Gastroduodenal Mucosal Alkaline Secretion and Mucosal Protection

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The gastroduodenal mucosa is a dynamic barrier restricting entry of gastric acid and other potentially hostile luminal contents. Mucosal HCO\(_3\) is a key element in preventing epithelial damage, and knowledge about HCO\(_3\) transport processes, including the role of the cystic fibrosis transmembrane conductance regulator channel, and their neurohumoral control are in rapid progress.

The human upper gastrointestinal tract from the mouth to the proximal duodenum takes a regular beating, with the ingestion of food, including hyper- and hyposmolar liquids at temperatures ranging from that of iced water to close to 90°C and with a pH extending from ~1.5 (such as a vinegar salad dressing) to ~11.0 (sodium bicarbonate for indigestion). In addition, the mucosa is exposed to noxious agents, including high concentrations of ethanol and medications such as aspirin and other nonsteroidal anti-inflammatory drugs. It is quite extraordinary that the mucosal layer does not more frequently develop erosions, ulcers, and hemorrhage.

Throughout the gastrointestinal tract, the mucosa provides a dynamic barrier within the host, allowing the passage of certain ions and molecules into the body and restricting the entry of other luminal contents. This maintenance of barrier function is not so much an anatomic barrier as it is a series of consecutive defense mechanisms, each of them finely regulated. The most proximal upper gut is anatomically and physiologically well prepared for the onslaught of potentially injurious agents. The tongue and esophagus are lined by an impermeable squamous epithelium, and each benefits from glandular secretions. In the mouth, the salivary glands provide considerable amounts of HCO\(_3\)-buffered liquid and mucoid secretions, whereas in the esophagus, scattered submucosal glands provide smaller amounts of HCO\(_3\) and mucus.

The stomach is particularly exposed to injury because the columnar mucosa is not only exposed to ingested potentially noxious agents but also secretes hydrochloric acid at a concentration of up to 145 mM (in humans) and proteolytic enzymes (pepsinogens). In particular, the fundic mucosa in the upper 80% of the stomach is a relatively “tight” epithelium, having a high electric resistance and, under normal conditions, being relatively impermeable to transport of luminal contents, including water. In contrast, the mucosa in the duodenum is a highly permeable, i.e., “leaky,” epithelium, whose major function, along with the remainder of the small intestine, is the absorption of nutrients and water. The duodenal mucosa is bathed in pancreatic enzymes (peptidases, lipases) and amphipathic bile salts and is intermittently exposed to acid discharged from the stomach. However, in spite of the potentially hostile exogenous and endogenous luminal contents, the respective epithelia normally maintain their integrity.

Gastroduodenal mucosal defense can be divided into preepithelial, epithelial, and subepithelial factors that function in concert with one another to prevent mucosal injury. This division is arbitrary, because an entire epithelium functions as a unit to prevent damage. The role of cytokines, growth factors, acid/base transporters, blood flow, adhesins, and microvascular integrity are the subjects of intense study. This review will focus on the factors that are responsible for the gastric and duodenal epithelium resistance to potential mucosal damage induced by acid and pepsin, certain drugs, and bacterial infection.

Gastric mucus layer and epithelial surface

The mucosa in the stomach and duodenum is covered by continuous and adherent layer of viscoelastic mucus gel that provides a physical barrier with low permeability for pepsin and other macromolecules between the lumen and the apical cell surfaces (3). The viscous and gel-forming properties of this mucus gel are derived from mucin glycoproteins (mucins), which constitute ~3-5% of the gel by weight, the remaining 95% being water, together with small amounts of lipids, nucleic acids, and proteins, including immunoglobulins. Studies using RNA analyses and immunohistochemistry have provided information about the types of mucin produced along the gastrointestinal tract. In the stomach (antrum, fundus, and cardia), the MUC5 mucin gene is present in the surface epithelial cells and the MUC6 gene is in mucous neck cells. Interestingly, MUC6 is also expressed in the Brunner's glands in the duodenum, whereas the duodenal surface epithelium (as well as the epithelium in more distal small intestine) expresses MUC2 and MUC3. Although separate gastrointestinal segments and cell types secrete different types of mucins, little is known about the possible different physiological actions or specific functions of the varying mucins. In the stomach as well as in the duodenum (3), convincing evidence has been provided that the adherent mucus gel layer and HCO\(_3\) secreted from the surface epithelium together provide a first line of defense against luminal acid (and pepsin). A pH gradient is formed, with pH at the epithelial cell surface considerably higher than that in acid-containing luminal bulk solutions, and diffusion of macromolecules, including...
pepsin, from the lumen to the epithelial surface is prevented or restricted.

