MINI-REVIEW

The salivary gland fluid secretion mechanism

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Abstract : Fluid secretion by exocrine glands requires the coordinated activity of multiple water and ion transporter and channel proteins. The molecular cloning of many of the transporter molecules involved in fluid secretion has yielded a better understanding of the fluid secretion process. Mouse salivary glands are easily accessible model systems for the study of exocrine gland secretion at the cellular and organ level. Indeed, the characterization of mice with null mutations in many of the water and ion transporter and channel genes has demonstrated the physiological roles of individual proteins. This overview will focus on recent developments in determining the molecular identification of the proteins that are involved in the fluid secretion process. J. Med. Invest. 56 Suppl. : 192-196, December, 2009

Keywords **:** *fluid secretion, NaCl absorption, salivary glands*

INTRODUCTION

Saliva is a mixture of fluids secreted by the three major salivary glands, i.e. parotid, submandibular and sublingual glands, with a slight contribution from many minor glands within the oral cavity. Human salivary glands secrete typically 0.5-1 liter of saliva per day in response to sympathetic and parasympathetic stimulation (1). The saliva secreted by the major glands differs in composition but the mechanism by which this fluid is secreted is highly conserved. Classical micropuncture experiments performed in rat submandibular glands demonstrated that saliva formation involves two stages (2) : acinar endpieces produce an isotonic plasma-like primary saliva (stage 1). This NaCl-rich fluid is modified during its passage along the ductal epithelium, where most of the NaCl is reabsorbed, while K^+ is usually secreted (stage 2). Because ductal epithelium is poorly permeable to water, the final saliva is usually hypotonic. Fig. 1 summarizes the saliva formation process.

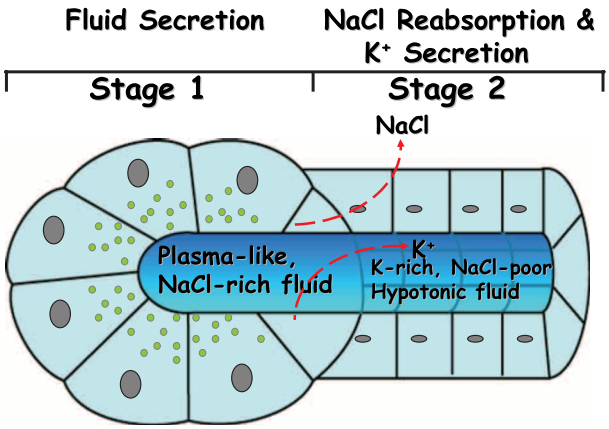

Fig. 1 Two stage salivary gland secretion model. In stage 1, acinar cell secrete a NaCl-rich fluid called primary saliva. In stage 2, the primary saliva is subsequently modified through its passage along the ductal tree mostly by reabsorbing NaCl and secreting $KHCO_3$. Because the ductal epithelium is poorly permeable to H_2O, the final saliva is hypotonic.

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PRIMARY SALIVA SECRETION MECHANISM

Secretion of the primary saliva fluid takes place in the secretory endpieces, also called acinar cells. There is a functional coupling mechanism between salt and fluid secretory processes. Ion channels and transporters expressed at the apical and basolateral membranes of the secretory cells play a key role in fluid secretion, their coordinated activity promotes a vectorial ion transport in the secretory direction, from the serosal (basolateral) to luminal (apical) side. An osmotic gradient is established upon ion secretion, thus promoting the transcellular movement of water through aquaporin 5 (Aqp5), the major water channel expressed in the apical membrane of secretory acinar cells. Consistent with transcellular water movement playing a major role in this process, fluid secretion and acinar cell volume regulation are dramatically impaired in mice lacking Aqp5 (3, 4). However, some contribution of a parallel, paracellular-driven water secretion mechanism cannot be ruled out (5, 6).

Water movement in salivary glands requires Cl^- secretion (7-9). Cl^- is secreted transcellularly by acinar cells, which indicates that Cl^--transporting proteins expressed at the basolateral membrane must accumulate intracellular Cl^- above its equilibrium potential. The basolateral Na^+-K^+-2 Cl^- cotransporter encoded by the *Nkcc1* gene (*Slc12a2*) is the main Cl^--concentrative component. Accordingly, fluid secretion is severely impaired (more than 70%) in salivary glands of mice lacking the Nkcc1 cotransporter (10). The residual fluid secretion observed in the $Nkcc1^{-/-}$ mice is HCO_3^--dependent, suggesting that there is a second Cl^- concentrating mechanism. This alternative Cl^- uptake mechanism is dependent on the coordinated activities of the basolateral Na^+/H^+ and Cl^-/HCO_3^- exchangers. Salivary gland fluid secretion is reduced by about 30% in mice lacking the basolateral Na^+/H^+ exchanger Nhe1, supporting the physiological significance of the HCO_3^--dependent fluid secretion mechanism (11). The molecular identity of the basolateral Cl^-/HCO_3^- exchanger involved in this process has not been directly demonstrated, but it is most likely encoded by the *Ae2* gene (12).

