Gastric exocrine and endocrine secretion

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Purpose of review

This review summarizes the last year's literature regarding the regulation and measurement of gastric exocrine and endocrine secretion.

Recent findings

Parietal cells, distributed along much of the length of the oxyntic glands, with highest density in the neck and base, secrete HCl as well as transforming growth factor-alpha, amphiregulin, heparin-binding epidermal growth factor-like growth factor, and sonic hedgehog. Acid facilitates the digestion of protein and absorption of iron, calcium, vitamin B_{12} as well as prevents bacterial overgrowth, enteric infection, and possibly food allergy. The major stimulants of acid secretion are gastrin, histamine, and acetylcholine. Ghrelin and orexin also stimulate acid secretion. The main inhibitor of acid secretion is somatostatin. Nitric oxide and dopamine also inhibit acid secretion. Although *Helicobacter pylori* is associated with duodenal ulcer disease, most patients infected with the organism produce less than normal amount of acid. The cytoskeletal proteins ezrin and moesin participate in parietal cell acid and chief cell pepsinogen secretion, respectively.

Summary

Despite our vast knowledge, the understanding of the regulation of gastric acid secretion in health and disease is far from complete. A better understanding of the pathways and mechanisms regulating acid secretion should lead to improved management of patients with acid-induced disorders as well as those who secrete too little acid.

Keywords

acetylcholine, acid secretion, gastrin, ghrelin, *Helicobacter pylori*, histamine, orexin, parietal cell, review, somatostatin

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Introduction

Histologically, the human gastric mucosa is divided into three zones: cardiac, fundus/corpus/oxyntic, and antral/pyloric [1]. There is some debate as to whether the cardia, a transition zone of 0–9 mm between the squamous mucosa of the esophagus and the oxyntic mucosa of the stomach, exists as a normal anatomic structure or develops as a result of abnormal reflux [2••]. Gastric glands have been functionally divided into four regions [3]: the pit region, consisting mainly of columnar surface mucous cells, the isthmus where the multipotent progenitor cells reside, the neck region abundant with mucous neck cells, which are distinct from surface mucous cells and are the transitioning cells destined to become chief cells as they divide and migrate downwards, and the base region abundant with chief cells. The hallmark of the oxyntic glands is the parietal cells that are distributed along much of the length of the gland with highest density in the neck and base. Parietal cells secrete HCl as well as transforming growth factor-alpha, amphiregulin, heparin-binding epidermal growth factor-like growth factor, and sonic hedgehog [4,5]. Pyloric glands, the hallmark of which is the gastrin cell, are branched and coiled at their basal ends with longer pits that occupy about half the thickness of the mucosa. The human pyloric mucosa has a higher turnover rate than the oxyntic mucosa [1].

Gastric acid secretion developed approximately 350 million years ago. Acid facilitates the digestion of protein and absorption of iron, calcium, and vitamin B_{12} as well as prevents bacterial overgrowth and enteric infection [2••,6•,7]. Acid may also reduce or eliminate the allergenicity of some food allergens. Dehlink *et al.* [8], using a population-based observational cohort formed by linking data from three Swedish national healthcare registers, report that maternal acid-suppressive drug use significantly increases the risk for developing childhood asthma (odds ratio, 1.51) but not other allergic diseases.

In newborns, gastric pH ranges from 6.0 to 8.0 [9]. This is followed by a burst of acid secretion 24–48 h after birth to

adult levels (pH 1.0–3.0). Acid secretion then returns to low levels during the next few months and adult pH levels are not again reached until the age of 2 years. In adults, parietal cells secrete HCl at a concentration of approximately 160 mmol/l or pH 0.8.

Milk–alkali syndrome consists of hypercalcemia, renal insufficiency, and metabolic alkalosis and is due to ingestion of large amounts of calcium and absorbable alkali [10]. The syndrome was first described in the 1930s when peptic ulcer disease was commonly treated with milk and sodium bicarbonate but has resurged as the result of the increasing use of calcium carbonate, mostly for osteoporosis prevention. It is now the third most common cause of hypercalcemia after hyperparathyroidism and malignant neoplasms. The precise pathogenesis of the syndrome is not known, as most patients ingesting large quantities of calcium and alkali do not develop hypercalcemia and alkalosis. However, preexisting renal disease and medications (e.g., thiazides) that decrease calcium excretion have been implicated. Alkalosis decreases calcium excretion and hypercalcemia impairs bicarbonate secretion, possibly setting up a vicious cycle in susceptible individuals.

