Revisiting the parietal cell

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The parietal cell is responsible for secreting concentrated hydrochloric acid into the gastric lumen. To fulfill this task, it is equipped with a broad variety of functionally coupled apical and basolateral ion transport proteins. The concerted scientific effort over the last years by a variety of researchers has provided us with the molecular identity of many of these transport mechanisms, thereby contributing to the clarification of persistent controversies in the field. This article will briefly review the current model of parietal cell physiology and ion transport in particular and will update the existing models of apical and basolateral transport in the parietal cell.

AS AN ORGAN the stomach fulfills a unique role in that it is the primary site for digestion and sterilization of food stuffs and water. To produce its acidic environment and at the same time generate a protective barrier, the gastric epithelium is composed of a complex collection of specialized cells that secrete acid, bicarbonate, mucus, hormones, and digestive enzymes. During the digestive process the interior pH of the stomach can fall to a pH of 1–2 so that a great deal of acid must be generated by the glands to reach this concentration. To generate the acid into the interior of the stomach, the gastric glands need to have specialized cells that can rapidly produce large quantities of hydrochloric acid (HCl) upon demand. These specialized cells are the parietal cells and are found in high abundance in the gastric gland, with typically 70–90 parietal cells per gland. Since secretion of HCl is an energy-consuming process, the parietal cell is dependent on the generation of large amounts of ATP. To meet these high demands, it is particularly rich in mitochondria; in fact it is the cell with one of the highest densities of mitochondria in the human body with fractional volumes reaching up to 40% of the total cell volume (30) compared with a mere 5% in chief cells (55). When the parietal cell is stimulated by secretagogues, the apical pole undergoes a morphological transformation as the acid-secreting pump (H+–K+–ATPase) traffics to the surface of the cell allowing for the secretion of a proton in exchange for a potassium ion at the expense of one ATP molecule. At the same time, the stomach has to prevent autodigestion by secreting protective mucus into the interior of the stomach. Surface cells that can also secrete mucus but have an additional capacity for HCO3– secretion aid in this process. This allows the pH of the stomach to remain acid without exposing the surface cells to the caustic acid environment coming from the orifice of the glands. Other specialized cells found in the gland are the chief cells that are located at the base of the gland and release pepsinogen, which is cleaved by the acid in the gland lumen to make pepsin, the active digestive enzyme that facilitates the digestion of the food stuffs (39, 125). In addition, histamine-secreting enterochromaffin-like (ECL) cells and gastrin-secreting G-cells, which both promote acid secretion, make the stomach an endocrine organ and clusters of immunocompetent cells that are exposed to early antigen contact round up the picture of the diverse tasks the stomach has to perform (39, 125).

The process of acid secretion is tightly regulated, partly by the aforementioned ECL and G cells, but also by neuronal stimulation through the vagus nerve and release of inhibitory substances like somatostatin. An imbalance in this regulation can lead to pathological conditions related to hypersecretion of acid such as gastroesophageal reflux disease (GERD) or gastric ulcer disease (GUD). Currently, up to 20% of people in Western countries suffer from GERD, experiencing symptoms such as heartburn, regurgitation, dysphagia, or a combination of the above at least three times per week (29). GERD thus not only represents a major health concern but also has a massive economical impact on our healthcare systems. Whereas short episodes of reflux of gastric acid into the esophagus are physiological, the basic pathophysiology of GERD can be attributed to prolonged exposure of the esophageal mucosa to acid loads. The most fatal consequence of this chronic acid exposure is esophageal cancer.

The current state-of-the-art treatment for GERD focuses on the reduction of gastric acid secretion by administration of proton pump inhibitors (PPIs) [although this approach has recently been criticized (87, 119)]. PPIs specifically impair the function of H+–K+–ATPase, which can be found at the apical membrane of parietal cells during the active acid secretory phase. However, recent studies suggest that up to 40% of patients on PPI therapy develop breakthrough acid secretion while taking these medications, thus remaining symptomatic and susceptible to long-term complications such as Barrett’s or oesophageal carcinoma (27). Despite recent insights into the
physiology of the parietal cell, this high percentage of breakthrough raises the question of whether the process of gastric acid secretion is entirely understood.

In this article we will review new transport proteins that have been discovered, new inhibitors of gastric acid secretion that have been developed, and discuss new receptors that have been identified (see Fig. 1, a–b). We will hopefully have a final aim of not only better understanding the physiological machinery in the parietal cell but to provide clues to why conventional therapies are not providing long-term relief for the vast population of patients suffering from potentially fatal conditions like GERD or GUD. This review is therefore meant to serve as an update on gastric acid secretion and will mainly focus on the parietal cell as the functional acid secretory unit.

