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Review

Aquaporin-5 water channel in lipid rafts of rat parotid glands

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Abstract

Aquaporin-5 (AQP5), an apical plasma membrane (APM) water channel in salivary glands, lacrimal glands, and airway epithelium, has an important role in fluid secretion. The activation of M3 muscarinic acetylcholine receptors (mAChRs) or α1-adrenoceptors on the salivary glands induces salivary fluid secretion. AQP5 localizes in lipid rafts and activation of the M3 mAChRs or α1-adrenoceptors induced its translocation together with the lipid rafts to the APM in the interlobular ducts of rat parotid glands. This review focuses on the mechanisms of AQP5 translocation together with lipid rafts to the APM in the interlobular duct cells of parotid glands of normal rats and the impairment of AQP5 translocation in diabetes and senescence.

Keywords: Aquaporin-5; Lipid rafts; Parotid glands; Diabetes; Aging; Interlobular ducts

Contents

1. Introduction

Rat parotid glands are innervated by both the sympathetic and parasympathetic nerves of the autonomic nervous system. Sympathetic stimulation induces the secretion of salivary proteins such as amylase and mucin from the secretory granules in the acinar cells by exocytosis due to activation of β2-adrenoceptors. Noradrenalin (Nor) released by sympathetic stimulation also activates α1-adrenoceptors on the cells and induces modest salivary fluid secretion [1]. Parasympathetic stimulation induces the largest salivary fluid secretion as a result of the activation of M3 muscarinic acetylcholine (ACh) receptors (mAChRs) on the cells [1]. The main component of

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saliva is water, and the molecular mechanisms by which water is secreted from the salivary gland cells remain unknown, because of the presence of the plasma membranes as a major barrier.

In 1988, a 28-kDa integral membrane protein, now known as aquaporin-1 (AQP1), was discovered as a water channel [2,3]. There have been many attempts at homology cloning to isolate water channel proteins and to identify sequence-related proteins in many kinds of living cells. AQP water channel proteins are abundant in a variety of cells for the transportation of fluid and have homology with the major intrinsic proteins of the lens, which form water channels [4]. Thirteen members of the AQP family, AQP0–AQP12, have been identified from many mammalian cells [5,6]. The AQP family consists of three subsets coresponding to AQPs, aquaglyceroporins, and super-aquaporins. AQPs selectively permeate water and consist of AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8. Aquaglyceroporins permeate water and glycerol and consist of AQP3, AQP7, AQP9, and AQP10 [5]. Superaquaporins have poorly conserved asparagin–proline–alanine (NPA) boxes and consist of AQP11 and AQP12 [6].

Recently, studies have focused on the mechanisms underlying the control of AQPs to clarify the molecular basis of water movement across biologic membranes in relation to disease [4–13]. Impaired AQP2 trafficking causes nephrogenic diabetes insipidus [8,9]. Changes in AQP1, AQP4, and AQP9 expression in the brain are correlated with edema formation [10,11]. Autoantibodies on the mAChRs in Sjogren's syndrome cause to decrease pilocarpine-induced AQP5 trafficking to APM of HSG cells [13], suggesting the importance of AQP5 trafficking to the APM of salivary glands in mACh agonist-induced exocytosis.

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. [14]. AQP5 is highly expressed in the apical domains of rat serous acinar cells of exocrine glands, such as the parotid and submandibular glands [15–17], lacrimal glands [18], and sweat glands [19], subepithelial glands of the upper airway [20], and rat interlobular duct cells of parotid glands [16]. AQP5 is predicted to have a significant role in fluid secretion from serous acinar cells of salivary glands on the basis of its abundance in the apical region of the cells. This is supported by the fact that knockout mice lacking AQP5 have markedly decreased rates of salivary secretion [21].

