REVIEW

Duodenal Carbonic Anhydrase: Mucosal Protection, Luminal Chemosensing, and Gastric Acid Disposal

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Abstract. The duodenum serves as a buffer zone between the stomach and jejunum. Over a length of only 25 cm, large volumes of strong acid secreted by the stomach must be converted to the neutral-alkaline chyme of the hindgut lumen, generating large volumes of CO_2, which the duodenum then absorbs. The duodenal mucosa consists of epithelial cells connected by low-resistance tight junctions, forming a leaky epithelial barrier. Despite this high permeability, the epithelial cells, under intense stress from luminal mineral acid and highly elevated Pco_2, maintain normal functioning. Furthermore, the duodenum plays an active role in foregut acid-base homeostasis, absorbing large amounts of H^+ and CO_2 that are recycled by the gastric parietal cells. Prompted by the high expression of cytosolic and membrane carbonic anhydrase (CAs) in duodenal epithelial cells, and the intriguing observation that CA activity appears to augment cellular acid stress, we formulated a novel hypothesis regarding the role of CA in duodenal acid absorption, epithelial protection, and chemosensing. In this review, we will describe how luminal CO_2/H^+ traverses the duodenal epithelial cell brush border membrane, acidifies the cytoplasm, and is sensed in the subepithelium. (Keio J Med 55 (3): 96–106, September 2006)

Key words: intracellular pH, carbonic anhydrase, duodenum, vanilloid receptor, carbon dioxide

Introduction

Located between the pylorus and ligament of Treitz, the duodenum routinely experiences 5-log changes of $[H^+]$ and PCO_2 > 500 Torr,[1] the most extreme environment in terms of rapid changes and absolute concentrations of H^+ and PCO_2 in the body.[2–5] This milieu places the mucosa at risk for irreversible cellular acidification with subsequent necrosis or apoptosis.[6–9] Furthermore, the duodenal mucosa is leaky (transepithelial resistance = ~50 Ω·cm,[2] com·pared with gastric transepithelial resistance ~1500 Ω·cm^2), the epithelial functions normally, due to the development of a potent defensive system geared towards preserving intracellular pH (pH_i) in the face of extreme environmental acid stress. While resisting this acid onslaught, the duodenum also functions to absorb virtually all of the acid secreted by the stomach, while sensing the acidity of the luminal content. This dichotomous role is unique for the duodenum, in that the other acid-exposed organs of the GI tract, although able to sense luminal acid, do not absorb significant amounts of acid or acid equivalents.[10–13]

Over its short length, the duodenum serves several essential functions. Unlike its more distal counterparts, the duodenum does not absorb significant quantities of fluid and electrolytes, although it is an important site for multivalent ion absorption.[14–17] The bulk of the ~400 mmol/24 hr of gastric H^+ secretion is neutralized in the duodenum by secreted HCO_3^-, generating equimolar amounts of CO_2. The duodenum also plays an important

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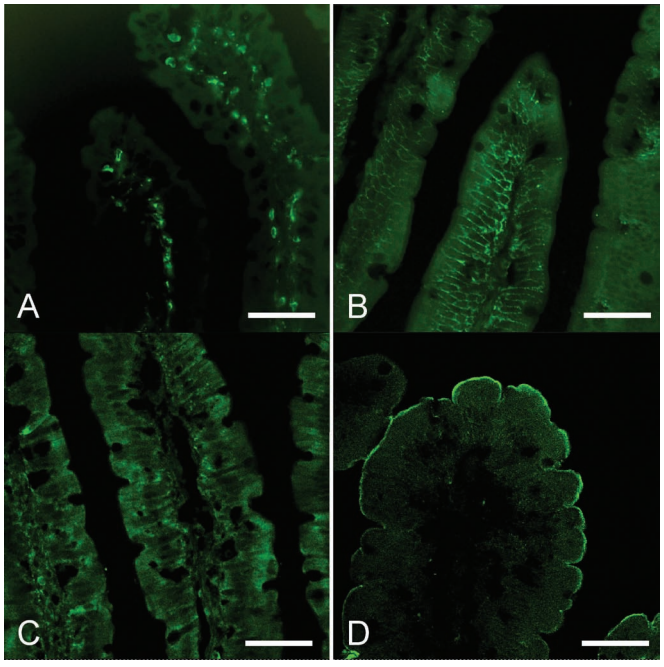

Fig. 1 *Expression of membrane-bound carbonic anhydrases (CAs) in duodenal villous epithelium.* Duodenal epithelial cells express CA IX on the basolateral membrane (B) and XIV on the apical membrane (D) of villous epithelial cells, whereas CA IV is expressed in the capillary endothelium and interstitial cells in the villi (A) and XII in the cytoplasm of the epithelial cells (C). *Bar* = 50 μm. (adapted from [32])

role in the regulation of traditional epithelial defense mechanisms, such as HCO_3^- and mucus secretion, and in the augmentation of mucosal blood flow.[18,19] Unlike the stomach or esophagus, the duodenum must absorb the gastric acid load while maintaining epithelial integrity. Furthermore, regulation of protective mechanisms is reliant on an ability of the mucosa to sense luminal acidity.[20] The need for acid absorption necessitates transmucosal permeability or 'leakiness' in the context of constant exposure to high H^+ and PCO_2 a situation generally associated with poor resistance to injury. In this review, we will describe hypotheses regarding the role of carbonic anhydrase (CA) in facilitating these dual, and seemingly contradictory functions.

