No Potassium, No Acid: \(K^+\) Channels and Gastric Acid Secretion

The gastric \(H^+\)-\(K^+\)-ATPase pumps \(H^+\) into the lumen and takes up \(K^+\) in parallel.

In the acid-producing parietal cells, luminal KCNE2/KCNQ1 \(K^+\) channels play a pivotal role in replenishing \(K^+\) in the luminal fluid. Inactivation of KCNE2/KCNQ1 channels abrogates gastric acid secretion and dramatically modifies the architecture of gastric mucosa.

**Luminal \(K^+\): A Critical Determinant of Gastric Acid Secretion**

No other tissue in the human body builds higher concentration gradients for \(H^+\) than the stomach mucosa by forming gastric acid. Parietal cells pump protons into the luminal space of gastric glands leading to a 1-million-fold enrichment of \(H^+\) in the gastric juice. The identification (18, 19, 23), characterization (42, 60), and cloning (11, 68) of the luminal \(H^+\)/\(K^+\) exchanging ATPase as a molecular principle of gastric acid secretion has revolutionized our understanding of this important physiological function. Since the \(H^+\)-\(K^+\)-ATPase is unable to pump \(H^+\) into the lumen without parallel uptake of \(K^+\), ongoing gastric acid secretion requires sufficient concentrations of \(K^+\) in the lumen. Twenty years ago, experiments on parietal cell vesicles have indicated that replenishment of luminal \(K^+\) and secretion of \(Cl^-\) are accomplished by ion channels as conductive pathways for \(Cl^-\) and \(K^+\) (55, 77, 78). However, other studies have questioned the idea of luminal \(K^+\) exit through \(K^+\) channels and have proposed a KCl symporter system instead (15, 54, 76). Apparently, no \(K^+\) conductance was present in vesicles harvested from resting parietal cells (60). However, in vesicles from prestimulated parietal cells, a \(K^+\) conductance has been described (77) that could be further augmented by the addition of phosphatidylinositol 4,5-bisphosphate (PIP_2). Interestingly, PIP_2 had no effect on vesicles obtained from nonstimulated parietal cells (51). These data suggested that, in \(H^+\)-\(K^+\)-ATPase-containing vesicles of resting cells, \(K^+\) channels are absent or are present but silenced. In vesicles of stimulated parietal cells, activity of \(H^+\)-\(K^+\)-ATPases is functionally coupled to \(K^+\) recycling through active \(K^+\) channels, whose molecular nature was unknown. (A simple scheme of the putative membrane topology of the various \(K^+\) channels is depicted in **FIGURE 1**.)

**Luminal \(Cl^-\) Secretion**

\(Cl^-\) leaves the cell across the luminal membrane through \(Cl^-\) channels that are believed to be activated by low extracellular pH and by cAMP (15). The luminal exit of \(Cl^-\) as a counter ion for \(K^+\) is a crucial step for luminal \(K^+\) secretion because the activity of \(Cl^-\) channels depolarizes the luminal membrane. This depolarization above the equilibrium potential of \(K^+\) energizes the exit of \(K^+\) through \(K^+\) channels; otherwise, the luminal membrane would hyperpolarize to the equilibrium potential of \(K^+\), and no net flux of \(K^+\) would be possible. CIC-2 channels were proposed to underlie luminal \(Cl^-\) conductance (44); however, two other studies were unable to confirm the CIC-2 localization in parietal cells by Western blotting and immunofluorescence (31, 52). Although CIC-2 knockout mice are available (9), no genetic phenotype has been reported to date. Parchorin (48) and the “cystic fibrosis transmembrane conductance regulator” CFTR are other candidates that could be related to the luminal \(Cl^-\) conductance. In mice exhibiting a cystic fibrosis-causing mutation of CFTR (ΔF508), gastric acid secretion is impaired (69). Intriguingly, this is not the case in mice lacking CFTR (47), which suggests the complete absence of CFTR may be compensated easier than expression of defective CFTR. CFTR has a single channel conductance smaller than the one reported for luminal \(Cl^-\) channels of parietal cells (7, 15). Therefore, it has been proposed that CFTR acts on acid secretion as a modulator of luminal \(K_{ATP}\) \(K^+\) channels (69). Despite a variety of regulatory functions attributed to CFTR, it has not been reported that CFTR exclusively exerts modulatory functions without inducing a cAMP-activated \(Cl^-\) conductance. Thus CFTR, CIC-2, and/or yet unidentified channels may be responsible for the luminal \(Cl^-\) conductance of parietal cells.