Measurement of gastric acid secretion

Gastric acid secretory testing, performed by placing a nasogastric tube into the most dependent portion of the stomach and aspirating gastric juice, is the gold standard for quantifying acid secretion but, because of complexity, is not widely performed. More commonly, a pH electrode is used to estimate acid secretion by measuring acid concentration. Using an esophageal pH catheter with an antimony sensor, Ayazi *et al.* [11] report that the median fasting gastric pH for 54 normal individuals is 1.5 with a normal range of 0.3–2.9. Similar results, mean fasting pH of 1.9 ± 0.2 ($n = 64$), were obtained by Hasler *et al.* [12] using a wireless transmitting capsule (SmartPill Corporation, Buffalo, New York, USA). The Smartpill records luminal pH, temperature, and pressure during transit through the gastrointestinal tract and transmits the data to a recorder. In that study [12], diabetic patients with gastroparesis exhibited reduced gastric acid secretion (mean fasting pH, 3.6 ± 0.4), an effect more pronounced in those with severely delayed gastric emptying, whereas those with idiopathic gastroparesis exhibited nearly normal gastric pH, except for those with severely delayed emptying. Thus, it appears that both the cause and degree of gastric stasis determine gastric acidity in patients with gastroparesis.

Two physicists, Giouvanoudi and Spyrou [13], used electrical impedance epigastrography, a technique that measures electrical impedance utilizing surface electrodes and a device that generates and applies alternating current of 1–4 mA rms at 32 kHz while simultaneously measuring the developed potential difference, to measure gastric acid concentration in male volunteers. Electrical impedance measurements change according to the conductivity in the gastric lumen, which depends upon the conductivity of the ingested meal and the conductivity of acid produced. Using complex mathematical formulas, the basal concentration of HCl in the empty stomach of a specific volunteer was 10.07 mmol or pH 1.997. Given the fact that electrogastrography, a method to assess gastric motor function, despite its noninvasive nature, has not gained widespread application, it is doubtful that electrical impedance epigastrography will be embraced.

A simple, noninvasive, and accurate method for measuring gastric acid secretion would be welcomed. Clough and Axon [14] describe a novel ^{13}C-labeled calcium carbonate breath test for the potential noninvasive measurement of stimulated gastric acid secretion. Ingested calcium carbonate is converted to calcium chloride, CO_2, and water by HCl. The CO_2 is rapidly absorbed by the gastric mucosa and delivered through the bloodstream to the lungs where it is excreted in the breath. Measurement of excess $^{13}CO_2$ in the breath enables the amount of acid that has been secreted by the stomach to be calculated.

Karamanolis *et al.* [15•] studied the acute effect of water as well as acid-neutralizing and acid-inhibiting drugs on gastric pH. A glass (200 ml) of water (tap water, pH 5.9; distilled water, pH 6.4; bottled water, pH 7.2) immediately increased gastric pH above 4 for a duration of 3 min. Antacid (15 ml) increased gastric pH above 4 within 2 min for a duration of 12 min. The onset and duration of gastric pH above 4 for ranitidine (150 mg) and omeprazole (20 mg) were 50/65 min and 171 min/>6 h, respectively. The findings indicate that water and antacid acutely raise intragastric pH, whereas antisecretory agents have a more delayed action. The findings suggest that 'on-demand' therapy for episodic heartburn should include water, antacid, or both in addition to antisecretory medication.

Neuroendocrine regulation of gastric acid secretion

A variety of neurocrine, paracrine, and endocrine signals regulate gastric acid secretion, including gastrin, histamine, acetylcholine, ghrelin, orexin, somatostatin, nitric oxide, and dopamine [2••,16]. In addition, infection with *Helicobacter pylori* may either inhibit or stimulate acid secretion, depending upon the circumstances.

