

PROCEEDING

Correlation between salivary secretion and salivary AQP5 levels in health and disease

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Abstract : Saliva samples are useful for noninvasive diagnosis of oral and systemic diseases. The water channel protein aquaporin-5 (AQP5) is released into human saliva. Salivary AQP5 levels show a diurnal variation with the secretion of high levels during the waking hours. An age-related decrease in salivary AQP5 levels parallels a decrease in the volume of saliva. Cevimeline, a muscarinic acetylcholine receptor (mAChR) agonist, induces the release of AQP5. Changes in salivary AQP5 levels after cevimeline administration occur simultaneously with changes in saliva flow rate. AQP5 and lipid rafts are released separately from human salivary glands upon M₃ mAChR stimulation. In patients with diabetes mellitus or Sjögren's syndrome, a decrease in salivary secretion occurs concomitantly with low salivary AQP5 levels. Salivary AQP5 levels correlate with salivary secretion in both healthy and disease states, suggesting that changes in salivary AQP5 levels can be used as an indicator of salivary flow rate and the effect of M₃ mAChR agonists on human salivary glands. *J. Med. Invest.* 56 Suppl. : 350-353, December, 2009

Keywords : salivary composition, water channel, aquaporin-5, lipid raft, salivary flow rate.

INTRODUCTION

The composition of saliva changes dynamically, varying through the course of day, and in response to physiological, psychological, and environmental stimuli, as well as disease states. Because saliva samples can be collected noninvasively, it is a suitable specimen for use in the diagnosis of disease and for monitoring physiologic status. Salivary cortisone, endorphin, and amylase levels are markers

of stress (1-3) and salivary mRNAs are used as markers of cancer (4). Recently, salivary proteomes and transcriptomes were shown to change in relation to disease (5, 6).

The plasma membrane is a major barrier to water transport. Aquaporins crucially regulate membrane permeability to water. Aquaporin-5 (AQP5) is highly expressed in salivary glands (7) and is important for salivary secretion (8). In response to the activation of M₃ muscarinic acetylcholine receptors (mAChR) and α_1 -adrenoceptors, AQP5 is translocated to the apical plasma membrane (APM) together with lipid rafts (9). Some parts of AQP5 and the lipid rafts are then released into the saliva (10).

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SALIVARY RELEASE OF AQP5

To investigate M_3 mAChR agonist-induced secretion of AQP5 into the saliva, we generated anti-AQP5 antisera against recombinant human AQP5 protein (anti-hAQP5 antibody ; 10) and synthetic C-terminus peptide of rat AQP5 (anti-rAQP5 antibody ; 9). Confocal microscopy revealed that AQP5 is located in the apical plasma membrane (APM) in the parotid glands in a diffuse pattern under resting conditions. After the injection of cevimeline, an M_3 mAChR agonist, AQP5 was predominantly associated with the APM and localized in the APM and lumen (Fig. 1). Immunohistochemical staining using anti-hAQP5 antibody (Fig. 1A) and anti-rAQP5

antibody (Fig. 1B in this review and Figs. 3B and 4B in Ref 9) showed AQP5 in the lumen of the ducts, suggesting that AQP5 was released into the saliva.

AQP5 LEVELS IN HUMAN SALIVA

To quantify the salivary AQP5 concentration, we developed an enzyme-linked immunosorbent assay. The amount of AQP5 in unstimulated saliva showed a diurnal variation with secretion of high levels during waking hours (1.59 ± 0.24 ng/ml) and low levels (0.63 ± 0.12 ng/ml) during sleeping hours (10). Salivary AQP5 levels decreased with an increase in age and the decrease coincided with a decrease in