Activation of M3 mAChRs and α1-adrenoceptors on the acinar cells of rat parotid glands increase the intracellular concentration of Ca^{2+}($[Ca^{2+}]_i$) and induces salivary secretion from the cells [1,22–25], indicating the importance of Ca^{2+}-mediated intracellular signal transduction for the secretion. Whether the subcellular distribution of AQP5 is regulated by Ca^{2+}-signaling through M3 mAChRs and α1-adrenoceptors in parotid glands, however, was unknown. We studied whether the stimulation of salivary fluid secretion is accompanied by the redistribution of AQP5 into the apical plasma membranes (APM) from the intracellular structures, the regulatory mechanisms of the redistribution of AQP5, and the characterization of the structures where AQP5 is located in the parotid gland cells [13–17,21,23–26]. We also focused here whether the induction of salivary fluid secretion is accompanied by the translocation of AQP5 together with lipid rafts into the APM via intracellular Ca^{2+}-signaling and dissociation of AQP5 from the lipid rafts at the APM, and the molecular mechanisms that underlie impaired responsiveness of AQP5 to neurotransmitters as a consequence of aging or diabetes-related xerostomia.

2. Distribution of AQP5 in salivary gland cells

AQP5 has been identified with AQP1, AQP3, and AQP8 in mammalian salivary glands [26]. AQP1 is expressed in the capillary endothelial cells of rat parotid and submandibular glands [27]. AQP8 is also expressed in rat salivary glands [28,29], but the localization of it is not clear. AQP5 is localized in the APM of serous acinar cells of rat submandibular [14,20] and parotid [15–17,21,23–26] glands, and of interlobular duct cells of rat parotid glands [16]. AQP5 is also localized in the intracellular structures in rat parotid gland cells [15,16,30,31]. To directly visualize the subcellular localization of AQP5 in rat parotid glands, a histologic approach was used [16]. Confocal immunofluorescence microscope images revealed that in the interlobular duct cells of rat parotid glands under resting conditions, AQP5 is located in the intracellular structures as well as in the APM (Fig. 1) [16]. To quantify the subcellular distribution of AQP5, the interlobular duct cells were analyzed in sections from control rat parotid glands with immunoelectron microscopy. Immunogold labeling at the APM and within the intracellular compartment was assessed on sections from unstimulated parotid glands of different rats. Under resting conditions, 90% of the immunogold particles were associated with intracellular compartments, the remaining 10% were associated with the APM. Thus, under resting conditions, AQP5 was mainly localized in intracellular vesicular structures in the interlobular duct cells of rat parotid glands. AQP5 is located in the cytoplasmic vesicles [30] and the secretory granule membranes [31] of rat parotid glands. AQP1 is localized in the zymogen granules in rat exocrine pancreas [32]. AQP6 is located in the intracellular vesicles in renal epithelia [33]. Thus, different AQP homologues are localized in different cell membranes and in various intracellular organelles in many kinds of cells.

3. Association of AQP5 with lipid rafts in rat parotid gland cells

Under resting conditions, AQP5 is localized in the intracellular structures of rat parotid gland cells (Fig. 1) [15,16]. The intracellular structures where AQP5 is located in rat parotid gland cells remain to be characterized. Cholesterol- and glycosphingolipid-enriched microdomains, commonly known as lipid rafts, were recently suggested to be involved in a number of cell functions, such as membrane sorting and trafficking [34–39], receptor signaling [40], and cholesterol homeostasis [41]. For example, transcytosis of IgA and exocytosis of newly made brush-border proteins in enterocytes occur through an apical lipid raft-containing compartment [42]. These findings suggest that lipid rafts are involved in sorting some apical resident proteins. In order to characterized the intracellular vesicles in rat parotid glands where AQP5 is located, flotillin-2 and GM1

ganglioside, which are a lipid raft-associated integral membrane protein and glycosphingolipid, respectively, were used as raft markers [38] to determine if AQP5 is located in lipid rafts in rat parotid gland cells. Confocal immunofluorescence microscope images revealed that under resting conditions, AQP5 was colocalized with flotillin-2 and GM1 in the cytoplasm of rat parotid gland cells, indicating that AQP5 is located in lipid rafts [16]. This result was further supported by sucrose density gradient experiments in which AQP5 in rat parotid gland tissues, under unstimulated conditions, fractionated to the same fractions containing flotillin-2 and GM1 [16]. Lipid rafts migrate to the lighter density fractions in an Opti-Prep discontinuous density gradient [39,43]. After separation of the rat parotid tissue homogenate on the Opti-Prep gradient, the amount of AQP5 in the tissues under resting conditions was highest in the lighter fractions, indicating that AQP5 is located with lipid rafts in resting parotid gland cells of rats. Next, to test the solubility of AQP5 in cold detergent, rat parotid slices were treated with solution containing 1% Triton X-100 for 30 min at 4 °C [35,39]. The homogenate was then centrifuged to separate TritonX-100-insoluble and -soluble fractions. Under resting conditions, the major portion of AQP5 was present in the Triton X-100-insoluble fraction, revealing the localization of AQP5 in lipid rafts [16]. These results indicated that AQP5 is located in lipid rafts in the cytoplasm of parotid gland cells under resting conditions.