Stimulants

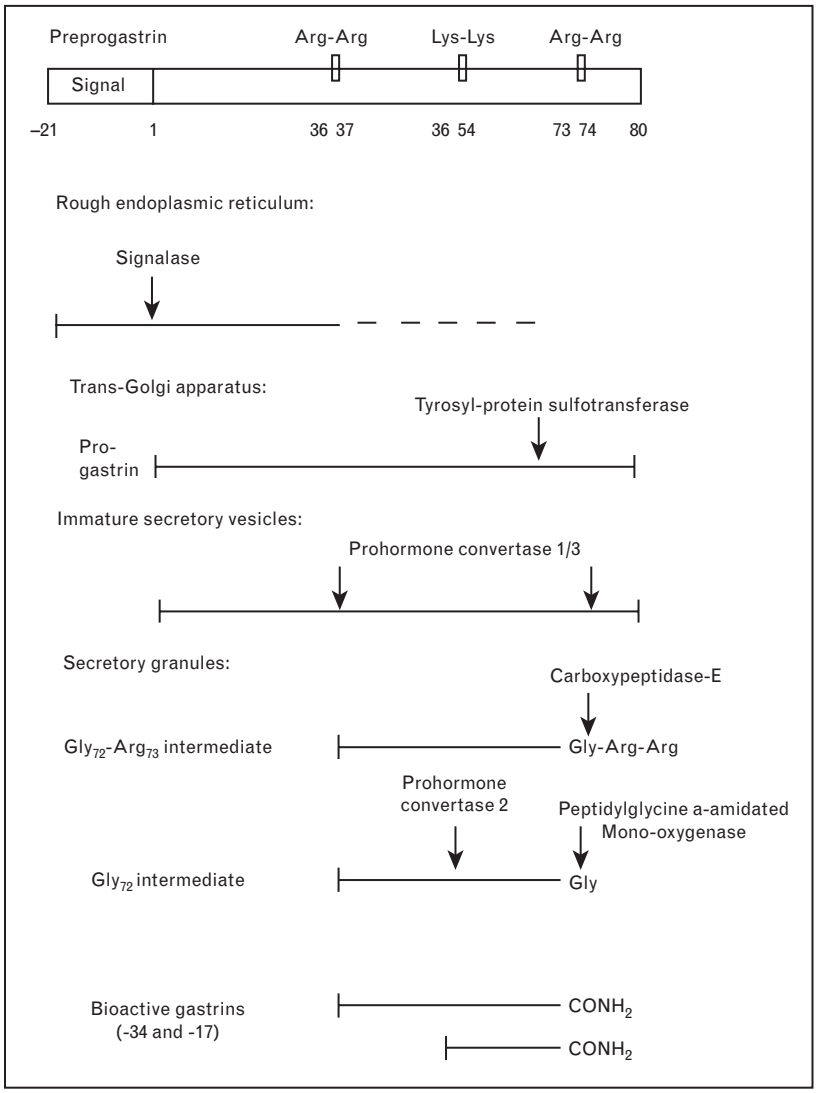
The major stimulants of acid secretion are acetylcholine, gastrin, and histamine [2••]. Acetylcholine, released from

postganglionic neurons, stimulates the parietal cell directly via M3 receptors and indirectly via M2 and M4 receptors coupled to inhibition of somatostatin secretion. Histamine, released from neighboring enterochromaffin-like (ECL) cells, stimulates the parietal cell directly via H2 receptors. Gastrin, released from antral G cells, stimulates the parietal cell mainly indirectly via cholecystokinin-2 (CCK-2) receptors on ECL cells coupled to histamine release. Other stimulants include ghrelin and orexin.