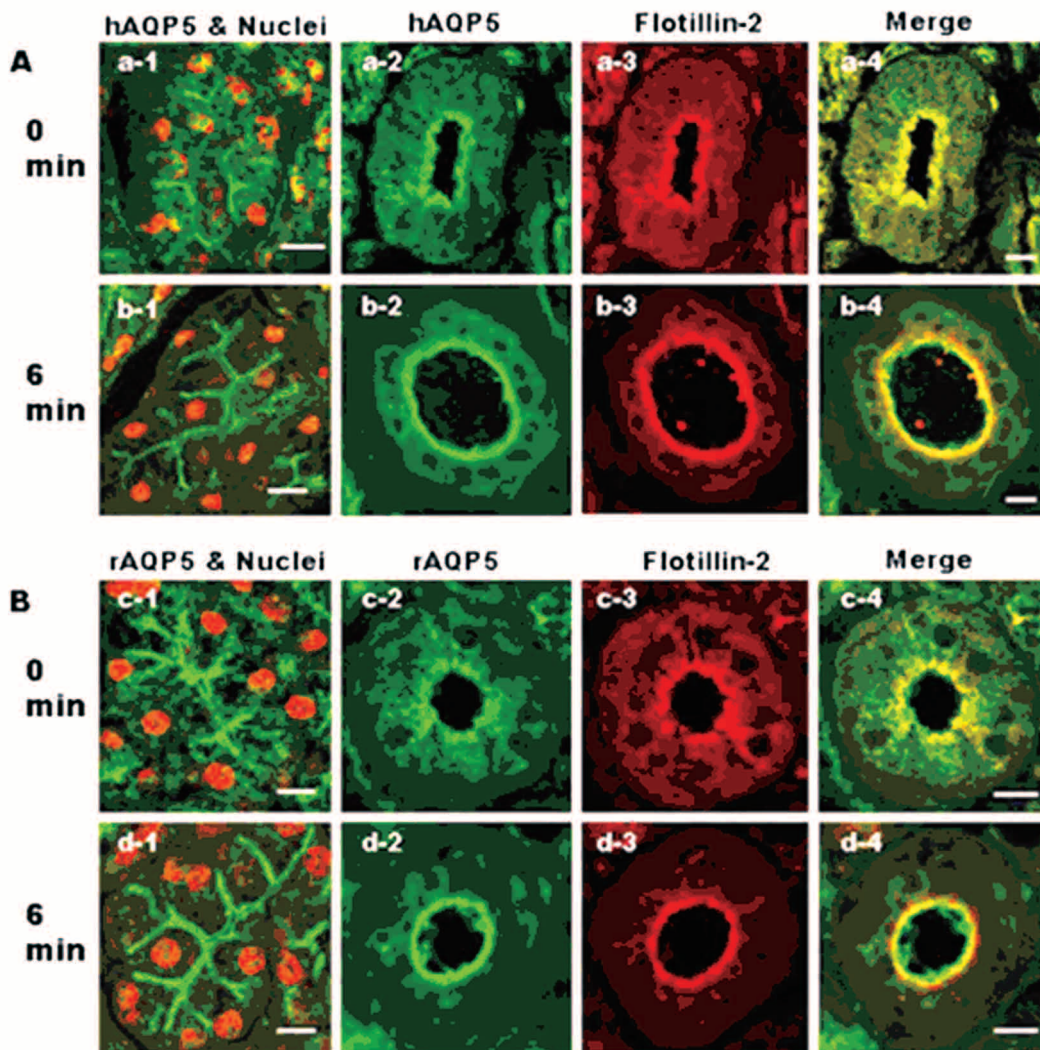


Fig. 1. Confocal immunofluorescence microscopic images of tissue slices showing changes in AQP5 in acinar and duct cells of rat parotid glands treated with cevimeline. The rat parotid gland was removed at 0 (a and c) and 6 (b and d) min after intravenous injection of cevimeline (5 mg/kg), rapidly frozen with liquid nitrogen. Frozen sections (7 μ m) were immediately fixed by prechilled ethanol. Sections were immunostained with anti-human (h) AQP5 antibody (A) and anti-rat (r)AQP5 antibody (B) to detect AQP5 in acinar (-1) and duct (-2, -3, and -4) cells using Alexa Fluor 488 (green). Nuclei were stained by propidium iodide (-1). Sections were also immunostained with anti-flotillin-2 antibody using Alexa Fluor 568 (-2, -3, and -4). Bars, 10 μ m.

the volume of unstimulated saliva secretion (10).

Cevimeline (*i.e.*, SNI-2011 or AF102B) induces long-lasting salivation (11). Forty minutes after cevimeline administration (30 mg/60 kg body weight), salivary flow rates increased, reaching maximum levels at 70 min, which then gradually declined to the levels found in resting saliva at 160 min. The changes in salivary AQP5 levels after cevimeline administration were parallel to the changes in salivary flow rates. Salivary flow rates were strongly correlated with the concentration of salivary AQP5 ($R=0.94$, $p<0.001$) and osmolality was strongly and negatively correlated with the concentration of salivary AQP5 in the saliva provoked by single administration of cevimeline (*p.o.*; $R=-0.81$, $p<0.001$). These findings indicate that in humans of the same age changes in salivary AQP5 levels can be used as an index of the salivary flow rate and the effect of M_3 mAChR agonists on salivary glands.

ASSOCIATION OF AQP5 WITH LIPID RAFTS IN HUMAN SALIVA

AQP5 localizes with lipid rafts in the cytoplasm of acinar and duct cells in rat parotid glands, and an increase in intracellular Ca^{2+} concentration mediates the effect of M_3 mAChR agonists on the translocation of AQP5 with lipid rafts to the APM, followed by the dissociation of AQP5 from the lipid rafts to non-rafts in the APM (9). The major portion of the AQP5 of unstimulated saliva and cevimeline-stimulated saliva was present in the supernatant fraction (Sup) (Fig. 2A) obtained by centrifugation at 100,000 g for 60 min of Sup (1), indicating that salivary AQP5 did not come from sloughed cells and zymogen granules. Lipid rafts are insoluble in 1% Triton X-100 at 4°C (12). As shown in Fig. 2B and 2C, 80% of the total amount of AQP5 in human saliva was detected in the 1% Triton X-100-soluble fraction (Sup(3)) (Fig. 2), whereas salivary GM1, a