Apical Membrane

Three independent apical mechanisms are necessary to successfully secrete HCl into the gastric lumen: 1) proton extrusion via H⁺-K⁺-ATPase, 2) Cl⁻ secretion, and 3) K⁺ recycling. Although one might intuitively consider H⁺-K⁺-ATPase to be the most important contributor, disruption of any of the aforementioned mechanisms leads to achlorhydria, which not only underlines the impressive degree of mutual regulation in this system but also opens new horizons in the possible future therapy of pathological conditions like GERD.

$H^+K^+ATPASE$. Parietal cell H⁺-K⁺-ATPase transports protons (H⁺) in exchange for K⁺ while consuming ATP. The product of this reaction releases concentrated H⁺ into the lumen of the stomach, and thus essentially contributes to the formation of concentrated HCl (134). Transport is achieved by conformational changes of the ATPase that are powered by cyclic phosphorylation and dephosphorylation of the catalytic α-subunit (134). H⁺-K⁺-ATPase consists of two subunits (α and β) and is a member of the alkali-cation P-type ion-motive ATPase family, which also includes Na⁺-K⁺-ATPase and Ca²⁺-ATPase (133). Although transport is specific for a particular cation, all ATPases share a significant degree of homology. Approximately 63% sequence homology is found between the α-subunits of H⁺-K⁺-ATPase and Na⁺-K⁺-ATPase, whereas the β-subunit genes share 35% of the nucleotides (81, 137). This homology also manifests in the functional characteristics of both proteins. The catalytic cycle of Na⁺-K⁺-ATPase, which was already established in the 1970s, thus shows significant similarity to that of H⁺-K⁺-ATPase (115, 134).

The α-subunit of gastric H⁺-K⁺-ATPase consists of 1,035 amino acids forming 10 transmembrane segments and the β-subunit of 291 amino acids with one transmembrane segment (81, 121). Both subunits are linked at the M7/loop/M8 region (M7 describes the seventh transmembrane region) of the α-subunit, with an additional attachment being present at the M5/loop/M6 region of the α-subunit when K⁺ is present (92, 136). A recent study demonstrates that an intact carboxy-terminal region of the α-subunit is required for structural and functional integrity (22). The entire enzyme was traditionally thought to be arranged as an (αβ)₂ heterodimer, as a result from α-α association (135). The latest observations however propose a tetrameric conformation (1), which was already speculated to exist earlier (135).

Because of the lack of a precise H⁺-K⁺-ATPase crystal model, the already known structure of Ca²⁺-ATPase and Na⁺-K⁺-ATPase remains the only vague approximations to the conformational changes of H⁺-K⁺-ATPase taking place during acid secretion (94, 152). Toyoshima et al. (152) were the first to analyze the crystal structure of Ca²⁺-ATPase. Ca²⁺-ATPase, which is also responsible for Ca²⁺ transport into the sarcoplasmatic reticulum of muscle cells, shares significant structural features with the α-subunit of H⁺-K⁺-ATPase. Several conformational states of the transporter were crystallized, which allowed observation of the structural changes in the action of Ca²⁺ transport (103, 152, 153). Munson et al. (95) recently postulated a homology model for H⁺-K⁺-ATPase identifying the exact binding sites for H₂O⁺ and K⁺. Binding of pharmaceutical agents at or near these sites, resulting in inhibition of H⁺-K⁺-ATPase activity, is the basis for contemporary treatment of GERD or GUD and remains a primary subject of current research.

Two pharmacological substance groups that interfere with proton extrusion by H⁺-K⁺-ATPase exist: 1) PPIs and 2) acid pump antagonists (APAs). Omeprazole was the first PPI in clinical use and has been succeeded by related substances differing in their pharmacokinetics and dynamics. Tenatoprazole is the latest addition to the PPI family and excels with