**Control of Acid Secretion**

A variety of hormonal, paracrine, and neuronal pathways are involved in the stimulation of gastric acid secretion. Acetylcholine, gastrin, and histamine are the key players directly stimulating acid secretion by binding to specific receptors on parietal cells (4, 65, 72, 80) (**FIGURE 2A**). Moreover, a plethora of mediators and hormones affect parietal cell function, either in a direct way or indirectly via stimulation of enterochromaffin-like (ECL) cells or gastrin-producing G-cells (12, 32, 80). Amino acids stimulate acid secretion of parietal cells in...
an indirect way (via allosteric activation of the Ca\(^{2+}\)-sensing receptor on G-cells, which induces release of gastrin) or directly by activating the Ca\(^{2+}\)-sensing receptor located on parietal cells (10, 25, 56). Additionally, amino acids taken up through system L-amino acid transporter can stimulate acid secretion (37).

**Parietal Cell Stimulation and Ion Transport**

Gastrin and acetylcholine lead to increases in cytosolic Ca\(^{2+}\) activity; histamine increases cAMP levels and in some species also intracellular Ca\(^{2+}\). Stimulation of these second messenger pathways induces two main cellular events (FIGURE 2B): 1) trafficking of the H\(^+-\)K\(^+\)-ATPase to the canalicular system of the luminal membrane (“canaliculi” are invaginations of the luminal membrane into which the gastric acid is secreted) and 2) activation of luminal membrane conductances for Cl\(^–\) and K\(^+\). In resting parietal cells, most of the H\(^+-\)K\(^+\)-ATPases are located in so-called tubulovesicles without access to the intracellular canaliculi system. On stimulation, the H\(^+-\)K\(^+\)-ATPase-carrying tubulovesicles fuse with the canaliculi via an exocytotic event involving a complex vesicular trafficking machinery (17, 20, 50, 63, 72). Translocation of the H\(^+-\)K\(^+\)-ATPase into the canalicular membrane together with the presence of luminal K\(^+\) suffices to induce activity of the enzyme. Apparently, the H\(^+-\)K\(^+\)-ATPase is not modified (e.g., by phosphorylation) to become active. For each H\(^+\) secreted into the lumen, a HCO\(_3\)^\(^–\) ion has to leave the cell across the basolateral membrane (49). This is accomplished by two types of Cl\(^–\)/HCO\(_3\)^\(^–\) exchangers: SLC4A2 (AE2) (35, 58) and SLC26A7 (38), whose transport rates are increased thermodynamically when cytosolic Cl\(^–\) decreases due to luminal Cl\(^–\) exit (70). Besides basolateral HCO\(_3\)^\(^–\) export, these Cl\(^–\)/HCO\(_3\)^\(^–\) exchangers, together with the Na\(^+/\)Cl\(^–\) cotransporter (NKCC1) serve as Cl\(^–\) uptake systems. During acid secretion, the stabilization of intracellular pH and cell volume is a continuous challenge for parietal cells. In this context, basolateral Na\(^+/\)H\(^+\) exchangers (NHE1, NHE2, and especially NHE4) have been shown to play pivotal roles for pH homeostasis, volume regulation, and survival of parietal cells (24, 59, 66). Basolateral K\(^+\) channels stimulated during secretion hyperpolarize the membrane voltage and provide a basolateral recycling pathway for K\(^+\) taken up by the Na\(^+/\)K\(^+\)-ATPase (14, 16).

Relatively little is known about the biophysical properties of K\(^+\) channels in the basolateral membrane of mammalian parietal cells. In rabbit parietal cells, a basolateral 230 pS K\(^+\) channel has been observed as well as nonselective cation channels (61). In guinea pig parietal cells, three types of K\(^+\) conductances have been observed: voltage-dependent inwardly rectifying, outwardly activating, and Ca\(^{2+}\)-activated K\(^+\) conductances (39). Unfortunately, no precise information is available concerning the molecular identity of those basolateral K\(^+\) channels.

