ACID-SENSING PROTECTIVE MECHANISMS OF DUODENUM

The proximal duodenal mucosa, exposed to frequent pulses of gastric acid, is functionally "leaky", increasing the importance of defense mechanisms such as the mucus gel layer, cellular acid/base transporters, bicarbonate secretion, and mucosal blood flow. Our laboratory has used a unique in vitro perfused microscopic system to measure thickness of the adherent mucus gel (MGT), intracellular pH (pHi), bicarbonate secretion, and mucosal blood flow in anesthetized rats. Exposure to pulses of luminal acid, mimicking the rapid physiologic shifts of luminal pH, increases MGT and blood flow, and induces cellular bicarbonate loading, the latter followed by augmented bicarbonate secretion. The mechanism by which the epithelium senses luminal acid includes capsazepine-inhibitable vanilloid receptors, presumably similar to the vanilloid receptor TPVR-1. CFTR, the cAMP-regulated anion channel mutated in the disease cystic fibrosis, plays an essential role in duodenal bicarbonate secretion. Our data are consistent with the hypothesis that cellular bicarbonate loading is an important means of preserving epithelial pH during luminal acid challenge. Increased MGT may damp rapid shifts of luminal pH. Enhanced mucosal blood flow plays a significant role in the removal of back-diffusing acid. These neurally coordinated systems act coherently to defend the vulnerable duodenal epithelial cells from concentrated gastric acid.

Key words: stomach, esophagus, duodenum, vanilloid receptor, blood flow

INTRODUCTION

The proximal duodenal mucosa is uniquely exposed to more than 7-log range of hydrogen ion concentrations. Gastric acid secretion, antral peristalsis, and duodenal and pancreatic bicarbonate secretion exposes the proximal
duodenum to cyclical and rapid variations of luminal pH. Unlike other acid-exposed organs such as the stomach or esophagus, the duodenum is has a high transepithelial permeability to water and solutes, necessitating the presence of non-structural defense mechanisms such as the mucus and bicarbonate secretion, and submucosal blood flow. We have shown previously that a brief exposure to intense luminal acidity, corresponding to physiological acid stress, induces alteration of all measured duodenal defense mechanisms, including mucus secretion/gel thickness increase, increased cellular bicarbonate concentration, and increased mucosal blood flow. The mucosal sensor underlying these rapid changes, however, remains unknown. In this review, we will show data suggesting that the mucosal acid sensing mechanism is a component of the well-known afferent branch of the enteric nervous system, with actual acid sensing transduced by a newly discovered acid-sensitive receptor.

Mechanisms of Transepithelial Acid movement

Transduction of luminal acid into submucosal mechanisms requires the movement of hydrogen ions from lumen to submucosal structures. Although our studies and others indicate that luminal acid acidifies epithelial cells and the submucosal, the entry mechanism for acid remains unclear. Although the duodenal mucosa has high paracellular permeability as one logical means for transepithelial proton transport, there are also data supporting the presence of transcellular mechanisms. One possibility involves the apical membrane sodium-hydrogen transporter 3 (NHE3), which resides in the epithelial cells apical membrane. This transporter, normally involved with intestinal sodium absorption, may be 'reversed' in the presence of strong luminal acid. Indeed, early studies demonstrated an increase of luminal sodium ion content during duodenal acid perfusion (1), consistent with a 'reversed' apical membrane sodium/proton exchange mechanism, whereby luminal protons exchange for cellular sodium ions. In any case this would also explain the prompt decrease of cellular pH during luminal acid exposure (2). A related, but much more common exchanger, NHE3, resides in the epithelial cell basolateral; membrane. Inhibition of this exchanger with dimethylamiloride abolishes the hyperemic response to cellular acidification, suggestive of the involvement of this exchanger in transduction of acid signals. Once in the submucosal, hydrogen ions presumably interact with acid-sensing receptors.

Defense mechanisms

The most studied duodenal defense mechanism is epithelial bicarbonate secretion. Other potential defense mechanisms include the mucus gel secretion and mucosal blood flow. As noted all of these defense mechanisms are acutely regulated by luminal acid exposure. Reparative processes such as restitution
from injury are beyond the scope of this review and will not be further addressed.