Fig. 1. Schematic model of ion transport in the parietal cell. A: old model. B: new model depicting recently characterized transport mechanisms (new transport mechanisms shown in white).
particularly long plasma half-life (36). All PPIs share the common mechanism of freezing H⁺-K⁺-ATPase in its E2 form by covalently binding at extracellular cysteine residues that are accessible from the gastric lumen (126). The covalent binding mechanism thus allows PPIs to act long after their plasma concentration levels have dropped (126). The pattern of the involved cysteine residues seems to determine the efficacy and length of inhibition by the individual substances (126). Since PPIs are delivered as prodrugs to the site of action and are being locally activated by the acidic environment surrounding the H⁺-K⁺-ATPase, they are characterized by a large therapeutic index, which makes them desirable for clinical use (126, 134). In contrast to PPIs, APAs are K⁺ competitive inhibitors at the K⁺ binding site of H⁺-K⁺-ATPase (155). The efficiency of the inhibition is very high (up to 95% decreased acid output) and the onset of action rapid (3, 67, 134). Since APAs do not form covalent bonds with H⁺-K⁺-ATPase, their efficacy is entirely dependent on and correlates with the plasma concentration of the drug (134). So far only revaprazam is in clinical use in certain countries, because other APAs proved to have toxicity issues (49, 134, 162).

Chloride secretion. Chloride is the second component, which has to be secreted into the gastric lumen, to allow formation of HCl. The significance of intact chloride secretion for successful proton extrusion was established very early (25, 26, 82). Pharmacological inhibition of chloride channels was shown to effectively inhibit gastric acid secretion (82, 139) suggesting a close functional coupling between these channels and H⁺-K⁺-ATPase.

Cystic fibrosis transmembrane regulator (CFTR) is a potential candidate for chloride secretion in the parietal cell. Although its degree of expression is lower than in other tissues, such as in intestinal or airway epithelia (145), it was shown to play a pivotal role during acid secretion in the murine stomach (139). H⁺-K⁺-ATPase activity could be inhibited in wild-type mice by exposure to a specific small-molecule CFTR inhibitor (139). In addition, mice carrying the homozygous ΔF508 mutation (the most common CFTR mutation responsible for cystic fibrosis) have a significantly decreased ability to secrete gastric acid (139).

Chloride channel type 2 (CIC-2) has been proposed as an alternative route for chloride secretion to CFTR and calcium-activated chloride channels in the intestine (35, 45). Málnowska et al. (83) were the first to clone a channel from rabbit canaliculi of rabbit parietal cells, these findings could neither be replicated in human nor in rat tissue (56, 132). Although its expression has been confirmed in the secretory canaliculi of rabbit parietal cells, these findings could neither be replicated in human nor in rat tissue (56, 132). In addition to these observations, CIC-2(−/-) mice showed normal gastric acidification as a response to histamine stimulation (10). The exact physiological role of CIC-2 in the parietal cell and its degree of involvement in the process of gastric acid secretion has thus still to be clarified.

Members of the SLC26 Cl⁻/HCO₃⁻ exchanger family represent the third possibility for Cl⁻ delivery into the gastric lumen. SLC26a6 was found to be colocalized with H⁺-K⁺-ATPase in the tubulovesicles of the mouse stomach (113). However, functional evidence for the involvement of these exchangers has so far only been obtained for another member of the SLC26 family, namely for SLC26a9. SLC26a9(−/-) mice present with an altered mucosal morphology and show both loss of tubulovesicles and impaired acid secretion on a cellular level (159). The same group had previously demonstrated SLC26a9 localization at the apical membranes of parietal cells (158).

Potassium recycling. K⁺ secretion into the gastric lumen is pivotal for maintaining sustained H⁺-K⁺-ATPase activity (54). In the activated parietal cell, opening of apical K⁺ channels enables the cell to maintain constant cytosolic K⁺ concentrations and provides the H⁺-K⁺-ATPase with enough substrate for reciprocal proton extrusion (54). One can think of this process as K⁺ recycling. Strong evidence supports the assumption that the K⁺ channel KCNQ1 is responsible for this task (54). KCNQ1 belongs to a large family of voltage-gated K⁺ channels and was originally identified in the heart, where it, if mutated, is responsible for the Long-QT-Syndrome (54, 156). The channel was subsequently found to be expressed in both mouse and human gastric mucosa (28, 43). The creation of KCNQ1(−/-) models has recently provided further insight into its functional role in the parietal cell (106, 141). Acid output in the affected mice was reduced by as much as 90% compared with wild-type animals (106). Mice lacking the regulatory subunit KCNE2 showed a similar degree of achlorhydria and developed both hypergastrinemia and massive glandular hyperplasia due to proliferation of nonparietal cells. (122). KCNE2 can dramatically change the conductance properties of KCNQ1 by decreasing its sensitivity to changes in voltage and by altering its biophysical response to low extracellular pH conditions (54). Whereas KCNQ1 alone is inhibited by a low extracellular pH, K⁺ conductance increases in an acidic environment when the KCNQ1/KCNE2 complex is formed (34, 53, 111). Naturally, this is of particular importance in the stomach. This interesting KCNE2-mediated change of KCNQ1 gating and conductance during external acidification may account for the observed lack of acid secretion in KCN2(−/-) mice.