4. Translocation of AQP5 together with lipid rafts to the APM and its dissociation to non-rafts in rat parotid gland cells

It is well documented that salivary fluid secretion is induced by the binding of neurotransmitters from autonomic nerve endings to M3 mAChRs or α1-adrenoceptors localized on the basolateral plasma membrane of parotid acinar cells [1]. The responsiveness of AQP5 in salivary gland cells to stimulation with M3 agonists or α1 agonists was investigated to evaluate the role of AQP5 in salivary secretion. Treatment of the tissues with ACh induces AQP5 translocation from the intracellular structures to the APM within 1 min, but treatment for more than 5 min results in AQP5 translocation from the APM to the intracellular structures [15]. SNI-2011, cevimeline, acting at M3 receptors induces a long-lasting increase in the amount of AQP5 in the APM of rat parotid glands [24]. Treatment of rat parotid tissues with epinephrine also induces transient and marked

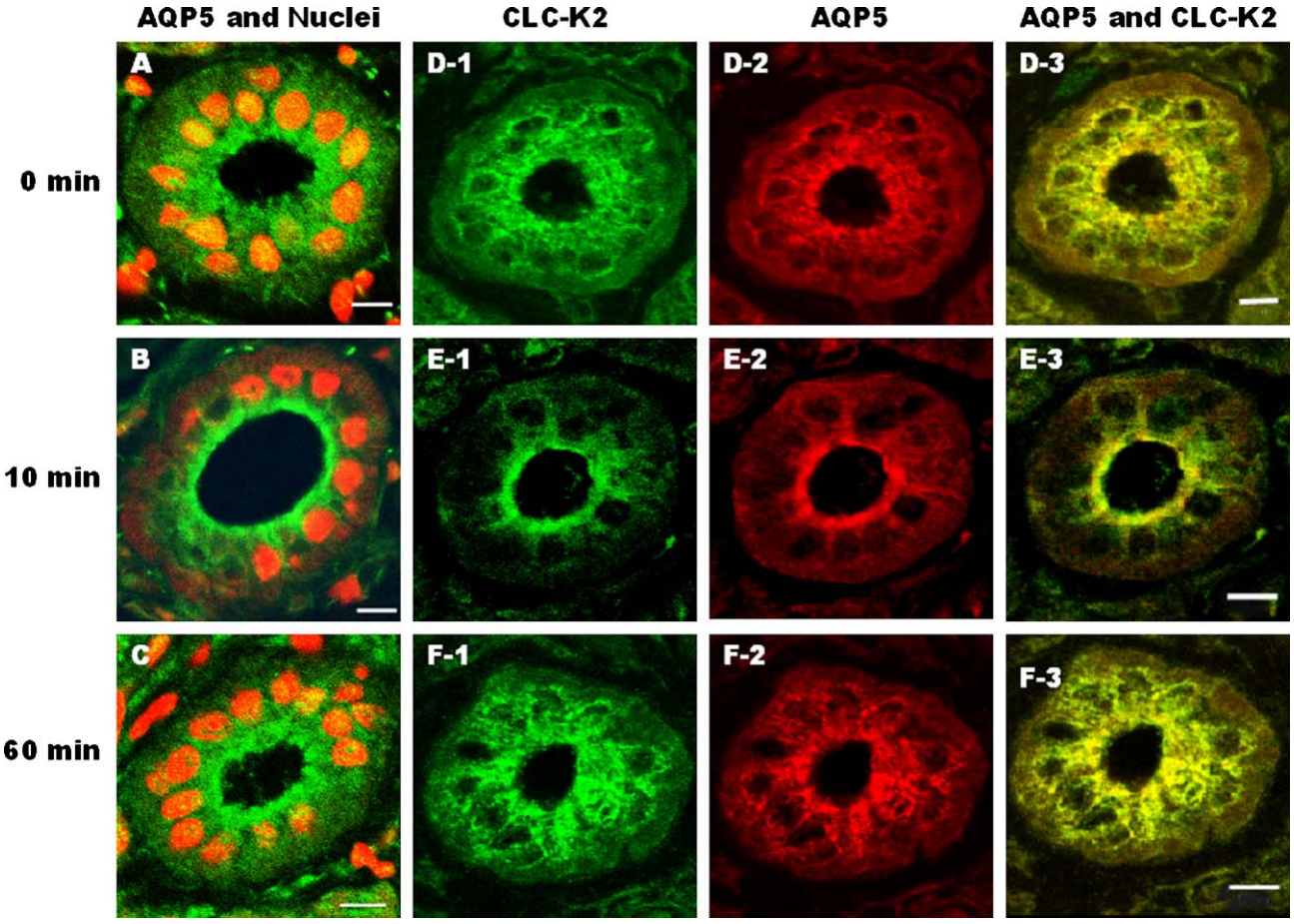

Fig. 1. Changes in confocal immunofluorescence microscope images of AQP5 and CLC-K2 in interlobular duct cells and acinar cells of parotid glands of rat injected with cevimeline. Parotid glands were obtained from rats injected with physiologic saline (A and D) and cevimeline (5.0 mg/kg) (B, C, E and F). The parotid glands were removed and embedded 0 min (A), 10 min (B and E), or 60 min (C and F) after injection. The section was immunostained to detect AQP5 using Alexa-488 (green, A, B, and C) and Alexa-568 (red, D-2, E-2, F-2) or CLC-K2 using Alexa-488 (green, D-1, E-1, and F-1). Nuclei were stained with ethidium bromide. Scale bars: 10 μm.

trafficking of AQP5 between intracellular structures and the APM [23]. Cytochalasin D and tubulozole-C inhibited the translocation of AQP5 to the APM, suggesting the importance of the cytoskeleton in this translocation [22,44]. The trafficking of AQP5 induced by cholinergic or adrenergic stimulation in rat parotid glands was directly visualized with immunohistochemistry [16]. Immunoelectron microscopy of AQP5 expression in interlobular duct cells of rat parotid gland revealed that 10 min after the intravenous injection of cevimeline, the percentage of gold particles in the APM rose to 70% from 10% (under resting conditions) and that of the remaining gold particles localized intracellularly fell to 30% from 90% (under resting conditions), indicating the translocation of AQP5 to the APM in response to cevimeline treatment. Confocal immunofluorescence microscope images revealed that cevimeline induced the translocation of the immunofluorescence of AQP5 together with that of flotillin-2 or GM1 towards the APM in rat interlobular duct cells 10 min after intravenous injection into the tail vein (Fig. 1B) [16]. Sixty minutes after the treatment, immunofluorescence was dispersed throughout the cell (Fig. 1C). These results indicate that AQP5 translocates with lipid rafts to the APM of interlobular duct cells of rat parotid glands, suggesting that water is secreted from interlobular duct cells of rat parotid glands as well as acinar cells. This suggestion is also supported by several cell lines of evidence of an important role for the ductal system in fluid secretion in pancreas [45].

As mentioned above, the major portion of AQP5 was present in the TritonX-100-insoluble fraction under resting conditions of rat parotid gland tissues. Conversely, after the treatment with cevimeline, the amount of AQP5 decreased in the TritonX-100-insoluble fraction and increased in the TritonX-100-soluble fraction. In contrast, cevimeline did not change the amount of flotillin-2 and GM1 in the Triton-X-100-insoluble fraction. These results indicate that M3 mAChR agonists and α1-adrenoceptor agonists induces the translocation of AQP5 with lipid rafts from the intracellular structures to the APM and the dissociation of AQP5 from lipid rafts to non-rafts at the APM in rat parotid glands [16], suggesting that the lipid-AQP5 interactions are important to play the function of AQP5 on the APM.

