Published in final edited form as:

Cell Calcium. 2014 June ; 55(6): 362–368. doi:10.1016/j.ceca.2014.01.005.

Ca²⁺-dependent K⁺ Channels in Exocrine Salivary Glands

Marcelo A. Catalán, Gaspar Peña-Munzenmayer, and James E. Melvin

Secretory Mechanisms and Dysfunction Section (SMDS), National Institute of Dental and Craniofacial Research, National Institutes of Health, 10 Center Drive, Building 10/Room 5N102, Bethesda, MD 20892 USA

Abstract

In the last 15 years, remarkable progress has been realized in identifying the genes that encode the ion-transporting proteins involved in exocrine gland function, including salivary glands. Among these proteins, Ca²⁺-dependent K⁺ channels take part in key functions including membrane potential regulation, fluid movement and K⁺ secretion in exocrine glands. Two K⁺ channels have been identified in exocrine salivary glands: 1) a Ca²⁺-activated K⁺ channel of intermediate single channel conductance encoded by the *KCNN4* gene; and, 2) a voltage- and Ca²⁺-dependent K⁺ channel of large single channel conductance encoded by the *KCNMA1* gene. This review focuses on the physiological roles of Ca²⁺-dependent K⁺ channels in exocrine salivary glands. We also discuss interesting recent findings on the regulation of Ca²⁺-dependent K⁺ channels by protein-protein interactions that may significantly impact exocrine gland physiology.

Keywords

Ca²⁺-dependent K⁺ channels; K⁺ secretion; exocrine glands; epithelial ion transport

Introduction

Saliva is a protein-rich fluid secreted by three paired sets of major glands (parotid, submandibular and sublingual) and numerous minor salivary glands. Saliva plays many physiological roles including, but not limited to, digestion of carbohydrates and lipids, demineralization and remineralization of teeth, and colonization of oral surfaces by bacteria [1–5]. The clinical importance of saliva is clearly observed in patients suffering from loss of salivary gland function as a consequence of diseases such as Sjögren's syndrome and diabetes, medication side-effects, and head and neck cancer therapies. Dry mouth is often associated with oral pain, dysphagia, reduced taste sensation and opportunistic infections [6].

Address correspondence to: Marcelo A. Catalán. National Institute of Dental and Craniofacial Research, National Institutes of Health, 10 Center Drive, Building 10/Room 5N102, Bethesda, MD 20892. Tel: (301)451-8707, Fax: (301)480-4455, marcelo.catalan@nih.gov Or James E. Melvin. National Institute of Dental and Craniofacial Research, National Institutes of Health, 10 Center Drive, Building 10/Room 5-2531, Bethesda, MD 20892. Tel: (301)827-1647, Fax: (301)480-4455, james.melvin@nih.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Humans typically secrete 0.5–1.5 liters of saliva into the oral cavity each day in response to sympathetic, parasympathetic and hormonal stimulation [3, 7]. The underlying fluid secretion mechanism is highly conserved in the different salivary glands and perhaps other exocrine glands such as sweat and lacrimal glands. The secretory acinar cells of salivary glands express numerous functionally distinct ion-transporting proteins that are primarily targeted to either the apical or basolateral domain to support fluid secretion, including Ca^{2+} -dependent K^+ channels [8].

In this review, we focus on the major roles that Ca^{2+} -dependent K^+ channels play in salivary gland function. We also discuss recent findings on the regulation of Ca^{2+} -dependent K^+ channels by protein-protein interactions. These studies have enhanced our understanding of how such protein complexes might regulate salivary gland function.

A K^+ channel-dependent acinar cell fluid secretion model

The pioneering work of Thaysen et al. provided important clues as to how saliva formation occurs in humans. Based on the observation that the ionic composition of saliva is dependent on the flow rate, they hypothesized that the salivary secretion process involves two different stages [9]. One decade later, Young and Schogel, and Martinez et al. performed micropuncture studies on rat salivary glands that directly supported Thaysen's findings [10, 11]. Together, these studies formed the basis of the current fluid secretion model, which predicts the following: acinar cells secrete a NaCl-rich, plasma-like isotonic fluid, the primary saliva (Stage 1). The primary saliva drains into the ductal system, which subsequently modifies the ion content of the saliva by reabsorbing most of the NaCl secreted by acinar cells and secreting KHCO_3 to form the final secreted saliva (Stage 2). NaCl reabsorption generally exceeds KHCO_3 secretion, and because the ductal epithelium is poorly permeable to H_2O , the resulting final saliva is hypotonic. Figure 1 summarizes the current saliva secretion model and shows the morphology of the human submandibular and parotid glands, which are mainly composed of acinar and duct cells.

It is well known that salivary gland acinar cells are primarily responsible for fluid secretion [8, 12, 13]. To generate saliva, acinar cells express several different ion-transporting proteins that are largely targeted to either the apical or the basolateral domain. The coordinated activity of these transporter proteins generates a net flux of ions in the secretory direction (i.e., from the serosal to the luminal side). The net transport of ions in the secretory pathway creates an osmotic gradient, which energizes water transport into the luminal space. It appears that water is predominantly secreted via a transcellular pathway that involves the apical Aqp5 water channel [14, 15], although some have suggested that water movement also occurs via a paracellular pathway [16, 17].

Transepithelial Cl^- movement drives salivary gland fluid secretion. This process can be explained by a simple mechanism originally proposed by Koefoed-Johnsen and Ussing to describe how Na^+ ions are absorbed across frog skin epithelium. This mechanism, known as the “pump-leak” or “two-membrane” mechanism, provides the basis for understanding ion transport processes across several epithelia, including the Cl^- transport that occurs in salivary glands [8, 12, 18, 19].