We now will show data to suggest that the ability of the duodenal mucosa to transport large amounts of acid across the mucosa, of the subepithelium to sense luminal acid, and of the epithelial cells to resist acid injury, is due to the function of membrane-bound and cellular CAs. In this fashion, CAs help the duodenum fulfill its unique dual purpose of efficiently absorbing luminal acid while preserving epithelial cell pH_i.

Duodenal CA

a) CA expression in duodenal epithelium

CAs are heavily expressed in duodenal epithelial cells. Histochemistry reveals high CA activity localized to the brush border and basolateral membranes, and in the cytoplasm.[21] CA II is believed to be the predominant cytoplasmic isoform, whereas CA IX and others are expressed in the epithelial cell plasma membranes.[22–25]

Traditionally, CAs have been classified into soluble and membrane-bound enzymes. Since activities were initially measured in red cells, until recently only four isoforms had been identified. Soluble CA II is the most accepted cytoplasmic CA in intestine, playing a major function in the hydration of cellular CO_2 to HCO_3^- and H^+, serving as a major source of cellular HCO_3^-, which is then transported into the lumen as HCO_3^- secretion, an accepted mucosal defense mechanism.[26–28]

Histochemical studies, however, have additionally identified CA activity closely linked with the brush border membrane of the intestinal epithelial cells.[21] The function of this membrane-bound (ecto) CA activity in intestine is not readily apparent. In the past years, many additional CA isoforms have been identified. Most importantly, several isoforms have been localized to the membranes of HCO_3^- transporting epithelia, such as renal proximal tubule[29,30] and in intestine.[22–24,31]

Although multiple CA isoforms have been discovered recently, there has been no systematic survey of intestinal CA expression. Using real-time RT-PCR and confocal microscopy of histologic sections with immunofluorescence staining for known CA isoforms, we conducted a survey of duodenal CA expression, focusing on the expression of membrane CA.[32] As seen in Fig. 1, known membrane CA isoforms IV, IX, XII, and XIV are expressed in the duodenum, with immunostaining indicating that CA XIV is the predominant apical isoform, whereas CA IX is the predominant basolateral isoform, similar to the expression pattern found in proximal tubule.[29,30] We hypothesize that the apical CA XIV facilitates CO_2 uptake across the apical membrane of duodenal epithelial cell, whereas the basolateral CA IX converts CO_2 diffusing across the basolateral membrane into interstitial H^+, or converts H^+ transported across the basolateral membrane into interstitial CO_2.

b) Role of CAs in epithelial protection

We here describe duodenal defenses in a novel manner, implicating the function of CAs in duodenal acid-base physiology. Interest in CAs in this regard originated with the realization that cytosolic and membrane bound CAs are highly expressed in the duodenal epithelium.[21,31] Cytosolic CA, by virtue of its conversion of CO_2 to H^+,

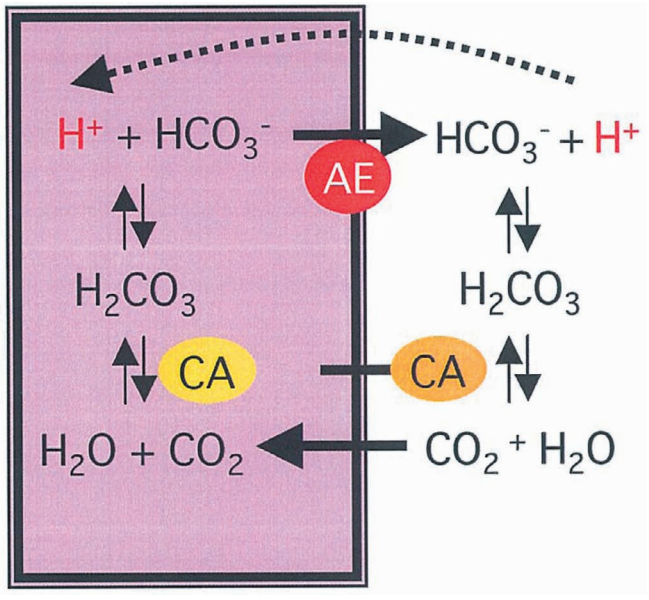

Fig. 2 *The Jacobs-Stewart Cycle.* Originally described in red cells, the Jacobs-Stewart cycle is a mechanism by which the net transfer of H^+ between medium and cytosol can occur via CO_2 uptake in the presence of extracellular and intracellular carbonic anhydrases combined with a plasma membrane anion exchanger. In the duodenal epithelium, the Jacobs-Stewart cycle is a likely means by which luminal CO_2 is absorbed as H^+ equivalents into the epithelial cells, from which it exits via the basolateral NHE1 into the interstitium and portal vein.

enhances cellular acidification in the presence of a high PCO_2 environment,[33,34] which would seem to be the last thing a cell would need in a strongly acid environment. This prompted the formulation and testing of a novel hypothesis regarding the overall role of CAs in duodenal epithelial defense, acid disposal, and chemosensing.

