Title
Prostaglandins and duodenal chemosensing

Journal
Journal of Gastroenterology and Hepatology, 29(0 4)

ISSN
0815-9319

Authors
Akiba, Yasutada
Kaunitz, Jonathan D

Publication Date
2014-12-01

DOI
10.1111/jgh.12731

Peer reviewed

HHS Public Access

Author manuscript

J Gastroenterol Hepatol. Author manuscript; available in PMC 2015 December 01.

Published in final edited form as:

J Gastroenterol Hepatol. 2014 December ; 29(0 4): 93–98. doi:10.1111/jgh.12731.

Prostaglandin pathways in duodenal chemosensing

Yasutada Akiba and **Jonathan D Kaunitz**

Veterans Affairs Greater Los Angles Healthcare System, Department of Medicine, School of Medicine, University of California Los Angeles, and Brentwood Biomedical Research Institute, Los Angeles, CA 90073, USA

Abstract

Acid-sensing pathways, which trigger mucosal defense mechanisms in response to luminal acid, involve the rapid afferent-mediated 'capsaicin pathway' and the sustained, 'prostaglandin (PG) pathway'. Luminal acid quickly increases protective PG synthesis and release from epithelia, although the mechanism by which luminal acid induces PG synthesis is still mostly unknown. Acid exposure augments purinergic ATP-P2Y signaling by inhibition of intestinal alkaline phosphatase (IAP) activity. Since P2Y activation increases intracellular Ca^{2+}, we further hypothesized that ATP-P2Y signals increase the generation of H_2O_2 derived from dual oxidase (Duox), a member of the NADPH oxidase family activated by Ca^{2+}. Our recent studies suggest that acid exposure increases H_2O_2 output, followed by phospholipase A_2 (PLA_2) and cyclooxygenase (COX) activation, increasing PG synthesis. Released PGE_2 augments protective HCO_3^- and mucus secretion via EP4 receptor activation. Thus, the PG pathway as a component of duodenal acid sensing consists of acid-related IAP inhibition, ATP-P2Y signals, Duox2-derived H_2O_2 production, PLA_2 activation, PGE2 synthesis and EP4 receptor activation. The PG pathway is also involved in luminal bacterial sensing in the duodenum via activation of pattern recognition receptors, including Toll-like receptors (TLRs) and NOD2. The presence of acute mucosal responses to luminal bacteria suggests that the duodenum is important for host defenses and may reduce bacterial loading to the hindgut using H_2O_2, complementing gastric acidity and anti-bacterial bile acids.

Keywords

acid sensing; ATP-P2Y signal; dual oxidase; bacterial sensing

INTRODUCTION

Prostaglandins (PGs) are a key component of mucosal defense, essential for maintaining the integrity of gastrointestinal (GI) tract. Nonsteroidal anti-inflammatory drugs (NSAIDs), through PG synthesis inhibition, injure the GI mucosa. In this review, we will discuss how

Corresponding author: Yasutada Akiba, MD, PhD, Bldg. 114, Suite 217, West Los Angeles VA Medical Center, 11301 Wilshire Blvd., Los Angeles, CA 90073 USA, Phone: 310-478-3711, Fax: 310-268-4811, yakiba@mednet.ucla.edu.

CONFLICT OF INTEREST

None

foregut chemosensors activate signaling pathways that enhance mucosal defense mechanisms via PG-related mechanisms.

The duodenal mucosa, regularly exposed to gastric acid, and endogenous and exogenous chemicals including nutrients, has a unique luminal chemosensing capacity that enables the mucosa to sense luminal chemicals followed by rapid mucosal responses that protect the mucosa from injury, releasing mediators and hormones that have local and systemic effects [1].