**Luminal KCNE2/KCNQ1 K\(^+\) Channels**

Although postulated for a long time, the molecular identity of the luminal K\(^+\) conductance is still a matter of debate. First evidence for the critical role of a distinct K\(^+\) channel protein for parietal cell function has been provided by Lee and coworkers (43). They observed an unexpected stomach phenotype in KCNQ1 knockout mice: The stomach was threefold enlarged due to hyperplasia of the antrum and fundus mucosa. The normal organization of the middle portion of the gastric glands (isthmic region) was severely disturbed, and the number of parietal cells was decreased. Functionally, KCNQ1 knockout mice displayed hypochlorhydria (pH
6–7 in knockout compared with pH 1–2 in wild-type mice) and elevated gastrin levels (43). Without knowing the subcellular distribution of KCNQ1, Lee and coworkers speculated that KCNQ1 might be required for acid secretion by “maintaining intracellular potassium at a relatively low level to permit H/K exchange” (43). KCNQ1 belongs to the large family of voltage-activated K+ channels (FIGURE 1). Biophysically, KCNQ1 is characterized by voltage dependence and slow activation kinetics. Originally, KCNQ1 was identified as a cardiac K+ channel mutated in the so-called “Long QT Syndrome Type I” (OMIM 607,542), and, therefore, it was named KvLQT1 (74). KCNQ1 is expressed in the heart muscle and in a variety of epithelial tissues (8, 75). In native tissues, the functional properties of KCNQ1 are modified by assembly with small regulatory subunits (KCNE1–5) (1, 2, 6, 62, 64). Shortly after the description of the gastric phenotype of KCNQ1 knockout mice, two independent immunofluorescence studies pointed to a localization of KCNQ1 in the luminal membrane compartment in mice and humans (13, 27). These data were suggestive for a role of KCNQ1 as luminal recycling pathway. In fact, inhibition of KCNQ1 by chromanols led to inhibition of acid secretion in mice, rats, and dogs in vivo and rabbit gastric glands in vitro (27). In a recent study, inhibition of KCNQ1 channels in isolated gastric glands blocked acid secretion with a similar efficiency as histamine receptor blockade or inhibition of the H+-K+-ATPase (41). In parietal cells, KCNQ1 and its subunit KCNE2 are strongly and specifically expressed (41), and both proteins are believed to assemble to form a luminal K+ channel (13, 27, 29). KCNQ1 expression in stomach mucosa is increased in response to gastrin (33); KCNE2 expression is decreased in gastric cancer tissue (which, at least in part, reflects the reduced number of differentiated parietal cells in tumor tissue) (79). The assembly of KCNQ1 and KCNE2 drastically modifies the functional properties of KCNQ1 (71): Heteromeric KCNE2/KCNQ1 channels are no longer voltage-dependent and slowly activating but rather voltage insensitive and constitutively open. In the luminal membrane of parietal cells, K+ channels face a specific challenge: They have to work under the extreme condition of pH 1 at the extracellular side. Homomeric KCNQ1 channels are inhibited by low extracellular pH, and, therefore, they are not able to conduct K+ under such conditions (21, 30, 53). Intriguingly, assembly with KCNE2 changes the biophysical properties of KCNQ1 and confers activation by external acidification on KCNQ1: Specific properties of the extracellular NH3 terminus of KCNE2 together with at least part of transmembrane domain transform the acid-inhibited KCNQ1 into an acid-activated channel complex (30). Taken together, these data strongly suggest that KCNE2 is the regulatory subunit assembling with KCNQ1 in the luminal membrane of parietal cells. Nevertheless, additional assembly of KCNQ1 with other members of KCNE family, especially KCNE1 and KCNE3, could not be fully excluded. Recently, a nice study by Roepke and coworkers on a KCNE2 knockout mouse model has finally demonstrated the pivotal role of KCNE2 as regulatory subunit of KCNQ1 in parietal cells (57). Similar to KCNQ1 knockout mice, KCNE2 knockout mice exhibited a severely impaired gastric acid secretion, abnormal parietal cell morphology, hypergastrinemia, and glandular hyperplasia.