Gastrin

Gastrin is a hormone that stimulates gastric acid secretion and mucosal cell growth. Most gastrin is synthesized in antral G cells via posttranslational processing of progastrin. The processing requires cleavage of dibasic arginine residues by prohormone convertases, prohormone convertase 1/3 followed by prohormone convertase 2 (Fig. 1) [17]. Although circulating gastrin is composed of a mixture of peptides of different lengths and amino acid derivations, more than 95% of secreted gastrins are α-amidated gastrins of which 85–90% are gastrin-17, 5–10% are gastrin-34, and the rest are a mixture of gastrin-71, gastrin-52, gastrin-14, and short C-terminal hepta-to tetrapeptide amide fragments [18•]. In serum, however, there are almost similar concentrations of gastrin-17 and gastrin-34 because the metabolic clearance of gastrin-17 is 10-fold faster than that of gastrin-34. It should be noted that in hypergastrinemic states, such as gastrinoma or achlorhydria, the translational activity in

Figure 1 Diagram illustrating the cotranslational and posttranslational modification of progastrin and antral G-cells



Through a series of C-terminal cleavages and modifications, progastrin is processed to yield bioactive α-amidated gastrins. In normal G cells, the predominant forms are gastrin-34 (5–10%) and gastrin-17 (85–90%) [17].

the gastrin-producing cells is so high and the cellular transport of secretory vesicles so fast that the processing enzymes cannot keep up with the progastrin maturation and large amounts of unprocessed and incompletely processed progastrin products, predominantly gastrin-71 and gastrin-34, are released [18•,19].

Gastrin, the main hormonal stimulant for acid secretion during meal ingestion, mediates its effects primarily through the CCK-2 receptor, a G protein-coupled receptor previously termed the CCKB or gastrin receptor. CCK-2 receptors are expressed on ECL cells. Whether a functional gastrin receptor exists on parietal cells is a matter of controversy [20]. Consequently, the acid secretory effect of gastrin is thought to be mediated primarily via release of histamine from ECL cells [16].

In addition to stimulation of acid secretion, gastrin exerts growth-promoting effects in normal and neoplastic tissues [21]. Almeida-Vega *et al.* [22] report that gastrin induces plasminogen activator inhibitor-2, a component of the urokinase activator system that acts extracellularly to inhibit urokinase plasminogen activator and intracellularly to suppress apoptosis. Ito *et al.* [23] report that the CCK-2 receptor is expressed in 65% of gastric cancers. In human gastric carcinoma cells transfected with the CCK-2 receptor, gastrin induces mcl-1 expression, an anti-apoptotic protein thought to inhibit apoptosis by inhibiting mitochondrial cytochrome *c* release [24]. Expression of this protein was also increased in hypergastrinemia-associated gastric carcinoid tumors that developed in patients with atrophic gastritis.

Histamine

The ECL cells, located at the base of the oxyntic glands, are the primary source of gastric histamine. Four distinct subtypes of histamine receptors have been identified and designated: H-1, H-2, H-3, and H-4. Gastrin stimulates acid secretion primarily by releasing histamine from ECL cells. Histamine reaches the parietal cell either by interstitial diffusion or capillary transport where it binds to H-2 receptors coupled to adenylyl cyclase activation, cyclic AMP (cAMP) production, and recruitment of H^+K^+-ATPase into the apical canalicular membrane. In contrast to gastrin, which induces sustained histamine secretion, ischemia, induced by clamping of the celiac artery, induces a large but short lasting burst of histamine release that might contribute to the pathogenesis of stress-induced gastric erosions and ulcers [25].

There has been some debate regarding the location and function of H-3 receptors in the stomach. Using immunohistochemistry, Grandi *et al.* [26] report that H-3 receptors are localized predominantly to ECL cells. The same group has localized the H-4 receptor to ghrelin cells [27].

Acetylcholine

Acetylcholine, released from postganglionic neurons, stimulates the parietal cell directly via M3 receptors coupled to increases in intracellular calcium concentration and indirectly via M2 and M4 receptors on somatostatin-containing D cells coupled to inhibition of somatostatin secretion [28]. Although there had been some debate as to the presence of M3 receptors on ECL cells, most data support the view that acetylcholine has no direct stimulatory effect on histamine secretion. In further support of this notion, Bitziou *et al.* [29], simultaneously using an iridium oxide pH microelectrode to measure hydrogen concentration and a boron-doped diamond microelectrode to detect histamine concentration in isolated guinea pig stomach, report that acetylcholine stimulates acid but not histamine secretion.

