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 CO2, 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 Pco2, maintain normal functioning. Furthermore, the duodenum plays an active role in foregut acid-base homeostasis, absorbing large amounts of H+ and CO2 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 CO2/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 PCO2 > 500 Torr,1 the most extreme environment in terms of rapid changes and absolute concentrations of H+ and PCO2 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 compared with gastric transepithelial resistance ~1500 Ω·cm2), the epithelial functions normally, due to the development of a potent defensive system geared towards preserving intracellular pH (pHi) 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 HCO3-, generating equimolar amounts of CO2. The duodenum also plays an important...
role in the regulation of traditional epithelial defense mechanisms, such as HCO$_3^-$ and mucus secretion, and in the augmentation of mucosal blood flow.\textsuperscript{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.\textsuperscript{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$_i$.

\textbf{Duodenal CA}

\textit{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.\textsuperscript{21} CA II is believed to be the predominant cytoplasmic isoform, whereas CA IX and others are expressed in the epithelial cell plasma membranes.\textsuperscript{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.\textsuperscript{26-28}

Histochemical studies, however, have additionally identified CA activity closely linked with the brush border membrane of the intestinal epithelial cells.\textsuperscript{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\textsuperscript{29,30} and in intestine.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 $CO_2$.

\textit{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.\textsuperscript{21,31}

Cytosolic CA, by virtue of its conversion of $CO_2$ to $H^+$,
enhances cellular acidification in the presence of a high P\textsubscript{CO}_2 environment,\textsuperscript{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\textsuperscript{+} to CO\textsubscript{2} through the intermediary H\textsubscript{2}CO\textsubscript{3}. CAs function in red cells to facilitate transmembrane H\textsuperscript{+} movement by conversion of H\textsuperscript{+} to CO\textsubscript{2} outside the cell, and CO\textsubscript{2} to H\textsuperscript{+} inside the cell, functionally transferring H\textsuperscript{+} from blood to cytoplasm through a plasma membrane anion exchanger.\textsuperscript{35} By transporting H\textsuperscript{+} as CO\textsubscript{2}, the cell is protected from irreversible acidification due to environmental H\textsuperscript{+}, but is able to transport large quantities of acid. CO\textsubscript{2}, being a membrane permeant gas, can readily cross most biological membranes, although recent studies are consistent with the presence of membrane CO\textsubscript{2} channels that facilitate CO\textsubscript{2} permeability.\textsuperscript{36-40} Furthermore, since the cell always contains \~25 mM HCO\textsubscript{3}\textsuperscript{-}, the lower limit of cellular acidification, even with saturating P\textsubscript{CO}_2 (e.g. \textgreek{>} 650 Torr), is \~pH 6.1. In this fashion, the cell can take up large amounts of acid while preserving pH 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\textsuperscript{+} and CO\textsubscript{2} are functionally equivalent. First described in 1942, the Jacobs-Stewart cycle is an accepted means of quantitative transmembrane transport of H\textsuperscript{+} by conversion to CO\textsubscript{2}, with transport of CO\textsubscript{2} across the cell membrane.\textsuperscript{35} According to the mechanism, plasma H\textsuperscript{+} is neutralized by HCO\textsubscript{3}\textsuperscript{-}, forming H\textsubscript{2}O and CO\textsubscript{2}. The CO\textsubscript{2} formed diffuses into the red cell, across the plasma membrane where it is hydrated to H\textsubscript{2}CO\textsubscript{3}, which dissociated to H\textsuperscript{+} and HCO\textsubscript{3}\textsuperscript{-}. HCO\textsubscript{3}\textsuperscript{-} is exchanged across the plasma membrane for Cl\textsuperscript{-}, where it can neutralize another H\textsuperscript{+} (Fig. 2). The H\textsuperscript{+} is carried by the red cell to the lung, where the process is reversed. In this fashion, H\textsuperscript{+} 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\textsuperscript{+} transport occurs according to the same mechanism. Since H\textsuperscript{+} does not actually cross the plasma membrane, and since CO\textsubscript{2} is buffered by cellular HCO\textsubscript{3}\textsuperscript{-}, the potentially deleterious effects of strong luminal acid on pH\textsubscript{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\textsuperscript{+}/day, almost all of which enters the duodenum.\textsuperscript{41} The stomach cannot absorb this acid to any great extent, based on studies of acid back-diffusion,\textsuperscript{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.\textsuperscript{44,45} Measured luminal P\textsubscript{CO}_2 is maximal in the duodenum, with P\textsubscript{CO}_2 rapidly normalizing to \~40 Torr in the distal intestine, indicating that HCO\textsubscript{3}\textsuperscript{-} neutralization by gastric H\textsuperscript{+} mostly occurs in the duodenum.\textsuperscript{4} These data imply that nearly the entire gastric acid load is absorbed by the duodenum. Although the mechanism of duodenal H\textsuperscript{+} absorption is controversial, a predominance of opinion has favored a combined mechanism of HCO\textsubscript{3}\textsuperscript{-} neutralization and H\textsuperscript{+} absorption in exchange for Na\textsuperscript{+}.\textsuperscript{46-49} No one, however, has previously addressed the mechanism by which luminal H\textsuperscript{+} or CO\textsubscript{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\textsuperscript{+} is absorbed by cells falls into at least four possibilities: direct diffusion across the...
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 CO₂ with diffusion of CO₂ 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. 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. NHE3 is present on the apical membrane of villous intestinal epithelial cells, including duodenum, 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⁺