In contrast, transport of acid and pepsin from the crypts into the gastric lumen is unimpeded by the mucus layer. Moreover, H+ secreted by the oxyntic cells within the gastric crypts appears to pass through small channels within the gel formed by the secretory hydrostatic pressure within the gastric glands (13). Furthermore, mucus secreted from cells within the gastric crypts has been proposed as a vehicle for transport of protons (and pepsinogen) from crypt to lumen (11). The latter mechanism, however, is likely quantitatively insufficient to deliver the large amounts of acid (20–40 mmol/h in humans) secreted at stimulated rates of acid secretion.

The thicknesses of the adherent mucus layers in the stomach and duodenum in humans are, in spite of the marked differences in epithelial surface topology, similar and range between 80 and 280 µm. The pH across the mucus gel layer to the epithelial cell surface has been measured precisely in vitro as well as in vivo using pH-sensitive microelectrodes (3). In spite of gastric luminal pHs as low as 2.0–3.0, there is a progressive increase in the pH within the mucus gel from the gastric lumen to the epithelial cell surface, where the pH is neutral. Higher luminal acidities tend to dissipate the pH gradient and therefore result in exposure of the gastric cell surface to the pH approaching that of the luminal bulk solution. However, these surface pH gradients were demonstrated in large part under experimental conditions in stomachs in which acidic solutions were used and intrinsic gastric acid secretion was low or absent.

Recent work indicates that the pH gradient at the surface of the rat stomach in vivo becomes thinner; yet it is not dissipated, even during maximal stimulation of acid secretion. This agrees with previous observations that stimulation of acid secretion enhances, rather than diminishes, the ability of the gastric mucosa to resist injury. The greater resistance to acid-induced damage while maintaining a pH gradient during periods of gastric acid secretion is likely secondary to the “alkaline tide” originating from the acid-secreting parietal cells. This represents the mole-for-mole formation of HCO₃⁻ simultaneously with H⁺ in the acid-secreting parietal cells followed by transport of HCO₃⁻ across the basolateral membrane by Cl⁻/HCO₃⁻ exchange, which results in increasing amounts of HCO₃⁻ available within the epithelial vasculature and mucosa for subsequent uptake and secretion by the surface epithelial cells. The role of the alkaline tide is strongly supported by findings that parenteral infusion of HCO₃⁻ in vivo animals, or serosal side application of HCO₃⁻ (not other buffer species) to gastric mucosa in vitro, protects the mucosa from injury, similar to that observed during acid secretion (7).

FIGURE 1. Mechanisms of HCO₃⁻ transport by duodenal enterocytes. The model is based on studies of isolated cells, membrane vesicles, and duodenal mucosa in vitro and in vivo in several species. Species differences, if any, seem small, but the mucosal location of the duodenal ion transport processes (villus and/or crypt cell) has not yet been fully determined. HCO₃⁻ reaches the epithelium via blood as well as secondary to the intracellular carbonic anhydrase conversion of CO₂ + H₂O to HCO₃⁻. In addition, HCO₃⁻ is imported via NaHCO₃ transport and extruded via Cl⁻/HCO₃⁻ exchange and ionic conductance. Any factors that diminish HCO₃⁻ availability may increase the vulnerability of the mucosa to acid injury. CFTR, cystic fibrosis transmembrane conductance regulator; AE, anion exchanger; NHE, Na⁺/H⁺ exchanger; CA, carbonic anhydrase; NBC, NaHCO₃ cotransporter.
Although neutralization of acid by HCO₃⁻ within the surface mucus gel provides a first line of gastric protection against acid, additional mechanisms are also operative. In the event that the surface pH gradient is dissipated, protons start entering the gastric surface epithelial cells, resulting in a decrease in intracellular pH (pHi). Intracellular neutralization of H⁺ occurs by HCO₃⁻ entry via the basolateral NaHCO₃ cotransporter (NBC) and by the export of protons by Na⁺/H⁺ exchange (NHE1), correcting the decrease in pH. The relatively low permeability of the apical cell membranes to H⁺ and the low conductance of the paracellular pathways between the cells are additional mechanisms for protection of the gastric surface. Furthermore, it should be noted that, unlike the gastric surface epithelium, most of the lumens of the gastric crypts within the mucosa are not covered by mucus gel. Luminal perfusion of isolated gastric crypts with solutions of varying pHs (15) has suggested that the apical membrane of parietal and chief cells that line the crypts are unusually resistant to low pH. Parietal cells are also likely to be protected by the apical membrane proton pump (H⁺-K⁺-ATPase activity), capable of exporting H⁺ at rapid rates.