Ghrelin

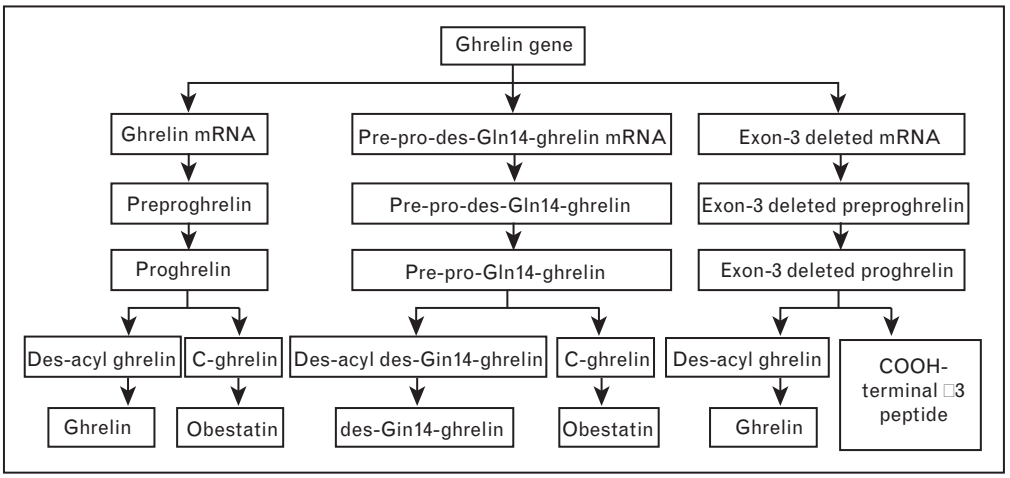
Ghrelin, the natural ligand for the orphan growth hormone secretagogue receptor type 1, was discovered in gastric extracts, localized to A-like or Gr cells in the basal part of oxyntic mucosa [30,31•]. Alternate splicing of the ghrelin gene yields ghrelin, des-acyl ghrelin, and obestatin (Fig. 2). Both plasma ghrelin and obestatin levels are decreased in patients with chronic atrophic gastritis [32]. The major active form of human ghrelin is a 28-amino acid peptide with an *n*-octanoyl modification at Ser3. The acyltransferase that catalyzes ghrelin octanoylation has recently been identified as ghrelin *O*-acyltransferase [33].

Ghrelin has multiple actions in multiple tissues including hyperphagia, inhibition of insulin release, and stimulation of gastrointestinal motility. Most, but not all studies, report that ghrelin stimulates gastric acid secretion, probably via vagal pathways and histamine release. Fukumoto *et al.* [34•] report, in rats, that gastrin receptors (i.e., CCK-2 receptors) are present on ghrelin cells by immunohistochemistry, gastrin stimulates ghrelin secretion in a dose-dependent manner, and gastrin and ghrelin act synergistically to stimulate gastric acid secretion. Bilgin *et al.* [35] report, in pylorus-ligated rats, that intravenous ghrelin stimulates gastric acid secretion as well as plasma nitrite levels. Both effects were abolished by an inhibitor of nitric oxide synthase, N^G-nitro-L-arginine methyl ester (L-NAME), indicating that the secretory effect was mediated via generation of nitric oxide.

Jang *et al.* [36] report that, in 22 patients with *H. pylori*-associated gastric and duodenal ulcers, eradication of the organism with healing of the ulcer is associated with increased expression of fundic ghrelin mRNA and increased appetite.

Ferrer-Lorente *et al.* [37] report that oral administration of oleoyl–estrone, a natural hormone secreted by adipose tissue that decreases food intake, decreases ghrelin

Figure 2 The sequential steps in the production of the three major ghrelin gene-derived products



Data from [30].

expression in rat oxyntic mucosa. Thus, a reduction in the ghrelin orexigenic signal may mediate the short-term decrease in food intake [38].

Orexin

Orexin-A and orexin-B are neuropeptides derived from propro-orexin by posttranslational processing. They bind and activate two G protein-coupled receptors termed orexin receptor 1 (OX1R) and orexin receptor 2 (OX2R). OX1R preferentially binds orexin-A, whereas OX2R has equal affinity for both peptides. Orexin and its receptors are present in the hypothalamus and gastric antrum.