The duodenal mucosa possesses three chemosensing modes (Fig. 1): 1) Luminal chemicals traverse epithelial cells, activating chemosensors expressed on subepithelial afferent nerves (Fig. 1A). This pattern includes luminal CO_2/H^+ sensing and spice sensing. Luminal CO_2 rather than H^+ traverses apical membrane of villous cells, acidifies the cytoplasm due to carbonic anhydrase activity, followed by H^+ extrusion through basolateral Na^+/H^+ exchanger-1 (NHE-1), which activates transient receptor potential vanilloid-1 (TRPV-1) expressed on capsaicin-sensitive afferent nerves [2, 3]. Luminal capsaicin or the TRP channel ankylin-1 (TRPA1) agonist also shares this pathway [4, 5]. 2) Luminal chemicals activate apical chemoreceptors, followed by mediator release from epithelial cells (Fig. 1B). Examples include luminal ATP-P2Y signaling or acid-induced PG release, stimulating protective HCO_3^- and mucus secretion [6, 7]. 3) Luminal chemicals activate G-protein coupled receptors (GPCR) expressed on enteroendocrine cells, followed by mediator or hormone release (Fig. 1C). Examples include luminal nutrient sensing by enteroendocrine cells. We have reported that luminal *umami* substances such as L-glutamate and 5′-inosine monophosphate (IMP) activate taste receptors expressed on enteroendocrine L cells, which release the incretin glucagon-like peptide-1 (GLP-1) and intestinotrophic GLP-2, the latter stimulating duodenal HCO_3^- secretion [8, 9].

The acid-sensing pathway in duodenum, the most important mucosal defense pathway, mediates PGE_2 release in response to luminal acid [10, 11]. Released PGE_2 then increases epithelial intracellular pH (pH_i), HCO_3^- secretion and mucus output, all important mucosal defense factors to luminal acid [7, 12, 13]. How luminal acid increases epithelial PGE_2 synthesis, is however still uncertain. Furthermore, whether other luminal stimuli increase PGE_2 synthesis and release is also unknown.

Here, we introduce our novel hypothesis that epithelial H_2O_2 production is related to duodenal acid-induced PGE_2 synthesis, a mechanism that can also be extrapolated to luminal bacterial sensing. We will show how the PG pathway is essential for duodenal acid and bacterial sensing, augmenting mucosal and host defense mechanisms.

Duodenal mucosal defense factors

Duodenal defense factors include HCO_3^- and mucus secretion (pre-epithelial), pH_i regulation with ion transporters and ecto- and cytosolic enzyme activities (epithelial), and blood flow regulated via afferent nerves and mediator releases (subepithelial). Rapid changes in these defense factors in response to topical application of luminal chemicals imply the presence of mucosal recognition of luminal chemicals via the pathways depicted in Fig. 1. We have assessed duodenal mucosal defense factors using microscopic mucosal

imaging *in vivo*, enabling the measurement of mucosal defense factors such as mucosal blood flow, mucus secretion, and enterocyte pH_i in response to luminal chemicals, in addition to measuring the rate of HCO_3^- secretion using a duodenal loop perfusion system. These approaches enable us to observe a rapid response to luminal compounds and identify the mechanisms using pharmacological or genetic tools.

Surface pH regulation via purinergic signaling

The second pattern of luminal chemosensing is brush border ecto-enzyme-related signals, including duodenal ATP-P2Y receptors and pH-dependent intestinal alkaline phosphatase (IAP) activity [14, 15] (Fig. 1B). Since the optimal pH of IAP is 8 – 9 and IAP activity is closely correlated to the HCO_3^- secretory rate [14], IAP may act as a surface pH sensor in the duodenum.

At neutral luminal pH, extracellular ATP, non-lytically released from the epithelial cells is rapidly degraded to adenosine (ADO), which is further degraded to inosine by adenosine deaminase. Once surface pH (pH_s) is lowered by gastric acid, surface ATP concentrations increase due to the decreased degradation by IAP or the increased release of ATP, since IAP activity is reduced at acidic pH. Ecto-ATPases, as known as ectonucleoside triphosphate diphosphohydrolases (ENTPDs; CD39 family), and 5′-nucleotidase (5′-NT; CD73) are also involved in the degradation of ATP to ADO. Increased surface ATP concentration stimulates P2Y receptors expressed on the apical membrane of epithelial cells, increasing HCO_3^- secretion. Increased surface HCO_3^- concentration increases the pH_s, increasing IAP activity, which degrades surface ATP, terminating ATP-P2Y signaling. Luminal ADO additionally increases HCO_3^- secretion via A_{2B} receptors [16]. These studies suggest that IAP acts as a pH sensor, regulating surface ATP concentrations according to its hydrolytic activity, serving a negative feedback loop. The mechanism of ATP-P2Y receptor signaling is implicated in other HCO_3^--secreting epithelia such as bile ducts, oviduct and bone [17].