In agreement with a pump-leak mechanistic model, Cl^- influx in salivary gland acinar cells occurs through a basolateral “pump”, while Cl^- ions are extruded via an apical “leak”. The primary basolateral Cl^- pump in salivary glands is a functional complex composed of Na^+ - K^+ - 2Cl^- co-transporters, Na^+/K^+ ATPases, and K^+ channels [20–23]. The coordinated activity of these proteins concentrates Cl^- above its equilibrium potential (E_{Cl}). Figure 2A shows the proposed Na^+ - K^+ - 2Cl^- co-transporter-dependent Cl^- pump mechanism that drives fluid secretion by salivary glands. An apical Cl^- leak pathway, Ca^{2+} -activated Cl^- channels, completes the acinar Cl^- secretory machinery required to produce the primary saliva secretion [24].

Although less active in fluid secretion than the Na^+ - K^+ - 2Cl^- co-transporter-dependent Cl^- pump, there is often another Cl^- uptake mechanism present in salivary gland acinar cells. In contrast to the Na^+ - K^+ - 2Cl^- co-transporter-dependent Cl^- pump, this secondary Cl^- influx pathway relies on the parallel activity of basolateral Na^+/H^+ and $\text{Cl}^-/\text{HCO}_3^-$ exchangers, along with Na^+/K^+ ATPases and K^+ channels [25–27]. It has been demonstrated that the Na^+/H^+ and $\text{Cl}^-/\text{HCO}_3^-$ exchangers and the Na^+ - K^+ - 2Cl^- co-transporters are co-expressed in the same, not two different, acinar cell populations [28]. Figure 2B shows the salivary gland fluid secretion model that utilizes a paired Na^+/H^+ and $\text{Cl}^-/\text{HCO}_3^-$ exchangers-dependent Cl^- pump mechanism.

Multiple research teams have recently made significant progress in determining the genes that encode the ion transporter and channel proteins involved in salivary gland fluid secretion. Table 1 summarizes gene names, transporting activity, localization and functional roles of the key proteins that have been identified to date [14, 15, 29–35]. Detailed descriptions of the physiological roles of the various ion transporting proteins involved in fluid secretion can be found elsewhere [8, 12].

As described above, K^+ channels are key components of the primary and the secondary basolateral Cl^- pump systems (Figures 2A & 2B). Na^+ -dependent Cl^- uptake by both of these pathways is driven by the ~15:1 inward-directed Na^+ chemical gradient created by the Na^+/K^+ ATPase. According to the current fluid secretion model, K^+ channels provide an essential basolateral pathway to recycle K^+ ions into the serosal space, which is necessary for Na^+/K^+ ATPase activity. Thus, K^+ ions are obligatory for efficient intracellular Na^+ /extracellular K^+ exchange via basolateral Na^+/K^+ ATPases [36, 37]. In addition to K^+ recycling, during stimulation K^+ channel opening hyperpolarizes the acinar cell membrane, which increases the electrochemical driving force for Cl^- exit via the apical Ca^{2+} -activated Cl^- channel [27, 34, 38].

Activation of the M3 muscarinic-type acetylcholine receptor, a G-protein coupled plasma membrane receptor, generates an increase in the intracellular Ca^{2+} concentration. This increase in the intracellular free $[\text{Ca}^{2+}]$ serves as the primary fluid secretion stimulatory signal in acinar cells [39] by activating Ca^{2+} -dependent K^+ and Cl^- channels. The binding of acetylcholine to M3 receptors activates phospholipase C (PLC), which cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and diacyl glycerol (DAG). Newly generated IP_3 diffuses through the cytosol and binds to IP_3 receptor- Ca^{2+} channels located in the endoplasmic reticulum. The activation of IP_3 receptor-

channels promotes Ca^{2+} movement from the endoplasmic reticulum to the cytosol, thereby causing an increase in cytosolic $[\text{Ca}^{2+}]$.

Ca^{2+} mobilization from intracellular stores during stimulation accounts for the initial rise in cytosolic Ca^{2+} levels. However, to support sustained fluid secretion, depletion of the IP_3 -sensitive intracellular stores stimulates prolonged Ca^{2+} influx mediated through Ca^{2+} -permeable store operated channels (SOCs) located in the plasma membrane. Intracellular Ca^{2+} store depletion induces SOCs activation and Ca^{2+} influx known as capacitative Ca^{2+} entry. This process has been extensively studied in salivary glands and is reviewed elsewhere [19, 40].

K^+ channels can regulate fluid secretion by modulating capacitative Ca^{2+} entry during sustained stimulation. Ca^{2+} influx via plasma membrane SOCs causes depolarization, and thus reduces the driving force for Ca^{2+} influx. Consequently, the coordinated opening of K^+ channels maintains the membrane potential close to E_K , thereby maintaining the driving force for Ca^{2+} influx. The regulation of capacitative Ca^{2+} entry by K^+ channels in acinar cells has not been directly tested. However, consistent with this role for K^+ channels, manipulations that cause depolarization inhibit Ca^{2+} entry during stimulation [41] and a similar effect has been reported in human macrophages [42].

Two different K^+ currents have been observed in human and rodent salivary gland acinar cells. One of these K^+ currents is generated by a Ca^{2+} -activated K^+ channel encoded by the *KCNN4* gene. This Ca^{2+} -activated K^+ channel has an intermediate single channel conductance (12–39 pS) that has been given numerous names such as $\text{K}_{\text{Ca}3.1}$, IK1, SK4 or the Gardos channel [43–45]. The second K^+ channel is referred to as $\text{K}_{\text{Ca}1.1}$, maxi-K, Slo1 or BK channel, has a large single channel conductance (200–272 pS), and is encoded by the *KCNMA1* gene [38, 46, 47]. The $\text{K}_{\text{Ca}1.1}$ channel is gated by membrane depolarization and is modulated by intracellular Ca^{2+} .