CAs are the only known enzymes that facilitate conversion of H^+ to CO_2 through the intermediary H_2CO_3. CAs function in red cells to facilitate transmembrane H^+ movement by conversion of H^+ to CO_2 outside the cell, and CO_2 to H^+ inside the cell, functionally transferring H^+ from blood to cytoplasm through a CO_2 surrogate.[35] By transporting H^+ as CO_2, the cell is protected from irreversible acidification due to environmental H^+, but is able to transport large quantities of acid. CO_2, being a membrane permeant gas, can readily cross most biological membranes, although recent studies are consistent with the presence of membrane CO_2 channels that facilitate CO_2 permeability.[36–40] Furthermore, since the cell always contains ~25 mM HCO_3^-, the lower limit of cellular acidification, even with saturating PCO_2 (e.g. > 650 Torr), is ~pH 6.1. In this fashion, the cell can take up large amounts of acid while preserving pH_i at an acceptable level. This mechanism was first discovered in the red cell, where the existence of soluble and membrane-bound CAs and plasma membrane anion exchangers was first hypothesized. In the presence of these three components, H^+ and CO_2 are functionally equivalent. First described in 1942, the Jacobs-Stewart cycle is an accepted means of quantitative transmembrane transport of H^+ by conversion to CO_2, with transport of CO_2 across the cell membrane.[35] According to the mechanism, plasma H^+ is neutralized by HCO_3^-, forming H_2O and CO_2. The CO_2 formed diffuses into the red cell, across the plasma membrane where it is hydrated to H_2CO_3, which dissociated to H^+ and HCO_3^-. HCO_3^- is exchanged across the plasma membrane for Cl^-, where it can neutralize another H^+ (Fig. 2). The H^+ is carried by the red cell to the lung, where the process is reversed. In this fashion, H^+ enters the red cell without having to cross the plasma membrane. Since the duodenal epithelium is equipped with the same array of transporters, we hypothesize that transapical H^+ transport occurs according to the same mechanism. Since H^+ does not actually cross the plasma membrane, and since CO_2 is buffered by cellular HCO_3^-, the potentially deleterious effects of strong luminal acid on pH_i are largely prevented.

Duodenal Acid Disposal

a) Overview

Duodenal acid disposal, though not commonly studied, plays an essential role in preserving the body's acid-base status, while transmitting luminal chemical signals to the subepithelium. The stomach secretes ~ 400 mmol H^+/day, almost all of which enters the duodenum.[41] The stomach cannot absorb this acid to any great extent, based on studies of acid back-diffusion,[12,42,43] and with the observation that in the case of pyloric obstruction, severe metabolic alkalosis develops, due to the lack of substantial acid absorption through the gastric wall.[44,45] Measured luminal PCO_2 is maximal in the duodenum, with PCO_2 rapidly normalizing to ~40 Torr in the distal intestine, indicating that HCO_3^- neutralization by gastric H^+ mostly occurs in the duodenum[1]. These data imply that nearly the entire gastric acid load is absorbed by the duodenum. Although the mechanism of duodenal H^+ absorption is controversial, a predominance of opinion has favored a combined mechanism of HCO_3^- neutralization and H^+ absorption in exchange for Na^+.[46–49] No one, however, has previously addressed the mechanism by which luminal H^+ or CO_2 traverses the mucosa, especially since the duodenum on the one hand must quantitatively absorb acid, while its cells are protected from irreversible acidification.

b) Mechanism

The mechanism by which H^+ is absorbed by cells falls into at least four possibilities : direct diffusion across the