H_2O_2 production via ATP-P2Y signals in the duodenum

Our recent studies further identify the mechanism underlying ATP-P2Y signal-induced HCO_3^- secretion [18]. We reported that luminal ATP-induced HCO_3^- secretion was inhibited by pretreatment with the cyclooxygenase (COX) inhibitor indomethacin (IND), suggesting that PG synthesis is a component of ATP-P2Y signaling. Indeed, luminal perfusion of ATP increased HCO_3^- secretion accompanied by increased mucosal PGE_2 content, similar to the response to luminal acid [18]. Since P2Y activation increases intracellular Ca^{2+} concentration [19], Ca^{2+}-sensitive mechanisms may be involved in the ATP-induced PG synthesis.

The NADPH oxidase family includes neutrophil NADPH oxidase (Nox2), which generates superoxide anion, contributing to host defense, but also to tissue damage during inflammation [20]. Among NADPH oxidase family members, dual oxidase (Duox) 1 and 2 are known as thyroid oxidases, which generate H_2O_2 essential for thyroid peroxidase to produce thyroid hormone [21, 22]. Duox2 is also localized to the gastrointestinal tract, especially in the apical membrane of epithelial cells [23]. Duox has an intracellular Ca^{2+} binding EF-hand motif, and is activated by intracellular Ca^{2+} [22]. Activated Duox transports an electron from

cytosolic NADPH to extracellular O_2, generating extracellular H_2O_2 [24]. Thus, Duox2 is a candidate for downstream of ATP-P2Y signals in duodenal epithelial cells.

Our study showed that Duox2 was predominantly expressed in rat duodenal mucosa with its accessary protein DuoxA2 [18], which localizes Duox2 to plasma membranes [25]. Using the H_2O_2-sensitive fluorogenic compound Amplex® Red, we reported that luminal perfusion of ATP increased H_2O_2 output accompanied by increased HCO_3^- secretion [18]. ATP-induced HCO_3^- secretion and H_2O_2 production were inhibited by co-perfusion of a P2Y receptor antagonist or a NADPH oxidase inhibitor, supporting the hypothesis that ATP-P2Y signals produce H_2O_2 via NADPH oxidase. Extracellular ATP increases H_2O_2 production in thyroid cells [26], and airway epithelial cells [27].

How does luminal H_2O_2 generated by ATP-P2Y signals induce PG synthesis? Previous in vitro studies demonstrate that extracellular H_2O_2 activates cytosolic phospholipase A_2 ($cPLA_2$), determined by radiolabeled arachidonic acid release [28–30], even in the intestinal cell line [31], suggesting that H_2O_2 activates epithelial PLA_2, which generates the COX substrate arachidonic acid. A $cPLA_2$ inhibitor reduced ATP-induced HCO_3^- secretion with no effect on H_2O_2 output. Furthermore, IND inhibited ATP-induced HCO_3^- secretion, whereas H_2O_2 output was increased. ATP-induced HCO_3^- secretion was then inhibited by co-perfusion with an EP4 receptor antagonist. These results suggest that H_2O_2 production by luminal ATP-P2Y signals precede PG synthesis, followed by HCO_3^- secretion due to EP4 receptor activation. Mucosal PGE_2 contents were also increased by luminal perfusion of ATP, reduced by P2Y receptor antagonists, NADPH oxidase inhibitors, or $cPLA_2$ inhibitors, further supporting our hypothesis that ATP-P2Y signal-induced H_2O_2 production increases PGE_2 synthesis, augmenting HCO_3^- secretion. H_2O_2 increases electrogenic Cl^- secretion via PGE_2 synthesis in rat colon [32] and in primary inner medullary collecting duct cells [33], consistent with our results.