Given that both K^+ channels are modulated by intracellular Ca^{2+} , it was hypothesized that $\text{K}_{\text{Ca}1.1}$ and/or $\text{K}_{\text{Ca}3.1}$ might be involved in fluid secretion. Unexpectedly, salivary gland fluid secretion was unaffected in mice lacking $\text{K}_{\text{Ca}1.1}$ or $\text{K}_{\text{Ca}3.1}$ channels [38, 48]. In contrast, fluid secretion was severely impaired in mice lacking both $\text{K}_{\text{Ca}1.1}$ and $\text{K}_{\text{Ca}3.1}$ channels ($\text{K}_{\text{Ca}1.1}^{-/-}/\text{K}_{\text{Ca}3.1}^{-/-}$), suggesting that either of these K^+ channels can independently support fluid secretion [38]. Moreover, membrane potential measurements of isolated acinar cells showed that $\text{K}_{\text{Ca}1.1}^{-/-}$ or $\text{K}_{\text{Ca}3.1}^{-/-}$ cells hyperpolarized toward the equilibrium potential of K^+ (E_K) in response to muscarinic stimulation, but hyperpolarization was not observed in cells deficient in both channels ($\text{K}_{\text{Ca}1.1}^{-/-}/\text{K}_{\text{Ca}3.1}^{-/-}$). Taken together, these findings demonstrate that K^+ channels play an essential role in acinar cell fluid secretion by increasing the electrochemical driving force required for Cl^- exit into the luminal space and that either the $\text{K}_{\text{Ca}1.1}$ or $\text{K}_{\text{Ca}3.1}$ channel is sufficient to hyperpolarize the acinar cell and support secretion.

K^+ channels and K^+ secretion in salivary glands

In humans, the K^+ concentration ($[\text{K}^+]$) of saliva is five to ten times as high as in plasma [49]. Salivary gland micropuncture studies revealed that the $[\text{K}^+]$ of saliva is inversely

proportional to the saliva secretion rate and that a nearly maximal $[K^+]$ is reached when the saliva passes through the early proximal segments of the ductal epithelium. This finding suggests that most K^+ ions are secreted by the intralobular ducts, although it has been shown that extralobular ducts also support K^+ secretion [11, 50, 51]. The lumen of intralobular ducts is quite narrow, making it technically difficult to perfuse intralobular ducts. Consequently, there are no reports of direct measurements of K^+ secretion by intralobular ducts, so what we know about K^+ secretion by salivary gland ducts comes from experiments performed on perfused main excretory ducts. Using this experimental approach, it was shown that K^+ secretion was dependent on an apical Na^+ channel [52], although another study showed that K^+ secretion in rat submandibular gland was not affected upon adrenalectomy, a maneuver that decreases the circulating levels of aldosterone and expression of the epithelial Na^+ channel ENaC [53].

Apical electroneutral K^+/H^+ exchanger- and/or $K^+-HCO_3^-$ co-transporter-dependent mechanisms have often been postulated to support K^+ secretion, but there is no direct functional or molecular evidence for these ion transporters in duct cells [25]. In fact, K^+ secretion was not dependent on HCO_3^- in perfused *ex vivo* mouse submandibular glands, suggesting that neither a K^+/H^+ exchanger nor a $K^+-HCO_3^-$ co-transporter is involved in K^+ secretion by mouse salivary glands [54]. Nevertheless, a minor physiological contribution by K^+ exchangers and co-transporters cannot be completely ruled out, especially at low flow rates [11].

Given that exchanger- and co-transporter-dependent mechanisms contribute little to K^+ secretion, it was postulated that an apical electrogenic K^+ efflux pathway, most likely a K^+ channel, may be required for K^+ secretion in salivary glands. Indeed, K^+ secretion is drastically reduced in mice lacking $K_{Ca1.1}$ K^+ channels, but not in mice deficient in $K_{Ca3.1}$ K^+ channels [34, 54]. Notably, pharmacological K^+ channel block by the $K_{Ca1.1}$ -selective blocker paxilline similarly inhibited K^+ secretion [54]. Together, these results suggest that the $K_{Ca1.1}$ channel is a major K^+ secretion pathway in mouse submandibular glands. Figure 3 shows three proposed mechanisms that account for K^+ secretion in salivary gland ductal epithelium.

Micropuncture studies on salivary glands also showed that the $[K^+]$ in the primary saliva is significantly higher than observed in plasma [11, 50, 51]. These observations suggest that acinar cells are capable of secreting K^+ ions into the primary saliva. However, the physiological relevance of the acinar-mediated K^+ secretion has not received much attention. Nevertheless, there is compelling evidence suggesting that acinar-mediated K^+ secretion might play an important role in salivary gland physiology. First, the inverse relationship between $[K^+]$ and the flow rate that is associated with ductal function appears to occur only at very low flow rates, even lower than those reported in a mouse hyposalivation model that lacks the Aqp5 channel, the key apical water pathway involved in salivary gland fluid secretion [11, 14, 50]. Second, Burgen et al. showed that salivary glands lose K^+ ions into the venous blood as well as into the saliva upon stimulation. It was shown that the initial rates of K^+ loss into the blood and saliva are directly related to each other and to the rate of saliva secretion [36]. We do not know the cellular source of the increase of K^+ in venous blood or saliva, i.e. acinar and/or ductal cells. However, considering that acinar cells

occupy ~80–90% of the volume of a salivary gland it is likely that acinar cells, in addition to their key role in fluid secretion, are involved in K^+ loss into the venous blood and saliva [55]. Third, stimulation induces the opening of an apical Cl^- channel and Cl^- efflux into the lumen. Unexpectedly, Lundberg found that the apical membrane potential of salivary gland acinar cells does not depolarize upon stimulation, but becomes hyperpolarized [56]. Hyperpolarization is inconsistent with the expected depolarization that should occur upon opening of an apical Cl^- channel. This result suggests that cations must also be exiting into the lumen. A K^+ channel expressed at the apical membrane could account for the stimulation-mediated hyperpolarization of the apical membrane potential.

Although K^+ channels were originally described in the basolateral domain of salivary gland acinar cells [37, 57, 58], the above evidence suggests that K^+ channels are also targeted to the apical membrane of acinar cells and likely may contribute to salivary gland K^+ secretion. Interestingly, mathematical models for fluid secretion incorporated a K^+ conductance in the apical membrane of acinar cells. These models predict that fluid secretion reaches maximal efficiency if 10–20% of the total acinar cell K^+ conductance is present in the apical membrane [59, 60]. Furthermore, recent work by Almasy et al. provided functional evidence for $K_{Ca1.1}$ and $K_{Ca3.1}$ channels in the apical membrane of mouse parotid acinar cells [61]. Considering that K^+ secretion is reduced by >75% in the submandibular salivary glands of $K_{Ca1.1}^{-/-}$ mice [54], it is plausible to speculate that acinar $K_{Ca1.1}$ channels significantly contribute to the K^+ secretion process in salivary glands.