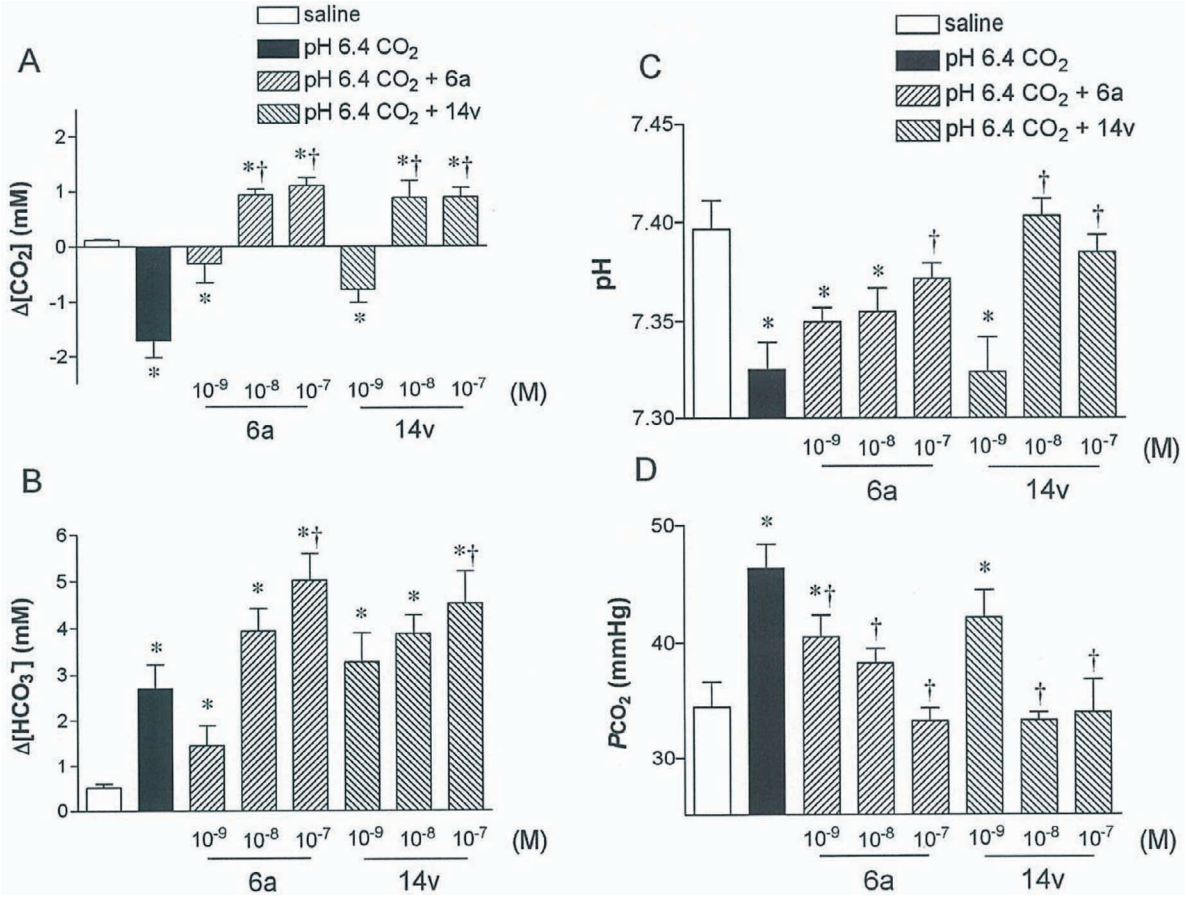

Fig. 3 *Effect of extracellular carbonic anhydrase (CA) inhibition on luminal high CO_2 challenge in rat duodenum.* **A, B:** The extracellular CA inhibitor 6a or 14v dose-dependently inhibited CO_2-induced CO_2 loss **(A)**, while enhancing CO_2-induced HCO_3^- secretion **(B)**. **C, D:** CO_2-induced portal venous acidification **(C)** and Pco_2 increase **(D)** were dose-dependently inhibited by 6a or 14v. All data represent the values at 10 min after the CO_2 challenge. Each datum is expressed as mean ± SEM (n=6). *$p < 0.05$ vs. saline group, †$p < 0.05$ vs. pH 6.4 CO_2 group. (Adapted from [58]).

apical membrane, diffusion through intercellular tight junctions and then facilitated transport across the basolateral membrane, facilitated transport across the apical membrane, and luminal conversion to CO_2 with diffusion of CO_2 across the apical membrane. In practice, these possibilities are difficult to differentiate, given the lack of effective means of differentiating bulk paracellular from transcellular transport.

i) Transport as H^+

Luminal H^+ may simply diffuse across the epithelial cell plasma membrane. Of all ions, protons are the only species small and permeant enough to cross the lipid bilayer without facilitation by membrane proteins. This possibility has been addressed directly by the measurement of ionic diffusion through artificial lipid membranes. The H^+ permeability of lipid bilayers (0.5-7 nS•cm^{-2}), is exceedingly low (typical ion channels have single conductance in the pS range). Furthermore, the H^+ diffusion rate decreases at low pH, and the apical membrane is less permeable than other membranes making the possibility of direct diffusion of significant H^+ directly through the apical membrane unlikely.[50] Protons may cross the membrane by an existing plasma membrane protein, the best candidate protein being Na^+/H^+ exchangers (NHE). NHEs comprise a family of membrane proteins, the most common of which is NHE1, which is one of the essential 'housekeeping' proteins of almost all cells, whose function is to maintain pH_i. NHE3 is present on the apical membrane of villous intestinal epithelial cells,[51] including duodenum,[52] and has been implicated in the mechanism of neutral NaCl uptake. These proteins, however, can work in the reverse direction, i.e. load H^+ while extruding Na^+ into the lumen, depending on the ion gradients to which they are exposed. When duodenal lumen pH is 2, for example, there is a 5 log order inward (lumen → cytoplasm) H^+