5. Water movements and the osmolarity generation in parotid interlobular ducts

Water moves passively in response to osmotic gradients generated by active Cl^- driven HCO_3^- secretion [46]. Salivary gland express multitype types of Cl^- channels including Ca^{2+}-dependent and volume-sensitive channels [47], the cystic fibrosis transmembrane conductance regulator (CFTR)[48] and the voltage-regulated ClC-2 and ClC-3 Cl^- channels [49]. CFTR is located in interlobular ducts of rat parotid glands [48] and cholangiocytes of rat liver [46]. Secretin and dibutyryl-cAMP stimulate the translocation of CFTR from intracellular vesicles to the APM [46]. Recently, we found that CLC-K2 (Cl^- channel) was also expressed in the cytoplasm of interlobular duct cells of rat parotid glands and colocalized with AQP5 in lipid rafts under unstimulated conditions (Fig. 1D-1) [61]. Ten minutes after the injection of cevimeline, CLC-K2 moved to APM together with AQP5 as shown in Fig. 1E-1 and E-2 [61], suggesting that CLC-K2 play a role to make osmotic gradients. Further experiments are, however, necessary to clarify the interaction Cl^- channel with the appearance of the function of AQP5 in salivary glands.

In the first stage, saliva is secreted initially from the acinar cells [50]. This saliva is called primary saliva and is plasma-like in concentration of Na^+, Cl^-, and HCO_3^-. In the second stage, the primary saliva is modified in duct cells. It was notified by the old theory that the ducts seem to be impermeability to water, but Na^+ is actively reabsorbed and K^+ secreted from the duct cells [50]. However, there are ample evidences for permeability to water in parotid interlobular ducts. For example, forskolin or carbachol evokes water secretion from rat parotid interlobular duct, associated with secretion through CFTR and diphenylamine-2-carboxylate-sensitive anion channels of carbonic anhydrase-dependent bicarbonate linked with the Na^+/H^+ exchange mechanism [51,52], suggesting that salivary fluid is secreted from parotid interlobular duct cells as well as acinar cells.

6. Mechanisms underlying the translocation of AQP5 towards the APM

Salivary fluid secretion is controlled through M3 mAChRs and α1-adrenoceptors [1]. AQP5 is likely to be a target molecule for the control of saliva production. The treatment of rat parotid tissues with ACh, cevimeline, or epinephrine induces the translocation of AQP5 between the APM and the intracellular structures [15,16,23,24]. The translocation of AQP5 induced by M3 mAChR agonists is inhibited by an inhibitor of calcium release from the intracellular compartment, TMB-8, but not by protein kinase C (PKC) inhibitors, H-7 and GF 109203X. Conversely, a PKC activator, phorbol 12-myristate 13-acetate, does not induce the translocation of AQP5 [24]. The calcium ionophore A-23187 induces the translocation of AQP5 between the APM and the intracellular structures [15,16]. Epinephrine acting at α1-adrenoceptors in parotid glands also induced the translocation of AQP5 from the intracellular structures to the APM [23]. The translocation of AQP5 induced by ACh or epinephrine is inhibited by a phospholipase C inhibitor, U73122, as well as the inhibitors of calcium release inhibition from intracellular stores, dantrolene, and TMB-8 [24]. Fluorescence studies with fura-2/AM demonstrate that $[Ca^{2+}]_i$ rapidly increases in a concentration-dependent manner with marked fluctuations after exposure of the isolated parotid acinar cells to ACh and cevimeline [24,25], and that the elevation of AQP5 levels in the APM coincided with the elevation of $[Ca^{2+}]_i$. The presence of extracellular Ca^{2+} was necessary for the maximum effect of ACh on the increase of AQP5 in the APM in parotid tissues [25]. An inhibitor of myosin light chain kinase (MLCK), ML-9, inhibited ACh- or pilocarpine-induced increases in the AQP5 levels in the APM [25]. MLCK was identified in parotid glands and regulates capacitative Ca^{2+} entry [53]. It is suggested that both the Ca^{2+} release from intracellular stores and the entry of Ca^{2+} into cells regulate the translocation of AQP5 from the intracellular structures to the APM in rat parotid glands. Furthermore, to confirm the involvement of Ca^{2+} in the translocation of AQP5, the Ca^{2+} ionophore, A-23187, was injected