Acinar cell Cl^- influx across the basolateral membrane is energized by the Na^+ electrochemical gradient. The Na^+, K^+ ATPase, which is highly expressed in the basolateral membrane of salivary glands secretory cells, maintains an inward-directed Na^+ electrochemical gradient. The Na^+, K^+ ATPase exports 3 Na^+ and imports 2 K^+ per hydrolysis of a molecule of ATP. As a consequence of the coordinated Nkcc1 and the Na^+, K^+ ATPase activities, Cl^- is concentrated in the intracellular space above its equilibrium potential. The importance of the Na^+, K^+ ATPase activity to salivary gland fluid secretion was demonstrated by Petersen and Poulsen. They showed that the fluid secretion by the cat submandibular gland was severely impaired by inhibiting the Na^+, K^+ ATPase activity with ouabain, a specific inhibitor of Na^+ pumps (13).

An apical Cl^- pathway is required to mediate Cl^- efflux across the luminal membrane. Iwatsuki, *et al.* first demonstrated an acetyl choline-evoked Ca^{2+}-dependent Cl^- conductance in whole cell recordings performed in mouse and rat parotid acinar cells (14). They argued that this Ca^{2+}-activated Cl^- conductance is associated with Cl^- secretion. Several Cl^- channels have been postulated to be the molecular counterpart of this Cl^- conductance, i.e. the cAMP-activated Cl^- channel CFTR, hyperpolarization-gated ClC-2, and Ca^{2+}-activated Cl^- channel Best2. However, salivary gland fluid secretion was not affected in CftrDF508, $ClC\text{-}2^{-/-}$ or $Best2^{-/-}$ knockout mice, suggesting that these channels do not play a major role in salivary gland fluid secretion (15, 16 and unpublished results). The molecular identification of the apical Cl^- channel mediating Cl^- secretion has been a major focus of the salivary gland research field for a number of years. Tmem16A, a Ca^{2+}-activated Cl^- channel highly expressed in several epithelia, including the salivary glands, was recently cloned and functionally characterized. The results from this study strongly suggest that the native CaCC expressed in salivary gland tissue is encoded by the *TMEM16A* gene (17). Unfortunately, mice lacking TMEM16A channels die a few days after birth apparently because of an airway malformation, making functional experiments to test the fluid secretion mechanism in adult salivary glands impossible (18). Using an alternative approach to modify expression, *in vivo* knockdown of Tmem16A by siRNA silencing resulted in a modest reduction in fluid secretion in mice (17). Moreover, we have demonstrated that a Ca^{2+}-dependent Cl^- conductance is the main Cl^- conductance in mouse, rat and human acinar cells (15, 19, 20). Interestingly, this Ca^{2+}-dependent Cl^- conductance disappears in acinar cells isolated from the mice lacking TMEM16A (unpublished results). Taken together, the results presented above suggest that TMEM16A is the apical Cl^- channel involved in salivary gland Cl^- secretion.

A K^+ channel located at the basolateral membrane of secretory cells is necessary to complete the Cl^- secretory molecular machinery. The primary saliva secretion mechanism is shown in the Fig. 2.

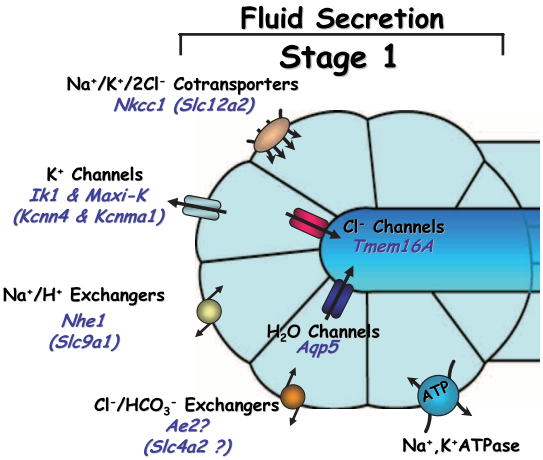

Fig. 2 Primary saliva secretion model. Acinar cells secrete fluid in a Cl^--dependent manner. The coordinated activity of ion channels, water channels, pumps, cotransporters and exchangers results in the primary saliva formation. The molecular components of this physiological process and their gene names are shown. Individual acinar cells express all of the different transport mechanisms, but are spread out for clarity.