Duodenal surface

The duodenal epithelium secretes HCO₃⁻ at higher rates (per unit surface area) than does the stomach (or more distal small intestine). Epithelial HCO₃⁻ secretion is currently accepted as the most important defense mechanism against acid discharged from the stomach, and the pH within the duodenal mucus gel is maintained at neutrality at acidities encountered in the healthy duodenum. In the first part of the duodenum, mucosal HCO₃⁻ is secreted not only in immediate proximity to the epithelial cell surface but also proximal to the entry of HCO₃⁻ from pancreaticobiliary ducts. The epithelial alkaline secretion in the duodenum is increased markedly by a low pH in the duodenal lumen (pH ~ 5 in rat and pH ~ 3 in human), a response mediated by neural reflexes and mucosal production of prostaglandins. Several transmitters, including vasoactive intestinal polypeptide (VIP) and acetylcholine, have been proposed as mediators of the efferent limb of the neural response. Chemical deafferentation by capsaicin inhibits the rise in HCO₃⁻ secretion in response to luminal acid. The response to exogenous prostaglandin (PG) E₂, in contrast, is not affected by the destruction of the afferent neurons. This suggests that luminal acidification involves the enteric nervous system, whereas PGE₂ acts directly on the HCO₃⁻-producing cell (14). Recent findings suggest that PGs stimulate duodenal HCO₃⁻ secretion by acting on the duodenal EP₃ receptors, whereas in the stomach they stimulated HCO₃⁻ by affecting the gastric EP₁ receptors. Notably, and affirming the role of PGs and epithelial HCO₃⁻ injury, the duodenal mucosa in EP₃ knockout mice have a markedly decreased ability to resist luminal acid, resulting in mucosal injury (14). It would seem rational physiologically that the presence of acid in the gastric lumen would result in an anticipatory rise in alkaline secretion by duodenal mucosa about to receive an acid load. However, instillation of acid into the ligated stomach, or conversely decreasing gastric acidity by inhibition of acid secretion, does not affect duodenal HCO₃⁻ secretion.

The presence of a pH gradient within the mucus gel adherent to both gastric and duodenal mucosae raises the interesting question of how acid present in the lumen resulting in an increase in epithelial HCO₃⁻ secretion is sensed by the secreting epithelium if the mucus pH gradient is indeed an effective barrier to H⁺. Speculations include the presence of acid-sensitive neural receptors or cell filaments protruding into the surface gel that sense the luminal pH; or, as recently proposed (6), the stimulus of alkaline secretion may not be due to H⁺ itself but due instead to the more rapidly diffusible CO₂ generated within the mucus gel during the reaction between secreted HCO₃⁻ and H⁺. The hormone secretin is released from the duodenal mucosa in response to duodenal acidification (pH ~ 4) and is the key mediator of acid-induced pancreatic and bile duct HCO₃⁻ secretion. However, it should be emphasized that secretin is without effect on the duodenal epithelial HCO₃⁻ secretion in all species tested both in vivo and in vitro.

The relative role of each HCO₃⁻-secreting tissue responding to acid (i.e., duodenal mucosa, pancreas, and bile ducts) in the duodenal neutralization of acid emptied from the stomach depends on the experimental conditions of the study and likely varies between species. In the pig, a decline in pancreaticobiliary HCO₃⁻ secretion results in a compensatory increase in duodenal epithelial HCO₃⁻ secretion. This may be important in patients with pancreatic secretory insufficiency. Overall, acid-stimulated mucosal HCO₃⁻ secretion likely accounts for ~40% of the neutralization of the gastric acid load in the duodenum.

The gastric HCO₃⁻ transport process

In vitro and in vivo mucosa have been used to study the properties of gastric alkaline secretion. The secretion by the gastric fundus and antrum display similar properties, including almost identical sensitivity to stimulants and inhibitors, suggesting that the surface epithelial cells (present in both fundus and antrum) are the origin. Secretion is stimulated by cholinergic stimuli (including sham feeding), by E-type PGs (acting via the EP₁ receptor), and the presence of a low luminal pH, whereas HCO₃⁻ secretion is inhibited by sympathetic stimuli acting at α₂-adrenoceptors.