Luminal acid exposure increases HCO_3^- secretion accompanied by increased H_2O_2 output into the perfusate, inhibited by co-perfusion of P2Y receptor antagonists or NADPH oxidase inhibitors. Furthermore, acid-induced HCO_3^- secretion was reduced by inhibition of $cPLA_2$ without affecting H_2O_2 output. Acid-augmented mucosal PGE_2 content was also reduced by these inhibitors, suggesting that the duodenal mucosa exposed to luminal ATP or acid generates H_2O_2 and PGE_2 via the same pathway. Therefore, acid exposure triggers ATP-P2Y signals, which activate Duox2 to generate extracellular H_2O_2, which activates epithelial $cPLA_2$, which increases PGE_2 synthesis via COX, followed by EP4 receptor activation, intracellular cAMP increase, and cystic fibrosis transmembrane conductance regulator (CFTR) activation, augmenting the rate of luminal HCO_3^- secretion (Fig. 2). This sequential pathway may explain the fundamental question of how luminal acid augments PG synthesis.

Duodenal epithelial cells possess high catalase activity [34–36], which may protect them from self-generated H_2O_2. Luminal exposure to $H_2O_2 \leq 0.3$ mM dose-dependently increased HCO_3^- secretion without epithelial injury or increasing mucosal permeability [18], consistent with the effect of H_2O_2 on rat colonic Cl^- secretion [32]. In contrast, 0.5 mM H_2O_2 inhibits cAMP-induced or Ca^{2+}-dependent Cl^- secretion in colonic T84 cells[37, 38]. H_2O_2 also increases epithelial permeability and cellular toxicity at higher concentration (≥0.5

mM) [39, 40], suggesting that the effect of luminal H_2O_2 is dependent on whether its concentration is in the physiological or pathological ranges.

Since generation of H_2O_2 by Duox2 requires sufficient luminal O_2, and since activation of HCO_3^- secretion consumes intracellular ATP, epithelial O_2 consumption may be increased during acid exposure. We reported that post-prandial epithelial hypoxia was present in duodenal villous cells, induced by acid exposure, and inhibited by pretreatment with proton pump inhibitor (PPI) or oral catalase [41]. Since duodenal hypoxia increases hypoxia-inducible factor-2α (HIF-2α) signaling, enhancing iron absorption [42, 43], and since PPI treatment decreased COX expression in the duodenal mucosa [41], acid exposure may maintain mucosal integrity by inducing villous hypoxia. This mechanism may be implicated in the clinical observation of PPI-induced iron deficiency [44, 45].

Duodenal bacterial sensing

The presence of H_2O_2 production in the duodenal lumen in response to luminal acid further suggests the presence of bacterial sensing, since H_2O_2 has anti-bacterial properties in and of itself or combined with other molecules [46, 47]. Orally ingested commensal bacteria in the food or drink, or derived from oral flora, may be killed by gastric acid or bile acids in the duodenum [48, 49], possibly explaining why the duodenal lumen is relatively sterile compared with lower small intestinal lumen. Further data obtained from drosophila suggest that Duox may also affect the composition of intestinal microbiota. Drosophila intestine expresses Duox, which generates superoxide anion via Ca^{2+}-sensitive NADPH oxidase activity [50]. Knockdown of intestinal Duox in drosophila using siRNA increases mortality due to intestinal bacterial overgrowth [50], suggesting that Duox-mediated intestinal epithelial H_2O_2 production affects the composition of the luminal microbiome. These data form the basis of the hypothesis that the duodenal mucosa senses luminal bacteria to produce H_2O_2, which complements gastric acid and bile acids to curb the viability of foregut microbiota.

Bacterial components are recognized by pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) or nucleotide-binding oligomerization domain-containing proteins (NODs) [51, 52]. TLRs and NODs, primarily studied in immune cells, are also expressed in intestinal epithelial cells [53], where they are expressed on the apical membrane of villous and Paneth cells [53, 54]. These results suggest that the duodenal mucosa may recognize luminal bacteria, generating anti-bacterial H_2O_2 in response.

