REVIEW —Current Perspective—

Aquaporin Water Channel in Salivary Glands

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ABSTRACT—Water secretion from salivary glands, which are innervated by parasympathetic and sympathetic nerves, occurs in response to the stimulation by neurotransmitters. In general, parasympathetic or sympathetic stimulation produces a high flow of saliva as a result of the activation of M_3 muscarinic or α_1 -adrenergic receptors, respectively. The secretory mechanisms of fluid secretion were osmotically regulated in response to a transepithelial ion gradient generated by ion transport systems that were located in the apical or basolateral membranes of the acinar cells. Recently, the identification of water-specific channels, or aquaporins (AQPs), in many mammalian tissue and cell types has provided insight into the molecular basis of water movement across biological membranes. It has been reported that several AQPs are expressed in salivary glands and especially AQP5 plays an important role in fluid secretion. This review will focus on the role of AQP5 in the movement of water across the apical plasma membrane in relation to the physiology and pathophysiology of salivary glands.

Keywords: Aquaporin-5, Salivary secretion, Neurotransmitter, Calcium mobilization, Cytoskeleton

I. Introduction

Water is an indispensable molecule for life. In general, the plasma membranes of mammalian cells are permeable to water and are able to maintain osmotic equilibrium between cells and stable external environments. However, the mechanisms by which water traverses cell membranes remained unclear until recently. The water permeability of the plasma membranes of certain epithelia (1) and erythrocytes (2) is so great that it was difficult to explain by diffusion alone. These observations have led to the suggestion that water-specific channels may exist in the plasma membranes of some cells to regulate water permeability and body fluid homeostasis (3).

In 1988, a protein with a molecular mass of 28-kDa that function as a water channel, designated CHIP28, was discovered in red cell membranes and identified as a member of the major intrinsic protein (MIP) family of lense fiber cells in the eye (4, 5). Subsequently, many members of the MIP family that exhibit channel activity specific for water, the channels being impermeable to small solutes and ions, have been cloned from many different tissues (4, 5). MIP family members that possess water-specific channel activity have been named aquaporins (AQPs) to distinguish them from the other MIP proteins that do not function as water

channels, and CHIP28 has been designated AQP1. More than 10 members of the AQP family have been identified to date in various mammalian cell types (6, 7). Recently, attention has been focused on the mechanisms that underlie regulation of the activity of AQPs in order to clarify the molecular basis of water movement across biological membranes under both physiological and pathological conditions.

Saliva has manifold functions in maintaining the integrity of the oral tissues, in protecting teeth from caries, in the tasting and ingestion of food, in speech and in the tolerance of dentures, for example. Xerostomia, or dry mouth, is caused by a reduction of salivary secretion associated with the malfunction of salivary glands. This condition often accompanies diseases of maturity such as diabetes insipidus and cardiac failure, psychological emotional states such as fear and depression, autoimmune diseases and the response to therapeutic irradiation of salivary glands. The use of anticholinergic drugs, anorectics, antihistamines, antidepressants, antihypertensives and calcium antagonists also often commonly causes xerostomia. This drug-induced side effect is a problem mostly in the elderly, who are principal consumers of such medications.

Saliva comprises approx. 99% water. The remaining 1%

consists of proteins such as α -amylase, mucin and epidermal growth factor; electrolytes such as Na^+ , Ca^{2+} , Cl^- , HCO_3^- and PO_4^{3-} ; and organic molecules such as glucose, urea and lipids. Salivary secretion is controlled by autonomic nerves. Whereas protein secretion from salivary glands is primarily under the control of β -adrenergic receptors, the activation of α -adrenergic or muscarinic receptors in these glands results in high rates of water and electrolyte secretion.

The AQP5 water channel has been cloned from the salivary glands of rats (8), humans (9) and mice (10). This review will focus on the role of AQP5 in the movement of water across the apical plasma membrane of salivary glands on the regulation of its activity in relation to the physiology and pathophysiology of salivary glands.