In summary, recent findings confirm that K^+ channels are not only targeted to the basolateral domain of acinar cells, but also to the apical domain of salivary gland acinar and duct cells. The fluid secretion models depicted in Figures 2A/2B include apical K^+ channels in acinar cells where they might be involved in both fluid and K^+ secretion. Additionally, models from Figures 3A–3C include apical K^+ channels that might be involved in ductal K^+ secretion.

Regulation of K^+ channels by protein-protein interactions

Considerable progress has been achieved in defining the important biophysical properties and physiological roles of K^+ channels in salivary glands [12, 13]. However, little is known about how K^+ channel activity is regulated by interacting proteins. This section focuses on how protein subunits interact with $K_{Ca1.1}$ and $K_{Ca3.1}$ channels to modulate their functional and kinetic properties in salivary glands.

Acinar $K_{Ca1.1}$ and $K_{Ca3.1}$ channels often have overlapping roles in fluid secretion, e.g. the absence of one channel compensates for the other K^+ channel [38, 48]. However, this is an oversimplification of the interaction of these K^+ channels that does not take into account the observation that $K_{Ca1.1}$ and $K_{Ca3.1}$ channel activities are interdependent. Interestingly, Thompson and Begenisich found that the opening of $K_{Ca3.1}$ channels leads to the closure of $K_{Ca1.1}$ channels in salivary gland acinar cells [62]. Further characterization of the interaction between $K_{Ca1.1}$ and $K_{Ca3.1}$ showed that it was dependent on cholesterol, an intact cytoskeleton, and significantly, that the N-terminus of $K_{Ca3.1}$ is able to block the $K_{Ca1.1}$ pore [63, 64]. Considering that $K_{Ca1.1}$ (but not $K_{Ca3.1}$) mediates K^+ secretion, it is

likely that the block of $K_{Ca1.1}$ by the N-terminus of $K_{Ca3.1}$ may regulate K^+ secretion by salivary gland acinar cells.

The α subunit of the $K_{Ca1.1}$ channel is encoded by the *KCNMA1* gene, which is composed of 27 constitutive exons and multiple alternative exons [65]. The parotid gland $K_{Ca1.1}$ α subunit is a parotid-specific variant termed parSlo, which has been found in human and mouse parotid glands [45]. The native $K_{Ca1.1}$ channels in many cell types, as well as $K_{Ca1.1}$ channels expressed in heterologous systems including the parSlo variant, require very high Ca^{2+} concentrations (in the μM range) for significant activation at physiological voltages. However, salivary gland acinar cells do not typically raise their intracellular Ca^{2+} levels to values that exceed $[Ca^{2+}]_{free} = 500$ nM during stimulation. This suggests that the $K_{Ca1.1}$ α subunit in salivary glands may require an interacting regulatory subunit to modulate its voltage and/or Ca^{2+} sensitivity. Consistent with this model, biophysical characterization by Romanenko et al. of native salivary gland acinar cell channels found that the voltage dependence of native $K_{Ca1.1}$ channel activation was shifted towards more negative values compared to parSlo expressed in CHO-K1 cells. The $V_{0.5}$, the voltage required to achieve 50% of maximal channel conductance, for native $K_{Ca1.1}$ channels was 46 mV compared to 164 mV for parSlo expressed in CHO-K1 cells at intracellular $[Ca^{2+}]_{free} = 80$ nM, and was 17 mV vs. 120 mV, respectively, at intracellular $[Ca^{2+}]_{free} = 500$ nM [66].

The difference in the voltage dependence between native $K_{Ca1.1}$ channels in salivary gland acinar cells and parSlo expressed in heterologous systems strongly suggests that $K_{Ca1.1}$ channels have a molecular partner that accounts for this functional difference. It has been shown that salivary glands express $\beta 1$ (encoded by *KCNMB1*) and $\beta 4$ subunits (encoded by *KCNMB4*). However, the voltage dependence of native $K_{Ca1.1}$ channel currents was unaffected in salivary gland acinar cells from $\beta 1/\beta 4$ double knockout mice [66]. Importantly, Yan and Aldrich recently discovered an accessory protein for $K_{Ca1.1}$ called LRRC26 [leucine-rich repeat (LRR)-containing protein 26] or BK channel $\gamma 1$ subunit. Strikingly, co-expression of $K_{Ca1.1}$ and LRRC26 generated K^+ channels that open at negative voltages [67]. The voltage dependence displayed by $K_{Ca1.1}/LRRC26$ channels mimicked the native $K_{Ca1.1}$ channels in LNCaP (lymph node carcinoma of the prostate) cells. Indeed, parSlo/LRRC26 co-expression in CHO-K1 cells shifted the voltage dependence of parSlo channels to more negative values, comparable to the voltage dependence in native acinar $K_{Ca1.1}$ channels [68]. Moreover, LRRC26 is highly expressed in salivary glands [69]. Taken together, it is quite likely that $K_{Ca1.1}$ channels in salivary gland acinar cells are primarily composed of $K_{Ca1.1}$ $\alpha 1$ subunit and LRRC26 proteins.

K^+ channels in other exocrine glands

Exocrine glands are multi-lobed glands mainly composed of acinar and duct cells. Lacrimal, exocrine pancreas and sweat glands, like the exocrine salivary glands, secrete large amounts of fluid each day. Experimental evidence suggests that acinar cells support the bulk of fluid secretion by most exocrine glands with one important exception. The duct epithelium of the exocrine pancreas secretes considerable amounts of bicarbonate-rich fluid per day [12].

