Potassium currents regulating secretion from Brunner’s glands in guinea pig duodenum

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Kovac, Jason, Beverley Moore, and Stephen Vanner. Potassium currents regulating secretion from Brunner’s glands in guinea pig duodenum. Am J Physiol Gastrointest Liver Physiol 286: G377–G384, 2004. First published November 6, 2003; 10.1152/ajpgi.00153.2003.—This study examined the role of outward K⁺ currents in the acinar cells underlying secretion from Brunner’s glands in guinea pig duodenum. Intracellular recordings were made from single acinar cells in intact acini in in vitro submucosal preparations, and videomicroscopy was employed in the same preparation to correlate these measures with secretion. Mean resting membrane potential was −74 mV and was depolarized by high external K⁺ (20 mM) and the K⁺ channel blockers 4-aminopyridine (4-AP), quinine, and clotrimazole. The cholinergergic agonist carbachol (60–2,000 nM; EC₅₀ = 200 nM) caused a concentration-dependent initial hyperpolarization of the membrane and an associated decrease in input resistance. This hyperpolarization was significantly decreased by 20 mM external K⁺ or membrane hyperpolarization and increased by 1 mM external K⁺ or membrane depolarization. It was blocked by the K⁺ channel blockers tetraethylammonium (TEA), 4-AP, quinine, and clotrimazole but not iberiotoxin. When videomicroscopy was employed to measure dilation of acinar lumen in the same preparation, carbachol-evoked dilations were altered in a parallel fashion when external K⁺ was altered. The dilations were also blocked by the K⁺ channel blockers TEA, 4-AP, quinine, and clotrimazole but not iberiotoxin. These findings suggest that activation of outward K⁺ currents is fundamental to the initiation of secretion from these glands, consistent with the model of K⁺ efflux from the basolateral membrane providing the driving force for secretion. The pharmacological profile suggests that these K⁺ channels belong to the intermediate conductance group.

exocytosis; cholinergergic; secretion

BRUNNER’S GLANDS RESEMBLE other exocrine glands, such as salivary and pancreatic glands, in the organization of their acini, but unlike these glands, which lie outside the intestinal tract, Brunner’s glands are uniquely positioned within the wall of the duodenum. Although first described in the 1600s (34), over 200 years elapsed before the functional significance of these glands was addressed, reflecting, in part, their relative inaccessibility. One of the first studies (5, 6) demonstrated that food in the “Brunner’s gland area” of the duodenum caused secretion of a mucoid material and perfusion of hydrochloric acid resulted in an increase in bicarbonate secretion. These studies drew attention to the role of Brunner’s gland in mucosal defense, and subsequent studies (13, 30, 31) have shown that in addition to contributing the secretion of mucus in the duodenum, these glands secrete numerous other substances. These include immunoglobulins and lysozymes (4) as well as growth factors and trefoil peptides (12, 28), which have been linked to epithelial proliferation and restitution (36). Together, these studies highlight that, similar to other exocrine glands, Brunner’s gland secretions play an important role in the gastrointestinal tract, but compared with other glands, there is a paucity of information concerning the intracellular mechanisms underlying this secretion.

Secretion of fluid and electrolytes from exocrine glands accompanies mucin and other substances (7, 27). Studies suggest that close integration of chloride secretion with exocytosis is necessary to maximize secretory responses (7, 16) and that these occur in a codependent fashion. Consequently, there have been numerous electrophysiological studies examining mechanisms underlying chloride and water secretion from acinar cells (3, 9, 23, 25). In some glands, agonists evoke hyperpolarization of acinar cells, and subsequent studies demonstrate that opening of K⁺ channels on the basolateral membrane underlies these findings and that this action is fundamental to providing the driving force for secretion. Some studies (3, 26) have suggested that large-conductance K⁺ (BK) channels are involved, but others imply that one or several other K⁺ conductances are important (3). Furthermore, in some glands, agonists evoke depolarization or an initial depolarization of acinar cells followed by hyperpolarization (3, 25). This depolarization appears to result from the opening of a nonselective Na⁺/K⁺ cation channel (26, 32). Overall, these studies demonstrate that there is considerable diversity in the nature of K⁺ channels that provide the driving force for electrolyte and fluid in exocrine glands.