II. Control of salivary secretion

The major salivary glands, comprising the submandibular, parotid and sublingual glands, are innervated by both sympathetic and parasympathetic nerves (11). The parasympathetic nerve that innervates the submandibular and sublingual glands is derived from the superior salivary nucleus, whereas that innervating the parotid glands is derived from the inferior salivary nucleus. The sympathetic nerve that innervates the major salivary glands is derived from the superior cervical ganglion. In general, parasympathetic stimulation produces the high flow of saliva as a result of the activation of M_3 muscarinic receptors on the salivary gland cells. In contrast, noradrenaline released from sympathetic nerve endings acts at both β - and α -adrenergic receptors in the salivary glands. Activation of β -adrenoceptors induces the secretion by exocytosis of salivary proteins contained in zymogen granules, whereas activation of α_1 -adrenoceptors induces fluid secretion (12–15).

Neuropeptides such as substance P and vasoactive intestinal peptide also contribute to the neuronal regulation of salivary secretion. Furthermore, autacoids such as histamine and bradykinin activate nociceptors in salivary glands and thereby stimulate salivary secretion (11, 16).

Fluid secretion from salivary gland cells actually consists of two processes (Fig. 1): transcellular and paracellular transports (12, 17, 18). Stimulation of M_3 muscarinic and α_1 -adrenergic receptors in the basolateral membrane of acinar cells induces activation of phospholipase C and a consequent increase in the cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$), in turn, results in the opening of Ca^{2+} -activated K^+ channels and Cl^- channels in the basolateral membrane and the apical plasma membrane, respectively. Secretion of Cl^- results in increases in the paracellular transport of Na^+ and in the water secretion by leakage through intercellular junctions. The increase in the $[\text{Ca}^{2+}]_i$ also stimulates Na^+/H^+ exchange activity. A $\text{Cl}^-/\text{HCO}_3^-$ exchanger acts in parallel with the Na^+/H^+ exchanger and stimulates HCO_3^- -dependent water secretion. It is reported that the ability of HCO_3^- to support secretion is about the same as that of Cl^- when the two anions are present in the same concentrations (19). Salivary secretion is thus thought to be promoted osmotically in response to a transepithelial ion gradient generated by ion transport systems located in the apical or basolateral membranes of the secretory cells.

III. The AQP family of water-specific channels

The passage of water across cell membranes is accomplished by two distinct routes: simple diffusion through the phospholipid bilayer, and rapid transit through members of the AQP family of specialized membrane-spanning proteins for water specific transport (4). The existence of water channels was first proposed to explain the observation that

Fig. 1. Schematic representation of mechanism of salivary secretion in parotid acinar cell. βR , β -adrenergic receptor; $\alpha_1\text{R}$, α_1 -adrenergic receptor; M_3R , M_3 muscarinic receptor; G_s , G protein that stimulates adenylyl cyclase (AC); G_q , G protein that stimulates phospholipase C (PLC); PIP_2 , phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; IP_3 , inositol 1,4,5-trisphosphate. IP_3R , IP_3 receptor; RyR , ryanodine receptor type 3. This scheme is based on that presented by Baum (12).

osmotic water permeability (P_f) is higher than diffusional water permeability in frog skin. It was suggested that the increase in P_f induced by exposure to antidiuretic hormone in this epithelium resulted from a widening of the diameter of the channel pore (5). Subsequently, it was demonstrated that whereas *p*-chloromercuribenzenesulfonate inhibited the permeation of urea, glycerol, and water through the plasma membrane of erythrocytes consistent with the reduction of the P_f of erythrocytes from approx. 200 to approx. 20 $\mu\text{m/s}$, β -(*p*-hydroxyphenyl) phloropropriophenol (phloretin) markedly inhibited urea and glycerol transport but did not affect water transport (2). These and other studies revealed that water and small nonelectrolytes are able to traverse the plasma membranes through specialized transporters whose selectivity is based on the physical properties of the transported substrates, rather than on their molecular size alone (2, 3).

Various proteins, including the Na^+ /glucose cotransporter 1 (SGLT1), the Na^+ -independent glucose transporter 1 (GLUT1) and the cystic fibrosis gene product (CFTR), have been proposed to function as water channels (4, 7, 20), but all of these proteins were subsequently shown to be lacking in some requisite characteristic. The AQP family of membrane proteins was eventually identified as being responsible for channel-mediated water transport across cell membranes. These proteins, all of which are smaller than 30 kDa, contain a pore that is selective for water.