Unlike the major salivary glands, the fluid secretion mechanism of most other exocrine acinar cells has not been extensively studied. Nevertheless, several ion-transporting proteins

involved in fluid secretion in salivary glands have been reported in acinar cells from pancreas, lacrimal and sweat glands. The ion-transporting proteins in these latter tissues include $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ co-transporters [70–73], Ca^{2+} -activated Cl^- channels [55, 74–76] and Ca^{2+} -dependent K^+ channels [55, 77–80].

The fact that acinar cells from different exocrine glands appear to express the same ion-transporting mechanisms strongly suggests that the fluid secretion machinery is highly conserved among exocrine glands. Future functional studies in other exocrine glands, especially those using genetically modified animal models lacking specific ion exchangers, co-transporters and channels are required to identify the proteins involved in fluid secretion and to confirm their functional importance at the molecular level.

Acknowledgments

The authors thank Frances Liu for critical reading of this manuscript and Dr. Ted Begenisich for discussions over the years. SMDS is supported by the Intramural Research Program of the NIH, NIDCR.

References

1. Amerongen AV, Veerman EC. Saliva--the defender of the oral cavity. *Oral Dis.* 2002; 8:12–22. [PubMed: 11936451]
2. Hay, DL.; Bowen, WH. The functions of salivary proteins. In: Edgar, WM.; O'Mullane, DM., editors. *Saliva and oral health.* 2. London, UK: British Dental Association; 1996.
3. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. *J Prosthet Dent.* 2001; 85:162–169. [PubMed: 11208206]
4. Mandel ID. Impact of saliva on dental caries. *Compend Suppl.* 1989:S476–481. [PubMed: 2691081]
5. Sonies BC, Ship JA, Baum BJ. Relationship between saliva production and oropharyngeal swallow in healthy, different-aged adults. *Dysphagia.* 1989; 4:85–89. [PubMed: 2640184]
6. Daniels TE, Fox PC. Salivary and oral components of Sjogren's syndrome. *Rheum Dis Clin North Am.* 1992; 18:571–589. [PubMed: 1496162]
7. Jensen SB, Pedersen AM, Reibel J, Nauntofte B. Xerostomia and hypofunction of the salivary glands in cancer therapy. *Support Care Cancer.* 2003; 11:207–225. [PubMed: 12673459]
8. Catalán, MA.; Ambatipudi, KS.; Melvin, JE. *Physiology of the Gastrointestinal Tract.* 5. 2012. Salivary Gland Secretion; p. 1229–1249.
9. Thaysen JH, Thorn NA, Schwartz IL. Excretion of sodium, potassium, chloride and carbon dioxide in human parotid saliva. *Am J Physiol.* 1954; 178:155–159. [PubMed: 13180731]
10. Martinez JR, Holzgreve H, Frick A. Micropuncture study of submaxillary glands of adult rats. *Pflugers Arch Gesamte Physiol Menschen Tiere.* 1966; 290:124–133.
11. Young JA, Schogel E. Micropuncture investigation of sodium and potassium excretion in rat submaxillary saliva. *Pflugers Arch Gesamte Physiol Menschen Tiere.* 1966; 291:85–98.
12. Lee MG, Ohana E, Park HW, Yang D, Muallem S. Molecular mechanism of pancreatic and salivary gland fluid and HCO_3^- secretion. *Physiol Rev.* 2012; 92:39–74. [PubMed: 22298651]
13. Melvin JE, Yule D, Shuttleworth T, Begenisich T. Regulation of fluid and electrolyte secretion in salivary gland acinar cells. *Annu Rev Physiol.* 2005; 67:445–469. [PubMed: 15709965]
14. Krane CM, Melvin JE, Nguyen HV, et al. Salivary acinar cells from aquaporin 5-deficient mice have decreased membrane water permeability and altered cell volume regulation. *J Biol Chem.* 2001; 276:23413–23420. [PubMed: 11290736]
15. Ma T, Song Y, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. *J Biol Chem.* 1999; 274:20071–20074. [PubMed: 10400615]