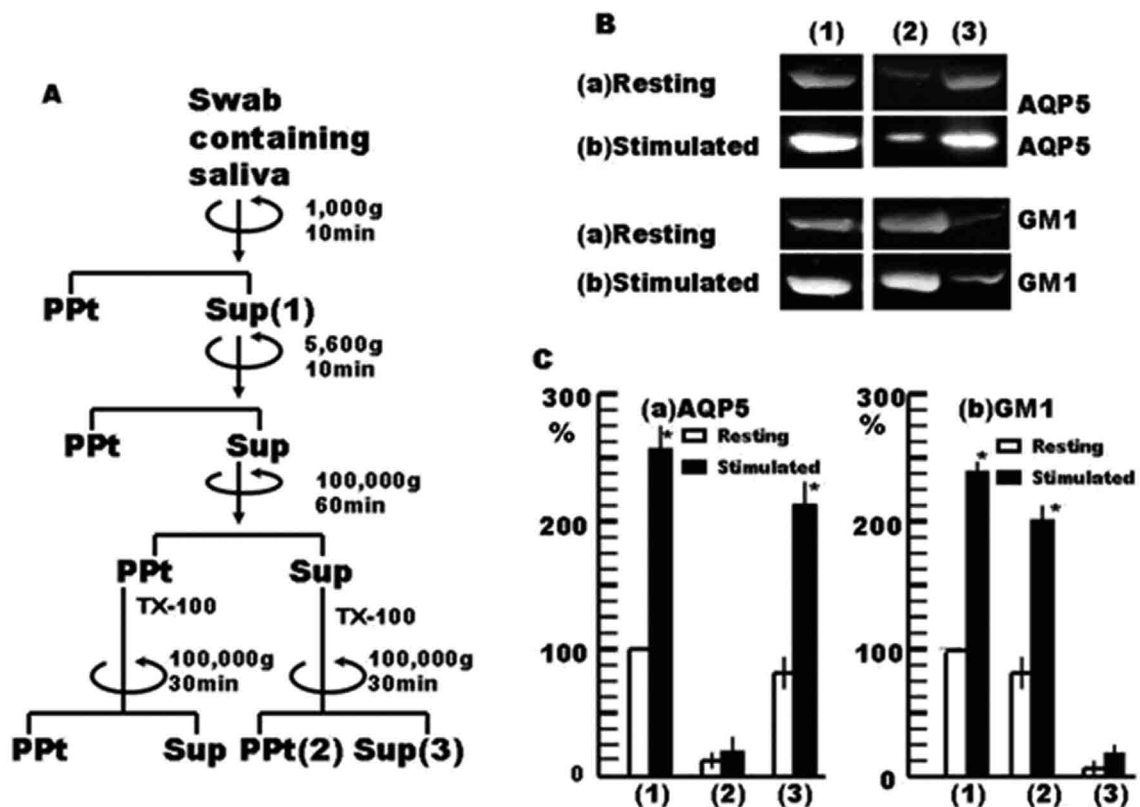


Fig. 2. Association of AQP5 and GM1 in human saliva.

Saliva was collected for 5 min from healthy subjects ranging in age from 20 to 39 years before and 60 min after a single oral dose of cevimeline (30 mg/60 kg of body weight). As shown in A, after centrifugation of saliva at 1000 g for 10 min, the resultant supernatant (Sup (1)) was centrifuged at 5600 g for 10 min to remove zymogen granules and their fragments (PPt). The resultant supernatant (Sup) was centrifuged at 100,000 g for 60 min to separate the membrane particles (PPt). The resultant supernatants (Sup) were treated with 1% TX-100 to separate the TX-100-soluble (Sup (3)) and -insoluble (PPt (2)) fractions. These fractions were analyzed by Western blotting with anti-human AQP5 and anti-GM1 antibodies (B). The intensity of chemiluminescence of AQP5 is expressed in (C) as the relative intensity of the chemiluminescence of AQP5, with the corresponding value for Sup (1), which was obtained from resting saliva and taken as 100%. The values and bars shown are the means of 4 to 6 separate experiments \pm S.E. A value of $p<0.01$ was considered significant. * <0.01 .

marker of lipid rafts, was detected in the 1% Triton X-100-insoluble fraction (PPt(2)) (Fig. 2), indicating that AQP5 in human saliva was not associated with lipid rafts.

SALIVARY AQP5 LEVELS IN DISEASE

It is well known that salivary flow rates are impaired in Type 1 diabetics (13) and Sjögren's syndrome (14). In patients with diabetes mellitus or Sjögren's syndrome, the decrease in the salivary secretion was concomitant with a decrease in AQP5 levels in the saliva (data not shown). In Alzheimer patients treated with donepezil, salivary secretion and salivary AQP5 levels were increased compared with those from same-age subjects without Alzheimer's disease (data not shown).

CONCLUSION

Changes in the amount of salivary AQP5 may be a useful index of the salivary flow rate and of the M₃ mAChR agonist effect in the human salivary gland. Salivary AQP5 levels may also be useful as a marker of xerostomia.

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