Digestion

Digestion 2011;83(suppl 1):25–31
DOI: 10.1159/000323401

Published online: March 10, 2011

Duodenal Chemosensing and Mucosal Defenses

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Key Words
Acid sensor · Vanilloid receptor · Carbonic anhydrase · Luminal nutrient · G-protein-coupled receptors

Abstract
The duodenal mucosa is exposed to endogenous and exogenous chemicals, including acid, CO_2, bile acids and nutrients. Mucosal chemical sensors are necessary to exert physiological responses such as secretion, digestion, absorption, and motility. We propose a mucosal chemosensing system by which luminal chemicals are sensed via mucosal acid sensors and G-protein-coupled receptors. Luminal acid/CO_2 sensing consists of ecto- and cytosolic carbonic anhydrases, epithelial ion transporters, and acid sensors expressed on the afferent nerves in the duodenum. Furthermore, a luminal L-glutamate signal is mediated via mucosal L-glutamate receptors, including metabotropic glutamate receptors and taste receptor 1 family heterodimers, with activation of afferent nerves and cyclooxygenase, whereas luminal Ca^{2+} is differently sensed via the calcium-sensing receptor in the duodenum. Recent studies also show the involvement of enteroendocrine G-protein-coupled receptors in bile acid and fatty acid sensing in the duodenum. These luminal chemosensors help activate mucosal defense mechanisms in order to maintain the mucosal integrity and physiological responses. Stimulation of luminal chemosensing in the duodenal mucosa may prevent mucosal injury, affect nutrient metabolism, and modulate sensory nerve activity.

Introduction

The duodenum, so named for its length of twelve fingers, is a prime segment of the gastrointestinal (GI) tract regarding luminal chemosensing due to its strategic positioning between the pylorus and the pancreaticobiliary ducts. The duodenal mucosa is regularly exposed to strong gastric acid (pH ~2), high PCO_2 (up to ~400 mm Hg) from the neutralization of secreted HCO_3^-, digestive enzymes, bile acids, and foodstuffs. Physiological processes such as secretion, digestion, absorption, and motility occur in response to these luminal substances, implying the presence of mucosal chemosensors, which evoke protective mucosal defense mechanisms [1]. The duodenal mucosa rapidly responds to luminal chemical stimuli not only by enhancing local defense factors such as mucosal blood flow, and HCO_3^- and mucus secretion [2], but also by inhibiting gastric emptying and secretion through the release of gastric inhibitory hormones including incretins, the latter known as 'duodenal brake' [3].

Mucosal defense mechanisms consist of pre-epithelial, epithelial and subepithelial defense factors. In vivo microscopy and duodenal loop perfusion systems enable us to study these factors, including HCO_3^- and mucus secretion (pre-epithelial), intracellular pH (pH_i) regulation with ion transporters and ecto- and cytosolic enzyme activities (epithelial), and blood flow regulated via afferent nerves and mediator releases (subepithelial). Since disruption of these defenses injures the mucosa, signals that enhance defense mechanisms may protect the mucosa

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from luminal substances in order to maintain epithelial integrity. Nevertheless, the mucosa needs to sense luminal acidity or substances in order to rapidly respond and enhance defense mechanisms.

Here, we will show how the duodenal mucosa senses luminal acidity under physiological conditions. We will also discuss the presence of chemosensing receptors for luminal nutrients in the duodenum, including amino acids and Ca^{2+}. Amino acids or Ca^{2+} may be physiologically sensed by GI mucosa. Understanding how the GI mucosa 'tastes' luminal chemicals may help identify novel molecular targets in the treatment of mucosal injury, nutrient metabolism and sensory sensitivity.

Duodenal Acid/CO_2 Sensing

The duodenal mucosa, which is constantly and cyclically exposed to luminal acid and high PCO_2 due to gastric acid and the secreted HCO_3^-, has multilayered, multistep defense mechanisms to counter acid-induced mucosal injury [4]. These mechanisms coordinately regulate mucus and HCO_3^- secretion, pH_i and cellular buffering, and submucosal neuronal activation and blood flow responses. Since duodenal luminal pH rapidly changes between 2 and 7 as a result of the constant mixture of secreted HCO_3^- with jets of antrally-propelled gastric acid, the duodenal mucosa must rapidly adjust its defense mechanisms according to luminal pH [5].