intravenously into rat tail vein [16]. Ten minutes after the injection, AQP5 fluorescence was predominantly associated with the APM of the interlobular duct cells of parotid glands. Treatment of the parotid tissues with A-23187 decreased the amount of AQP5 in the TritonX-100-insoluble fraction [16]. These findings suggest that increases in $[Ca^{2+}]_i$ mediate the effects of cevimeline on the movement of AQP5 with lipid rafts from the cell cytoplasm to the APM and subsequently the dissociation of AQP5 from lipid rafts to non-rafts within the APM. The site of action of Ca^{2+} for the movement and dissociation of AQP5 in parotid gland cells has not been clarified. In Ca^{2+}-mediated intracellular signal transduction, an increase in $[Ca^{2+}]_i$ has an important role in the activation of Ca^{2+}/calmodulin (CaM)-dependent proteins, such as CaM kinase, MLCK, and nitric oxide synthase (NOS). CaM kinase is a multifunctional enzyme required for both granule mobilization under stimulation conditions and maintenance of secretory capacity under resting conditions in pancreatic β-cells [54]. MLCK regulates capacitative Ca^{2+} entry [53] and is involved in the Ca^{2+}-dependent secretion of insulin [55] and rennin [56]. Nitric oxide (NO) increases cGMP formation through the stimulation of guanylyl cyclase (GC) [57,58]. The possible roles of CaM kinase, NOS, MLCK, and protein kinase G (PKG) in the regulation of the function of AQP5 were investigated to clarify the molecular basis of water movement across the biologic membranes in parotid gland cells [25]. Western blot analysis demonstrated that neuronal (n) NOS is expressed in isolated parotid acinar cells, but endothelial and inducible NOS are not [22]. Carboxy-PTIO, an NO scavenger, inhibits ACh- and pilocarpine-induced increases in AQP5 in the APM in rat parotid glands. SIN-1 and SNAP, NO donors, mimic the effects of ACh [25]. KT5823 and L-Nil, inhibitors of PKG and NOS, respectively, block NO donor-induced increases in AQP5 in the APM. KN-62, an inhibitor of CaM kinase II, decreases the pilocarpine-induced increase in AQP5 in the APM [25]. A study using diaminofluorescein-2 diacetate demonstrates enhanced NOS activity in isolated parotid acinar cells in real time after ACh-treatment. Treatment with dibutyryl cGMP, but not dibutyryl cAMP, induces an increase in AQP5 in the APM. BAPTA-AM, a cell-permeable Ca^{2+} chelator prevents the cGMP-induced increase in AQP5 in the APM. Pretreatment of the parotid tissues with ML-9, an MLCK inhibitor, inhibited the ACh-induced increase in AQP5 in the APM. AQP1 has a cyclic nucleotide binding domain in the C terminus [59]. PKG phosphorylates AQP2 on the C terminal residue and increases the insertion of AQP2 into renal epithelial cells [60]. The PKG regulatory mechanism on AQP5 proteins in parotid glands, however, remains unknown. These results are summarized in Fig. 2, suggesting that NO/cGMP signal transduction has a crucial role in Ca^{2+} homeostasis in the ACh-stimulated increase in AQP5 in the APM of rat parotid gland.

7. The mechanisms underlying age- and diabetes-related impairment of the responsiveness of AQP5 to cholinergic stimulation

The importance of AQPs in human diseases is recently clarifying [4,5,8–13]. The study of subcellular localization of AQP5 provides details of the molecular mechanisms for xerostomia. Xerostomia is characterized by oral dryness and difficulty performing oral functions and in tolerating dentures. Diabetic patients or aged people often complain of xerostomia, but the mechanisms have not been clarified.