To date, more than 10 AQPs have been cloned from various mammalian tissues. Thus, AQP0 is expressed in eye lens; AQP1 in proximal tubules of kidney and in intrahepatic cholangiocytes; AQP2 and AQP3 in kidney collecting ducts; AQP3, AQP4, AQP7 and AQP8 in the gastrointestinal tract; AQP5 in salivary and lacrimal glands, corneal epithelium and lung; AQP6 in renal epithelia; AQP8 in liver and pancreas; and AQP9 in liver (6).

IV. AQPs in salivary glands

The AQP5 cDNA, which encodes a 265-residue polypeptide, was isolated from rat submandibular gland with the use of a homology-based cloning approach by Raina et al. (8). In vitro transcription and translation of this cDNA yielded a 27-kDa polypeptide, and expression of the corresponding cRNA in *Xenopus* oocytes resulted in a 20-fold increase in P_f that was reversibly inhibited by HgCl_2 , but which was not accompanied by increases in the membrane transport of urea or glycerol. Hydropathy analysis of the deduced amino acid sequence of AQP5 predicted a protein with six transmembrane domains (7, 8) (Fig. 2). The amino acid sequence contains two tandem repeats corresponding approximately to the NH_2 -terminal and COOH -terminal halves of the polypeptide (7, 8). Northern blot analysis revealed that AQP5 mRNA is present in submandibular, parotid and sublingual glands, lacrimal gland, trachea, eye

Fig. 2. Proposed membrane topology of AQP5. The topology is based on sequence analysis and the hourglass model proposed by Raina et al. (8). The AQPs molecule is thought to consist of six bilayer-spanning domains and five connecting loops (loop A through loop E). The aqueous pore is thought to form when loops B and E fold together into an "hourglass". The protein contains characteristic NPA motifs in the loops B and E. Loop C contains an *N*-glycosylation site (ϵ) and loop E contains a mercury-sensitive residue (C). Loop D has phosphorylation sites for PKA.

and distal lung, but not in kidney, brain or intestine (8). The AQP5 protein is located in the apical plasma membrane of acinar cells and intercellular secretory canaliculi, but is not present in the basolateral membrane (21, 22). Adenovirus-mediated transfer of AQP5 cDNA to submandibular glands of rats resulted in a 12-fold increase in the abundance of AQP5 at 1 day postinfection; the amount of AQP5 was increased threefold at 2 days and remained slightly increased above basal values at 7 days after infection, suggesting that adenovirus-mediated transfer of AQP5 cDNA may provide a useful means to increase water permeability of cells (23). The increase in AQP5 expression induced by this approach in the submandibular glands of dexamethasone-treated rats was greater than that in rats not treated with this steroid (23).

AQP1 is a 28-kDa protein that was first isolated from human red blood cells (4). Antibodies to AQP1 react with endothelial cells and erythrocytes in capillaries and venules within the salivary glands, but not with the gland cells themselves (21). However, adenovirus-mediated transfer of AQP1 cDNA to salivary glands of irradiated rats resulted in a 2- to 3-fold increase in salivary secretion compared with secretion from glands exposed to a control virus (24).

AQP4 is a 28-kDa protein that was cloned from lung and brain (4) and is located in the basolateral membranes of columnar cells of bronchial, tracheal and nasopharyngeal epithelia. Although AQP4 has been reported to be expressed in the basolateral membrane of submandibular gland cells (25), another study failed to detect AQP4 in these cells by immunofluorescence microscopy (23).

The AQP8 cDNA, which encodes a 263-amino acid pro-

tein with a predicted molecular size of 28 kDa, was isolated from rat pancreas (26). AQP8 was shown to be abundant in pancreas and liver and to be present in smaller amounts in salivary glands. The cellular and subcellular localization and functional regulation of this AQP in salivary glands remain unclear.

Insight into the roles of AQP1, AQP4 and AQP5 in salivary secretion has been provided by characterization of the corresponding knockout mice (25). In AQP5 knockout mice, although protein secretion by salivary glands and amylase activity in saliva were not affected, the body growth rate was reduced by 20% and pilocarpine-stimulated saliva production was reduced by more than 60% compared with those in wild-type mice. In contrast, AQP1 and AQP4 knockout mice showed no defects in the volume or composition of saliva. On the basis of observations, Ma et al. concluded that AQP5 plays an important role in salivary fluid secretion (25).