Several other groups have proposed an involvement of channels from the Kir family in the process of gastric acid secretion (65, 84). This hypothesis is sustained by the findings that the parietal cell expresses Kir2.1 and Kir4.1, which colocalized in tubulovesicles with H⁺-K⁺-ATPase and that pharmacological inhibition of KCNQ1 does not completely abolish acid output (65, 84). In addition, Kir4.1 was shown to be trafficked together with H⁺-K⁺-ATPase to the apical membrane upon stimulation, whereas KCNQ1 remained confined to cytosolic compartments (65). Although controversy exists concerning which K⁺ channel is responsible for K⁺ recycling, the latest KCNQ1(−/-) data suggests that KCNQ1 is the primary pathway of K⁺ extrusion.

Basolateral Membrane

The purpose of basolateral ion transport is to compensate for apically secreted ions and to maintain intracellular pH homeostasis and cell volume during active acid secretion. The parietal cell is therefore equipped with basolateral capacities for K⁺ and Cl⁻ uptake in the form of the Na⁺-2Cl⁻ K⁺ cotransporter (NKCC), Cl⁻/HCO₃⁻ exchangers, and the Na⁺-K⁺-ATPase. pH regulation mainly occurs through Cl⁻/HCO₃⁻ exchangers and sodium-hydrogen exchangers (NHE). Basolat-
eral ion transport therefore provides the basis for functional HCl formation in the stomach and thus plays an integral role in the process of acid secretion.

**Chloride entry.** The SLC4 transporter family comprises three different exchange types, one of which is the electroneutral and Na\(^+\)-independent Cl\(^-\)/HCO\(_3^-\) exchanger subgroup that includes SLC4A1 (AE1), SLC4A2 (AE2), and SLC4A3 (AE3) (117). As a consequence of multiple observations, the basolateral Cl\(^-\)/HCO\(_3^-\) exchange of parietal cells has mainly been assigned to the activity of AE2 (24, 60, 123, 157). AE2 expression was demonstrated to be at higher levels in parietal cells than in any other cell type (123, 157), and localization of three different variants of AE2 (AE2a, b, c) has been confirmed at the basolateral membrane (146). Furthermore, parietal cells exhibit a 4,4-diisothiocyanostilbene-2,2-disulfonic acid (DIDS)-inhibitable Cl\(^-\) and HCO\(_3^-\) transport pattern that is in accordance with that of AE2 (59, 74). The importance of AE2 as a main participant in gastric acid secretion is additionally emphasized by studies on AE2 knockouts. AE2\(^{-/-}\) mice presented with achlorhydria and chronic mild gastric mucosal degeneration (38), whereas later experiments in AE2a,b\(^{-/-}\) demonstrated normal basal acid secretion but a 70% reduced response in acid output to stimulants such as histamine or carbachol. All three AE2 polypeptide variants were shown to be inhibited by protons with slightly different but yet overlapping ranges in extracellular and intracellular pH. Activation of AE2 can occur via hypertonicity, NH\(_4\)\(^+\), and calmidazolium (73, 144). In summary, the AE2 family appears to be the main player in parietal cell Cl\(^-\)/HCO\(_3^-\) exchange and, as demonstrated by the knock-out experiments, can be seen as a necessary prerequisite for functional acid secretion.

SLC26A7, a member of the SLC26 gene superfamily is also believed to contribute to acid secretion. SLC26A7 was identified by Northern hybridization and immunofluorescent staining to be exclusively located at the basolateral membrane of gastric parietal cells as well as acid-secreting cells of the renal outer medullary collecting duct (112). Functional studies in oocytes microinjected with SLC26A7-cRNA suggested that SLC26A7, like AE2, is a DIDS-sensitive Cl\(^-\)/HCO\(_3^-\) exchanger. However, unlike AE2, which is inhibited under acidic conditions (73, 144), SLC26A7 activity is unaffected by acidic and alkaline surroundings (112). A recent study demonstrated impaired gastric acid secretion in SLC26A7\(^{-/-}\) mice without histological changes in the gastric mucosa (160). This finding underlines the possible contribution of SLC26A7 to acid-secretion-related anion exchange, which would explain the partial impairment of HCl formation that has been observed in AE2a,b knockouts, where SLC26A7 was believed to contribute to the maintenance of a neutral intracellular pH in resting conditions (118). The assumption that SLC26A7 is only conductive to basal pH regulation has been opposed by Kosiek et al. (71) and Xu et al. (160) who demonstrated an involvement of SLC26A7 in secretagogue-stimulated Cl\(^-\) uptake and acid secretion. It still remains to be clarified whether SLC26A7 acts as a Cl\(^-\)/HCO\(_3^-\) exchanger or a bona fide Cl\(^-\) channel, because a recent work by Kim et al. conducted in Xenopus oocytes and HEK293 cells showed SLC26A7 to function as a pH\(_7\)-regulated chloride channel with minimal HCO\(_3^-\) permeability (66).