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**Fig. 3 Effect of extracellular carbonic anhydrase (CA) inhibition on luminal high CO₂ challenge in rat duodenum.** A, B: The extracellular CA inhibitor 6a or 14v dose-dependently inhibited CO₂-induced CO₂ loss (A), while enhancing CO₂-induced HCO₃⁻ secretion (B). C, D: CO₂-induced portal venous acidification (C) and Pco₂ increase (D) were dose-dependently inhibited by 6a or 14v. All data represent the values at 10 min after the CO₂ challenge. Each datum is expressed as mean ± SEM (n=6). *p < 0.05 vs. saline group, †p < 0.05 vs. pH 6.4 CO₂ group. (Adapted from 54).
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, with the lower limit of the fall of pH\(_i\) limited by [Na\(^+\)]. This would keep [Na\(^+\)] 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. 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 co-workers tested this hypothesis by examining the effect of luminal perfusion of solutions of varying pH and CO\(_2\) content on alkaline secretion in rats, finding that solutions with calculated P\(_{CO_2}\) > 120 Torr stimulated HCO\(_3^-\) secretion to the same extent as did exposure to pH 2.0 solutions. Since P\(_{CO_2}\) in the duodenum due to mixture of concentrated HCl with HCO\(_3^-\) can be > 400 Torr, 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

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 P\(_{CO_2}\). 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 \(\rightarrow\) mucosa, whereas net HCO\(_3^-\) movement is mucosa \(\rightarrow\) 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)
occurs by conversion of H⁺ to CO₂ with secreted HCO₃⁻ in the lumen, diffusion of CO₂ into the epithelial cells, re-conversion of CO₂ to H⁺ in the cytoplasm, and transport of H⁺ via basolateral NHE1 into the subepithelial interstitium. Recently, we have studied H⁺ and CO₂ movement among lumen, mucosa, and portal vein in an in vivo perfused rat preparation. Measuring input (perfusate) and output (effluent) pH and [CO₂], we were able to measure uptake and secretion of H⁺ and CO₂ by the mucosa, and also measure the transmucosal absorption of H⁺ and CO₂ into portal vein. Our data powerfully implicates membrane-bound and cytosolic CAs in net transmucosal acid movement. One particularly striking finding was the reversal of net H⁺ absorption to net CO₂/HCO₃⁻ secretion with selective inhibition of membrane CAs (Fig. 3). Note that superfusion of a high PCO₂ solution over the mucosa was associated with net loss of CO₂ from lumen (3A), net HCO₃⁻ secretion to lumen (3B), decreased portal venous pH (3C) and increased portal venous PCO₂ (3D), consistent with prior observations of high PCO₂ solutions augmenting HCO₃⁻ secretion, although this happens later period and the assumption that CO₂ diffuses down its concentration gradient. Unexpectedly, selective inhibition of membrane CA with experimental CA inhibitors, provided by Prof. C. Supuran (Firenze, Italy) reversed CO₂ absorption to net secretion, increased net HCO₃⁻ secretion, and inhibited portal venous acidification and PCO₂ increase. These effects are most consistent with membrane CAs facilitating CO₂ uptake by the mucosa and conversion of HCO₃⁻ to CO₂ in the lumen.

Once transported into the subepithelial interstitium, H⁺ may interact directly with subepithelial acid sensors, or may be further converted to CO₂ by basolateral membrane CAs such as CA IX or vascular CA such as CA IV. Possible mechanisms of CO₂/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. 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 mucous gel thickness and mucus secretion, and augmented HCO₃⁻ secretion. Clinically, excess luminal acid is associated in some cases with functional symptoms, such as heartburn and dyspepsia. 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. 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 TRPV1 serving as the primary acid sensor.

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). Furthermore, TRPV1 is present in the submucosa and villous core of the duodenal epithelium. The net effect of the abolition of these compensatory mechanisms by capsaicin is the enhancement of the susceptibility of the duodenum to acid injury. With cell acidification in response to elevated luminal CO₂ and H⁺, the next step is to transduce the signal through the epithelium. With a fall in pH₄, 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. Activation of TRPV1 onafferent 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 HCO₃⁻ and mucus secretion. Furthermore, TRPV1 activation is likely to produce clinical sensations such as heartburn and dyspepsia. Hyperemia in response to luminal perfusion of a high CO₂ solution in rat duodenum, a response inhibited by the inhibition of membrane-bound and cytosolic CAs, NHE1 and TRPV1. These data support our hypothesis that luminal H⁺ traverses the apical membrane as CO₂ facilitated by membrane-bound CA activity. CO₂ 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, further suggesting that a transport metabolon consisting
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Fig. 5 Foregut acid-base balance. CO₂ 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 HCO₃⁻, 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. HCO₃⁻ alkalinizes the circulation, creating the “alkaline tide”. This HCO₃⁻ 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 CO₂. Luminal CO₂ and H⁺ are absorbed by the mucosa, enter the portal vein, and are taken up by the parietal cells.