16. Kawedia JD, Nieman ML, Boivin GP, et al. Interaction between transcellular and paracellular water transport pathways through Aquaporin 5 and the tight junction complex. *Proc Natl Acad Sci U S A*. 2007; 104:3621–3626. [PubMed: 17360692]
17. Murakami M, Murdiastuti K, Hosoi K, Hill AE. AQP and the control of fluid transport in a salivary gland. *J Membr Biol*. 2006; 210:91–103. [PubMed: 16868676]
18. Koefoed-Johnsen V, Ussing HH. The nature of the frog skin potential. *Acta Physiol Scand*. 1958; 42:298–308. [PubMed: 13544986]
19. Reuss L. Ussing's two-membrane hypothesis: the model and half a century of progress. *J Membr Biol*. 2001; 184:211–217. [PubMed: 11891544]
20. Compton J, Martinez JR, Martinez AM, Young JA. Fluid and electrolyte secretion from the isolated, perfused submandibular and sublingual glands of the rat. *Arch Oral Biol*. 1981; 26:555–561. [PubMed: 6947759]
21. Martinez JR, Cassity N. Effect of transport inhibitors on secretion by perfused rat submandibular gland. *Am J Physiol*. 1983; 245:G711–716. [PubMed: 6314825]
22. Turner RJ, George JN, Baum BJ. Evidence for a $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransport system in basolateral membrane vesicles from the rabbit parotid. *J Membr Biol*. 1986; 94:143–152. [PubMed: 3560199]
23. Nauntofte B, Poulsen JH. Effects of Ca^{2+} and furosemide on Cl^- transport and O_2 uptake in rat parotid acini. *Am J Physiol*. 1986; 251:C175–185. [PubMed: 2426956]
24. Iwatsuki N, Maruyama Y, Matsumoto O, Nishiyama A. Activation of Ca^{2+} -dependent Cl^- and K^+ conductances in rat and mouse parotid acinar cells. *Jpn J Physiol*. 1985; 35:933–944. [PubMed: 3938826]
25. Cook DI, Young JA. Fluid and electrolyte secretion by salivary glands. *The Gastrointestinal System III*. 1989; Chapter 1:1–23.
26. Martinez JR. Cellular mechanisms underlying the production of primary secretory fluid in salivary glands. *Crit Rev Oral Biol Med*. 1990; 1:67–78. [PubMed: 1966239]
27. Nauntofte B. Regulation of electrolyte and fluid secretion in salivary acinar cells. *Am J Physiol*. 1992; 263:G823–837. [PubMed: 1476190]
28. Turner RJ, George JN. $\text{Cl}^-/\text{HCO}_3^-$ exchange is present with $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransport in rabbit parotid acinar basolateral membranes. *Am J Physiol*. 1988; 254:C391–396. [PubMed: 3348383]
29. Evans RL, Park K, Turner RJ, et al. Severe impairment of salivation in $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter NKCC1-deficient mice. *J Biol Chem*. 2000; 275:26720–26726. [PubMed: 10831596]
30. Nakamoto T, Srivastava A, Romanenko VG, et al. Functional and molecular characterization of the fluid secretion mechanism in human parotid acinar cells. *Am J Physiol Regul Integr Comp Physiol*. 2007; 292:R2380–2390. [PubMed: 17347411]
31. Nguyen HV, Stuart-Tilley A, Alper SL, Melvin JE. $\text{Cl}^-/\text{HCO}_3^-$ exchange is acetazolamide sensitive and activated by a muscarinic receptor-induced $[\text{Ca}^{2+}]_i$ increase in salivary acinar cells. *Am J Physiol Gastrointest Liver Physiol*. 2004; 286:G312–320. [PubMed: 12958022]
32. Park K, Evans RL, Watson GE, et al. Defective fluid secretion and NaCl absorption in the parotid glands of Na^+/H^+ exchanger-deficient mice. *J Biol Chem*. 2001; 276:27042–27050. [PubMed: 11358967]
33. Romanenko VG, Catalan MA, Brown DA, et al. Tmem16A encodes the Ca^{2+} -activated Cl^- channel in mouse submandibular salivary gland acinar cells. *J Biol Chem*. 2010; 285:12990–13001. [PubMed: 20177062]
34. Romanenko VG, Nakamoto T, Srivastava A, Begenisich T, Melvin JE. Regulation of membrane potential and fluid secretion by Ca^{2+} -activated K^+ channels in mouse submandibular glands. *J Physiol*. 2007; 581:801–817. [PubMed: 17379640]
35. Soltoff SP, Asara JM, Hedden L. Regulation and identification of Na,K-ATPase $\alpha 1$ subunit phosphorylation in rat parotid acinar cells. *J Biol Chem*. 2010; 285:36330–36338. [PubMed: 20841356]
36. Burgen AS. The secretion of potassium in saliva. *J Physiol*. 1956; 132:20–39. [PubMed: 13320370]
37. Petersen OH. Some factors influencing stimulation-induced release of potassium from the cat submandibular gland to fluid perfused through the gland. *J Physiol*. 1970; 208:431–447. [PubMed: 4250905]

38. Romanenko V, Nakamoto T, Srivastava A, Melvin JE, Begenisich T. Molecular identification and physiological roles of parotid acinar cell maxi-K channels. *J Biol Chem.* 2006; 281:27964–27972. [PubMed: 16873365]
39. Putney JW. Calcium signaling system in salivary glands. *The Gastrointestinal System III.* 1989; Chapter 3:51–61.
40. Smyth JT, Dehaven WI, Jones BF, et al. Emerging perspectives in store-operated Ca^{2+} entry: roles of Orai, Stim and TRP. *Biochimica et biophysica acta.* 2006; 1763:1147–1160. [PubMed: 17034882]
41. Zhang GH, Chang B, Melvin JE. Mediation of the depolarization-induced $[\text{Ca}^{2+}]_i$ increase in rat sublingual acini by acetylcholine released from nerve terminals. *Archives of oral biology.* 1996; 41:85–90. [PubMed: 8833595]
42. Gao YD, Hanley PJ, Rinne S, Zuzarte M, Daut J. Calcium-activated K^+ channel $\text{K}_{\text{Ca}3.1}$ activity during Ca^{2+} store depletion and store-operated Ca^{2+} entry in human macrophages. *Cell calcium.* 2010; 48:19–27. [PubMed: 20630587]
43. Ishii TM, Silvia C, Hirschberg B, Bond CT, Adelman JP, Maylie J. A human intermediate conductance calcium-activated potassium channel. *Proc Natl Acad Sci U S A.* 1997; 94:11651–11656. [PubMed: 9326665]
44. Joiner WJ, Wang LY, Tang MD, Kaczmarek LK. hSK4, a member of a novel subfamily of calcium-activated potassium channels. *Proc Natl Acad Sci U S A.* 1997; 94:11013–11018. [PubMed: 9380751]
45. Nehrke K, Quinn CC, Begenisich T. Molecular identification of Ca^{2+} -activated K^+ channels in parotid acinar cells. *Am J Physiol Cell Physiol.* 2003; 284:C535–546. [PubMed: 12388098]
46. Butler A, Tsunoda S, McCobb DP, Wei A, Salkoff L. mSlo, a complex mouse gene encoding “maxi” calcium-activated potassium channels. *Science (New York, NY).* 1993; 261:221–224.
47. Tseng-Crank J, Foster CD, Krause JD, et al. Cloning, expression, and distribution of functionally distinct Ca^{2+} -activated K^+ channel isoforms from human brain. *Neuron.* 1994; 13:1315–1330. [PubMed: 7993625]
48. Begenisich T, Nakamoto T, Ovitt CE, et al. Physiological roles of the intermediate conductance, Ca^{2+} -activated potassium channel $\text{K}_{\text{Ca}4}$. *J Biol Chem.* 2004; 279:47681–47687. [PubMed: 15347667]
49. Ferguson, DB. Salivary Electrolytes. In: Tenovuo, J., editor. *Human Saliva: Clinical Chemistry and Microbiology.* Boca Raton, Florida: CRC Press, Inc; 1989. p. 75-99.
50. Mangos JA, McSherry NR, Nousia-Arvanitakis S, Irwin K. Secretion and transductal fluxes of ions in exocrine glands of the mouse. *Am J Physiol.* 1973; 225:18–24. [PubMed: 4714400]
51. Mangos JA, Braun G, Hamann KF. Micropuncture study of sodium and potassium excretion in the rat parotid saliva. *Pflügers Arch Gesamte Physiol Menschen Tiere.* 1966; 291:99–106.
52. Schneyer LH. Amiloride inhibition of ion transport in perfused excretory duct of rat submaxillary gland. *Am J Physiol.* 1970; 219:1050–1055. [PubMed: 5459467]
53. Gruber WD, Knauf H, Fromter E. The action of aldosterone on Na^+ and K^+ transport in the rat submaxillary main duct. *Pflügers Arch.* 1973; 344:33–49. [PubMed: 4797974]
54. Nakamoto T, Romanenko VG, Takahashi A, Begenisich T, Melvin JE. Apical maxi-K ($\text{KCa}1.1$) channels mediate K^+ secretion by the mouse submandibular exocrine gland. *Am J Physiol Cell Physiol.* 2008; 294:C810–819. [PubMed: 18216162]
55. Marty A, Tan YP, Trautmann A. Three types of calcium-dependent channel in rat lacrimal glands. *J Physiol.* 1984; 357:293–325. [PubMed: 6096532]
56. Lundberg A. Anionic dependence of secretion and secretory potentials in the perfused sublingual gland. *Acta Physiol Scand.* 1957; 40:101–112. [PubMed: 13469517]
57. Ishikawa T, Murakami M, Seo Y. Basolateral K^+ efflux is largely independent of maxi- K^+ channels in rat submandibular glands during secretion. *Pflügers Arch.* 1994; 428:516–525. [PubMed: 7530839]
58. Maruyama Y, Gallacher DV, Petersen OH. Voltage and Ca^{2+} -activated K^+ channel in baso-lateral acinar cell membranes of mammalian salivary glands. *Nature.* 1983; 302:827–829. [PubMed: 6302513]

