Abstract

Introduction

Main function of salivary gland is saliva secretion. This function is essential for proper oral homeostasis. Aquaporins are water-permeable trans-membrane proteins involved in trans-cellular water flow. Aim of this review is to give an overview of the expression of aquaporins in the salivary gland; their role in saliva secretion and in pathophysiological conditions.

Discussion

Several aquaporins are expressed in salivary glands, amongst which aquaporin-5, plays an essential physiological role. Consequently, modulation of aquaporin expression and/or trafficking may contribute to the pathogenesis of diseases affecting salivary glands such as xerostomia conditions. Indeed, therapeutic tools increasing aquaporins expression might be valuable for the treatment of xerostomia conditions.

Conclusion

Aquaporins are involved in salivary gland physiological and pathophysiological processes. Therefore, they could represent novel therapeutic targets for the treatment of diseases affecting the salivary glands.

Introduction

The existence of water channels was anticipated based on the existence of biological membranes presenting high-membrane water permeability that could not be explained solely by passive water diffusion though a lipid bilayer. The existence of water channels, also called aquaporins, was discovered following the isolation of a 28 kDa membrane protein from red blood cells termed channel-forming integral protein of 28 kDa (CHIP28), conferring increased water permeability to Xenopus laevis oocytes following microinjection with an in vitro-transcribed CHIP28 RNA, and to proteoliposomes containing pure CHIP28 protein. Later, related mammalian and plant proteins were sequenced and shown to allow water transport. The term aquaporin (AQP) was proposed for the family of water channel and the name CHIP28 was changed to AQP1. CHIP28 was predicted to have a homotetrameric structure, each monomer containing six trans-membrane domains and the N- and C-termini of the proteins located intra-cellularly. As two halves of the AQP1 protein are symmetric forming a channel pore in the membrane bilayer, with two highly conserved asparagine-proline-alanine (NPA) motifs juxtaposed within the channel, serve as a water selective gate. AQP1’s three-dimensional structure resembles a hourglass. Some AQPs transport only water, while other transport other solutes such as glycerol, urea, anions, ammonia, hydrogen peroxide, arsenite, antimonite, carbon dioxide and nitric oxide. According to their permeability and structure, the 13 mammalian AQPs have been subdivided into classical AQPs, primarily permeable to water but also to gasses and ions (AQ0, AQP1, AQP2, AQP4, AQP5, AQP6, AQP8); aquaglyceroporins are also permeable to glycerol and other small solutes (AQP3, AQP7, AQP9, AQP10); and non-classical AQPs with permeability specificity that has not been clearly established. The review discusses the expression and roles of AQPs in salivary secretion.

Discussion

The author has referenced some of its own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies. Animal care was also in accordance with the institution guidelines.

Salivary glands morphology

Salivary glands (SG) consist of acinar (serous, mucous or seromucous), ductal and myoepithelial cells. The acinar cells secrete a primary fluid, composed of proteins and fluid, into ducts. The ductal cells then modify the primary fluid secreted by the acinar cells. Contraction of myoepithelial cells surrounding the basal area of acinar and ductal cells forces fluid out of the ducts.

Mammals possess three major pairs of SG: parotid (PG), submandibular (SMG) and sub-lingual glands (SLG), as well as minor labial SG (LSG) scattered throughout the oral cavity. Rodent and human PGs are exclusively composed of serous acinar cells, whereas SMG and LSG contain both serous and mucous acinar cells. Human SMG contain more serous acinar cells than mucous acinar cells, while it is opposite in LSG. Still, both human SMG and LSG contain some seromucous acinar cells.

Expression and localisation of AQPs in SGs

In rats, AQP1, AQP3, AQP4 and AQP5 are expressed in SMG. AQP1 is expressed in blood vessels.
expression of AQP4 in SMG acinar cells remains controversial, as for the expression of AQP5 in ductal cells\(^\text{13}\). In PG acinar cells, AQP5 is expressed at the apical membrane, while AQP6 is expressed at the plasma membrane near tight junctions and the secretory granule membranes\(^\text{13}\). AQP8 is located in myoepithelial cells from SG acinar cells\(^\text{13}\).