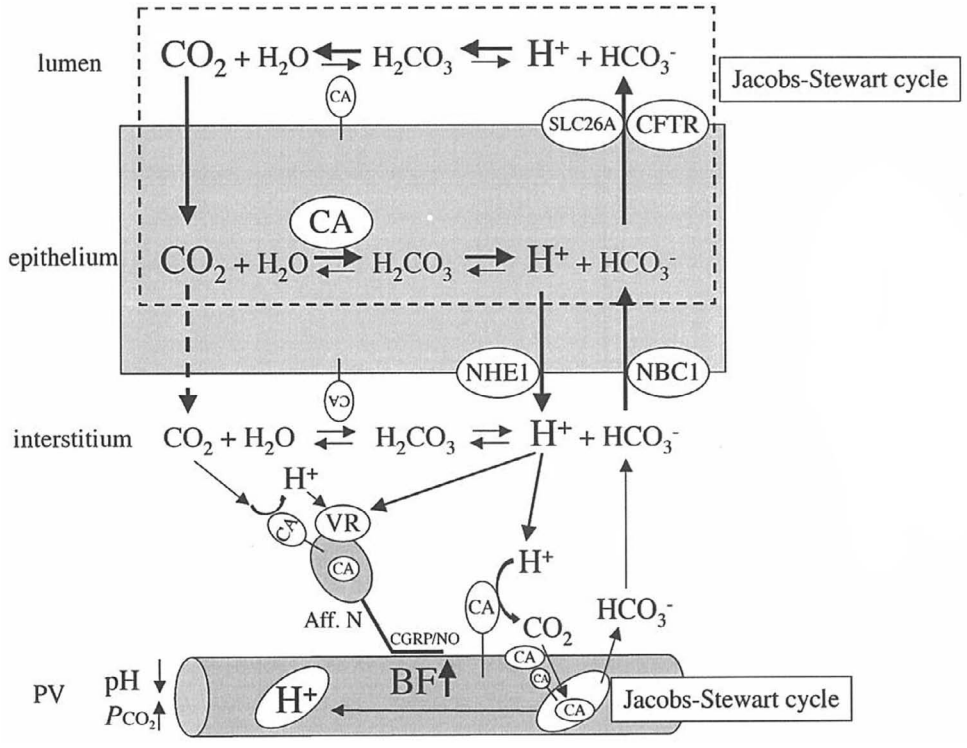

Fig. 4 *Model of transmucosal duodenal CO_2/H^+ movement and sensing.*
Luminal H^+ from the stomach is neutralized with the secreted HCO_3^- in the duodenum, generating CO_2. CO_2 diffuses into the cytoplasm through the apical membrane of the epithelial cells. CO_2 is converted into H^+ and HCO_3^- by cytosolic CA. H^+ acidifies cells and is extruded into the subepithelium via NHE1. H^+ stimulates acid sensors such as VR, followed by the release of CGRP and NO, producing blood flow increase. H^+ is also converted to CO_2 by sub-epithelial CA, including the basolateral CA or vascular CA. CO_2 enters the PV blood and is carried by red blood cells, acidifying PV blood and increasing PV Pco_2. Cytoplasmic HCO_3^- generated from CO_2 and loaded via NBC1, is secreted through an apical SLC26Ax anion exchanger or CFTR to the lumen. Luminal HCO_3^- is converted to CO_2 by apical extracellular CA. Note that net movement of CO_2 is lumen → mucosa, whereas net HCO_3^- movement is mucosa → lumen. These simultaneous movements result in net H^+ absorption and net HCO_3^- secretion consistent with the function of the Jacobs-Stewart cycle. Abbreviations: CA: carbonic anhydrase, NHE: Na^+/H^+ exchanger, NBC: Na^+:HCO_3^- cotransporter, SLC26A: solute carrier family 26Ax, CFTR: cystic fibrosis transmembrane conductance regulator, PV: portal vein, VR: vanilloid receptor, CGRP: calcitonin gene-related peptide, NO: nitric oxide, BF: blood flow. (Adapted and modified from [58])

gradient placed across the apical cell membrane, which far exceeds the ~10-fold inward sodium gradient. Under such conditions, the apical NHE-3 might reverse, loading H^+ into the cell while extruding Na^+ into the lumen. This would lower pH_i, as has been observed previously,[6,53–55] with the lower limit of the fall of pH_i limited by $[Na^+_i]$. This would keep $[Na^+_i]$ low, driving HCO_3^- uptake via the basolateral sodium-bicarbonate cotransporter (NBC), which would further limit the drop of pH_i. There is thus a 'safety' mechanism that, even in the presence of an apical proton transporter, would limit cellular acidification by depleting intracellular Na^+ and activating HCO_3^- loading. Our data, however, does not support the role of NHE3 in the mechanism of luminal H^+ entry into duodenal epithelial cells. We found that basal pH_i, measured *in vivo* when perfusing the duodenum with a pH 7 solution, did not change when the selective NHE3 inhibitor S3226 was added to the perfusate.[56] Further preliminary studies have revealed that mild acid (pH 4)-induced intracellular acidification of duodenal villus cells is enhanced by S3226, whereas S3226 has no effect on intracellular acidification in the presence of strong luminal acid (pH 2.2), suggesting that NHE3 in the 'reverse mode' is not involved in H^+ absorption in the presence of luminal acid (unpublished observations).