59. Cook DI, Young JA. Effect of K^+ channels in the apical plasma membrane on epithelial secretion based on secondary active Cl^- transport. *J Membr Biol*. 1989; 110:139–146. [PubMed: 2553974]
60. Palk L, Sneyd J, Shuttleworth TJ, Yule DI, Crampin EJ. A dynamic model of saliva secretion. *Journal of theoretical biology*. 2010; 266:625–640. [PubMed: 20600135]
61. Almasy J, Won JH, Begenisich TB, Yule DI. Apical Ca^{2+} -activated potassium channels in mouse parotid acinar cells. *The Journal of general physiology*. 2012; 139:121–133. [PubMed: 22291145]
62. Thompson J, Begenisich T. Membrane-delimited inhibition of maxi-K channel activity by the intermediate conductance Ca^{2+} -activated K channel. *The Journal of general physiology*. 2006; 127:159–169. [PubMed: 16418402]
63. Romanenko VG, Roser KS, Melvin JE, Begenisich T. The role of cell cholesterol and the cytoskeleton in the interaction between IK1 and maxi-K channels. *Am J Physiol Cell Physiol*. 2009; 296:C878–888. [PubMed: 19176762]
64. Thompson J, Begenisich T. Mechanistic details of BK channel inhibition by the intermediate conductance, Ca^{2+} -activated K channel. *Channels (Austin, Tex)*. 2009; 3:194–204.
65. Lu R, Alioua A, Kumar Y, Eghbali M, Stefani E, Toro L. MaxiK channel partners: physiological impact. *J Physiol*. 2006; 570:65–72. [PubMed: 16239267]
66. Romanenko VG, Thompson J, Begenisich T. Ca^{2+} -activated K channels in parotid acinar cells: The functional basis for the hyperpolarized activation of BK channels. *Channels (Austin, Tex)*. 2010; 4:278–288.
67. Yan J, Aldrich RW. LRRC26 auxiliary protein allows BK channel activation at resting voltage without calcium. *Nature*. 2010; 466:513–516. [PubMed: 20613726]
68. Almasy J, Begenisich T. The LRRC26 protein selectively alters the efficacy of BK channel activators. *Molecular pharmacology*. 2012; 81:21–30. [PubMed: 21984254]
69. Yan J, Aldrich RW. BK potassium channel modulation by leucine-rich repeat-containing proteins. *Proc Natl Acad Sci U S A*. 2012; 109:7917–7922. [PubMed: 22547800]
70. Zhao H, Muallem S. Na^+ , K^+ , and Cl^- transport in resting pancreatic acinar cells. *The Journal of general physiology*. 1995; 106:1225–1242. [PubMed: 8786358]
71. Walcott B, Birzgalis A, Moore LC, Brink PR. Fluid secretion and the Na^+ - K^+ -2Cl cotransporter in mouse exorbital lacrimal gland. *Am J Physiol Cell Physiol*. 2005; 289:C860–867. [PubMed: 15917300]
72. Nejsun LN, Praetorius J, Nielsen S. NKCC1 and NHE1 are abundantly expressed in the basolateral plasma membrane of secretory coil cells in rat, mouse, and human sweat glands. *Am J Physiol Cell Physiol*. 2005; 289:C333–340. [PubMed: 15843440]
73. Cui CY, Childress V, Piao Y, et al. Forkhead transcription factor FoxA1 regulates sweat secretion through Bestrophin 2 anion channel and Na-K-Cl cotransporter 1. *Proc Natl Acad Sci U S A*. 2012; 109:1199–1203. [PubMed: 22223659]
74. Huang F, Rock JR, Harfe BD, et al. Studies on expression and function of the TMEM16A calcium-activated chloride channel. *Proc Natl Acad Sci U S A*. 2009; 106:21413–21418. [PubMed: 19965375]
75. Evans MG, Marty A. Calcium-dependent chloride currents in isolated cells from rat lacrimal glands. *J Physiol*. 1986; 378:437–460. [PubMed: 2432252]
76. Inoue R, Sohara E, Rai T, et al. Immunolocalization and translocation of aquaporin-5 water channel in sweat glands. *Journal of dermatological science*. 2013; 70:26–33. [PubMed: 23473857]
77. Maruyama Y, Petersen OH, Flanagan P, Pearson GT. Quantification of Ca^{2+} -activated K^+ channels under hormonal control in pig pancreas acinar cells. *Nature*. 1983; 305:228–232. [PubMed: 6310413]
78. Suzuki K, Petersen OH. Patch-clamp study of single-channel and whole-cell K^+ currents in guinea pig pancreatic acinar cells. *Am J Physiol*. 1988; 255:G275–285. [PubMed: 2458682]
79. Hayashi M, Wang J, Hede SE, Novak I. An intermediate-conductance Ca^{2+} -activated K^+ channel is important for secretion in pancreatic duct cells. *Am J Physiol Cell Physiol*. 2012; 303:C151–159. [PubMed: 22555847]
80. Henderson RM, Cuthbert AW. A high-conductance Ca^{2+} -activated K^+ channel in cultured human eccrine sweat gland cells. *Pflugers Arch*. 1991; 418:271–275. [PubMed: 1857635]