V. Structure of the AQP5 water channel

All AQPs comprise a single polypeptide chain that is thought to span the cell membrane six times, with the NH₂- and COOH-termini located in the cell interior. There are three extracellular loops (loops A, C and E) and two intracellular loops (loops B and D). The two halves of each AQP share substantial sequence similarity but are oriented in the plasma membrane in such a manner that corresponding regions are located on opposite sides of the membrane. Both loops B and E, which contain the Asp-Pro-Ala (NPA) sequence that is characteristic of AQPs and are intracellular and extracellular, respectively, have been shown to be critical for formation of functional water-selective pores (4, 8).

In polarized mammalian cells, proteins anchored to the cell membrane by glycopospholipids are generally localized to the apical plasma membrane (27). Glycosylation may, therefore, play an important role in the function of integral membrane proteins. AQP5 as well as AQP2, AQP3 and AQP4 each contain an N-glycosylation site in loop C (8), whereas AQP1 contains such a site in loop A. However, elimination of the N-glycosylation site in AQP1 had no effect on the trafficking of this protein when expressed in *Xenopus* oocytes (4).

AQP5 contains a mercury-sensitive cysteine residue at position 182, which is located just upstream of the NPA sequence in loop E (8–10). Treatment of *Xenopus* oocytes expressing AQP5 with 1 mM HgCl₂ resulted in a marked decrease in P_f , which was reversed by incubation with β -mercaptoethanol (8–10). The AQP5 amino acid sequence also contains potential phosphorylation sites for protein kinase (PK) A, PKC and casein kinase II (8–10).

VI. Regulation of AQP5 water channel activity

Immunoblot analysis with antibodies to AQP5 revealed that exposure of rat parotid tissues to acetylcholine or epinephrine induced rapid increases in the amount of AQP5 in the apical plasma membrane (Fig. 3); the effects of these agents were maximal (fourfold and twofold increases, respectively) at 15 s and 1 min, respectively (28–30). The muscarinic receptor agonists, pilocarpine and ($\pm$)-*cis*-2-methylspilo(1,3-oxathiolane-5,3')quinuclidine hydrochloride hemihydrate (SNI-2011), induced effects similar to that of acetylcholine on the amount of AQP5 in the apical plasma membrane (31). Furthermore, the effects of acetylcholine and epinephrine were blocked by M₃ muscarinic and α_1 -adrenergic receptor antagonists, respectively. Im-

Fig. 3. Time courses for the effects of acetylcholine, epinephrine, SNI-2011 and pilocarpine on the amount of AQP5 in the apical plasma membrane of rat parotid glands. Rat parotid tissue was incubated with acetylcholine (10 μ M) plus eserine (10 μ M) (○), with epinephrine (10 μ M) (●), with SNI-2011 (10 μ M) (△) or with pilocarpine (10 μ M) (▲) for 0, 0.25, 1, 3, 10 or 30 min (corresponding to lanes 1 to 6, respectively, in the inset). The apical plasma membrane fraction was then prepared and subjected to immunoblot analysis with the antibody to AQP5. Data were quantitated by densitometry and are means $\pm$ S.E.M. of three to six independent experiments (28–31). The position of the 27-kDa immunoreactive protein is indicated. * P <0.05, ** P <0.01, *** P <0.001 versus the value for zero time.

munohistochemical analysis with the antibody to the COOH-terminus of AQP5 revealed that pilocarpine induced an increase in the amount of AQP5 in the apical plasma membrane of lacrimal gland cells (32). Taken together, the amount of the functional water channel AQP5 in apical plasma membrane is regulated by neurotransmitters.

The increases in the abundance of AQP5 in the apical plasma membrane of parotid gland cells induced by M_3 muscarinic and α_1 -adrenergic agonists were accompanied by corresponding decreases in the amount of AQP5 associated with intracellular vesicles. These results suggest that the accumulation of AQP5 in the apical plasma membrane induced by these agonists results from the fusion of AQP5-containing intracellular vesicles with the apical plasma membrane. The decrease in the amount of AQP5 in the apical plasma membrane apparent after exposure of parotid gland tissue to acetylcholine for >15 s was accompanied by a corresponding increase in the amount of this water channel associated with intracellular vesicles, suggesting that AQP5 is retrieved from the apical plasma membrane by endocytosis. Thus, the "shuttle" hypothesis (33) proposed that the rapid regulation of water secretion by neurotransmitters is mediated by the redistribution of a pool of AQP5 water channels between intracellular vesicles and the apical plasma membrane.