Information on the cellular mechanisms of gastric HCO₃⁻ transport was obtained initially in isolated mucosal sheets and isolated cells, and, more recently, the genetic expression of transporters has been characterized. Export by Cl⁻/HCO₃⁻ exchange is likely the principal mechanism for exit of HCO₃⁻ into the mucus gel. Cellular uptake of base occurs by Na⁺/Cl⁻ cotransport (NBC). Interestingly, NBC1 and NBC2 were recently reported to show higher expression in gastric surface epithelial cells than in parietal cells (10). Their presence would facilitate surface epithelial
The pH-sensitive fluoroprobe 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) confirmed three of the mechanisms previously proposed for duodenal enterocyte acid/base transport. An amiloride-sensitive NHE extrudes acid. Duodenal enterocytes import HCO₃⁻ at the basolateral membrane by Na⁺-(n)-HCO₃⁻ cotransport and export HCO₃⁻ by Cl⁻/HCO₃⁻ exchange as well as via an apical anion conductive pathway. Recent evidence indicates that the cystic fibrosis transmembrane conductance regulator (CFTR) is the ubiquitous membrane-spanning conductance that transports HCO₃⁻ as well as Cl⁻ (12). CFTR knockout mice, as well as patients with cystic fibrosis, have decreased resting and stimulated duodenal HCO₃⁻ secretion (9). This secretory abnormality may not only explain diminished duodenal pH in cystic fibrosis patients but may contribute to the secretory abnormalities in several other organs (e.g., lung, pancreas, gall bladder, and vas deferens).

cAMP-stimulated HCO₃⁻ transport across murine duodenum in vitro involves electrogenic transport via CFTR channels as well as electroneutral transport via a CFTR-dependent Cl⁻/HCO₃⁻ exchange process that is associated with epithelial carbonic anhydrase activity (2). The latter finding may be of particular interest with respect to the mucosal location (crypt and/or villus) of the transporters. Suppression of carbonic anhydrase activity, which is contained in both duodenal epithelial and Brunner’s gland cells, decreases duodenal mucosal HCO₃⁻ secretion. Furthermore, the carbonic anhydrase isoenzyme (CA II) associated with HCO₃⁻ transport is located mainly in the villi and not in the duodenal crypts (8). This suggests that part of duodenal alkaline secretion originates from the villi, in contrast to the earlier hypothesis that intestinal secretions were of crypt origin and absorptive function resided in the villi.

Anion channel-dependent transport of HCO₃⁻ may, however, as suggested for secretion of anions (predominantly Cl⁻) by more distal small intestine, be a property of crypt cells in which CFTR is also expressed at the greatest levels. In contrast, villus cells might export HCO₃⁻ mainly by Cl⁻/HCO₃⁻ exchange. Recent studies indicate that both villus and crypt cells respond to the duodenal secretagogues VIP and dopamine (acting at D₁ receptors), with an increase in intracellular cAMP production. Similarly, both cell types respond to carbachol (acting at muscarinic M₃ receptors) with a rise in intracellular calcium ([Ca²⁺]ᵢ) (1). This suggests that the functions of the villus and crypt cells may be more similar than dissimilar. The specific absorptive and secretory events that occur in villus versus crypt cells, however, require additional study, as does a better understanding of the intramucosal (cell-to-cell) signaling and stimulus-secretion coupling events.

### Neurohumoral control of gastroduodenal HCO₃⁻ secretion

As mentioned above, HCO₃⁻ secretion by the duodenal mucosa is markedly, and that by gastric mucosa is more moderately, stimulated by a low intraluminal pH. In addition to luminal acid, some physiological conditions and a variety of agents influence the secretion in the duodenum (Table 1) as well as in the stomach. Secretion is influenced by the central and enteric nervous systems, by local mucosal production of eicosanoids, and by some hormones. The presence of differ-
ences in control between the duodenum and the stomach should be noted. Some more recently detected agents are mentioned here in particular.

Guanylin and uroguanylin are endogenous ligands for the apical membrane receptor for *Escherichia coli* heat-stable enterotoxin (STa). These peptides are secreted to the intestinal surface, and binding to the receptor increases intracellular cGMP. Both are potent stimuli of HCO3⁻ secretion and act by inducing CFTR-dependent electrogenic transport of HCO3⁻ (12). Interestingly, uroguanylin mRNA is most abundant in the proximal small intestine, and acidification of the duodenal lumen enhances release and stimulation of duodenal HCO3⁻ secretion by uroguanylin.