Figure 1.

A. Two stage salivary gland secretion model. In Stage 1, acinar cells secrete a NaCl-rich fluid called primary saliva. In Stage 2, the primary saliva is modified through its passage along the duct epithelium by reabsorbing NaCl and secreting KHCO_3 . Due to the poor permeability of the ducts for water, the resulting final saliva is hypotonic. **B.** The morphology of human submandibular (left panel) and parotid (right panel) glands. Hematoxylin and Eosin stained submandibular gland section shows that the gland is mainly composed of serous and mucous acinar cells (SA and MA, respectively) and duct cells (Dc). In contrast, human parotid glands are mainly composed of serous acinar cells (SA) as well as duct cells (Dc).

Figure 2.

Acinar Cl⁻ “pump-leak” models. Fluid secretion by salivary glands is driven by transcellular Cl⁻ movement. The primary Cl⁻ secretion mechanism relies on the basolateral Na⁺-K⁺-2Cl⁻ co-transporter (Figure 2A) to mediate Cl⁻ influx, whereas the parallel activities of Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchangers mediate Cl⁻ influx in the secondary Cl⁻ secretion mechanism (Figure 2B).

Primary and secondary Cl⁻ uptake (pump) mechanisms depend on the large inward-directed Na⁺ electrochemical gradient generated by the basolateral Na⁺/K⁺ ATPase to promote Na⁺ extrusion in exchange for extracellular K⁺, which is recycled into the serosal space by basolateral K⁺ channels. K⁺ channel opening also supports Cl⁻ secretion (leak) by hyperpolarizing the membrane potential. Cl⁻ efflux into the luminal space occurs through

apical Cl^- channels. Apical K^+ channels enhance Cl^- efflux by hyperpolarizing the apical plasma membrane.

Figure 3.

K^+ secretion models by salivary gland ducts. The current model for K^+ secretion predicts that salivary gland duct cells secrete K^+ and HCO_3^- . Two molecular mechanisms have been proposed for K^+ - and HCO_3^- -coupled secretion by salivary gland ducts. **A.** Primary K^+ and HCO_3^- secretion model, in which basolateral Na^+/K^+ ATPase promotes K^+ accumulation in duct cells above its equilibrium potential (E_K) while apical K^+ channels are responsible for K^+ efflux into the ductal lumen. Na^+/HCO_3^- co-transporters may mediate HCO_3^- uptake across the basolateral membrane and apical Cl^-/HCO_3^- exchangers mediate HCO_3^- efflux into the ductal lumen. An apical Cl^- channel recycles Cl^- ions into the lumen. **B.** Secondary K^+ and HCO_3^- secretion model is identical to the primary secretion model, except that an

apical HCO_3^- - permeable channel is responsible for the HCO_3^- efflux into the ductal lumen. Note that apical HCO_3^- - permeable channel replaces the parallel activities of apical $\text{Cl}^-/\text{HCO}_3^-$ exchangers and Cl^- channels shown in the primary secretion model. **C.** In addition to the K^+ - and HCO_3^- -coupled secretion models, there is also experimental evidence supporting a Na^+ -reabsorptive –dependent K^+ secretion process, in which the electrogenic Na^+ influx via ENaC channels depolarizes the apical membrane of duct cells thus increasing the driving force for K^+ secretion through apical K^+ channels. K^+ uptake and Na^+ efflux across the basolateral membrane occurs via Na^+/K^+ ATPase.

Table 1

Gene	Activity	Localization	Functional Role	References
K _{Ca} 3.1 (<i>KCNN4</i>)	K ⁺ channel	Apical Basolateral?	Fluid secretion Em	34
K _{Ca} 1.1 (<i>KCNMA1</i>)	K ⁺ channel	Apical Basolateral	Fluid and K ⁺ secretions Em	34
TMEM16A (<i>ANO1</i>)	Cl ⁻ channel	Apical	Fluid secretion Cl ⁻ efflux	33
Aquaporin 5 (<i>AQP5</i>)	Water channel	Apical	Fluid secretion Water efflux	14,15
NHE1 (<i>SLC9A1</i>)	Na ⁺ /H ⁺ exchanger	Basolateral	Fluid secretion pH _i regulation	32
AE2 (<i>SLC4A2</i>)	Cl ⁻ /HCO ₃ ⁻ exchanger	Basolateral?	Fluid secretion? Cl ⁻ uptake?	31
NKCC1 (<i>SLC2A2</i>)	Na ⁺ -K ⁺ -2Cl ⁻ Co-transporter	Basolateral	Fluid secretion Cl ⁻ uptake	29
Alpha 1 Na ⁺ /K ⁺ ATPase (<i>ATP1A1</i>)	Na ⁺ /K ⁺ ATPase	Basolateral	Fluid secretion Na ⁺ extrusion Em	35

Proteins involved in acinar fluid secretion are listed (their corresponding human gene name is also given between parenthesis), transporter activity, localization, and role in acinar function. Em = membrane potential.