Using an in vivo microscopic system, we have studied, in addition to measuring the rate of HCO_3^- secretion using duodenal loop perfusion system, the integrated regulation of mucosal defense factors such as mucosal blood flow, mucus secretion, and enterocyte pH_i in response to luminal acid in rat duodenum [2]. Luminal acid is sensed by the 'capsaicin pathway', which is comprised of epithelial cell acidification due to in-diffusing luminal acid, and H^+ extrusion across the basolateral membrane via the Na^+/H^+ exchanger-1. The extruded H^+ activates the cation channel transient receptor potential vanilloid 1 (TRPV1) on capsaicin-sensitive afferent nerves. Activated afferents release vasoactive mediators such as calcitonin gene-related peptide and nitric oxide. Finally, mucosal blood flow and mucus secretion are increased, followed by cyclooxygenase (COX)-dependent mucus and HCO_3^- secretion [2, 4]. These results demonstrate that the duodenal mucosa senses luminal acidity using epithelial ion transporters and neuronal acid sensors, and that intracellular acidification triggers the enhancement of mucosal defense mechanisms [1].

How does luminal acid acidify the epithelial cells in order to trigger mucosal defense mechanisms? The high level of PCO_2, generated in the proximal duodenum, gradually declines in the jejunum [6], consistent with rapid CO_2 absorption by the duodenal mucosa. Since the duodenal mucosa has the highest carbonic anhydrase (CA) activity in the GI tract [7], which rapidly equilibrates $H^+ + HCO_3^- \leftrightarrow CO_2 + H_2O$, we hypothesize that the duodenal mucosa absorbs luminal CO_2 effectively by cytosolic and membrane-bound CA activities. Using duodenal loop perfusion with flow-through pH and CO_2 electrodes, and simultaneous portal venous blood gas monitoring, we have reported that luminal CO_2 is CA-dependently absorbed by the duodenal epithelium with stimulated HCO_3^- secretion, accompanied by portal venous acidification [8]. Furthermore, CO_2-induced intracellular acidification of epithelial cells is also CA-dependent and accompanied by a TRPV1-dependent hyperemic response [9]. These results suggest that luminal H^+ is actively absorbed into the epithelium as CO_2, which is converted into H^+ and HCO_3^-, facilitated by membrane-bound and cytosolic CAs. Intracellular H^+ is extruded via Na^+/H^+ exchanger-1, and sensed by the 'capsaicin pathway'. This suggests that luminal H^+ and CO_2 provide equivalent acid loads, in terms of intracellular acidification, that trigger protective effector mechanisms. The duodenum absorbs luminal H^+ secreted by the stomach in order to maintain the acid-base balance between the stomach and duodenum. Acid-base balance between the stomach and duodenum is clinically important, since loss of gastric content by vomiting in the patients with the pyloric obstruction induces acute metabolic alkalosis and hypochloremia [10, 11].

High PCO_2-induced HCO_3^- secretion has also been confirmed by Sasaki et al. [12], reporting that CO_2-induced HCO_3^- secretion is differently regulated in the stomach and duodenum. Acid-induced intracellular acidification of the duodenal epithelial cells is also reported by Sjöblom et al. [13], confirming the importance of CA activities in the acid-sensing pathway of the duodenum using knockout mice.

H^+-sensitive G-protein-coupled receptors (GPCRs) are also candidates of acid sensors. So far, 4 GPCRs, GPR4, OGR1, TDAG8 and G2A, are the known H^+-sensitive GPCRs [14], implicated in the detection of tissue or blood acidosis, although still controversial whether H^+ is a receptor ligand or a modifier [15, 16]. These GPCRs are also activated by phospholipids, whereas low pH-induced activation of these GPCRs is inhibited by phospholipids. H^+-sensitive GPCRs are expressed in the afferent neurons as well as vasculature and immune cells [17–19], suggest-

ing that H^+-sensitive GPCRs may be involved in mucosal defense mechanisms in the upper GI tract, in addition to the acidosis and inflammation.