Our recent studies (19, 20) have shown that agonist-evoked secretion in Brunner’s glands can be recorded in in vitro duodenal submucosal preparations from the guinea pig using videomicroscopy, but the role of K⁺ channels in this secretion is unknown. The aim of this study was to examine this role using intracellular recording techniques and pharmacological blockers of K⁺ channels in single acinar cells. Videomicroscopy was then employed in the same preparation to directly correlate electrophysiological recordings with secretion.

METHODS

Experiments were approved by the Queen’s University Animal Care Committee. Guinea pigs (150–225 g; either sex) were obtained from Charles River Laboratories (Quebec, PQ, Canada). Animals were anesthetized by isoflurane inhalation and killed by cervical transection. The duodenum was removed from ~1 cm distal to the pylorus to the ligament of Treitz, and submucosal preparations (~1 × 1 cm) containing Brunner’s glands were dissected, as previously described (19, 20).

Intracellular Recordings

Submucosal duodenal preparations were pinned in small organ baths (~1.0 ml) and superfused with a physiological saline solution...
containing (in mM): 120 NaCl, 1.5 NaH2PO4, 1.5 MgCl2, 2.5 CaCl2, 5 KCl, 25 NaHCO3, and 11 glucose gassed with 95% O2-5% CO2 at 35–36°C. Stable impalements of acinar cells lasting for hours were obtained using glass microelectrodes (tip resistances of 55–105 MΩ) filled with 2 M KCl. Changes in membrane potential were recorded with an Axoclamp 2A amplifier and displayed on a Gould TA240 chart recorder. When the effects of antagonism were examined, agonist responses were studied first in the presence of the antagonist, and control responses were then obtained following a 10-min washout period. Antagonists were applied by continuous superfusion for 3 min before studying the effects of agonists. Drugs were added to the bath by superfusion in most studies. In a small series, carbachol (CCh; 1 mM) was applied by pressure-pulse application (3–5 ms) using small glass micropipettes positioned close to the acinar cell from which recordings were made.

**Videomicroscopy**

Preparations were pinned in organ baths as described for electrophysiological experiments and superfused with identical solutions. Dilations of acinar lumen were recorded with videomicroscopy, as previously described (19, 20). Briefly, a camera image of the acinus is projected to a television screen, and cursors are placed on the outside edges of the acinus lumen (dark lines). The diameter is continuously monitored using a computer-assisted videomicroscopy system (Di-amtrak). This system uses an Imaging Technology PC VisionPlus framegrabber board in an IBM PC/AT computer to digitize television images of the acinar lumen and converts this result to an analog signal, which is displayed on a chart recorder (Graphtec Linearacorder Mark 8). The resolution of the system is ~1 μm; the sampling rate is 15 Hz. Agonist and antagonists were applied in a similar fashion to that described for electrophysiological experiments.

**Drugs and Materials**

CCh was obtained from Aldrich (Oakville, ON, Canada); 4-aminopyridine (4-AP) and clotrimazole (Clot) were from Sigma (Oakville, ON, Canada); 4-diphenylacetoxy-N-(2-chloroethyl)-piperdine hydrochloride (4-DAMP) and tetraethylammonium (TEA) were from Research Biochemicals International (Oakville, ON, Canada); quinine was obtained from ICN Biomedicals (Costa Mesa, CA); iberiotoxin (IbTX) was purchased from Bachem (Torrence, CA). Clot was dissolved in methanol, and all other agents were dissolved in water. Solutions with 4-AP were titrated with HCl to maintain a pH of 7.4.

**Statistics**

Results were expressed as means ± SE. Data were compared using a two-tailed Student’s t-test for paired values obtained from tissues of the same animal. An associated probability (P) value of <0.05 was considered significant unless otherwise stated.

**RESULTS**

**Electrophysiological Studies**

**Passive membrane properties.** Stable intracellular recordings (n = 139) were obtained from acinar cells for 30 min to several hours (Fig. 1A). Only cells that had stable membrane potentials and reproducible agonist-evoked responses were studied. The mean resting membrane potential was −74.3 ± 0.8 mV.