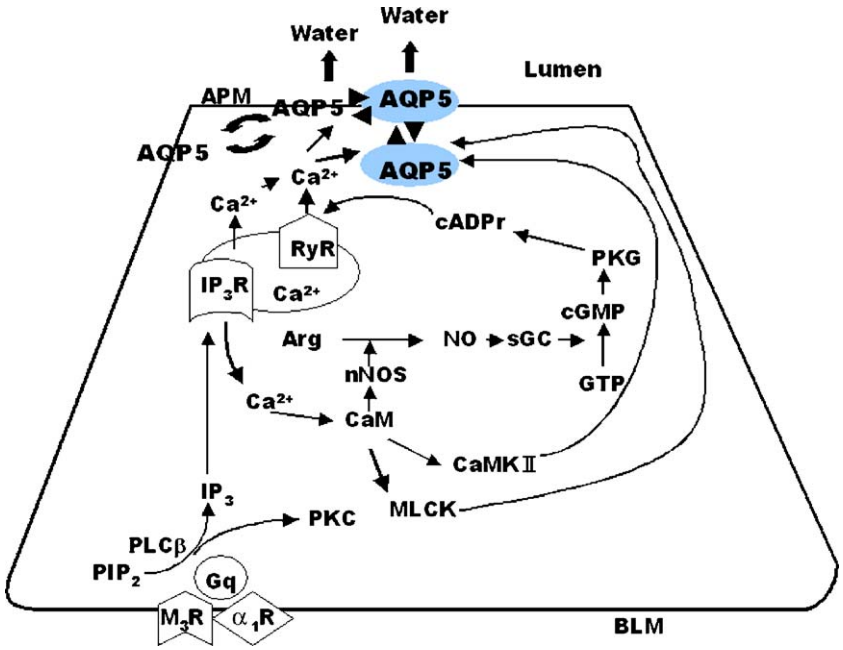

Fig. 2. Schematic representation of signal transduction in M3 muscarinic receptor-induced the translocation of AQP5 in rat parotid glands. M_3R; M_3 muscarinic receptor, α_1R; α_1 adrenergic receptors, Gq, G protein that stimulates phospholipase C (PLC); PIP2, phosphatidylinositol 4, 5-bisphosphate; DAG, diacylglycerol; IP3, inositol 1,4,5-trisphosphate. IP_3R, IP_3 receptor; Ry_3R, Ryanodine receptor type 3; Arg, Arginine; CaM, calmodulin; CaMKII, calmodulin-dependent kinaseII; nNOS, neuronal, nitric-oxide synthase; sGC, soluble guanylate cyclase; PKG, cGMP-dependent protein kinase; cADPr, cADP-ribose; APM, apical plasma membrane; BLM, basolateral plasma membrane. Gray circle is lipid rafts.

The changes in the distribution of AQP5 in rat parotid cells in response to M3 agonists have been studied to clarify the mechanisms underlying age-related xerostomia [62]. ACh and epinephrine induce increases in AQP5 levels in the APM of parotid cells of both young adult and senescent rats. The stimulatory effect of ACh, but not that of epinephrine, on AQP5 levels in the APM of the cells decreases markedly during aging. The amounts of AQP5, M3 mAChRs, inositol trisphosphate (IP3), and Gq/11α proteins do not decrease during aging. This finding indicates that AQP5 responsiveness to cholinergic, but not adrenergic, stimulation is markedly decreased in the parotid cells of senescent rats. The changes in NOS activities measured in real time using 4,5-diaminofluorescein/diacetate in isolated parotid acinar cells from young adult and senescent rats treated with ACh or epinephrine coincides with those of the responsiveness of AQP5 in these cells. Confocal images revealed that under unstimulated conditions, AQP5 fluorescence locates in a diffuse pattern in the cytoplasm of parotid acinar cells of both young adult and senescent rats. Ten minutes after the intravenous injection of cevimeline, AQP5 fluorescence does not localize in the APM of parotid acinar cells of senescent rats, in contrast with that of young adult rats [56]. These findings indicate that the age-related impairment of the responsiveness of AQP5 in parotid cells to muscarinic stimulation might account for the concomitant changes in NOS activity in the cells, and might induce age-related xerostomia [62,63].