pH-Sensitive Surface pH Regulation via Purinergic Signaling

Another pH-sensitive luminal chemosensor is the brush border ecto-enzyme-related signaling system including ATP-P2Y receptor signals with intestinal alkaline phosphatase (IAP) activity in the duodenum [20, 21]. At neutral luminal pH, extracellularly, non-lytically released ATP from the epithelial cells is rapidly degraded to adenosine (ADO), which is further degraded to inosine by adenosine deaminase. Once the surface pH is lowered by gastric acid, surface ATP concentration increases due to the accumulation of ATP and/or the stimulated ATP release, according to the decreased IAP activity at acidic pH. The increased surface ATP stimulates $P2Y_1$ receptor on the apical membrane of epithelial cells, and then increases HCO_3^- secretion. The increased surface HCO_3^- concentration raises surface pH, close to the optimal pH for IAP. The recovered IAP activity degrades the surface ATP to terminate ATP-P2Y signals. The surface ADO-P1 receptor signal is also present to regulate HCO_3^- secretion [22]. These studies suggest that IAP acts as a pH sensor to modify the surface ATP concentration and serve negative feedback loop. The mechanisms of ATP-P2Y receptor signals are implicated in other epithelium secreting HCO_3^-, such as bile ducts, oviduct and bone [23].

Esophageal and Gastric Acid Sensing

Similar to the duodenal acid sensing, the esophageal mucosa also senses luminal acidity as the permeant gas CO_2 with help of epithelial and neuronal CA activities and acid sensors, TRPV1 and acid-sensing ion channels (ASICs), in order to maintain the interstitial pH at a physiological level via the hyperemic response to luminal H^+/CO_2 [1, 24]. The concept that CA activity and acid sensors are the essential components of H^+/CO_2 sensing is supported by the observation that taste of carbonation on the tongue is mediated and sensed by CA activity and an acid sensor [25].

Gastric acid sensing should be separately discussed as intragastric pH monitoring and the mucosal response to back-diffused acid. Intragastric pH monitoring, in terms of physiological gastric acid sensing, may be mediated by the calcium-sensing receptor (CaSR) expressed on gastric endocrine cells [for review, see 26]. This hypothesis is supported by the following observations: extracellular Ca^{2+} stimulates gastrin release from antral G cells [27]. CaSR is pH-sensitive and stimulated by L-type amino acids [28, 29]. Finally, CaSR knockout stomach lacks gastrin release response to luminal pH increase, Ca^{2+} or peptone [30].

Once pathological acid back-diffuses into subepithelium or submucosa, acid may stimulate acid sensors TRPV1 or ASICs on afferent nerves with COX activation, implicated in not only protective afferent responses, but also noxious sensation conducted to the central nervous system [31, 32].

Nutrient Sensing in the Duodenum

Recent molecular studies have identified the structure of specific receptors on the tongue for the basic tastes (sweet, sour, salty, bitter, umami) [33]. These receptors have also been found in the GI tract. In addition to the 'salty' taste sensed by epithelial Na^+ channels and 'sourness' by H^+-gated ion channels such as ASIC, the 'sweet' receptor heterodimer (T1R2/T1R3) is expressed in the small intestinal mucosa [34–36]. Bitter taste receptors of the type 2 taste receptor (T2R) family are also expressed in the GI tract [37]. The expression of these taste receptors in the GI mucosa suggests the need to sense the luminal contents, presumably to detect the presence of nutrients and unfavorable substances, in order to optimize digestion, absorption, secretion, and motility.

Luminal chemosensing has been reported for glucose, bitter substances and fatty acids in the GI tract [35, 38, 39]. We thus hypothesized that mucosal defense factors may be modulated by luminal nutrients, acting via taste receptors in the duodenum.

Luminal Amino Acid Sensing and Mucosal Defenses

The receptor on the tongue for L-glutamate (L-Glu), which is the primary nutrient conferring *umami* taste, is a heterodimer of taste receptor type 1 T1R1 and T1R3 [40, 41] and/or a metabotropic L-Glu receptor (mGluR) [42, 43]. These receptors, which belong to the GPCR superfamily, are localized, along with the specific G protein, α-gustducin in the epithelial cells of the GI tract [34, 35, 39, 44]. Another candidate for L-Glu receptor is CaSR,

whose activity is modified by aromatic amino acids [29]. CaSR is also localized in the duodenal epithelial cells [45]. These observations suggest that the mucosa directly senses luminal L-Glu or other amino acids, and may directly or indirectly conduct luminal information to the internal signaling systems. Indeed, luminal L-Glu stimulates gastric vagal afferents through the release of nitric oxide and 5-hydroxytryptamine [46]. Our study suggests that luminal L-Glu enhances mucosal defense mechanisms via multiple L-Glu receptors in the duodenum [47].