**Bicarbonate secretion**

Luminal acid exposure is thought to be the principal endogenous trigger for duodenal bicarbonate secretion. A brief exposure to physiological duodenal pH ~2 will increase bicarbonate secretion for the following 90-120 min (3). Although we have shown that bicarbonate secretion does not increase during actual exposure but subsequent to it, it is clear that luminal acid acutely upregulates epithelial cellular transport mechanisms involved with bicarbonate secretion. Our studies indicate that one of the net effects of repeated exposure to luminal acid pulses is an increase of cellular bicarbonate concentration.

**Blood flow**

Mucosal blood flow is an accepted component of upper gastrointestinal barrier function. In the stomach, for example, interventions that attenuate the hyperemic response to acid perfusion increase mucosal injury (4 - 6). The data derived from studies of duodenum are less conclusive, however. One potential confounder is that stimuli that bicarbonate secretion and blood flow are co-regulated, making it difficult to determine the relative importance of blood flow in terms of overall barrier function.

Our studies revealed some novel observations about the nature of duodenal blood flow and its regulation. For example, inhibition of sodium-proton

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**Fig. 1. Trans- and subepithelial pathways involved in the hyperemic response to luminal acid.** According to this scheme, luminal acid enters the duodenal epithelial cells by the 'reversed' action of the apical membrane exchanger NHE3, and exits the cell via NHE1. VR-1 acts as a submucosal acid sensor that transduces the acid signal into a neural signal which releases CGRP and enhances NO activity in the smooth mucosal of the submucosal microcirculatory regulatory elements.
exchange (NHE) with the potent amiloride analog dimethylamiloride inhibited the hyperemic acid response. Interestingly, acidification of the cytoplasm by alternate means such as with ammonium pre-pulse or valinomycin increased blood flow, also inhibitable by dimethylamiloride (2). These studies suggested that acid must pass through the epithelial cell and exit via by NHE prior to eliciting a hyperemic response. In further studies, we examined the sensing mechanisms underling the hyperemic response. Capsazepine (CPZ), an antagonist to the recently cloned vanilloid receptor, abolished the hyperemic response to acid, confirming the involvement of vanilloid receptors in the acid response. Further studies also confirmed that the hyperemic response was mediated by a well-known pathway that includes afferent sensory nerves, nitric oxide release the neuropeptide calcitonin gene-related peptide (CGRP), but was not inhibited by indomethacin, a non-selective inhibitor of cyclooxygenase. These studies provided data supporting our proposed mechanism of duodenal acid-induced hyperemia, including acid diffusion into the epithelial cell, basolateral extrusion via NHE1, activation of vanilloid receptors on afferent nerves, CGRP release, with activation of endothelial nitric oxide synthesis, with production of vasodilatory nitric oxide. A scheme transmucosal acid transport and sensing mechanisms involved in the sensing of luminal acid and regulation of mucosal blood flow is shown in Fig. 1.

**Mucus Secretion**

The role of mucus in duodenal mucosal defense is the subject of only a few studies. The most accepted hypothesis is that mucus stabilizes the pre-epithelial pH gradient, with neutral pH measured near the mucosa, preventing acid from entering the epithelial cells (7 - 10). Mucus secretion is also co-regulated by the same neurohormonal and pharmacologic stimuli that increase other defense mechanisms such as bicarbonate secretion and blood flow, making it a likely candidate for being a secondary defense mechanism.

With our technique, we could optically and non-invasively measure mucus gel thickness in our anesthetized preparation (11). We found, for example, that mucus gel thickness rapidly increases in response to perfused acid, but equally rapidly decreases in thickness when the acid challenge is removed. Measurement of effluent mucus glycoprotein content was consistent with increased sloughing of mucus into the perfusate when mucus was rapidly secreted, indicating that there is a dynamic relation between mucus secretion and erosion, as has been previously hypothesized (12). Inhibitors of elements the capsaicin pathway, such as capsazepine, the CGRP inhibitor CGRP8-37, and de-afferentation with neurotoxic doses of capsaicin slow the rate of mucus gel thickening after luminal acid exposure (11). Coupled with measurement of effluent glycoprotein content, these studies indicate that the initial rapid thickening of the duodenal mucus gel in response to luminal acid is a result of goblet cell phase II degranulation,
a slower contribution from Brunner's gland secretions. These studies again reinforce the importance of the capsaicin pathway in the regulation of duodenal defense mechanisms. When the secretion slowed, the rapid sloughing remained, thinning the gel until a new steady state occurred. Further studies showed that the capsaicin pathway, involving acid-sensing vanilloid receptors, afferent nerves, CGRP, and nitric oxide, regulates mucus gel secretion and that non-selective COX inhibition with indomethacin abolishes the mucus secretory response to all secretagogues, suggesting a fundamental role of prostaglandins in duodenal mucus secretion (13).