Alterations in external K+ were examined to demonstrate a role for K+ permeability in the resting membrane potential. Superfusion of high external K+ (20 mM) evoked a mean depolarization of −29 ± 3 mV (Fig. 1B; n = 10). Solutions containing low K+ (1 mM) had no effect (n = 5). Membrane potential was also depolarized by 4-AP (1–5 mM, n ≥ 7), quinine (250 μM, n = 9), and Clot (10 μM, n = 6; Fig. 1C) but not IbTX (100 nM, n = 4), pinacidil (300 nM, n = 3), or TEA (1–5 mM, n ≥ 5). TEA at higher concentrations (10 mM, n = 6) caused a modest depolarization (Fig. 1C).

**CCh-evoked hyperpolarizations.** Superfusion of CCh (60 mM-2 μM) evoked a concentration-dependent hyperpolarization (EC50 = 200 mM; Fig. 2, A and B). CCh-evoked responses

![Fig. 1. K+ conductances and the resting membrane potential (RMP) of acinar cells. A: schematic diagram showing a submucosal Brunner’s gland preparation. Single acinar cells were impaled with intracellular electrodes, and electrical changes were monitored in response to agonists, antagonists, and altered extracellular K+ (K+)o. "Stimulated" refers to activation of secretion with carbachol. B: representative trace showing that perfusion of 20 mM K+o for the duration indicated by the arrow depolarized the RMP. Horizontal dashed line represents RMP in this and subsequent electrophysiological recordings. C: summary of the results for experiments performed, as shown in A. K+o (20 mM, n = 10) and the K+ channel blockers 4-aminopyridine (4-AP; 1 mM, n = 7; 5 mM, n = 9), quinine (Quin; 250 μM, n = 9), and clotrimazole (Clot; 10 μM, n = 6) significantly depolarized the RMP with tetraethylammonium (TEA; 1 mM, n = 5; 5 mM, n = 7; 10 mM, n = 6), and iberiotoxin (IbTX; 100 nM, n = 4) had no effect. Values are means ± SE; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.**
(600 nM) were blocked by the muscarinic antagonist 4-DAMP (1 μM, n = 3). In all subsequent experiments examining hyperpolarizations, 600 nM CCh was employed to allow comparisons with videomicroscopic studies of secretion, because this concentration evoked consistent and robust secretory responses (see Videomicroscopy). CCh-evoked hyperpolarizations were followed by concentration-dependent depolarizations (Fig. 2, A and B; see also Fig. 8).

Changes in input resistance evoked by CCh-induced hyperpolarizations were measured by comparing the magnitude of the voltage deflection caused by brief hyperpolarizing pulses before the superfusion of CCh (600 nM) and during the hyperpolarization. The membrane potential was manually reset at the resting potential during the hyperpolarization to control for the effects on voltage-sensitive conductances. CCh-evoked hyperpolarizations were associated with a decrease in input resistance in six of seven cells (mean = 19.4%, n = 7), consistent with the opening of K+ channels. To further establish that the opening of K+ channels underlies the CCh-evoked hyperpolarizations, the effects of altering external K+ were examined (Fig. 3). Changes in membrane potential evoked by altering external K+ were manually corrected to the resting potential before examining their effects on CCh responses. The changes in membrane potential in high-K+ (20 mM) solutions mean CCh-evoked hyperpolarizations were inhibited by 80% (P < 0.05, n = 4; Fig. 3, B and C), whereas in low-K+, solutions mean responses were increased by 171% (P < 0.01, n = 6; Fig. 3, A and C). The effect of altering membrane potential on CCh-evoked responses was also studied to determine whether they behaved as predicted by K+ equilibrium potentials (Fig. 4). Resting membrane potential was altered by passing depolarizing or hyperpolarizing current through the intracellular electrode, and CCh was applied by pressure-pulse application (Fig. 4, A and B). Membrane hyperpolarization caused a significant reduction (mean = 56% for 20-mV hyperpolarization), whereas depolarization resulted in a marked enhancement (mean = 259% for 20-mV depolarization) of the CCh-evoked hyperpolarizations.

Pharmacological actions of K+ channel blockers on CCh-evoked hyperpolarizations. The effects of K+ channel blockers on CCh-evoked hyperpolarizations were examined by studying...
the effects of CCh superfusion (600 nM) after a 3-min superfusion of the blocker and comparing these responses compared with the application of CCh alone following a 10-min washout of the K⁺ channel blocker (Fig. 5A). Membrane depolarization evoked by K⁺ blockers (see Fig. 1) was compensated by passing current through the intracellular electrode and hyperpolarizing the membrane potential back to the resting potential before applying CCh. CCh-evoked hyperpolarizations were significantly inhibited by TEA, 4-AP, and quinine (Fig. 5B). They were also blocked by the intermediate K⁺ channel blocker Clot (10 μM) but not by the specific BK channel blocker (10), IbTX (100 nM; Fig. 5C).