Immunohistochemical analysis with the antibody to AQP5 revealed that stimulation of parotid gland cells with isoproterenol resulted in the dispersal of AQP5 immunoreactivity into clusters of dots in the cytoplasm (22). Cytochalasin D and tubulozole C inhibit strongly acetylcholine- or epinephrine-induced increase in the amount of AQP5 in the apical plasma membrane, showing that AQP5 translocates between the apical plasma membrane and cytoplasm in the cells (29, 30). These findings support that AQP5 traffics between the apical plasma membrane and the cytoplasm in these secretory gland cells. Thus, it appears likely that the chief means of regulation of water secretion in salivary glands is the regulation of trafficking of AQP5 water channel. Judging from numerous data that AQP5 is located in the apical plasma membrane under the physiological condition of the salivary gland (21, 22), which is tonic-innervated, AQP5 may be located in the apical plasma membranes.

Regulation of AQP5 activity by $[Ca^{2+}]_i$: The increases in the amount of AQP5 in the apical plasma membrane of parotid gland cells induced by M_3 muscarinic or α_1 -adrenergic agonists are blocked by U-73122, an inhibitor of phospholipase C, as well as by TMB-8 or dantrolene, both of which inhibit the mobilization of Ca^{2+} from intracellular stores (28, 29). These observations suggest that the activation of phospholipase C and the consequent increase in the $[Ca^{2+}]_i$ triggered by activation of inositol 1,4,5-

trisphosphate (IP_3) and ryanodine receptors of intracellular Ca^{2+} stores mediated by M_3 muscarinic or α_1 -adrenergic agonists induced the translocation of AQP5 to the apical plasma membrane of parotid gland cells. This conclusion is supported by the observation that A23187, a Ca^{2+} ionophore, also induces a rapid increase in the amount of AQP5 in the apical plasma membrane of parotid cells (28).

Exposure of pancreatic acinar cells to acetylcholine results in an increase in $[Ca^{2+}]_i$ that is initiated at a small trigger zone (T-zone) in the apical region, which also contains high sensitivity IP_3 receptors and secretory granules, and that spreads rapidly within this region; at high concentrations of agonist, the increase in $[Ca^{2+}]_i$ propagates slowly toward the basal region (34). Such $[Ca^{2+}]_i$ dynamics are thus consistent with a role for cytosolic Ca^{2+} in the translocation of AQP5-containing vesicles to the apical plasma membrane of exocrine gland cells in response to neurotransmitters.

Regulation of AQP5 activity by phosphorylation: In several nonexcitable cell types, the activation of cell surface receptors by hormones results in the trafficking of AQPs. Thus, AQP1 in rat bile duct cells and AQP2 in rat renal collecting duct cells translocate from intracellular vesicles to the plasma membrane in response to secretin (35) and vasopressin (36), respectively; moreover, this translocation is accompanied by a simultaneous increase in fluid transport. Vasopressin binds to receptors on the basolateral membrane of collecting duct principal cells and initiates a cascade of events that result in the insertion of AQP2 into the apical plasma membrane. These events include phosphorylation by PKA of the serine residue at position 256 in the cytoplasmic COOH-terminus of AQP2 located in intracellular vesicles, which is thought to trigger the exocytosis of these vesicles and their fusion with the apical plasma membrane (37, 38). Phosphorylation of AQP2 expressed in *Xenopus* oocytes resulted in an increase in water permeability.

AQP5 contains the consensus phosphorylation sequence for PKA in loop D (4, 8, 9) and the cytoplasmic COOH-terminus (10). β -Adrenergic agonists, dobutamine, salbutamol and isoproterenol, have no effect on AQP5 trafficking to the apical plasma membrane of parotid gland cells (26), suggesting that phosphorylation of AQP5 by PKA does not play an important role in the activity of the AQP5 water channel. This conclusion is supported by the observation that acetylcholine- or epinephrine-induced increases in the amount of AQP5 in the apical plasma membrane of parotid gland cells are not blocked by inhibitors of either PKA or PKC (28–30).