The Na\(^+\)-2Cl\(^-\)/K\(^+\) cotransporter 1 (NKCC1) represents the third potential candidate for basolateral Cl\(^-\) entry that has been described to date. NKCC1 is part of the solute carrier family 12 (SLC12) and allows for electroneutral transport of Na\(^+\), K\(^+\), and 2Cl\(^-\) into the cell (46, 80, 89, 143). A contribution of NKCC1 to gastric acid secretion has been suggested for a long time as it is highly expressed in the basolateral membrane of parietal cells in a broad variety of animal species (33, 69, 80, 89, 109, 143). Earlier studies on guinea pig and amphibian mucosa with the NKCC1 inhibitors furomethide and bumetamide showed a decrease in electrical conductance and subsequent acid secretion and therefore proposed NKCC1 as a major mechanism for Cl\(^-\) entry (4, 143). However, studies on NKCC1 null mutants failed to confirm these observations as these knockouts were capable of acidifying gastric contents following stimulation with histamine (33, 90). Furthermore, studies on isolated rabbit and rat gastric glands (71, 91), as well as gastric mucosa isolated from bullfrog (47) and eel (154), failed to show an inhibitory effect of bumetanide on gastric acid secretion. In summary, Cl\(^-\) uptake by NKCC1 in the parietal cell seems to play no major role in the process of HCl secretion but appears mainly to contribute to nonacidic electrolyte secretion in the stomach as proposed and demonstrated lately by McDaniel et. al (88, 90).

**Sodium-hydrogen exchanger.** Almost all prokaryotic and eukaryotic cells display activity of the NHE, providing for the electroneutral exchange of hydrogen and sodium (23, 99). NHE isoforms NHE1–4 have been shown to be expressed in gastric parietal cells and to contribute to multiple cellular functions (5, 6, 8, 37, 68, 114, 124, 131, 142).

NHE1 was recently shown to play a key role in maintaining parietal cell volume homeostasis after stimulated acid secretion and subsequent secretagogue-induced cell shrinkage (5). However, expression of this transporter in parietal cells is relatively low (124). NHE1-knockout studies suggest that this isoform does not appear to be directly involved in gastric acid secretion (8, 131).

NHE2, like NHE1, does not appear to be essential for HCl production but seems to play an important role in the long-term viability and survival of parietal cells as NHE1, NHE2 knockouts display gastric mucosal atrophy (124).

The localization and functional activity of NHE3 in the process of parietal cell proton extrusion has lately been examined by Kirchhoff et al. (68). The authors were able to localize the protein at the apical membrane of parietal cells and demonstrate its putative function. NHE3 was shown to be down-regulated by histamine in the active phase of H\(^+\)-K\(^+\)-ATPase stimulation and to be active under resting conditions, thereby providing a potential auxiliary mechanism for proton secretion in the nonstimulated parietal cell. (Despite apical localization, NHE3 was covered in this section for the sake of simplicity).

NHE4 exhibits very high expression in the basolateral membrane of the stomach with particularly high levels in parietal and chief cells (124). Targeted disruption of NHE4 leads to a reduced number of parietal cells, an impaired differentiation and maturation and defective secretory membrane development (37). It is the most abundant NHE isofrom in parietal cells, and in light of current evidence, clarifies its role as a major protein responsible for basolateral Na\(^+/\)H\(^+\)-exchange activity (37, 124). NHE4 is functionally coupled to AE2-mediated Cl\(^-\)/HCO\(_3^-\) exchange (38). Thus NHE4 in connection with AE2 offer a mechanism for NaCl entry into the parietal cell, providing a major electrochemical driving force for HCl secretion (37).
Na⁺-K⁺-ATPase. The ubiquitous Na⁺-K⁺-ATPase, originally described in 1957 (140), is, like the gastric H⁺-K⁺-ATPase, a member of the P-type ATPase family (105). Its crystal structure has recently been unveiled by Morth et al. (94). The sodium pump is responsible for the generation of the electrochemical gradient across the cellular membrane, which in turn fuels nutrient absorption, pH regulation, and ion transport in the parietal cell. Removal of Na⁺ from the serosal bathing solution or inhibition of the Na⁺-K⁺-ATPase by ouabain thus leads to a diminution of HCl secretion (57, 58).