ii) Transport as CO_2

Acid equivalents may enter the cell as CO_2 gas, which is then hydrated by CA to HCO_3^- and H^+. Holm and coworkers tested this hypothesis by examining the effect of luminal perfusion of solutions of varying pH and CO_2 content on alkaline secretion in rats,[57] finding that solutions with calculated $PCO_2 > 120$ Torr stimulated HCO_3^- secretion to the same extent as did exposure to pH 2.0 solutions. Since PCO_2 in the duodenum due to mixture of concentrated HCl with HCO_3^- can be > 400 Torr[1], acid entry as CO_2 may play a role in duodenal defense by maintaining pH_i in the face of extreme acid stress. We hypothesize that transepithelial duodenal H^+ transport

occurs by conversion of H^+ to CO_2 with secreted HCO_3^- in the lumen, diffusion of CO_2 into the epithelial cells, re-conversion of CO_2 to H^+ in the cytoplasm, and transport of H^+ via basolateral NHE1 into the subepithelial interstitium.[20,32,33,58] Recently, we have studied H^+ and CO_2 movement among lumen, mucosa, and portal vein in an *in vivo* perfused rat preparation. Measuring input (perfusate) and output (effluent) pH and [CO_2], we were able to measure uptake and secretion of H^+ and CO_2 by the mucosa, and also measure the transmucosal absorption of H^+ and CO_2 into portal vein. Our data powerfully implicates membrane-bound and cytosolic CAs in net transmucosal acid movement.[58] One particularly striking finding was the reversal of net H^+ absorption to net CO_2/HCO_3^- secretion with selective inhibition of membrane CAs (Fig. 3). Note that superfusion of a high P_{CO_2} solution over the mucosa was associated with net loss of CO_2 from lumen **(3A)**, net HCO_3^- secretion to lumen **(3B)**, decreased portal venous pH **(3C)** and increased portal venous P_{CO_2} **(3D)**, consistent with prior observations of high P_{CO_2} solutions augmenting HCO_3^- secretion, although this happens later period[33,57] and the assumption that CO_2 diffuses down its concentration gradient. Unexpectedly, selective inhibition of membrane CA with experimental CA inhibitors, provided by Prof. C. Supuran (Firenze, Italy) reversed CO_2 absorption to net secretion, increased net HCO_3^- secretion, and inhibited portal venous acidification and P_{CO_2} increase. These effects are most consistent with membrane CAs facilitating CO_2 uptake by the mucosa and conversion of HCO_3^- to CO_2 in the lumen.

Once transported into the subepithelial interstitium, H^+ may interact directly with subepithelial acid sensors, or may be further converted to CO_2 by basolateral membrane CAs such as CA IX or vascular CA such as CA IV. Possible mechanisms of CO_2/H^+ diffusion into duodenal mucosa and portal venous blood with epithelial and sub-epithelial CAs are summarized in Fig. 4. Subepithelial acidity is presumably the chemical signal that is transduced into efferent protective responses.

Luminal chemosensing

The mucosa can 'taste' the luminal contents with multiple sensors that identify specific components.[59] One well-established luminal component is acidity, which is sensed by the upper gut. Responses to luminal acid include reflex, protective responses such as augmentation of mucosal blood flow (hyperemia), increased mucus gel thickness and mucus secretion, and augmented HCO_3^- secretion.[60,61] Clinically, excess luminal acid is associated in some cases with functional symptoms, such as heartburn and dyspepsia.[62] If luminal acid provokes these responses, a mechanism must exist that senses luminal acid and coordinates the protective responses. The mechanism must be comprised of a means to transport luminal acid or acid equivalents across the mucosa, acid sensors, and an efferent system to convert afferent acid-induced signals into responses such as increased blood flow.

Our laboratory, having measured mucosal responses to acid perfusion in a variety of systems, has been actively involved in identifying the nature of this sensing mechanism.[20,32,63,64] Through these studies, we have shown evidence that NHE1 and the vanilloid receptor (VR1) (now termed transient receptor potential vanilloid-1 or TRPV1) play important roles in acid chemosensing, with NHE1 transporting H^+ out of the cells, and TRPV-1 serving as the primary acid sensor.[55,64] Data supporting this hypothesis include inhibition of efferent responses by the NHE1 inhibitor dimethylamiloride (DMA), selective de-afferentation with capsaicin, and with the capsaicin receptor antagonist capsazepine (CPZ).[55,64] Furthermore, TRPV1 is present in the submucosa and villous core of the duodenal epithelium.[20] The net effect of the abolition of these compensatory mechanisms by capsaicin is the enhancement of the susceptibility of the duodenum to acid injury.[65] With cell acidification in response to elevated luminal CO_2 and H^+, the next step is to transduce the signal through the epithelium. With a fall in pH_i, the basolateral membrane NHE1 is activated, serving in its well-accepted role of eliminating excess cellular acid. The acid is thus transported to the subepithelial interstitium, where it then presumably interacts with acid sensors. The precise identity of these sensors is unknown, although there are accumulating data that these are likely to be vanilloid receptors (TRPV1), which are acid- and heat-sensing cation channels that are present in the gastroduodenal lamina propria mucosae and submucosa.[20,66–68] Activation of TRPV1 on afferent nerves produces effector responses such as nitric oxide (NO) generation and the release of the vasoactive peptide calcitonin gene-related peptide (CGRP) or prostaglandins, eventuating in hyperemia, and HCO_3^- and mucus secretion. Furthermore, TRPV1 activation is likely to produce clinical sensations such as heartburn and dyspepsia.[62,69,70] Hyperemia in response to luminal perfusion of a high CO_2 solution in rat duodenum, a response inhibited by the inhibition of membrane-bound and cytosolic CAs, NHE1 and TRPV1.[32] These data support our hypothesis that luminal H^+ traverses the apical membrane as CO_2 facilitated by membrane-bound CA activity, CO_2 acidifies the cells facilitated by cytosolic CA activity, H^+ exits via NHE1, acidifying the interstitium, and finally subepithelial interstitial H^+ activates TRPV1 on afferent nerves followed by the release of vasodilatory CGRP and NO, producing hyperemia. Intrinsic and extrinsic afferent neurons express membrane-bound CA and TRPV1,[32] further suggesting that a transport metabolon consisting

