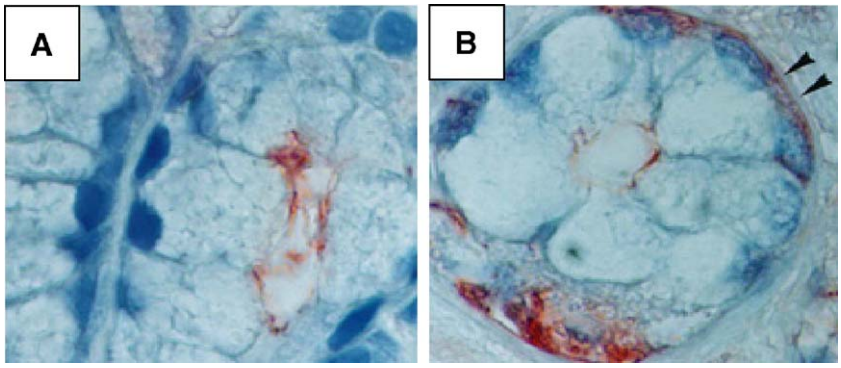

Fig. 3. AQP5 localization in human minor salivary gland. AQP5 was localized in minor salivary gland sections from a control subject (A) (×40), or a patient with Sjögren's syndrome (B) (×40), using affinity-purified anti-hAQP5 antibodies and a Histostain SP kit (Zymed Lab. Inc., San Francisco, CA) [55].

patients, computer-assisted microscopy revealed that AQP5 labelling was higher at the basal membrane, and lower at the apical membrane of acinar cells, compared with biopsies from normal subjects ([55]; Fig. 3). In each case, Western blot analysis revealed the presence of a 27-kDa band, corresponding to the expected molecular weight of AQP5 [55]. From this study, it was proposed that abnormal distribution of AQP5 could contribute to the deficiency of fluid secretion observed in Sjögren's syndrome patients. The administration of anti-TNF-α antibodies (infliximab) to Sjögren's syndrome patients dramatically improved unstimulated salivary flow [55] and restored proper apical localization of AQP5 in acinar cells from minor salivary glands [78]. However, studies performed with *anti-rat AQP5* antibodies were not able to detect any AQP5 distribution modification in labial salivary gland biopsies from Sjögren's syndrome patients, compared to control subjects [79]. These apparent contradictory data might be explained by the use of *anti-rat AQP5* antibodies that were shown to not cross-react with the human AQP5 protein [78]. It is significant to note that *anti-human AQP5* antibodies altered AQP5 trafficking in lacrimal gland biopsies from Sjögren's syndrome patients [80].

In salivary glands from non-obese diabetic mice, generally considered as a good animal model for Sjögren's syndrome [81,82], AQP5 distribution was not restricted to the apical membrane of acinar cells as in control mice, but rather present at both basolateral and apical membrane [83]. These data support observations that implied a similar loss of the ordered and polarized expression of AQP5 in human minor salivary glands and lacrimal glands from patients with Sjögren's syndrome [55,80].

4.4.4. Diabetes

In streptozocin-induced diabetic rats, SNI-2011 was unable to induce the AQP5 translocation from lipid rafts to the apical membrane in interlobular duct cells of parotid glands as of control rats [62]. The defect in AQP5 trafficking could be responsible for the xerostomia.

5. AQP6 and AQP7

Although AQP6 and AQP7 mRNAs were detected by RT-PCR in human parotid gland [27], this has not been confirmed by other studies. It is generally accepted that AQP6 is strictly expressed in kidney [4,84]. AQP7 mRNA detection in human parotid gland tissue [27] might be due to a sample contamination with adipocytes which are known to express AQP7 [85]. No data are currently available concerning possible functional role of AQP6 or AQP7 in the salivary secretion process.

6. AQP8

6.1. Salivary glands

AQP8, originally cloned from rat pancreas and liver by homology cloning, was detected by in situ hybridization in the acinar cells from rat salivary glands [86]. By immunohistochemistry, AQP8 originally was reported as detected in the basolateral membrane of acinar cells from rat submandibular glands [87]. However, Elkjaer et al. [88] reported a different distribution: in myoepithelial cells around the acini and the intercalated duct, but not in acinar or ductal cells, of rat parotid, submandibular and sublingual cells. Wellner et al. [89] recently have provided new data that indicate their agreement with the conclusions of Elkjaer et al. [88].

Microarray analysis of gene expression during aging in rat submandibular gland revealed a decrease in AQP8 (2.3-fold) gene expression [90]. Further studies will be required to analyze the consequence of decreased AQP8 gene expression in senescent rats.

Transgenic mice lacking AQP8 compared to wild-type mice, as well as AQP5/AQP8 double knockout mice compared to AQP5-null mice, did not display any modified agonist-driven saliva secretion [93]. Therefore, AQP8 does not appear to play a major role in salivary secretion in mice.

6.2. Salivary gland cell lines

In the rat salivary epithelial SMIE cell line, AQP8 mRNA as well as AQP8 protein were detected by RT-PCR and confocal microscopy [91]. SMIE cells cultured on collagen-coated polycarbonate filters can form a monolayer and exhibit polarized morphology, authentic tight junctions and a polarized membrane protein distribution [92]. On polarized SMIE cells, AQP8 was detected at the apical membrane and its expression was responsible for the fluid movement measured in response to modified osmotic gradient [91].

7. Conclusions

In salivary glands, AQP1 was localized to endothelial and myoepithelial cells (Table 1), and AQP8 in myoepithelial cells. The data concerning the presence of AQP3, AQP4, AQP6 and AQP7 are still controversial (Table 1). These AQPs do not appear to play any important role in the salivary secretion process. AQP5 was identified in acinar and intercalated duct cells. Functionally, AQP5 seems to be the only salivary AQP clearly playing a major role in the salivary secretion process. Indeed, in normal physiological conditions, due to its presence in acinar cells, AQP5 participates in water movement to the lumen (Introduction, [37,53,69]). Agonist-stimulated saliva secretion probably requires AQP5 trafficking from intracellular vesicles or secretory granules to the apical plasma membrane of acinar cells. However, some discrepancies exist between in vitro experiments performed on salivary gland sections using biochemical methods [51,57–60] and in vivo experiments using immunohistological methods [61]. Several reasons could account for those discrepancies: AQP5 trafficking in acinar and ductal cells cannot be distinguished using tissues slices and biochemical methods; the duration of the gland stimulation and the anaesthetic chosen to study AQP5 trafficking in vivo may not be optimal. Further studies need to be performed to clarify whether AQP5 trafficking occurs in physiological conditions. For example, analysis of AQP5 trafficking by confocal microscopy following agonist addition to acinar or ductal cell

preparations, as well as additional determination of AQP5 localization following distinct kinetics of stimulation and using other anaesthetics, could be valuable to address the issue. The fact that trafficking might be an important modulator of AQP5 localization, and therefore of AQP5 physiological function in vivo, seems to be confirmed by the presence of a defect in AQP5 translocation under various xerostomic conditions such as senescence [59,71], Sjögren's syndrome [55,69,78,80,83], and diabetes [62].

In ductal cells, shown as water impermeable [13–17], AQP5 is unlikely to be physiologically relevant to the saliva secretion mechanism. Therefore, further studies need to be done to investigate the functional role of AQP5 trafficking in ducts.

The secretion of a plasma-like primary fluid by the acinar cells to the lumen requires the presence of AQP5 at the apical membrane, and, evidently, of another water channel that remains to be identified at the basolateral membrane. Further studies will be necessary to identify the AQP present at the basolateral membrane of acinar cells and determine its functional role in the saliva secretion process.

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