Using in vivo microscopic techniques, we have recently reported that luminal L-Glu (0.1–10 mM) dose-dependently increases epithelial pH_i and mucus gel thickness, but not blood flow, in rat duodenum [48]. The gastric mucosa also similarly responds to luminal L-Glu [1]. These effects of L-Glu are mediated by capsaicin- and indomethacin-sensitive pathways in the duodenum, suggesting the involvement of capsaicin-sensitive afferent nerves and COX activity, respectively. Co-perfusion of L-Glu and 5′-inosine monophosphate (IMP) increases duodenal HCO_3^- secretion. Furthermore, pre-perfusion with L-Glu inhibits supraphysiological acid-induced epithelial injury in the duodenum [48], assessed by in vivo in situ propidium iodide staining [49]. These results suggest that luminal L-Glu enhances duodenal mucosal defenses possibly via L-Glu or amino acid receptor.

T1R1/R3 is activated by certain L-amino acids in the mM range with synergistic enhancement by the presence of IMP [40, 41], representing the specific characteristic of *umami* taste [50]. The T1R1/R3 *umami* receptor and the T1R2/R3 sweet receptor are expressed in the intestinal mucosa [51–53]. Since the luminal concentration of free amino acids reaches mM levels (which is the optimal concentration for T1R1/R3), after a protein-rich meal in human jejunum and ileum [54], the presence of amino acid sensing is predicted in the intestinal epithelium.

We have demonstrated that luminal co-perfusion of L-Glu and IMP synergistically stimulates duodenal HCO_3^- secretion in rat duodenum [48], although L-Glu or IMP alone has a little effect, consistent with the activation of T1R1/R3. Furthermore, other amino acids, such as L-aspartate, L-leucine or L-alanine, increase HCO_3^- secretion, enhanced by the addition of IMP [48], also supporting the presence of T1R1/R3 in the duodenum. However, these amino acids do not mimic the effects of L-Glu on pH_i and mucus gel thickness, suggesting that L-Glu-induced cellular alkalinization and mucus secretion are mediated via different pathways from T1Rs signaling.

We have also demonstrated that luminal perfusion of a mGluR1/5 agonist or mGluR4 agonist increases pH_i and mucus gel thickness, and an mGluR4 antagonist inhibits L-Glu-induced increases of pH_i and mucus gel thickness [48]. In contrast, mGluR agonists fail to affect duodenal HCO_3^- secretion, unlike L-Glu. These results suggest that L-Glu enhances duodenal mucosal defenses via mGluR4 activation, separately from T1R-mediated HCO_3^- secretion.

Calcium Sensing via Calcium-Sensing Receptors

CaSR is another candidate for an L-Glu or amino acid receptor. CaSR is directly activated by extracellular Ca^{2+} and positively modulated by L-amino acids [29]. Although calcium absorption is believed to occur mostly in the ileum [55], the duodenal mucosa also absorbs luminal Ca^{2+} via apical transporter TRPV6 (previously known as CaT1) and the intracellular carrier calbindin-D9k [56–58]. Calcium absorption and luminal sensing may thus also occur in duodenum.

We have examined the effect of high luminal Ca^{2+} or a CaSR agonist spermine on pH_i, mucus gel thickness, blood flow and HCO_3^- secretion in rat duodenum. The CaSR agonists acidify epithelial cells, and increase blood flow, mucus gel thickness and HCO_3^- secretion [48]. These effects of CaSR activation on duodenal mucosal defenses are similar to the effects of luminal acid [1], but unlike the effects of luminal L-Glu. Selective CaSR agonists and antagonists will clarify the role of CaSR in L-Glu-induced mucosal protection. Nevertheless, luminal Ca^{2+} sensing is present in the duodenum and enhances mucosal defense mechanisms.

Recent Advances in Enteroendocrine GPCRs and Clinical Perspectives

Recent studies implicate the chemosensing GPCRs localized in the enteroendocrine cells in the release of peptide hormones [59]. Especially primary isolated L cells tagged with fluorescent protein-labeled proglucagon express GPR41 and 43, GPR40 and 120, GPR119, and TGR5 (GPR131), whose ligands are short-chain fatty acids, long-chain fatty acids, N-acylethanolamines, and bile acids, respectively [60]. Since K and L cells secrete the incretins gastric inhibitory peptide/glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), the ligands for the chemosensing GPCRs may be new nutrient-based therapeutic tools for diabetes. α-Linolenic acid increases GLP-1 release via GPR120 [61].