Dopaminergic compounds ameliorate mucosal damage in animal models of ulcer disease, and, conversely, mucosal depletion of dopamine has been related to the appearance of ulcerations. Dopamine D₁ receptor agonists as well as peripheral catecholamine-O-methyl transferase (COMT) inhibition stimulate duodenal mucosal HCO3⁻ secretion in the rat; similar stimulation of the HCO3⁻ secretion has been observed in human volunteers. The D₂ agonist bromocriptine, in contrast, causes a modest decrease in secretion. These findings that the duodenal secretion is stimulated via peripheral dopamine D₁ receptors are supported by the finding that D₁ (but not D₂) receptor agonists increase the production of cAMP in duodenal crypt and villus enterocytes. The role of dopamine in the control of mucosal integrity is an interesting topic for further studies.

It is likely that stress-induced reactions contribute to gastrointestinal damage elicited by infection with *Helicobacter pylori* and other agents. Splanchnicotomy or adrenergic blockade also ameliorate stress-induced gastroduodenal ulceration in animals, and increased plasma levels of norepinephrine are reported in patients with duodenal ulcer disease. These observations stimulated studies of the sympathetic influence on gastroduodenal HCO3⁻ secretion. Norepinephrine and epinephrine inhibited HCO3⁻ secretion by frog gastric mucosa in vitro, and effects were prevented by the antagonist phentolamine, thus suggesting an α-adrenoceptor-mediated action directly on HCO3⁻-secreting cells or, possibly, on local neural tissue remaining in the in vitro preparation. More recently, mucosal HCO3⁻ secretion has been studied in splanchnicotomized and/or adrenal-ligated animals and by elicitation of sympathetic reflexes in animals and humans (3). The use of subtype-selective adrenoceptor ligands has demonstrated α₂-adrenoceptor-mediated inhibition of the HCO3⁻ secretion in the stomach as well as in the duodenum.

However, activation of the sympathetic nerves releases neuropeptide Y (NPY) as well as norepinephrine. Recent studies of duodenal secretion in rats have shown that local (close intra-arterial) infusion of low doses of NPY to the duodenum, although without effect on basal HCO3⁻ secretion, inhibits the secretion stimulated by PGE₂ and VIP. Furthermore, administration of neuropeptide Y₁ receptor antagonists markedly increases (basal) HCO3⁻ secretion. Combined
administration of neuropeptide Y<sub>1</sub> receptor antagonist BIBP-3226 and the adrenoceptor antagonist phentolamine results in potent, up to threefold, increases in duodenal secretion, as illustrated in Fig. 2. Duodenal mucosal secretion of HCO<sub>3</sub>⁻ is thus under potent sympathetic inhibition mediated by norepinephrine acting at α<sub>2</sub>-adrenoceptors, as well as NPY acting at Y<sub>1</sub> receptors, and this likely influences the ability of the epithelium to resist intraluminal acid.

**Role in disease**

Surface epithelial HCO<sub>3</sub>⁻ secretion is likely to be of key importance in mucosal protection within the upper gastrointestinal tract (i.e., stomach, duodenum, and, to some extent, esophagus), and in each organ HCO<sub>3</sub>⁻ secretion protects against acid-induced injury. Stimulating the duodenal secretion with some drugs (PG analogs, VIP, α-receptor ligands) enhances mucosal resistance to luminal acid. In the human duodenum, mucosal HCO<sub>3</sub>⁻ secretion and in particular the ability of this mucosa to respond to luminal acid with a rise in HCO<sub>3</sub>⁻ secretion are decreased in patients with acute and chronic duodenal ulcer disease. Examples by association include 1) the marked increase in gastroduodenal ulcers associated with the ingestion of aspirin and other nonsteroidal anti-inflammatory drugs that suppress mucosal PG formation and mucosal HCO<sub>3</sub>⁻ production; 2) cigarette smoking, another inhibitor of HCO<sub>3</sub>⁻ secretion that is strongly associated with ulcer disease; and 3) H. pylori-associated duodenal ulcers (5). Following eradication of the bacterium, duodenal HCO<sub>3</sub>⁻ secretion normalizes and ulcers rarely recur. The precise processes responsible for these events, either by the organism or the host, are the subject of intense study.

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**References**