To study the mechanisms underlying diabetic xerostomia, changes in the distribution of AQP5 in the interlobular duct cells of parotid glands were investigated after the administration of cevimeline into the tail vein of control and streptozotocin-induced diabetic (diabetic) rats [64]. Confocal images revealed that under unstimulated conditions, AQP5 colocalized with flotillin-2 and GM1, lipid raft markers, with a diffuse pattern in the cytoplasm of interlobular duct cells of parotid glands of both control and diabetic rats. Ten minutes after intravenous injection of cevimeline, AQP5 levels dramatically increased together with flotillin-2 and GM1 in the APM of the cells of control rats, and then AQP5 was again colocalized with flotillin-2 and GM1 in a diffuse pattern in the cytoplasm 60 min after the injection. The cevimeline-induced trafficking of AQP5 between the APM and intracellular structures are not observed in the cells of diabetic rats. Treatment of the parotid tissues with cevimeline induces a decrease in the TritonX-100 insolubility of AQP5 in control rats, but not in diabetic rats. These findings indicate the impairment of AQP5 trafficking between APM and intracellular structures with lipid rafts and that of AQP5 dissociation from lipid rafts to non-rafts on the APM in the interlobular duct cells of diabetic rat parotid glands. The amount of AQP5 does not decrease in parotid glands of diabetic rats compared with that of control rats [64]. In contrast, there is a 50% decrease in M3 mAChRs parotid glands of diabetic rats. Administration of insulin to diabetic rats recovers the distribution and dissociation of AQP5 as compared to control rats. Treatment with cevimeline of isolated parotid acinar cells from diabetic rats loaded with DAF-2/DA did not induce a fast and significant increase in the fluorescent triazol formed by DAF-2 and NO in the presence of oxygen as observed in control rats, demonstrating that cevimeline did not stimulate NOS activity in parotid acinar cells of diabetic rats. Injection of insulin to diabetic rats showed the same distribution and dissociation of AQP5 as those observed in normal control rats [57]. These findings indicate that the decrease in the amount of Ca^{2+} released from intracellular Ca^{2+} stores via IP3 receptors according to the decrease in the amount of IP3 synthesized through agonist-induced activation of M3 mAChRs decreased in parotid glands of diabetic rats. Finally, the impairment of translocation of AQP5 to the APM in parotid gland cells induced by the decline in Ca^{2+} signaling via M3 mAChRs in parotid glands is important in the mechanisms underlying diabetes-related xerostomia. These findings support also that water is secreted from not only acinar cells but also interlobular duct cells of rat parotid glands.

8. Conclusion

The increases in $[Ca^{2+}]_i$ in salivary gland cells by the activation of M3 mAChRs and α1-adrenoceptors play a crucial role on salivary fluid secretion. It is documented that water, the main component of saliva, is secreted with Na^+ and K^+ into the duct system from serous acinar cells in salivary glands as the primary saliva component. Subsequently, water and K^+ are also secreted from the interlobular duct cells in the glands and in contrast, Na^+ is absorbed into the cells as they pass along the duct. It was also shown that water was secreted from not only the acinar cells but also the interlobular duct cells of parotid glands by the translocation of AQP5 water channel together with lipid rafts to the APM from intracellular vesicles in these cells by Ca^{2+} signaling through M3 mAChRs or α1 adrenoceptors [16]. Therefore, the principal sites for water transport are both the acinar cells and interlobular duct cells in the salivary glands.

AQP5 is a target molecule for sympathetic or parasympathetic control of saliva production. As shown in Fig. 2, the activation of M3 mAChRs and α1-adrenoceptors induced the interaction of IP_3 receptors and ryanodine receptors with IP3 and cADP ribose, respectively, via sGC/PKG signaling. The increase in $[Ca^{2+}]_i$ released from intracellular storage sites by the activation of IP_3 receptors and ryanodine receptors induced translocation of AQP5 together with lipid rafts from the intracellular structures to the APM in the interlobular duct cells of rat parotid glands. Then, at the APM, AQP5 located with lipid rafts was dissociated to non-rafts via the increase in $[Ca^{2+}]_i$.

Thus, AQP5 in salivary glands [16] and lung [65] is subjected to trafficking between the intracellular structures and the APM in response to neurotransmitters or hormones as well as AQP2 ileal collecting duct cells [8], AQP1 in rat cholangiocytes [66], and AQP8 in hepatocytes [67]. At the APM, AQP5 moves between lipid rafts and non-rafts, suggesting that protein–lipid interaction is important to play a role of AQP5. It is very interesting that the impaired responsiveness of AQP5 to M3 mAChRs results in xerostomia. Furthermore, it is important to study the mechanisms that control the polarization of AQPs coupled with fluid flow and the network of the AQPs involved in fluid secretion from salivary glands. Recently, in order to search the domain in AQP5 structures to facilitating to target the APM, much interest has been focused on N- or C-termini of AQP5 [68]. Further

experiments are necessary to clarify the mechanisms in the translocation of AQP5 in relation with those.

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