Signaling

Gastric acid secretion is stimulated by a variety of endocrine, paracrine, and neuronal signals in vivo. Two intracellular signaling pathways have been postulated to play a key role in the action of H⁺-K⁺-ATPase recruitment: the cAMP-mediated PKA activation pathway, typically induced by histaminergic stimulation of the H₂ receptor, and the Ca²⁺ pathway, typically induced by cholinergic stimulation of the muscarinic M₃ receptor or gastrin binding to the CCK₈ receptor (129, 161). However, some overlap between the two pathways is speculated to exist and may even be necessary to effectively trigger acid secretion (78, 129). Although cholinergic stimulation by the vagus nerve plays an important role in the stimulation of acid secretion, which is reflected by the fact that surgical vagotomy was a widely used treatment for GUD, histamine released from surrounding gastrin-stimulated ECL cells is thought to be the essential activating agent for the parietal cell in vivo (116).

The significance of histamine for intact acid secretion is underlined by the inability of H₂₋(−/−) mice to secrete acid in response to gastrin and histamine (70). Pharmacological blockade of the H₂ receptor was thus the state-of-the-art therapy for diseases related to a hypersecretion of acid before PPIs were developed. Although their efficacy in abolishing acid output is lower compared with PPIs, H₂ blockers are still in clinical use for patients experiencing breakthrough under PPI therapy and for patients on a regime of the platelet aggregation inhibitor clopidogrel. The latter is a result of an adverse pharmacodynamic interaction between PPIs and clopidogrel. Although this interaction is proven to exist, it does not seem to impose an increased risk in terms of a worse clinical outcome, which is why the current clinical guidelines on coadministration of PPIs and clopidogrel are in the process of being revised (100).

Histamine was first identified by Chew et al. (16, 19) to increase intracellular cAMP levels and thereby to provoke activation of PKA type I. Today it is known that several proteins serve as downstream phosphorylation targets following histamine-induced PKA activation in parietal cells (for a detailed review please refer to Ref. 161): the 80-kDa protein ezrin, which is thought to be a membrane cytoskeletal linker crucial for the formation of the characteristic apical microvilli of the secreting parietal cell (51); the recently cloned 65-kDa protein parchlorin (98), which has significant homology to the family of Cl⁻ intracellular channels (CLIC) and is implicated in Cl⁻ and water transport in a variety of secretory glands (93, 98); and the 40-kDa protein lasp-1 (21), which is an F-actin binding protein involved in the process of cell migration and tumor formation (44, 79, 164). Following stimulation with histamine, lasp-1 was shown to be translocated from the basolateral membrane to the apical membrane where it is speculated to play a role in the regulation of secretion by the parietal cell (20). Functional evidence for its regulatory role was gained by genetic disruption of lasp-1, which leads to an increase in acid output in response to histamine (18). A recent knockdown model has also underlined the importance of ezrin in the process of gastric acid secretion. Affected mice were achlorhydric and presented with defects in the formation of the canalicular apical membrane (149). Interestingly, a different study has proposed that ezrin also serves as a target for gastrin stimulation, which further illustrates the overlap between the intracellular signaling pathways (104). Although a common tendency of PKA targets to rearrange both membrane and cytoskeleton in the preparation of acid secretion can be seen, it is still unclear how an elevation of cAMP levels directly affects H⁺-K⁺-ATPase activity.