Videomicroscopy

Our previous studies (20) have shown that 0.1–10 μM CCh evokes a concentration-dependent dilation of Brunner’s gland acini (Fig. 6A). CCh 600 nM (~EC₅₀) provides the lowest concentration that gives consistent and robust responses (20). This concentration was employed because it was similar to the EC₅₀ for the CCh-evoked hyperpolarizations (200 nM), thus allowing direct comparisons with electrophysiological studies (Fig. 6B).

Alteration in extracellular K⁺. Increases (20 mM) or decreases (1 mM) in extracellular K⁺ had no effect on the resting diameter of acinar lumen (Fig. 6, C and D). In the presence of high extracellular K⁺, dilations evoked by superfusion of CCh (600 nM) were inhibited by 33% (P < 0.05; Fig. 6E). When extracellular K⁺ was decreased (1 mM), CCh-evoked (600 nM) dilations were increased by 60% (P < 0.05; Fig. 6E).

Fig. 4. Membrane potential alters CCh-evoked hyperpolarizations. A: representative traces of CCh-evoked responses from the same cell. Membrane potential was altered by passing current through the intracellular electrode. CCh (1 mM) was applied by pressure-pulse application (>). A 10-min washout occurred between each application. B: summary of data obtained from experiments shown in A: n ≥ 3 for each point. ∆, change.

Fig. 5. Pharmacology of K⁺ channels involved in CCh-evoked hyperpolarizations. A: representative trace showing that TEA inhibited hyperpolarizations that returned following 10-min washout. B: summary of the data obtained from experiments in A. TEA (1 mM, n = 5; 5 mM, n = 4) and 4-AP (1 mM, n = 5; 5 mM, n = 4) significantly inhibited CCh-evoked hyperpolarizations. C: summary of data obtained from experiments described in A employing more selective K⁺ channel blockers. IbTX had no effect on hyperpolarizations (n = 3); Quin (n = 4) and Clot (n = 4) inhibited hyperpolarizations. *P < 0.05.
Effects of K⁺ channel blockers on secretion. CCh-evoked (600 nM) dilations were blocked by TEA (1 mM; Fig. 7B). 4-AP at 1 mM inhibited CCh-evoked dilations >90% and at 5 mM almost completely blocked responses (Fig. 7).

Fig. 6. Brunner’s gland secretion is regulated by K⁺o. A: schematic diagram showing the videomicroscopy technique used for measuring changes in lumen diameter of Brunner’s gland acini as an index of secretion. In preparations identical to those used in electrophysiological experiments, cursors were placed on either side of the lumen and dilations were tracked as an index of secretion. B: representative traces showing that superfusion of low K⁺o (1 mM) enhanced and high K⁺o (20 mM) decreased CCh (600 nM)-evoked lumen dilations. After a 10-min washout, lumen diameter returned to baseline and CCh-evoked control responses were recorded. Curved dotted line shows that the imaging system did not track lumen diameter for a brief period in the low-K⁺o experiment due to a rapid dilation. The horizontal dotted line highlights the baseline before dilation. Superfusion of CCh (600 nM) dilated acini for the duration of stimulation. C: summary of the data obtained in B for low (n = 5, left bars) and high K⁺o (n = 6, right bars). Control responses were obtained following washout of altered K⁺o solutions. *P < 0.05.

Quinine (250 μM) completely blocked CCh dilations. Clot, an intermediate-conductance K⁺ channel blocker (10, 15), inhibited CCh-evoked dilations by 40% at 10 μM and at 30 μM blocked dilations by 75% (Figs. 7C and 8). The BK channel blocker IbTX (100 nM, n = 5) had no effect with Quin (250 μM, n = 5) and Clot (10 μM, n = 7) inhibiting CCh-evoked responses.