Role of the cytoskeleton in AQP5 trafficking: Membrane trafficking in polarized epithelial cells is thought to require both actin microfilaments and microtubules. Cytochalasin D and tubulozole C inhibit the translocation of AQP5 to

the apical plasma membrane of rat parotid cells induced by epinephrine, acetylcholine or A23187, suggesting that both microfilaments and microtubules are necessary for the AQP5 trafficking (29, 30). However, the mechanisms by which AQP5 interacts with microfilaments and microtubules remain unknown. AQP2 was recently shown to be an actin-binding protein, and the microtubule motor protein dynein has also been implicated in the trafficking of AQP2 in response to vasopressin (37). Microtubules are necessary for AQP5 movement toward the apical plasma membrane and microfilaments are involved in the trafficking of AQP5 via the elevation of $[Ca^{2+}]_i$ (29, 30).

Changes in AQP5 expression with aging: The effect of aging on salivary flow rate in humans is controversial. Thus, the unstimulated and stimulated flow rates for whole saliva have been reported either to decrease with age (39) or to be unaffected by age (40, 41). The incidence of xerostomia increases with age, however, because of the age-related increase in the use of drugs that cause this condition as a side effect (42).

The pilocarpine-stimulated salivary flow rate of the parotid in aged rats is 50% of that in young animals, with the flow rate of young females being less than that of young males (43). The amount of AQP5 in the apical plasma membrane of parotid gland cells treated with 10 μ M acetylcholine for 1 min was 4 times greater for aged rats than for young adult rats, with this neurotransmitter inducing a twofold increase in this parameter in the tissue from old rats (Y. Ishikawa et al., unpublished data).

Expression of AQP5 in rat lung occurs within 2 weeks after birth (44) and increases continuously until adulthood (45). The time course of AQP5 expression has not been described.

VII. Conclusion

Since the discovery of AQP water channels in 1988, the number of such proteins identified in various cell types and organisms has increased to more than 10. On the basis of gene structure, sequence homology, and function, the AQPs have been divided into two groups: those that are expressed in the apical plasma membrane of polarized cells and are regulated by hormones or neurotransmitters, and those that are expressed constitutively in the basolateral membrane of such cells. AQP5 is a neurotransmitter-regulated water channel that is expressed in salivary glands and whose trafficking between intracellular vesicles and the apical plasma membrane is controlled by $[Ca^{2+}]_i$. Further characterization of the role of AQP5 in both the normal physiology and pathophysiology of the salivary gland should also provide insight into the molecular basis of water movement across biological membranes.

