

Duodenal Intracellular Bicarbonate and the 'CF Paradox'

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Summary

HCO₃⁻ secretion, which is believed to neutralize acid within the mucus gel, is the most studied duodenal defense mechanism. In general, HCO₃⁻ secretion rate and mucosal injury susceptibility correlate closely. Recent studies suggest that luminal acid can lower intracellular pH (pH_i) of duodenal epithelial cells and that HCO₃⁻ secretion is unchanged during acid stress. Furthermore, peptic ulcers are rare in cystic fibrosis (CF), although, with impaired HCO₃⁻ secretion, increased ulcer prevalence is predicted, giving rise to the 'CF Paradox'. We thus tested the hypothesis that duodenal epithelial cell protection occurs as the result of pH_i regulation rather than by neutralization of acid by HCO₃⁻ in the pre-epithelial mucus. Cellular acidification during luminal acid perfusion, and unchanged HCO₃⁻ secretion during acid stress are inconsistent with pre-epithelial acid neutralization by secreted HCO₃⁻. Furthermore, inhibition of HCO₃⁻ secretion by 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) despite preservation of pH_i and protection from acid-induced injury further question the pre-epithelial acid neutralization hypothesis. This decoupling of HCO₃⁻ secretion and injury susceptibility by NPPB (and possibly by CF) further suggest that cellular buffering, rather than HCO₃⁻ exit into the mucus, is of primary importance for duodenal mucosal

protection, and may account for the lack of peptic ulceration in CF patients.

The location of the duodenum just distal to the gastric antrum and proximal to the pancreaticobiliary ducts uniquely exposes its leaky epithelium to a low pH environment due to peristaltically conveyed pulses of concentrated gastric acid, which vary luminal pH between two and seven on a scale of minutes [1, 2, 3]. Rapid shifts of duodenal pH are likely to create intense stress on the epithelial cells to maintain constant intracellular pH (pH_i) in order to maintain function and prevent irreversible necrosis due to intracellular acidification [4, 5]. The currently held explanation as to why the cells are protected is that active epithelial bicarbonate secretion forms a neutralizing barrier in the pre-epithelial mucus, preventing acid penetration into the cells [6].

Cystic fibrosis (CF) is an inherited disease caused by mutations of the cystic fibrosis transmembrane regulator or cystic fibrosis transmembrane conductance regulator (CFTR). Impaired duodenal and pancreatic HCO₃⁻ secretion characterizes patients afflicted with this disease, which, combined with normal or supernormal gastric acid secretion, produces abnormally high acidity in the upper gastrointestinal tract. Consequences of this

acidity are erosive esophageal disease, pulmonary acid reflux, and inactivation of secreted pancreatic enzymes. Despite these marked acid-related abnormalities, these patients are remarkably resistant to peptic duodenal ulceration, a phenomenon yet unexplained. In this report, we will propose and we will experimentally test what we will term the 'CF Paradox' in an attempt to explain this apparent protection from duodenal injury.

Bicarbonate has been thought to be the major means by which the duodenal epithelium was protected from acid-induced injury. It is a logical duodenal defense mechanism for the following reasons: 1) Duodenal bicarbonate secretion/cm² epithelium much greater than gastric bicarbonate secretion [7, 8, 9, 10]; 2) pH electrode studies suggest that epithelial bicarbonate secretion creates a layer of neutral pH next to the mucosa [11, 12, 13]; 3) *Helicobacter pylori* infection complicated by duodenal ulcers is associated with diminished bicarbonate secretion, and eradication of *Helicobacter pylori* infection restores duodenal bicarbonate secretory capacity [14, 15]. Furthermore, a strong correlation between bicarbonate secretion and mucosal injury susceptibility has been found in experimental animal models [16, 17, 18]. The mechanism by which bicarbonate is secreted from the epithelial cell is controversial. Bicarbonate is transported from the blood across the epithelial cell basolateral membrane by a variant of the sodium-bicarbonate transporter (NBC), in response to decreased pH_i resulting from exposure to luminal acid. Alternatively, bicarbonate can be formed in the epithelial cell cytoplasm from condensation of gaseous indiffusing CO₂ and water catalyzed by carbonic anhydrase, with generated protons exiting via a basolateral Na⁺/H⁺ exchanger 1 (NHE1) [19, 20]. Since inhibiting or eliminating the apical membrane CFTR greatly attenuates bicarbonate secretion [21, 22], the CFTR has been implicated in the mechanism of bicarbonate secretion, although it is unknown whether it

serves directly as a bicarbonate channel, or indirectly to preserve transmembrane electrical or ion gradients.