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 CO₂, which diffuses into the cells and is converted to HCO₃⁻ and H⁺ by cytosolic CA. H⁺ is secreted into the secretory canaliculus, which is contiguous with the gastric lumen. HCO₃⁻ exits the basolateral membrane of the cell via an anion exchanger and enters the circulation as the ‘alkaline tide’. The estimated amount of H⁺ and HCO₃⁻ secretion, as judged by gastric secretory rates, is approximately 400 mmol/24 hr. With antral peristalsis, the gastric acid content enters the duodenum, where it combines with HCO₃⁻ secreted by the duodenal epithelium and by the liver and pancreas. Estimates of total duodenal HCO₃⁻ secretion rate are not available, but, on the basis of measured human HCO₃⁻ secretory rates of ~700 μmol/hr and a pancreatic secretory volume of ~ 100 ml/hr of 75 mM HCO₃⁻, a total pancreatic-duodenal HCO₃⁻ output of 200 mmol/24 hrs or higher is easily justified. With the mixture of gastric HCl and secreted HCO₃⁻ in the duodenal lumen, CO₂ is generated in equimolar amounts. We have postulated that this generated CO₂ 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 CO₂ uptake using two approaches: measurement of portal vein blood gas composition, and simultaneous and continuous measurement of perfusate pH and P CO₂ during luminal CO₂ perfusion. Our data indicate that net CO₂ and H⁺ loss from the lumen correlates with net H⁺ and CO₂ gain in the portal vein, strongly consistent with transmucosal CO₂ and H⁺ movement from the lumen to the portal vein. This net transmucosal movement of H⁺ and CO₂ explains why the elevated P CO₂ of the duodenal lumen does not occur in more distal segments, and suggests the near-quantitative duodenal absorption of acid. Clinical correlate- Helicobacter pylori, pyloric obstruction, and CO₂-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). 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.\textsuperscript{78–81} Since Hp colonizes the antrum, and can colonize duodenal gastric metaplasia, this mechanism may explain some aspects of Hp-related gastroduodenal injury.\textsuperscript{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\textsubscript{3}\textsuperscript{-} secretion.\textsuperscript{85} Furthermore, since active Hp infection reduces acid-induced duodenal HCO\textsubscript{3}\textsuperscript{-} secretion,\textsuperscript{86} and since HCO\textsubscript{3}\textsuperscript{-} secretion is needed for transmucosal H\textsuperscript{+} absorption\textsuperscript{58} and above, Hp might affect duodenal protective and chemosensing mechanisms.

Hp infection can be complicated by perforation, pyloric obstruction, or hemorrhage.\textsuperscript{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\textsubscript{3}\textsuperscript{-} is still present in the circulation. Since renal compensation for excess filtered HCO\textsubscript{3}\textsuperscript{-} is inefficient, a hypochloremic metabolic alkaloisis develops, demonstrating the importance of duodenal acid and base transport in terms of overall acid-base homeostasis,\textsuperscript{45, 90} and also the importance of the gastro-duodenal ‘carbon cycle’. The acid-base disturbance also underscores the physiological concept that the gastric mucosa is unable to effectively absorb H\textsuperscript{+} or CO\textsubscript{2},\textsuperscript{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\textsubscript{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,\textsuperscript{93, 94} the colocalization of membrane-bound CA and TRPV1 in duodenal afferent neurons and nerves,\textsuperscript{32} suggests that CA and TRPV1 may form a transport metabolon that senses CO\textsubscript{2}. This sensor may be analogous to chemosensors in the central nervous system, in avian pulmonary chemosensors, and in the nasal epithelium.\textsuperscript{95–99} In epithelial cells, CA may form a ‘transport metabolon’ with HCO\textsubscript{3}\textsuperscript{-} transporters to facilitate net CO\textsubscript{2} transport through conversion to HCO\textsubscript{3}\textsuperscript{-} by CA and HCO\textsubscript{3}\textsuperscript{-} transport by anion exchange or cotransport.\textsuperscript{71, 72} In this fashion, submucosal sensors could detect changes of Pco\textsubscript{2} through conversion of CO\textsubscript{2} to H\textsuperscript{+} and HCO\textsubscript{3}\textsuperscript{-}, with subsequent activation of H\textsuperscript{+} 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\textsubscript{2} insufflation prior to laparoscopic surgery.\textsuperscript{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\textsuperscript{+} 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\textsuperscript{+} via transepithelial H\textsuperscript{+} and CO\textsubscript{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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