In a mouse, AQP1, AQP3, AQP4 and AQP5 mRNA have been detected\(^\text{14}\). Mouse PG expresses AQP5 at the apical membrane and basolateral membrane of acinar cells\(^\text{15}\). In a human, AQP1 is localised on capillaries and myoepithelial cells\(^\text{13}\) of all SG. AQP3 is expressed at the basolateral membrane of both serous and mucous acinar cells, and not on ductal cells of all SG\(^\text{13}\). Despite the presence of AQP4 mRNA in all SG, AQP4 protein expression has not been confirmed\(^\text{13}\). AQP5 is expressed and localised to the APM of acinar cells, but not on the ductal cells of all SG\(^\text{13}\). Furthermore, AQP5 expression is confined to the serous acinar cells and absent from the mucous acinar cells\(^\text{13}\). AQP6 and AQP7 mRNAs are present in SMG\(^\text{13}\).

**Physiology of saliva secretion**

In humans, SGs secrete daily 750 to 1,000 ml of saliva. Mainly, SMG and LSG secrete saliva under resting conditions, while PG secrete saliva upon stimulation. Endocrine, paracrine and neuronal inputs control saliva secretion\(^\text{16}\). Neuronal regulation of saliva secretion is controlled by parasympathetic and sympathetic nerve endings. In response to acetylcholine, activating M3 and M1 (to a lesser extent) receptors in both acinar and ductal cells, intra-cellular calcium release leads mainly to fluid secretion\(^\text{17}\). On the other hand, noradrenaline, activating β-adrenergic receptors, induces intra-cellular cAMP increase leading mainly to enzyme secretion from acinar cells and fluid and electrolyte transport in ductal cells\(^\text{16}\).

Several important physiological functions, such as protection and hydration of mucosal structures, initiation of digestion and antimicrobial defences, are ensured by saliva. Therefore, multiple clinical manifestations are encountered in response to dysfunction of saliva secretion\(^\text{18}\).

Saliva secretion results from a two step process (Figure 1). During the first step of the saliva secretion process, an isotonic-like fluid rich in NaCl is secreted by acinar cells\(^\text{19}\). Accumulation of ions in the acinar lumen generates a trans-epithelial osmotic gradient driving large water

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**Figure 1:** Molecular mechanisms of saliva secretion. Acinar cells secrete a large volume of isotonic-like fluid rich in NaCl. The generation of a trans-epithelial osmotic gradient drives water flow through apical AQP5 and possibly paracellular pathways. Ductal cells, relatively impermeable to water, re-absorb most of the NaCl and secrete K\(^+\) and HCO\(_3^-\).

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eflux to the acinar lumen via apical AQP5 channels and possible paracellular pathways. This resulting primary fluid then flows into the ductal lumen. During the second step of the saliva secretion process, the ductal cells, relatively impermeable to water, modify the composition of the fluid by reabsorbing most of the Na⁺ and Cl⁻, while secreting HCO₃⁻ and K⁺19. Upon reaching the mouth, the final saliva is hypotonic19.

Role of AQP5s in saliva secretion

The involvement of AQP5 in transcellular water movement during saliva secretion has been exposed using AQP5 knockout mice30,31. Indeed, the knockout mice displayed a 60% decrease in pilocarpine-stimulated saliva production and a more viscous and hypertonic saliva than their wild-type littermates30,31. Furthermore, PG and SLG acinar cells, prepared from AQP5-knockout mice, presented, respectively, a 65% and a 77% decrease in water permeability in response to osmotic challenge, compared to acinar cells prepared from wild-type littermates30,31. AQP1, AQP4 and AQP8 seemly do not participate to saliva secretion as knocking out their expression had no impact on mice pilocarpine-induced saliva, compared to wild-type littermates30,31. The implication of other AQP5s in salivation has not been demonstrated either. In light of the still debated expression of AQP5 in ductal cells, further studies will be required to establish its possible functional role and regulation in that cell type.