**Relationship between CCh-evoked depolarizations and secretory responses.** CCh-evoked hyperpolarizations were followed by a prolonged depolarization (Figs. 2 and 8A). The conductance underlying this latter voltage change was not examined, but the relationship between the hyperpolarization–depolarization and secretion was studied to determine whether the conductance underlying the depolarization was essential to...
the initiation of secretion. The effects of Clot (30 μM) and TEA (5 mM) on both the CCh-evoked electrophysiological and secretory response were compared, because at these concentrations, hyperpolarizations were completely blocked. Clot (30 μM, n = 4) completely blocked the hyperpolarization but had no effect on the amplitude of the depolarization (Fig. 8). In a parallel series of experiments, Clot (30 μM) blocked the majority of the secretory response (∼75%) despite the absence of significant effect on the depolarization (n = 4; Fig. 8). TEA (5 mM) essentially blocked hyperpolarizations (93%; see Fig. 5) and secretions (90%; see Fig. 7) but only partially reduced depolarizations (control 600 nM CCh peak amplitude = 30.6 ± 2.6 mV vs. 600 nM CCh + 5 mM TEA peak amplitude = 13.6 ± 4.1 mV). CCh-evoked hyperpolarizations and depolarizations were both completely blocked by the M1 muscarinic antagonist 4-DAMP (n = 4) and quinine (250 μM, n = 6). This latter effect on the depolarization presumably reflects additional nonselective effects of quinine.

**DISCUSSION**

There have been very few studies concerning the cellular mechanisms underlying secretion from Brunner’s glands, largely because of the relative inaccessibility of these glands. In addition, the few studies that have addressed these mechanisms have been limited by the lack of correlation with functional data. Our in vitro model (19, 20) not only allowed the electrophysiological properties of single cells in these glands to be studied, but in the same model, the relevance of these findings was examined in studies of secretion using videomicroscopy. The results have demonstrated that K+ conductances play a fundamental role in regulating secretion from these glands.

Measurement of the passive membrane properties of glandular cells provides some insights into the types of membrane conductances present and active at rest. In general, the membrane potential of glandular cells resulting from a membrane permeability dominated by K+ has resting membrane potentials that approach the K+ equilibrium predicted by the Nernst equation (25, 26). In the current study, the mean resting membrane potential (−74 mV) falls within the high range (−57 to −73 mV) (3, 21, 25) for most studies of salivary glands, suggesting a particularly dominant K+ membrane permeability. In keeping with this finding, increases in extracellular K+ (20 mM) caused a significant depolarization of Brunner’s gland acinar cells. However, the resting membrane potential is below the expected Nernst potential for K+, as found in studies of other glands, reflecting smaller permeability to other ions. This is typically due to a small chloride permeability (3).

The findings obtained from intracellular and whole cell patch-clamp recordings obtained from single acinar cells have contributed significantly to our current understanding of agonist-secretion coupling in exocrine acinar glands (3, 24, 25).

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On the basis of these studies, models suggest that K+ conductances on the basolateral membrane provide the driving force for fluid and electrolyte secretion. These conductances can involve either a nonselective cation (Na+/K+) channel or a selective K+ conductance. In those glands with nonselective cation channels, agonists evoked depolarizations in intracellular recordings (11, 22, 25), which reversed in polarity near −30 mV, whereas those with selective K+ conductances had agonist-evoked hyperpolarizations, which reversed in polarity −90 mV (25). In the current study, CCh evoked hyperpolarizations with associated decreased input resistance. Moreover, they responded to changes in extracellular K+, changes in membrane potential (see Figs. 3 and 4), as predicted by the Nernst equilibrium potential for K+, and were blocked by K+ channel blockers. Together, these findings are consistent with activation of a selective K+ conductance, which provides the driving force for fluid and electrolyte secretion in Brunner’s gland acinar cells.

Current models suggest that basolateral K+ conductance provides the driving force for sustained electrogenic flux of anion and water at the apical membrane and regulates intracellular K+ concentrations, which are important for maintenance of the Na+/K+/2Cl transporter and the Na+/K+/ATPase (3, 25). The present study demonstrated that changes secretory responses correlated closely with electrophysiological responses following alterations in extracellular K+ or K+ chan-
nel blockers, providing functional data consistent with the role of K+ conductance on the basolateral membrane. An unanticipated finding of this study, however, was that inhibition of K+ conductances almost completely blocked dilation and, therefore, may block both ion and water secretion and exocytosis. Although the relationship between mucin exocytosis and fluid and electrolyte secretion has been poorly studied, there is evidence to suggest a dependence on these two secretory pathways (7). In studies of monolayers of HT29/B6 mucin-containing cells, Ussing chamber studies demonstrated that Cl– and K+ channel blockers inhibited Cl– and mucin secretion (14, 16).