We have re-examined the role of bicarbonate secretion in overall duodenal defense from acid, and, in doing so, have formulated a novel hypothesis with regard to the role of bicarbonate transport. To test these possibilities, we developed a technique for the measurement of pH_i in the duodenum of anesthetized rats [23]. With this system, we could perfuse solutions of varying pH through a chamber placed over the exposed duodenal mucosa, thereby simulating changes in luminal pH. With this system, we exposed the mucosa to a brief pulse of acid, which promptly decreased pH_i. This fall of pH_i, even with mildly acidic perfusates, suggested that acid could readily penetrate the overlying mucus gel and the mucosa, therefore calling into question the role of pre-epithelial bicarbonate neutralization in duodenal mucosal defense. With removal of the acid challenge, pH_i was elevated to supernormal values, which indicated that cellular buffering power has increased, not decreased, during acid challenge. Furthermore, a second acid challenge acidified pH_i less than the first; further confirming that acid exposure was associated with increased cellular buffering power. This somewhat surprising finding was confirmed by comparison with prior studies conducted in a variety of systems, in which acid pulses were followed by pH_i overshoot, indicative of increased buffering power in cells containing a plasma membrane base-loading mechanism such as sodium-bicarbonate cotransport [24].

Further studies indicated that this buffering power increase was inhibited by the stilbene anion transport inhibitor 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS). When exposed to two short acid pulses, pH_i decreased less during the second challenge; again strongly suggestive that cellular buffering power was increased during acid exposure. DIDS inhibited this adaptive

effect. Our studies were thus consistent with bicarbonate uptake being induced by luminal acid exposure by a DIDS-inhibitable mechanism, which is most likely a sodium-bicarbonate cotransporter (NBC), presumably located on the basolateral, blood-facing cellular pole. This finding was expected insofar as primary isolated duodenal epithelial cells recovered from acid exposure by a mechanism consistent with the activity of an NBC [25], and that bicarbonate-secreting pancreatic duct cells have a basolateral membrane NBC [26]. A recent study confirms the presence of a NBC1 in the basolateral membrane duodenal epithelial cells [27].

Base loading during acid challenge, which increases cellular buffering power and attenuates the fall of pH_i , is an attractive means of defending the epithelium from acid challenge. To address how bicarbonate secretion is related to this observation, we performed parallel experiments in which bicarbonate secretion was measured in a perfused duodenal loop exposed to the same pH perfusion sequence as the measurements of pH_i . We found that titratable alkalinity increased substantially during acid perfusion, although, total CO_2 content decreased somewhat at the same time. The best explanation for these data was that although acid back-diffusion increased markedly during luminal acid perfusion, bicarbonate secretion was unchanged, inconsistent with its putative protective function. The implications of these data, combined with our measurements of pH_i , support our hypothesis that increased cellular buffering, and not bicarbonate secretion, is the primary duodenal defense mechanism from acid. Acid was not neutralized at the duodenal surface, since cellular pH_i clearly decreased during acid challenge, and since acid back-diffusion was the major means of acid loss when perfused over the mucosa. Furthermore, since bicarbonate secretion was unchanged during acid perfusion, and only increased after acid removal, bicarbonate cannot be a major