**FIGURE 2.** Simplified models for gastric acid secretion

A: control of gastric acid secretion (left: basolateral; right: luminal). Stimulation of acetylcholine and gastrin receptors leads to increases in cytosolic Ca2+, and stimulation of histamine receptors leads to generation of cAMP (and in some species also to increases in Ca2+). Distension of the filled stomach, amino acids, and peptides stimulate G-cells, which release gastrin. Additionally, amino acids directly stimulate parietal cells via allosteric activation of the Ca2+-sensing receptor and/or after uptake by system L-amino acid transporter. Somatostatin, prostaglandin E2, and low pH within the stomach lumen inhibit acid secretion. B: ion transport in parietal cells. After activation of Ca2+ and cAMP pathways, H+-K+-ATPases (red circles) carrying vesicles fuse with the luminal membrane compartment. In the presence of luminal K+, H+-K+-ATPases pump H+ into the lumen in exchange for K+. Luminal KCNE2/KCNQ1 K+ channels (violet) recycle K+ across the luminal membrane. They are activated by cAMP, PIP2, and the acidic pH of the luminal fluid. Cl− channels (green) are also activated by cAMP and low pH at the external side of apical membrane. Red arrows indicate stimulation.
How Are KCNE2/KCNQ1 Channels Activated During Acid Secretion?

As postulated for the luminal K⁺ conductance of parietal cells (51, 77), KCNE2/KCNQ1 channels are activated by stimuli that induce acid secretion: cAMP increased the KCNE2/KCNQ1 current by 50–70%, and PIP₂ increased the current by some 20%. Additionally, low extracellular pH strongly increased the KCNE2/KCNQ1 current (threefold increase by extracellular acidification pH from 7.4 to 4.5) (29, 30).

As mentioned above, the localization of the H⁺-K⁺-ATPase undergoes dramatic changes during stimulation of acid secretion. Without stimulation, H⁺-K⁺-ATPases are located in intracellular vesicles (tubulovesicles) that do not have a substantial K⁺ conductance. On stimulation, these tubulovesicles are targeted to the luminal membrane compartment (canaliculi) by an exocytotic event. In resting parietal cells, KCNQ1 also appears to be localized in an intracellular vesicular membrane compartment. The subcellular distribution of KCNQ1 has a similar appearance as H⁺-K⁺-ATPase, but co-staining experiments disclosed that the staining patterns of both proteins are not identical. After stimulation of secretion, active KCNE2/KCNQ1 channels and H⁺-K⁺-ATPases have to be localized in the canalicular membrane to act in concert. Therefore, it is tempting to speculate that KCNQ1 is regulated by membrane targeting as observed for the H⁺-K⁺-ATPase. In fact, Lambrecht and coworkers have described a co-localization of KCNQ1 and H⁺-K⁺-ATPase only after stimulation of acid secretion (41). However, KCNQ1 staining in stimulated parietal cells does not change dramatically compared with resting cells. In addition, it is not as focused to the apical pole of parietal cells as it is the case for the H⁺-K⁺-ATPase (FIGURE 3A).

Thus we proposed a secretion model with KCNQ1 already sitting in the canalicular membrane compartment at resting conditions. On stimulation, the H⁺-K⁺-ATPase carrying vesicles fuse with the canaliculi, and the H⁺-K⁺-ATPase is directed to the central canaliculi as well as the apical pole. KCNE2/KCNQ1 channels are activated by cAMP and PIP₂-dependent mechanisms rather than by membrane targeting. They produce a K⁺-rich secretion in the depth of the canalicular system, which is turned into an H⁺-rich secretion by H⁺-K⁺-ATPase at the apical pole (29).

The above-mentioned studies on KCNQ1 (43, 73), KCNE2 knockout mice (57), and the pharmacological inhibition of KCNE2/KCNQ1 channels in various species (5, 27, 41) highlight the importance of this heteromeric channel for gastric acid secretion. However, the ratio of KCNQ1 and H⁺-K⁺-ATPase protein abundance appears to be variable and shows a gradient along the gland axis (FIGURE 3B): KCNQ1 staining is very strong in parietal cells at the base of the gland, and the H⁺-K⁺-ATPase-specific signal is strongest in parietal cells from the upper part of the glands. One might speculate that parietal cells at the base of gland produce a K⁺-rich secretion and that parietal cells in the upper gland regions exchange this K⁺ for H⁺, resulting in more acidic secretion in the upper gland region. Moreover, differences in KCNQ1 and H⁺-K⁺-ATPase expression might reflect functional and developmental diversity of parietal cells as has been observed in other respects (34, 36, 67).