A basolateral K^+ channel recycles K^+ ions to the extracellular space and its opening hyperpolarizes the membrane, thus increasing the driving force for Cl^- exit. Two different K^+ channels have been characterized in salivary gland acinar cells, one of which is a Ca^{2+}-activated K^+ channel of intermediate single channel conductance commonly named IK1 or SK4, and is encoded by the *Kcnn4* gene. The second K^+ channel is both Ca^{2+}- and voltage-activated with a large single channel conductance. It is named maxi K or Slo and is encoded by the *Kcnma1* gene (21). Surprisingly, salivary gland fluid secretion was not affected in mice lacking either the IK1 channel or the Slo channel (22, 23). In contrast, fluid secretion was severely impaired in mice lacking both IK1 and Slo channels, suggesting that either type of K^+ channel can independently support fluid secretion (23).

PRIMARY SALIVA IS MODIFIED BY SALIVARY GLAND DUCTS

The final ionic composition of saliva is the result of transport processes in the acini as well as the ducts system. Salivary gland ducts are composed of several different cell types and their composition differs between salivary glands. Basically there are three main types of ducts in salivary glands : intercalated, striated and excretory ducts. Intercalated and striated ducts are intralobular and excretory ducts are primarily extralobular. A comparison of the ion composition and osmolality of the saliva collected in intralobular and extralobular ducts suggests that the NaCl reabsorption takes place in both intra and extralobular ducts (2, 24-26). Fig. 3 summarizes the ion channels and transporters involved in salivary gland ductal function.

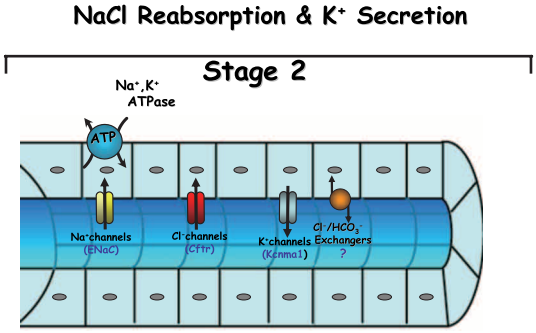

Fig. 3 Ductal function model. Salivary gland duct cells reabsorb NaCl and secrete $KHCO_3$. NaCl reabsorption exceeds $KHCO_3$ secretion resulting in a hypotonic final saliva. Ion channels, pumps and exchangers involved in ductal function are shown in this figure.

It has been proposed that the epithelial Na^+ channel ENaC, which is expressed in the apical membrane of salivary gland ducts, plays a key role in ductal Na^+ reabsorption. ENaC blockade by low doses of amiloride (10 μM) impaired Na^+ reabsorption as well as the transepithelial potential difference in the main duct of the rat submandibular gland (27). The functional expression of ENaC was also demonstrated at the single cell level by whole-cell recordings in intralobular duct cells isolated from the mouse submandibular gland (28). A Na^+/H^+ exchanger expressed at the apical membrane of salivary gland duct cells is also thought to be involved in the Na^+ reabsorption process (29). Immunolocalization studies performed in both rat and mouse salivary glands show that the Na^+/H^+ exchangers Nhe2 and Nhe3 are expressed in the apical pole of duct cells (11, 30). However, Na^+ reabsorption is not impaired in mice lacking either Nhe2 or Nhe3 (11). Together, the experimental evidence strongly suggests that ENaC Na^+ channels are the main molecular component involved in Na^+ reabsorption by the salivary gland duct epithelium.

Cl^- is also actively reabsorbed in salivary gland ducts. Apical Cl^- channels and Cl^-/HCO_3^- exchangers have been postulated to mediate Cl^- reabsorption in salivary gland duct epithelium (29). The Cl^- channel Cftr, which is mutated in the disease cystic fibrosis (CF), appears to play a key role in Cl^- reabsorption. Cftr is expressed in the apical membrane of rat and mouse submandibular gland ducts (31, 32). It has been postulated that Cftr is involved in Cl^- reabsorption by modulating the activity of an apical Cl^-/HCO_3^- exchanger, the activity of which is severely impaired in ducts from the mice expressing the most common CF mutation (DF508) (31). It has been suggested that the apical Cl^-/HCO_3^- exchanger in the mouse parotid gland is encoded by the *Slc26a6* gene (33).

The K^+ concentration of saliva is higher than the concentration found in plasma. K^+ is secreted in response to secretagogues by intra and extralobular salivary gland ducts (2, 24-26). Apical K^+/H^+ exchangers, K^+-HCO_3^- cotransporters have been suggested to play a role in K^+ secretion (29). Recently, it was demonstrated that K^+ secretion is severely impaired in mice lacking Slo K^+ channels (34, 35). A similar effect was obtained upon pharmacological Slo channel blockade with paxilline, a Slo-selective blocker. On the other hand, the same study demonstrated, using a perfused *ex vivo* mouse submandibular gland, that the K^+ secretion process is not dependent on HCO_3^- secretion, suggesting that neither a K^+/H^+ exchanger nor a K^+-HCO_3^- cotransporter is involved in K^+ secretion (34). Taken together, the experimental evidence suggests that most K^+ secretion requires the functional presence of Slo K^+ channels.

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