Intraventromedial hypothalamic injection of orexin-A stimulates gastric acid secretion in pyloric-ligated conscious rats in a concentration-dependent manner [39]. The effect is blocked by the OX1R receptor antagonist, SB-3345867, given intraperitoneally. The antagonist, by itself, inhibits acid secretion, implying that endogenous orexin-A, acting via OX1R receptors, stimulates acid secretion.

Inhibitors

Inhibitors include somatostatin, *H. pylori*, nitric oxide, and dopamine.

Somatostatin

Somatostatin is the main inhibitor of acid secretion. Acting in a paracrine fashion, it directly inhibits acid secretion from the parietal cell, histamine secretion from the ECL cell, and gastrin secretion from the G cell. The inhibitory effect of somatostatin on gastrin secretion is mediated via the somatostatin subtype 2 receptor that is coupled to suppression of cAMP and induction of menin [40]. *Menin*, a tumor suppressor gene, directly inhibits gastrin gene expression.

Helicobacter pylori

H. pylori colonizes the gastric epithelium of one-half of the world's population and is a cause of gastritis, peptic ulcer, mucosa-associated lymphoid tissue lymphoma, and gastric cancer. Acute infection is associated with hypochlorhydria. The mechanisms by which *H. pylori* inhibits acid secretion are multifactorial and include production of the proinflammatory cytokine interleukin-1β, repression of H^+K^+-ATPase α-subunit promotor activity, and vacuolating cytotoxin A (VacA)-induced proteolysis of ezrin, an actin-binding protein that plays a role in trafficking H^+K^+-ATPase-containing tubulovesicles to the apical membrane of the parietal cell [41,42].

Chronic infection with *H. pylori* results in either hypochlorhydria or hyperchlorhydria depending upon the severity and distribution of gastritis. Most patients chronically infected manifest a pangastritis and produce less than normal amount of acid. Eradication of the organism in these patients normalizes acid secretion, not only in the intact stomach but also in the remnant stomach after antrectomy [43].

Approximately 12% of patients chronically infected with *H. pylori* have antral predominant inflammation and it is these patients who are predisposed to duodenal ulcer. These patients produce increased amounts of acid as a result of reduced somatostatin content and elevated basal and stimulated gastrin secretion. Eradication of the organism normalizes somatostatin, gastrin, and acid secretion [44].

Nitric oxide

The influence of nitric oxide on gastric acid secretion has been somewhat controversial with most studies showing inhibition. In anesthetized rat, Ito *et al.* [45] report that gastric distension by instillation of isotonic saline through an acute fistula increases luminal acid secretion and nitric oxide production. The acid response was augmented in the presence of the nitric oxide synthase inhibitor, L-NAME, implying that endogenous nitric oxide inhibits acid secretion. Consistent with this notion, FK409, a nitric oxide donor, dose-dependently decreased acid secretion.

Dopamine

Dopamine signaling is mediated by at least five cloned receptors that have been grouped into two main families, namely D1-like (D1 and D5) and D2-like (D2, D3, and D4); D2 receptors have been identified in stomach. Using anesthetized rats, Eliassi *et al.* [46] show that the D2 receptor-like agonist, quinpirole, inhibits histamine, pentagastrin, and carbachol-stimulated acid secretion in a concentration-dependent manner, and that the effects are blocked by domperidone, a peripheral D2 receptor antagonist.

Intracellular regulation of gastric acid secretion

During acid secretion, there is a profound morphological transformation of the parietal cell with translocation of cytoplasmic tubulovesicles to the apical membrane with the formation of multiple secretory canaliculi. Coincident with this transformation, the H^+K^+-ATPase is inserted into the apical canalicular membrane where it transports protons out of the cell in exchange for luminal potassium.