Whereas histamine-mediated activation is a paracrine process, cholinergic stimulation occurs via the vagus nerve, which releases ACh at the presynaptic terminus (129). In the 1980s Chew et al. (17) demonstrated, by use of the Ca²⁺-sensitive fluorescent dye fura2, that carbachol, an activator of muscarinic M₃ receptors, triggered an increase in intracellular Ca²⁺ concentrations. The elevation of intracellular Ca²⁺ manifested as an initial spike, representing Ca²⁺ release from intracellular stores, followed by a lower-level Ca²⁺ plateau caused by an inward Ca²⁺ current (97). Although a concomitant rise of intracellular inositol trisphosphate (IP₃) concentrations has been observed during carbachol stimulation (17), the role of PKC in this pathway is still controversial (2, 7, 12, 50, 161). Phosphorylation of the 28-kDa calcium-sensitive protein CSPP28 is reported to be a direct effect of cholinergic stimulation (107). CaM kinase might serve as a mediator for this process, since CSPP28 phosphorylation rates were increased by incubation of parietal cells with purified CaM kinase II (107, 161). Mamiya et al. (85) showed that inhibition of CaM kinase II by the specific inhibitor KN-62, despite high intracellular Ca²⁺ concentrations, abolished carbachol-induced acid secretion, which further supports the possibility that CaM kinase might serve as a downstream effector of cholinergic stimulation. Carbachol also induces a variety of other kinases: IkB, mitogen-activated protein kinases (MAPKs), extracellular signal-regulated protein kinases (ERKs), and the c-Jun NH₂-terminal protein kinases (JNKs) (96, 147, 148, 151, 161). Their contribution to the action of gastric acid secretion, however, is still unclear. Recent findings suggest that stimulation of the calcium-sensing receptor (CaSR) represents a novel mechanism in the process of H⁺-K⁺-ATPase activation. CaSR was first cloned in the 1990s and was speculated to sense extracellular Ca²⁺ concentrations on parathyroid and kidney cells (11). Now it is known that CaSR expression spans a broad spectrum of cell types throughout the gastrointestinal tract, including the parietal cell (15). It was shown that stimulation of CaSR by L-type amino acids or polyvalent cations, such as Ca²⁺, Mg²⁺, and Gd³⁺, increases acid secretion rates in the absence of other stimulatory agents (13, 40). Exposure to the secretagogue histamine in low extracellular calcium concentrations significantly reduces the anticipated secretory response implying that the receptor may also operate as a regulator in the process of secretagogue-triggered proton extrusion (40). In an attempt to uncover the intracellular mechanisms underlying CaSR stimulation, Remy et al. (120) suggested coupling of CaSR to a
pertussis toxin-sensitive G protein and subsequent PKC-, PLC-, ERK-, and MAPK activation to be parts of the downstream signaling cascade. However, the exact intracellular processes remain to be elucidated.

The inhibitory pathways affecting gastric acid secretion are even less well characterized. Somatostatin, which is known to be released by D cells in the antrum and fundus upon stimulation by cholecystokinin (CCK) and vasoactive intestinal peptide (VIP) (9, 163), has a direct effect on parietal cells by lowering intracellular cAMP concentrations via an inhibitory G protein that attenuates adenylyl cyclase activity (108). Inhibition of the G protein by pertussis toxin made this process reversible and rendered the parietal cells resistant to the effects of somatostatin (108, 130). A somatostatin receptor (SSTR2) knockout model has further shown an increase in acid output in response to vagal and gastrin stimulation (165).

Recently, AMP-activated protein kinase (AMPK) has emerged as a possible intracellular off-switch for acid secretion (138). AMPK is a multisubunit protein that senses intracellular energy levels (52). In response to certain stress factors, it adapts mostly metabolic pathways to prevent ATP depletion of the cell. Common stresses that induce AMPK activation are glucose deprivation (128), ischemia (72), hypoxemia (86), and exercise (127). The primary sensing mechanism for AMPK is an alteration in the AMP:ATP ratio, as a result of all of the aforementioned stresses (52). AMPK thus functions as an emergency off-switch to avoid ATP depletion of the cell by downregulating energy-consuming processes (52). Pharmacological activation of AMPK in the murine parietal cell results in a significant decrease in acid output (138). Since the process of acid secretion is particularly energy demanding, an interaction between H+-K+-ATPase and AMPK is plausible. However, the exact intracellular mechanism between AMPK and H+-K+-ATPase activity are so far unidentified. In summary, one can say that despite our in depth understanding of how acid secretion is initiated, we are far from comprehending the mechanisms involved in the termination of these effects.

H+-K+-ATPase Trafficking

In the nonsecreting parietal cell, H+-K+-ATPase is stored in intracellular tubulovesicles, which, upon stimulation of acid secretion, fuse with the apical canalicular membrane (64, 77), to enable proton transport into the lumen of the stomach (for a detailed review please refer to Ref. 101).