The biophysical properties of the selective K+ conductances underlying secretory responses in exocrine glands have been extensively studied using pharmacological and electrophysiological approaches. Many of the earlier studies that examined the properties of K+ channels concluded that these responses were mediated by the BK, voltage-activated, and Ca2+-activated K+ channels (17, 18, 26, 35). Subsequent studies, however, suggest that these channels may not provide the dominant route for K+ efflux and identified several other K+ channels in the membrane (3, 17). Our pharmacological studies of both electrophysiological and secretory responses also do not support the contention that BK channels are the predominant channel underlying the K+ conductance changes. We found that IbTX, a selective BK channel blocker (10), had no effect on either the CCh-evoked hyperpolarizations or secretions. Similarly, we found that the small-conductance K+ channel blocker apamin (2) had no effect. In contrast, the intermediate-conductance blocker Clot (29) blocked both electrophysiological and secretory responses. We also found that TEA, 4-AP, and quinine inhibited these responses, but these agents are significantly less selective in their actions. Although selectivity is a potential limitation of all K+ channel blockers, these latter agents have not only been shown to affect multiple different K+ channels in acinar cells, but they have been reported to have other nonselective actions. For example, TEA has also been reported to be a competitive muscarinic antagonist in some acinar cells (3), and quinine can block Cl– channels (8). This latter action does not appear to account for the effects of Clot, because studies of intestinal T84 cells have shown that Clot blocks K+ conductances on the basolateral membrane and has no effect on apical membrane chloride conductances (29). We also found that both Clot and quinine not only blocked secretion but also blocked CCh-evoked hyperpolarizations, thus providing direct evidence that K+ currents were involved. Furthermore, whole cell patch-clamp studies are needed to confirm the biophysical properties of K+ channels on Brunner’s gland acinar cells, but, given our pharmacological data taken together with the current literature, intermediate-conductance Ca2+-activated K+ channels are likely candidates.

The conductances that underlie the depolarization that follows the CCh-evoked hyperpolarization (Figs. 1 and 8) were not examined in this study, but experiments were performed to determine whether the conductance(s) underlying the depolarization were a major driving force for the initiation of secretion. In these studies, Clot (30 μM) and TEA (5 mM) completely blocked the hyperpolarization but either had no effect or only a partial effect on the depolarization (see Fig. 8). In parallel studies, Clot blocked ~75% of secretion and TEA blocked 90%. Thus effects on secretion closely parallel the blockade of the hyperpolarization and not the depolarization. The fact that Clot did not completely block secretion, despite blocking the hyperpolarization, may suggest that additional conductances play a role in regulating secretion. Further studies are needed to determine the nature of the conductances underlying the depolarization and what their role is in secretion.

Brunner’s glands, in concert with the duodenal epithelium, provide an important secretory process that generates the alkaline mucus gel layer. This protects against the potential injurious actions of gastric acid and digestive enzymes (1). Previous functional studies examining the role of cellular mechanisms regulating mucous secretion from Brunner’s glands have been limited by the inability to separate secretion from the glands and the epithelium. A recent study in porcine Brunner’s gland identified cholinergic-evoked calcium-dependent K+ currents, but parallel in vivo studies were unable to demonstrate CCh-evoked mucin depletion from Brunner’s glands (33). The current study has employed an in vitro submucosal duodenal model that is devoid of epithelium and, hence, the confounding influences of secretion from enterocytes. Our previous studies demonstrated using electron microscopic, immunohistochemical, and videomicroscopic techniques that CCh evokes exocytosis of mucin from Brunner’s glands (20) and that this effect was mimicked by cholinergic vagal stimulation (19). The results of the present study directly demonstrate that selective K+ conductances play a major role in regulating secretion from these glands. Further studies are needed to determine the more precise nature of these channels and whether they are targets for modulation that might limit the ability of these glands to respond to potentially injurious challenges from the lumen.

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REFERENCES
9. Hayashi T, Hirono C, Young JA, and Cook DI. The ACh-induced whole-cell currents in sheep parotid secretory cells. Do BK channels really