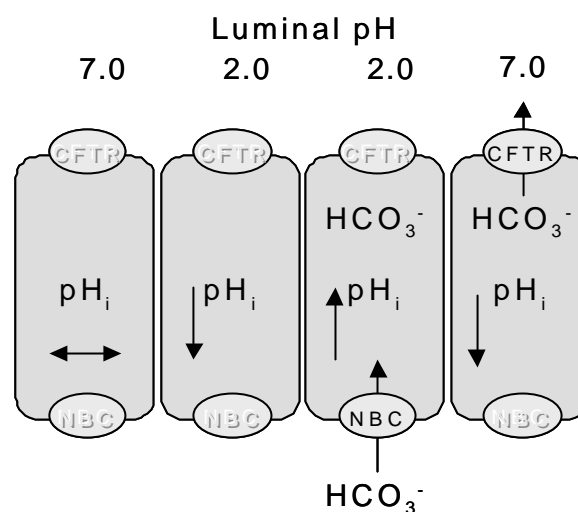


Figure 1. Sequential response of duodenal epithelial cells to luminal acid. In the left panel, steady-state pH_i about 7.1 when no acid is present. In the succeeding panels to the right, luminal acid rapidly acidifies the epithelial cells. Low pH_i increases the activity of the basolateral sodium-bicarbonate cotransporter (NBC), which increases cellular bicarbonate concentration, increasing cellular buffering power. When luminal pH returns to neutrality, acid diffuses out of the cell. The excess intracellular base produces a supernormal pH or overshoot. This in turn activates the bicarbonate secretory mechanism (CFTR), which produces bicarbonate secretion.

protective mechanism, since its increased secretion is present only when it is not needed i.e. when acid is no longer present. In this proposed mechanism, shown in Figure 1, bicarbonate secretion occurs to remove excess alkali from the cell when excess intracellular bicarbonate is no longer needed after acid challenge.

In our most recent studies, we have used the inhibitors DIDS and NPPB in order to further test our hypothesis. Both inhibitors abolished bicarbonate secretion, as has been published previously [28, 29]. DIDS decreased pH_i of the duodenal cells whereas NPPB increased pH_i . These effects on pH_i were consistent with inhibition of base uptake and exit from the cell, respectively. We then showed that susceptibility of the epithelial cells to acid injury was enhanced by DIDS but decreased by

NPPB. We thus were able to uncouple bicarbonate secretion from mucosal protection, since NPPB inhibited bicarbonate secretion while enhancing injury susceptibility. These data added further evidence that intracellular pH regulation, and not secreted bicarbonate, appears to be of prime importance for duodenal mucosal protection.

We have formulated the 'CF Paradox', in which we pose the question: why is the prevalence of duodenal ulcers not increased in patients with CF? Patients with CF, for example, have high normal acid secretion [30], and must take gastric antisecretory medications in order to diminish esophageal acid reflux and to prevent acid-mediated inactivation of pancreatic enzymes [31, 32]. Furthermore, pancreatic and duodenal bicarbonate secretion are presumably impaired by the disease [22], and duodenal pH is lower than normal [33]. *Helicobacter pylori* infection prevalence resembles that of the unaffected population [34]. Combined with the frequent prevalence of chronic lung disease, these patients should be a high risk for peptic ulceration. Clinical experience, and the literature, however, does not support an increased incidence of peptic ulceration in this population, but rather, it appears that the prevalence of peptic ulceration may actually be diminished [35, 36].

We propose that the impairment of duodenal bicarbonate secretion in the disease may explain why this population may be protected from peptic ulceration. Recall that the CFTR is needed for duodenal bicarbonate secretion. If the cells can base load normally via a basolateral NBC, or CO₂ diffusion, but cannot secrete bicarbonate across the apical membrane due to defective CFTR functioning, 'bicarbonate trapping' may occur. Elevated intracellular bicarbonate concentrations would create a new set point in which cellular buffering was elevated compared with non-affected cells, protecting the cytoplasm from irreversible acidification during acid stress.

Key words Bicarbonates; Cystic Fibrosis; Fluorescence; Hydrochloric Acid; Hydrogen-Ion Concentration; Ion Channels; Ion Transport; Microscopy; Rats

Abbreviations CF: cystic fibrosis; CFTR: cystic fibrosis transmembrane conductance regulator; DIDS: 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid; NBC: sodium-bicarbonate cotransporter; NHE: Na⁺/H⁺ exchanger; NPPB: 5-nitro-2-(3-phenylpropylamino) benzoic acid; pH_i: intracellular pH

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