A complex interaction between both vesicle- and outer membrane-bound proteins is necessary for the membrane fusion process to take place. The cluster of proteins that mediates the process of vesicle docking and fusion is called soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex and basically consists of three proteins: SNAP-25, the vesicle-associated membrane protein (VAMP), alternatively named synaptobrevin, and syntaxin. Syntaxin 3 and VAMP2 were both shown to be specifically associated to H+-K+-ATPase-containing tubulovesicles (110). Syntaxin 3 was also identified by Karavar et al. (63) to translocate from the cytosolic compartment to the apical membrane, where it colocalizes with H+-K+-ATPase, syntaxin 1, and F-actin in stimulated parietal cells (63). By fluorescent tagging of vesicle-bound VAMP2 and apical membrane-bound SNAP-25, the same group demonstrated merging of both fluorescent signals at the apical pole of the parietal cell after stimulation with histamine (62), consistent with SNARE complex formation in the process of vesicle fusion. Additionally, defective SNAP-25 mutants showed a significantly decreased acid secretion capacity (62). A recent proteomic investigation of tubulovesicles from human parietal cells has established a vast number of additional proteins involved in the process of H+-K+-ATPase trafficking, including Rab10, VAMP8, syntaxin 7, and syntaxin 12/13, all of which were shown to colocalize with H+-K+-ATPase (75).

GTP-binding rab proteins are involved in various phases of vesicle processing and regulation. In the 1990s, rab2, rab11, and rab25 were identified in the tubulovesicles of the parietal cell (41, 42, 150). During acid secretion, rab11a was later shown to redistribute to the apical canalicular membrane. Its essential function in the action of vesicle trafficking was underlined by the incapability of defective rab11a mutants to recruit H+-K+-ATPase (14, 31). Upon stimulation with histamine, the mutant cells showed a H+-K+-ATPase distribution pattern similar to that in resting parietal cells. Incubation with tetracycline, blocking the expression of mutant rab under the control of the “tet-off,” restored the secretory function of the cells (31). Hales et al. (48) described four proteins as downstream rab11a effectors: rab11-family interacting protein 1 (rab11-FIP1), rab11-FIP2, rab11-FIP3, and pp75/rip11, all of which, except rab11-FIP3, coenriched with rab11a and H+-K+-ATPase in tubulovesicles (48). Rab11-FIP1 and rab11-FIP2 were additionally shown to translocate together with rab11a and H+-K+-ATPase to the apical membrane following histamnergic stimulation (48). However, rab11b localization patterns differ from rab11a. Rab11b is not associated with H+-K+-ATPase and, in contrast to H+-K+-ATPase containing tubulovesicles, is not dependent on microtubules (76). It therefore probably does not directly participate in H+-K+-ATPase trafficking.

Huntingtin-interacting protein related protein 1 (Hip1R) is thought to be a linking protein between clathrin and F-actin in the early stages of vesicle budding (32). An involvement of clathrin during H+-K+-ATPase recycling has already been demonstrated (102). Disruption of Hip1R results in phenotypical changes of the gastric mucosa with glandular hyperplasia and loss of tubulovesicles in the parietal cell as the most prominent features. Functionally, Hip1R-deficient mice present with an impaired ability to extrude acid, which is thought to be a consequence of a decreased total number of parietal cells and increased rates of apoptosis (61).

Conclusion

Considering the plethora of interactions among receptors, messengers, transport proteins, channels, and ion pumps that are a prerequisite for acid secretion and to maintain secretion, one cannot help but to marvel at the ingenuity of the highly specialized parietal cell machinery. As we have seen, disruption of a single component in this complex cascade can render the parietal cell functionally useless in terms of its function to secrete. The scientific effort of the past years has provided us with the potential identities of previously anonymous ion channels and transporters. It has thus for example been possible to identify KCNQ1 as the primary channel responsible for K+ recycling and to establish the contribution of diverse...
players such as CFTR or SLC26A9 to the process of chloride secretion. With the advent of various knockout mouse models, new antibodies, and functional studies, our understanding of the physiological regulation of acid secretion has notably improved. These models have significantly contributed to our knowledge concerning apical membrane regulation of acid secretion and have helped to uncover novel potential therapeutic targets for the management of acid-related disorders. In light of the high breakthrough rates occurring under conventional PPI therapy, an alternative or complementary pharmacological approach is highly desirable and would provide relief for the increasing population of patients who suffer from diseases like GERD. Although the APAs showed promise, these drugs were limited by pharmacokinetic and pharmacodynamic insufficiencies. There is no question about the high degree of PPI efficacy; however, it is also obvious that room for improvement still exists and that this potential needs to be exploited with some of the new transport proteins and receptors that have been outlined in this review.

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