GPR119 agonists are already in clinical trials. GPR40 and 120, and GPR41 are also expressed in cholecystokinin-expressing I cells, and implicated in host adiposity [62]. GPR40 mediates cholecystokinin release in response to luminal long-chain fatty acid in murine duodenum [63]. Activation of TGR5 improves glucose metabolism via GLP-1 release [64]. These studies suggest that luminal fatty acids and endogenous bile acids are sensed by corresponding GPCRs to release peptide hormones regulating energy balance and satiety.

Not only for diabetes and obesity studies, but also for GI research, luminal nutrient-induced gut hormone release may also affect mucosal integrity (fig. 1). Enteroendocrine L cells also release GLP-2, another derivative from proglucagon, which mediates intestinal ion secretion [65] and intestinal cell growth [66], further suggesting that the activation of duodenal chemosensing GPCRs may increase HCO_3^- secretion. We found that L-Glu/IMP-induced HCO_3^- secretion was reduced by a GLP-2 receptor antagonist, accompanied by GLP-1 and GLP-2 release, and not by GIP release [67], supporting this hypothesis. The multiple effects of GLP-2 [68] may lead to oral nutritional therapies for short bowel syndrome and intestinal mucosal injury in addition to the metabolic syndrome, especially diabetes mellitus via GLP-1.

Duodenal chemosensing has been implicated in the pathophysiology of functional dyspepsia [69]. Many clinical studies have related abnormal chemosensing to dyspeptic symptoms. The strategy includes the increased stimulus intensity (increased acid secretion or decreased HCO_3^- secretion), increased expression of chemosensors (epithelial or afferent nerve endings), hypersensitivity of chemosensors (sensitization), and functional modulation of chemosensors (mucosal mediator release). Nevertheless, a recent pilot study of real-time pH monitoring using a wireless pH meter has demonstrated that duodenal acidification is implicated in the onset of epigastric pain in healthy subjects [70], suggesting that duodenal chemosensing may control epigastric sensation via neurohormonal pathways [71]. Studying the physiological roles of luminal chemosensing may help to understand the pathophysiology of dyspeptic symptoms.

In conclusion, the upper GI mucosa 'tastes' luminal chemicals such as H^+, CO_2, amino acids, especially L-Glu, and Ca^{2+}, and enhances mucosal defense mechanisms through specific signaling cascades, including epithelial ion transporters, enzymes and receptors, and capsaicin-sensitive afferent nerves and the COX pathway. The result is the protection of the duodenal mucosa from acid injury. Understanding luminal chemosensory mechanisms may help to identify novel molecular targets for treating and preventing mucosal injury, metabolic disorder and abnormal visceral sensation.

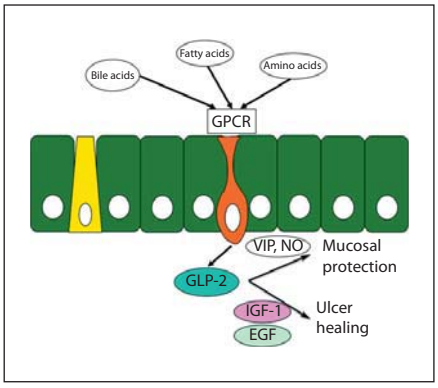

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Fig. 1. Luminal chemosensing and mucosal defenses via GLP-2 in the duodenum. The scheme shows our hypothesis that luminal chemicals/nutrients enhance the duodenal mucosal defenses partly via GLP-2 release. Luminal chemicals activate GPCRs expressed on the apical membrane of the enteroendocrine cell, which secretes gut hormones including GLP-2. GLP-2 may stimulate duodenal HCO_3^- secretion via vasoactive intestinal peptide (VIP) and nitric oxide (NO) pathways. Since GLP-2 is intestinotropic, the stimulated GLP-2 release may enhance mucosal repair via growth factor, such as epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1).

Acknowledgements

We thank Drs. Misa Mizumori, Maggie Ham, Chikako Watanabe, Takanari Nakano, Joonho Wang and Takuya Inoue for their research contributions. Supported by a research grant from Ajinomoto Inc., Japan (Y.A.), Investigator-Sponsored Study Program of AstraZeneca IRUSESOM0424 (Y.A.), Department of Veterans Affairs Merit Review Award (J.K.), and NIH-NIDDK R01 DK54221 (J.D.K.).

Disclosure Statement

No conflicts of interest to disclose.

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