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Evidence for Other Luminal K⁺ Channels

There is convincing evidence that KCNE2/KCNQ1 form luminal K⁺ channels in parietal cells that are activated during secretion and whose functional integrity is a prerequisite for gastric acid secretion and normal parietal cell morphology. However, as mentioned in the last section, not all H⁺-K⁺-ATPase-positive parietal cells show strong KCNQ1 staining. Nevertheless, KCNE2 and KCNQ1 knockout mice have a very severe gastric phenotype that could have at least three reasons:

1) KCNE2/KCNQ1 channels are crucial for acid secretion in the large majority of parietal cells and loss of function of this channel complex cannot be compensated by other K⁺ channels.

2) Other K⁺ channels also facilitate acid secretion, but they need initial co-activation of KCNE2/KCNQ1. This explanation may hold true for inwardly rectifying K⁺ channels, whose conductance is strongly decreased when external K⁺ (in the canaliculi) is very low.

3) KCNE2/KCNQ1 channels have essential roles for normal development of parietal cells, for the maintenance of their structural integrity, and for cell survival.

Taken together, it could well be that K⁺ channels other than KCNE2/KCNQ1 are also localized in the luminal membrane of parietal cells. These alternative K⁺ channels could be localized in all parietal cells or only in a subset of cells. In the past, several K⁺ channels have been proposed as luminal channels. Besides KCNQ1, members of the inward rectifier family (FIGURE 1) appear to be localized in the luminal membrane of parietal cells. KCNJ10 (Kir4.1) is expressed in parietal cells but probably not at very high levels (22, 41). The acid-resistant KCNJ10 (Kir4.1) channel protein has been shown to co-localize with the H⁺-K⁺-ATPase in the luminal membrane compartment (22). However, no specific pharmacological data or evidence from knockout mice is yet available. Based on PCR, immunofluorescence, Western blots, and patch-clamp data, KCNJ2 (Kir2.1) has been proposed as another luminal K⁺ channel in rabbit parietal cells (45). KCNJ2 has been reported to be activated by acidic extracellular pH and by protein kinase A. In the rat stomach, KCNJ2 gene expression appears to be low or absent (22, 41). KCNJ16 (Kir5.1), probably co-assembling with KCNJ15 (Kir4.2), has been found to be very specifically expressed in parietal cells (41). At present, it is unclear whether KCNJ16/KCNJ15 channels are localized in the basolateral or luminal membrane of parietal cells. Studies are underway to obtain functional data from knockout mice. KCNJ1 (ROMK) has also been submitted as a luminal parietal cell K⁺ channel in one review article (3). Functional data pointing to a possible gastric phenotype of KCNJ1 knockout have not been published so far. In addition, several members of the 2-P-domain K⁺ channel family are expressed in stomach mucosa (EST data, Unigene); however, their functional role has not been investigated so far.

What is the Physiological Significance for the Putative Luminal K⁺ Channels?

The data on KCNE2/KCNQ1 as a K⁺ channel of the luminal membrane are compelling. This channel complex is crucial for physiological acid output under stimulated conditions and for the maintenance of normal parietal cell morphology. One could speculate that KCNE2/KCNQ1 channels act in concert with inward rectifiers or other K⁺ channels. Alternatively, the nature of the luminal K⁺ conductance might be dependent on the developmental or functional status of the parietal cells. Further studies on genetically modified animals will be needed to evaluate the physiological contribution of the various candidates to the luminal K⁺ conductance of gastric parietal cells. A better knowledge of the nature of the luminal K⁺ conductance will improve our understanding concerning the principles of gastric acid secretion, parietal cell biology, and functional morphogenesis of gastric mucosa. It is envisioned that, in the future, this information may offer new perspectives for the treatment of diseases caused by dysfunction of the gastric oxyntic mucosa.

The authors thank S. Bandulik and M. Reichold for fruitful discussions.

This work was supported by the “Deutsche Forschungsgemeinschaft” and the SFB699.

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