REFERENCES

- 1 Levine SD, Levine RD, Worthington RE and Hays RM: Selective inhibition of osmotic water flow by general anesthetics in toad urinary bladder. *J Clin Invest* **58**, 980–988 (1976)
- 2 Macey RI and Farmer REL: Inhibition of water and solute permeability in human red cells. *Biochim Biophys Acta* **211**, 104–106 (1970)
- 3 Verkman AS: Mechanisms and regulation of water permeability in renal epithelia. *Am J Physiol* **257**, C837–C850 (1989)
- 4 King LS and Agre P: Pathophysiology of the aquaporin water channels. *Annu Rev Physiol* **58**, 619–648 (1996)
- 5 Knepper MA: The aquaporin family of molecular water channels. *Proc Natl Acad Sci USA* **91**, 6255–6258 (1994)
- 6 Ma T and Verkman AS: Aquaporin water channels in gastrointestinal physiology. *J Physiol (Lond)* **517**, 317–326 (1999)
- 7 Lee MD, King LS and Agre P: The aquaporin family of water channel proteins in clinical medicine. *Medicine* **76**, 141–156 (1997)
- 8 Raina S, Preston GM, Guggino WB and Agre P: Molecular cloning and characterization of an aquaporin cDNA from salivary, lacrimal, and respiratory tissues. *J Biol Chem* **270**, 1908–1912 (1995)
- 9 Lee MD, Bhakta KY, Raina S, Yanesco R, Griffin CA, Copeland NG, Gilbert DJ, Jenkins NA, Preston GM and Agre P: The human aquaporin-5 gene. *J Biol Chem* **271**, 8599–8604 (1995)
- 10 Krane CM, Towne JE and Menon AG: Cloning and characterization of murine Aqp5: evidence for a conserved aquaporin gene cluster. *Mamm Genome* **10**, 498–505 (1999)
- 11 Bradley RM: Salivary secretion. In *Essentials of Oral Physiology*, Edited by Bradley RM, pp 161–186, Mosby-Year Book, Inc, St Louis, Missouri (1995)
- 12 Baum BJ: Principles of saliva secretion. *Ann NY Acad Sci* **694**, 17–23 (1993)
- 13 Hata F, Ishida H, Kagawa K, Kondo E, Kondo S and Noguchi Y: β -Adrenoceptor alterations coupled with secretory response in rat parotid tissue. *J Physiol (Lond)* **341**, 185–196 (1983)
- 14 Ishikawa Y, Amano I, Eguchi T and Ishida H: Mechanism of isoproterenol-induced heterologous desensitization of mucin secretion from rat submandibular glands; regulation of phosphorylation of Gi proteins controls the cell response to the subsequent stimulation. *Biochim Biophys Acta* **1265**, 173–180 (1995)
- 15 Amano I, Ishikawa Y, Eguchi T and Ishida H: Regulation of phosphorylation of Gi2 α protein controls the secretory response to isoproterenol in rat parotid tissues. *Biochim Biophys Acta* **1313**, 146–156 (1996)
- 16 Eguchi T, Ishikawa Y and Ishida H: Mechanism underlying histamine-induced desensitization of amylase secretion in rat parotid glands. *Br J Pharmacol* **124**, 1523–1533 (1998)
- 17 Turner BJ: Mechanisms of fluid secretion. *Ann NY Acad Sci* **694**, 24–35 (1993)
- 18 Nauntofte B: Regulation of electrolyte and fluid secretion in salivary acinar cells. *Am J Physiol* **263**, G823–G837 (1992)
- 19 Melvin JE, Moran A and Turner RJ: The role of HCO_3^- and Na^+ / H^+ exchange in the response of rat parotid acinar cells to muscarinic stimulation. *J Biol Chem* **263**, 19564–19569 (1988)
- 20 Diamond JM: Wet transport proteins. *Nature* **384**, 611–612 (1996)
- 21 He X, Tse CM, Donowitz M, Alper SL, Gabriel SE and Baum BJ: Polarized distribution of key membrane transport proteins in