**Immunolocalization of VR-1**

Recent studies using VR-1 antibodies have revealed that there is intense staining of VR-1-immunoreactive nerves in the duodenal epithelium, including the lamina propria mucosa up to the villous tips and down to the pericryptal regions, the submucosal layer and intrinsic nerves (myenteric plexus) (14). These VR-1-positive nerves highly colocalize with CGRP. Furthermore, VR-

![Diagram](image.png)

*Fig. 2. The capsaicin pathway. We have depicted a scheme for the regulation of upper gastrointestinal defense mechanisms in response to luminal acid. Inhibitors are depicted in red. Abbreviations: CGRP: calcitonin gene-related peptide; COX: cyclooxygenase; NSAIDs: non-steroidal anti-inflammatory drugs; L-NAME: Nω-nitro-L-arginine methyl ester; hCGRP<sub>8-37</sub>: inhibitor of human calcitonin gene-related peptide.*
1-positive neurons are present not only in the dorsal root ganglion (splanchnic afferent center), but also in the nodose ganglion (vagal afferent center) and in the myenteric plexus (intrinsic afferent center), and sympathectomy, not vagotomy, not abolishes the acid-induced hyperemic response in duodenum, suggesting that VR signaling projects to vagal afferents and intrinsic afferents, unlikely to splanchnic afferents (15). These histological and surgical studies confirm the physiological observations reported above and have helped confirm our supposition regarding the nature of the acid-sensing protective upregulation of duodenal defense mechanisms, differently from the gastric defenses in which the splanchnic afferents contribute to the acid-induced hyperemia (16).

**Acid-sensing mechanism**

Integrating these observations, we have hypothesized the 'capsaicin pathway' as a plausible means by which luminal acid regulate duodenal defense mechanisms. Based on some data, we have hypothesized that luminal protons enter epithelia; cells via the apical membrane NHE3, acidify the cytoplasm, and exit the cell via the basolateral NHE1. These protons acidify the submucosal space and villous core, interacting with acid sensing VR-1 or similar receptors. These receptors are on capsaicin-sensitive CGRP-contain afferent nerves, which, though CGRP and NO release dilate the submucosal microvasculature, increasing mucosal blood flow, and also degranulate goblet cells, producing a burst of mucus secretion. The mechanism by which epithelial bicarbonate secretion is concurrently increased is even less well understood, but appear to involve the release of melatonin by submucosal melatonergic nerves, interacting with MT-2 receptors presumably residing on the basolateral membrane of the epithelial cells (17). The net result of all of these mechanisms is a rapid upregulation of mucosal defense mechanism, in effect matching low luminal pH with an enhanced state of preparedness by the mucosa, but we term the capsaicin pathway (Fig. 2).

**Clinical Importance of the Capsaicin Pathway and Future Directions**

Many studies using experimental systems have documented the importance of the capsaicin pathway in that the susceptibility of the upper gastrointestinal mucosa to standard injurious stimuli is increased with interruption of any component of the system, such as chemical de-afferentation, CGRP antagonism, and NOS inhibition. Thus far, there is no known clinical correlate to these studies in that there is no known disease state or intervention that involves interruption of this pathway. Nevertheless, with the availability of VR-1 knockout mice, many studies can be made into the specific contributions made by this receptor in terms of gastroprotection. Furthermore, non-neurotoxic doses of capsaicin, the pungent component of red peppers, should actually enhance mucosal defense
mechanisms, contrary to the former dietary recommendations for ulcer patients (18).

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REFERENCES