H^+K^+-ATPase

The gastric H^+K^+-ATPase is an electroneutral transmembrane pump that transports hydronium ions from the cytoplasm into the canaliculus of the parietal cell in exchange for potassium [47]. Using ATP hydrolysis as energy source, it generates an extracellular proton concentration that can be as much as a million times higher than the intracellular concentration [48]. The enzyme consists of a catalytic α-subunit and a regulatory β-subunit. The heavily glycosylated β-subunit is necessary for endoplasmic reticulum to Golgi trafficking as well as apical delivery and membrane retention of the enzyme [49]. H^+/K^+ counter-transport activity is acquired only when the enzyme is transferred from the tubulovesicles to the secretory apical membrane.

Recent studies suggest that cytoskeletal proteins such as ezrin (a cytoskeletal linker protein) and LASP-1 (LIM and SH3 domain protein 1, an actin-binding protein) participate in proton pump trafficking to and from the parietal cell apical membrane. Studies in gastrin null mice suggest that gastrin acts via CCK-2 receptors on parietal cells as well as transactivation of the epidermal growth factor receptor to stimulate ezrin expression and distribution to tubulovesicles [50]. Studies in LASP-1 null mice suggest that LASP-1 may modulate endocytic retrieval of H^+K^+-ATPase from the apical membrane back to the cytosolic tubulvesicular compartment [51].

Channels and receptors

Acid secretion by the parietal cell requires a functional H^+K^+-ATPase as well as apical potassium and chloride channels [52]. KCNQ1 assembles with its β-subunit, potassium voltage-gated channel, Isk-related family member 2 or KCNE2, to function as a constitutively open, voltage-insensitive, and acid-resistant luminal potassium channel that is activated during stimulation of acid secretion. Other luminal potassium channels may include members of the inward rectifier family (e.g., KCNJ10, KCNJ15, and KCNJ16).

Glucocorticoids stimulate gastric acid secretion, at least in part, by upregulating serum and glucocorticoid-inducible kinase (SGK1), which, in turn, stimulates the activity of KCNQ1.

Adenomatous polyposis coli (*APC*), a tumor suppressor gene inactivated in familial adenomatous polyposis, fosters degradation of β-catenin, which, in turn, interferes with the WNT/β-catenin pathway and upregulates SGK1. Mice carrying a loss of function mutation in the *APC* gene spontaneously develop gastrointestinal tumors and manifest increased acid secretion; the latter is at least partially due to enhanced SGK1-dependent upregulation of KCNQ1 [53].

A calcium-sensing receptor has been identified on the apical and basolateral membranes of gastrin-secreting cells (G cells) and basolateral membrane of parietal cells [54]. In G cells, activation of this receptor leads to an increase in intracellular calcium and gastrin release. This receptor may be responsible for the rebound acid secretion observed with ingestion of calcium-containing antacids.

Pepsinogen

Pepsinogen is synthesized primarily not only in chief cells but also in mucous neck cells. Group I pepsinogens (PGIs) are expressed in oxyntic mucosa, whereas group II pepsinogens (PGII) are expressed in both oxyntic and pyloric mucosa. Tashima *et al.* [55] report a novel method to isolate and culture rat chief cells using hepatocyte growth factor. The cell layers display high transepithelial resistance, continuous tight junctions, and low permeability.

Zhu *et al*. [3] report that moesin, a member of a family of proteins involved in the establishment and maintenance of polarity as well as the membrane activities of secreting cells, is expressed in chief cells, exclusively on the apical membrane.

Acid is produced from the hydration of CO_2 to form H^+ and $HC0_3^-$, a reaction catalyzed by carbonic anhydrase. In an intriguing paper, Steer [56] provides preliminary evidence that the source of CO_2 may be the decarboxylation of arginine residues derived from PGII contained within parietal cell canaliculi. As pepsinogen is not produced by parietal cells, precisely how it gains access to the canaliculi requires further study.

Stimulants for pepsinogen secretion include acetylcholine, cholecystokinin, gastrin, gastrin releasing peptide, secretin, vasoactive intestinal peptide, epidermal growth factor, and nitric oxide [45].

Conclusion

Gastric acid secretion remains an important pathogenic factor for a variety of common upper gastrointestinal disorders. We continue to make progress in our understanding of gastric acid secretion in health and disease. It is anticipated that this knowledge will be used to develop new and more effective strategies to prevent and manage these disorders.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 588–589).

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