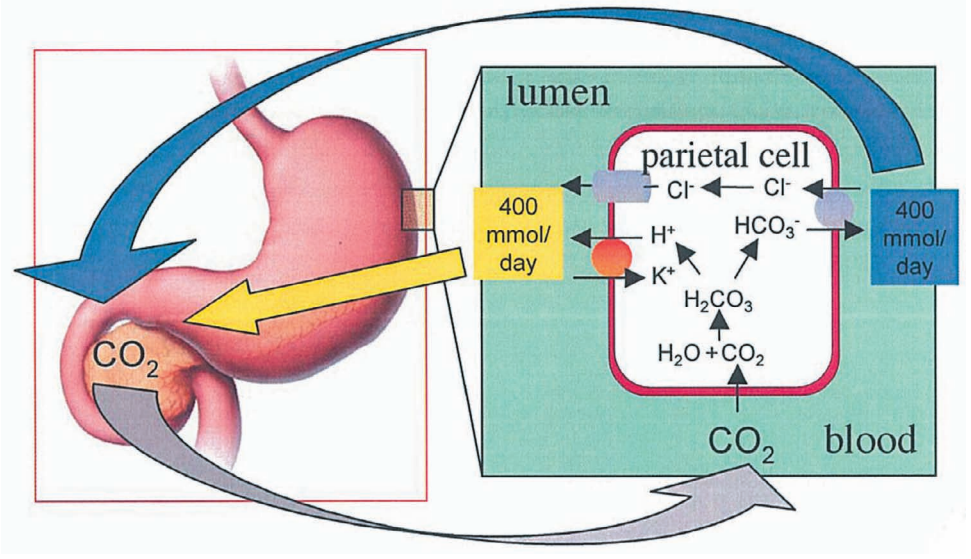

Fig. 5 *Foregut acid-base balance.* CO_2 from the systemic circulation enters gastric parietal cells, where it is hydrated to carbonic acid by carbonic anhydrase. Carbonic acid is dissociated into a proton which is secreted by the apical membrane H, K ATPase, and HCO_3^-, which is secreted into the circulation via a basolateral anion exchanger, most likely AE2. Secreted acid enters the gastric lumen, where peristalsis carries it to the duodenal lumen. HCO_3^- alkalinizes the circulation, creating the "alkaline tide". This HCO_3^- is secreted by the pancreatic duct cells, duodenal epithelial cells and possibly by the Brunner's glands into the duodenal lumen, where it combines with H^+, generating CO_2. Luminal CO_2 and H^+ are absorbed by the mucosa, enter the portal vein, and are taken up by the parietal cells.

of CA and TRPV1 serves as a CO_2/H^+ sensor, similar to the metabolon of CA and anion exchanger.[71,72] A schematic illustrating our hypotheses regarding luminal acid chemosensing in duodenum is provided in Fig. 4.

The gastroduodenal 'carbon cycle'

Due to the massive amounts of acid and base that flow past its mucosa every day, the duodenum is a major acid-base regulatory organ. To understand upper GI acid-base balance, we will describe the basic mechanisms underlying gastric acid secretion and duodenal function.

Parietal cells secrete concentrated HCl derived from circulating CO_2, which diffuses into the cells and is converted to HCO_3^- and H^+ by cytosolic CA. H^+ is secreted into the secretory canaliculus, which is contiguous with the gastric lumen. HCO_3^- exits the basolateral membrane of the cell via an anion exchanger and enters the circulation as the 'alkaline tide' .[73] The estimated amount of H^+ and HCO_3^- secretion, as judged by gastric secretory rates, is approximately 400 mmol/24 hr.[41] With antral peristalsis, the gastric acid content enters the duodenum, where it combines with HCO_3^- secreted by the duodenal epithelium and by the liver and pancreas. Estimates of total duodenal HCO_3^- secretion rate are not available, but, on the basis of measured human HCO_3^- secretory rates of ~700 µmol/hr[74] and a pancreatic secretory volume of ~ 100 ml/hr of 75 mM HCO_3^-,[75] a total pancreatico-duodenal HCO_3^- output of 200 mmol/24 hrs or higher is easily justified. With the mixture of gastric HCl and secreted HCO_3^- in the duodenal lumen, CO_2 is generated in equimolar amounts. We have postulated that this generated CO_2 is transported from the lumen to the subepithelial circulation, circulated through the pulmonary and systemic circulations, where it is then taken up by parietal cells to complete the cycle.