In addition to the well-documented transcellular water movement, via AQP5, occurring during saliva secretion, several experiments have provided evidences for the existence of a concurrent paracellular water flow, via tight junctions21. The data generated from such experiments suggested an osmosensor feedback model in which osmosensor, likely AQP5 in SGs, controls the tonicity of the transported fluid by mixing trans-cellular and paracellular flows21,22. However, another secretory model based a trans-cellular-only osmotic mechanism is sufficient to predict the results obtained from AQP5-knockout mice studies, despite the fact that AQP5-knockout mice studies do not give enough information to definitively rule out the existence of an additional paracellular water pathway23. Indeed, whether paracellular water flow also participates remains an ongoing debate. Therefore, further studies will be required to ultimately assess which of the two proposed secretory models accounts best for saliva secretion.

Role of AQP5 trafficking in saliva secretion

Sub-cellular localisation of plasma membrane proteins is dynamically regulated by post-translational modifications such as phosphorylation and ubiquitination occurring on sorting signal located into the cytosolic domains of the transported proteins. Phosphorylation-dependent trafficking of AQP5 in SGs remains poorly understood as compared to that of its most closely sequence-related family member AQP2 in the kidney24. AQP5 shares homologous structural and amino acid features with AQP2, namely in the carboxyterminus domain, the latter being involved in AQP2 cellular trafficking and apical membrane targeting in the kidney24. Targeting of AQP5 to the apical membrane of epithelial cells seemingly result from the presence of a specific targeting signal present in the C-terminal part of the protein25. AQP5 trafficking, resulting from the fusion of intra-cellular vesicles expressing AQP5 to the apical membrane, can be induced by cAMP and acetylcholine24,25 (Figure 2). AQP5 amino acids Ser15630 and Thr25931 have been shown to be phosphorylated by a cAMP-dependent mechanism but are not involved in AQP5 trafficking. So far, in vivo trafficking of AQP5 could not be detected in SGs32. Additional studies will be necessary to identify the phosphorylated amino acids involved in stimuli-induced AQP5 trafficking and concomitant saliva secretion.

Implication of AQP5s in xerostomia conditions

With senescence, declined salivation occurs gradually as age increases in humans and mice. In senescent rats, decreased AQP5 translocation results from a reduction in PG nitric oxide synthase33. Civimeline could be used as a therapeutic agent for the treatment of age-related xerostomia as it increases AQP5 expression at the acinar apical membrane34. DNA demethylation agents increased AQP5 expression and restored salivation in a murine aging model35. Therefore, increase of AQP5 expression by analogs of acetylcholine, activators of nitric oxide synthase and DNA demethylation agents could be useful for xerostomia treatment in elderly individuals.

Radiation therapy is often used as a therapeutic component for patients diagnosed with head and neck cancer. However, this therapy damages the SG acinar cells lying in the radiation field and induces loss of salivation36. This loss of salivation could result from decreased AQP5 expression37-41 and AQP5 trafficking39. Based on the physiological mechanisms of saliva secretion, it was hypothesised that the introduction of a facilitated water pathway in irradiated ductal cells could generate an osmotic gradient (extra-cellular lumen > intra-cellular) allowing fluid secretion37. The introduction of such facilitated water pathway was performed using a recombinant adenoviral vector coding for human AQP1 (AdhAQP1) that drove both AQP1 expression and osmotically driven fluid secretion in epithelial cells37,42,43, and restored saliva secretion in irradiated SMG from rats37, non-human primates44 and miniature pigs45. Furthermore, a clinical trial showed that AdhAQP1...
Review


All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.

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and to evaluate the secretory benefits induced by pharmacological or genetic approaches increasing AQP expression.

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**Abbreviations**

AdhAQP1, recombinant adenoviral vector coding for human AQP1; AQP, aquaporin; AQPs, aquaporins; CHIP28, channel-forming integral protein of 28kd; LSG, minor labial glands; PG, parotid glands; SG, salivary glands; SLG, sublingual glands; SMG, submandibular glands; SS, Sjögren's syndrome.

**References**