- rat submandibular gland. *Pflugers Arch* **433**, 260–268 (1997)
- 22 Matsuzaki T, Suzuki T, Koyama H, Tanaka S and Takata K: Aquaporin-5 (AQP5), a water channel protein, in the rat salivary and lacrimal glands: immunolocalization and effect of secretory stimulation. *Cell Tissue Res* **295**, 513–521 (1999)
 - 23 Delporte C, Redman RS and Baum BJ: Relationship between the cellular distribution of the $\alpha_3\beta_5$ integrins and adenoviral infection in salivary glands. *Lab Invest* **77**, 167–173 (1997)
 - 24 Delporte C, Hoque ATNMS, Kulakusky JA, Braddon VR, Goldsmith CM, Wellner RB and Baum BJ: Relationship between adenovirus-mediated aquaporin 1 expression and fluid movement across epithelial cells. *Biochem Biophys Res Commun* **246**, 584–588 (1998)
 - 25 Ma T, Song Y, Gillespie A, Carlson EJ, Epstein CJ and Verkman AS: Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. *J Biol Chem* **274**, 20071–20074 (1999)
 - 26 Koyama Y, Yamamoto T, Kondo D, Funaki H, Yaoita E, Kawasaki K, Sato N, Hatakeyama K and Kihara I: Molecular cloning of a new aquaporin from rat pancreas. *J Biol Chem* **272**, 30329–30333 (1997)
 - 27 Ramamoorthy S, Melikian H, Qian Y and Blakely RD: Biosynthesis, N-glycosylation, and surface trafficking of biogenic amine transporter proteins. *Methods Enzymol* **296**, 347–370 (1998)
 - 28 Ishikawa Y, Eguchi T, Skowronski MT and Ishida H: Acetylcholine acts on M_3 muscarinic receptors and induces the translocation of aquaporin5 water channel via cytosolic Ca^{2+} elevation in rat parotid glands. *Biochem Biophys Res Commun* **245**, 835–840 (1998)
 - 29 Ishikawa Y, Skowronski MT, Inoue N and Ishida H: α_1 -Adrenoceptor-induced trafficking of aquaporin-5 to the apical plasma membrane of rat parotid cells. *Biochem Biophys Res Commun* **265**, 94–100 (1999)
 - 30 Ishikawa Y, Skowronski MT, Eguchi T and Ishida H: Trafficking of AQP5 water channel in parotid glands by neurotransmitters. *In* Control and Diseases of Sodium Dependent Transport Proteins and Ion Channels, Edited by Suketa Y, Elsevier Science Publishing Co, Amsterdam, The Netherlands (in press)
 - 31 Ishikawa Y, Skowronski MT, Eguchi T and Ishida H: SNI-2011 acts on M_3 -receptors and induces the long-lasting increase in the amount of aquaporin-5 in the apical plasma membrane of rat parotid glands. *Jpn J Pharmacol* **79**, Suppl I, 92P (1999)
 - 32 Ishida N, Hirai SI and Mita S: Immunolocalization of aquaporin homologs in mouse lacrimal glands. *Biochem Biophys Res Commun* **238**, 891–895 (1997)
 - 33 Wade JB, Stetson DL and Lewis SA: ADH action: evidence for a membrane shuttle mechanism. *Ann NY Acad Sci* **372**, 106–117 (1981)
 - 34 Kasai H, Li YX and Miyashita Y: Subcellular distribution of Ca^{2+} release channels underlying Ca^{2+} waves and oscillations in exocrine pancreas. *Cell* **74**, 669–677 (1993)
 - 35 Malinelli RA, Pham L, Agre P and LaRusso NF: Secretin promotes osmotic water transport in rat cholangiocytes by increasing aquaporin-1 water channels in plasma membrane. *J Biol Chem* **272**, 12984–12988 (1997)
 - 36 Fushimi K, Uchida S, Hara Y, Hirata Y, Marumo F and Sasaki S: Cloning and expression of apical membrane water channel of rat kidney collecting tubule. *Nature* **361**, 549–552 (1993)
 - 37 Brown D, Katsura T and Gustafson CE: Cellular mechanisms of aquaporin trafficking. *Am J Physiol* **275**, F328–F331 (1998)
 - 38 Sasaki S, Ishibashi K and Marumo F: Aquaporin 2 and -3: Representatives of two subgroups of the aquaporin family colocalized in the kidney collecting duct. *Annu Rev Physiol* **61**, 199–220 (1998)
 - 39 Cowman RA, Frisch M, Lasseter CJ and Scarpace JP: Effects of beta-adrenergic antagonists on salivary secretory function in individuals of different ages. *J Gerontol* **49**, B208–B214 (1994)
 - 40 Ship JA and Baum BJ: Is reduced salivary flow normal in old people? *The Lancet* **336**, 1507 (1990)
 - 41 Koller MM, Maeda N, Purushotham KR, Scarpace PJ and Humphreys-Beher MG: A biochemical analysis of parotid and submandibular salivary gland function with age after simultaneous stimulation with pilocarpine and isoproterenol in female nia Fischer 344 rats. *Archs oral Biol* **37**, 219–230 (1992)
 - 42 Sreebny LM and Schwartz SS: A reference guide to drugs and dry mouth. *Gerontology* **5**, 75–99 (1986)
 - 43 Bodner L and Baum BJ: Characteristics of stimulated parotid gland secretion in the aging rat. *Mech Ageing Dev* **31**, 337–342 (1985)
 - 44 King LS, Nielsen S and Agre P: Aquaporins in complex tissues. I. Developmental patterns in respiratory and glandular tissues of rat. *Am J Physiol* **273**, C1541–C1548 (1997)
 - 45 Yasui M, Serlachius E, Lofgren M, Belusa R, Nielsen S and Aperia A: Perinatal changes in expression of aquaporin-4 and other water and ion transporters in rat lung. *J Physiol (Lond)* **505**, 3–11 (1997)