We have addressed the mechanism of duodenal CO_2 uptake using two approaches: measurement of portal vein blood gas composition, and simultaneous and continuous measurement of perfusate pH and PCO_2 during luminal CO_2 perfusion. Our data indicate that net CO_2 and H^+ loss from the lumen correlates with net H^+ and CO_2 gain in the portal vein, strongly consistent with transmucosal CO_2 and H^+ movement from the lumen to the portal vein.[58] This net transmucosal movement of H^+ and CO_2 explains why the elevated PCO_2 of the duodenal lumen does not occur in more distal segments, and suggests the near-quantitative duodenal absorption of acid[1,76] (Fig. 5).

Clinical correlate- *Helicobacter pylori,* pyloric obstruction, and CO_2-induced visceral pain

Helicobacter pylori (Hp) infection is implicated in the pathogenesis of peptic ulcer disease in patients not taking nonsteroidal anti-inflammatory drugs (NSAIDs).[77] The pathogenesis of gastroduodenal injury due to Hp remains incompletely understood. One possibility is that

inflammation due to Hp infection releases neutrophil hypochlorous acid, which, combined with Hp-derived ammonia, yields highly toxic monochloramine.[78–81] Since Hp colonizes the antrum, and can colonize duodenal gastric metaplasia, this mechanism may explain some aspects of Hp-related gastroduodenal injury.[77,82–84] How these Hp-related disturbances affect mucosal protective and signaling mechanisms remains conjectural, although recent data suggest that ammonium ion inhibits the SLC26A9 anion exchanger, a paralog of the duodenal anion exchangers implicated in HCO_3^- secretion.[85] Furthermore, since active Hp infection reduces acid-induced duodenal HCO_3^- secretion,[86] and since HCO_3^- secretion is needed for transmucosal H^+ absorption[58] and above, Hp might affect duodenal protective and chemosensing mechanisms.

Hp infection can be complicated by perforation, pyloric obstruction, or hemorrhage.[87–89] Obstruction of the pyloric valve or proximal duodenum, as a result of an acute pyloric channel ulcer, scarring from prior ulcer disease, or duodenal compression from a pancreatic mass, diverts the acidic gastric content from neutralization by the duodenum to elimination by vomiting. Despite the loss of HCl, alkaline tide HCO_3^- is still present in the circulation. Since renal compensation for excess filtered HCO_3^- is inefficient, a hypochloremic metabolic alkalosis develops, demonstrating the importance of duodenal acid and base transport in terms of overall acid-base homeostasis,[45,90] and also the importance of the gastroduodenal 'carbon cycle'. The acid-base disturbance also underscores the physiological concept that the gastric mucosa is unable to effectively absorb H^+ or CO_2;[91,92] Fig. 5.

CA and TRPV1 may also function as pain sensors, producing burning or dyspeptic symptoms in response to luminal acidity or CO_2 in acid-related symptomatic gastrointestinal diseases such as functional dyspepsia and irritable bowel syndrome. Although we will not include a detailed discussion about the role of TRPV1 function as a pain sensor in this review, due to the availability of excellent reviews,[93,94] the colocalization of membrane-bound CA and TRPV1 in duodenal afferent neurons and nerves,[32] suggests that CA and TRPV1 may form a transport metabolon that senses CO_2. This sensor may be analogous to chemosensors in the central nervous system, in avian pulmonary chemosensors, and in the nasal epithelium.[95–99] In epithelial cells, CA may form a 'transport metabolon' with HCO_3^- transporters to facilitate net CO_2 transport through conversion to HCO_3^- by CA and HCO_3^- transport by anion exchange or cotransport.[71,72] In this fashion, submucosal sensors could detect changes of P_{CO_2} through conversion of CO_2 to H^+ and HCO_3^-, with subsequent activation of H^+ sensors such as TRPV1 or acid-sensing cation channels (ASIC). Further evidence supporting the presence of a TRPV1-CA metabolon is that acetazolamide decreases pain associated with abdominal CO_2 insufflation prior to laparoscopic surgery.[100]

Summary and conclusions

Study of duodenal acid movement and CA expression and function has yielded fresh insights into how mucosal surfaces protect themselves from a hostile environment while absorbing large quantities of H^+ and sensing the luminal pH. The duodenum is also an important component of a larger system of acid-base balance involving gastric acid secretion and carbonic anhydrases. In the duodenal subepithelium, sensors detect interstitial acidification that is transduced from luminal H^+ via transepithelial H^+ and CO_2 movement.

Through study of the duodenal mucosa, we hope to understand how environmentally exposed epithelia deal with hostile conditions, with the hope that this may provide further understanding and insight into inflammatory and neoplastic epithelial diseases.

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