When evoking a mucosal response to bacterial components, we observed that TLR ligands or a NOD2 ligand alone had no effect, whereas the combination of a TLR with NOD2 ligands stimulated HCO_3^- secretion, accompanied by increased H_2O_2 output and mucosal PGE_2 synthesis [55], akin to the mucosal response to luminal acid. Ligands for TLR and NOD2 synergistically increase inflammatory responses in murine macrophages [56], consistent with our results. Although a delayed (hours-days) inflammatory response to TLR or NOD2 activation is well described, presumably due to genomic activation, this is the first description in mammals of an acute epithelial response to luminal bacterial components, reinforcing the notion that PRR sensing mediates rapid anti-bacterial mucosal responses (Fig. 2). Extracellular ATP activates Duox1, mediating airway epithelial pro-inflammatory responses to bacterial stimuli [27], similar to our results.

Clinical relevance of duodenal H_2O_2 production

Mucosal H_2O_2 production via Nox1/Duox2 in response to bacterial exposure was reported in human duodenal biopsies [57], suggesting the anti-bacterial activity of duodenal Duox2. Compared with the lower intestine with its abundant flora, the duodenal lumen is 'clean' from bacterial residency. It is possible that, in addition to gastric acid and bile acids, toxic to microorganisms, duodenal H_2O_2 may participate in sterilizing the duodenal lumen. PPI-induced bacterial overgrowth, the presence of which is controversial [58–60], may be also affected by duodenal H_2O_2 production.

NSAID-induced enteropathy is associated with bacterial translocation [61]. PPI pretreatment aggravates NSAID-induced enteropathy by inducing dysbiosis [62], suggesting that disruption of sterility in the foregut lumen by inhibition of PG synthesis combined with gastric acid suppression may also induce bacterial overload in the hindgut lumen, with resultant dysbiosis.

Enteric pathogens such as salmonella, campylobacter, or listeria possess catalase activity. *Helicobacter pylori*, a unique pathogen limited to the gastric mucosa, also possesses catalase and superoxide dismutase activity, as one explanation for its long-term survival *in situ*. Pathogenic bacteria can resist the deleterious effects of H_2O_2, gastric acid and bile acid, whereas some commensal bacteria and eukaryotes such as fungi and yeast are H_2O_2 sensitive [63, 64]. Therefore, relative sterility of the duodenal lumen may be achieved by duodenal epithelial H_2O_2 production in addition to gastric acid and bile acid toxicity.

The duodenal mucosa also senses luminal nutrients via nutrient sensors in order to rapidly control gastric emptying, bile and pancreatic secretion, and intrinsic mucosal defenses through augmented ion secretion [1, 65]. Since luminal bacteria may disrupt nutrient sensors by taking up nutrients or by their metabolites interfering with nutrient detection, Duox2-mediated H_2O_2 release may repel bacteria from the epithelial surface, enhancing nutrient chemosensing and nutrient-evoked mucosal responses [66]. Luminal nutrients may also be important instigators of antibacterial foregut mucosal responses. Duodenal bacterial overload potentiates mucosal secretory responses*, further suggesting that the luminal bacterial environment affects the duodenal physiology.

In conclusion, acid-induced PG synthesis may be mediated by luminal ATP-P2Y signals, Duox2-mediated H_2O_2 production, and $cPLA_2$ activation, followed by COX activation. Released PGE_2 stimulates basolateral EP4 receptors, augmenting protective HCO_3^- secretion via CFTR activation. This pathway forms one of the most important regulatory schemes coordinating duodenal mucosal defense mechanisms in response to luminal acid. Furthermore, the PG pathway, including anti-bacterial H_2O_2 production is also an important component of foregut mucosal defenses. Therefore, the duodenal PG pathway not only protects the foregut from mucosal injury, but also contributes to host defenses to luminal dysbiosis.

*Akiba Y, Strugatsky D, Kaji I, Kaunitz JD, unpublished observation

Acknowledgments

Grant supports

We thank Drs. Masaaki Higashiyama, Izumi Kaji and David Strugatsky for their research contributions, and Bea Palileo for her assistance with manuscript preparation.

Supported by a research grant from Department of Veterans Affairs Merit Review Award (J. Kaunitz), and NIH-NIDDK R01 DK54221 (J. Kaunitz).

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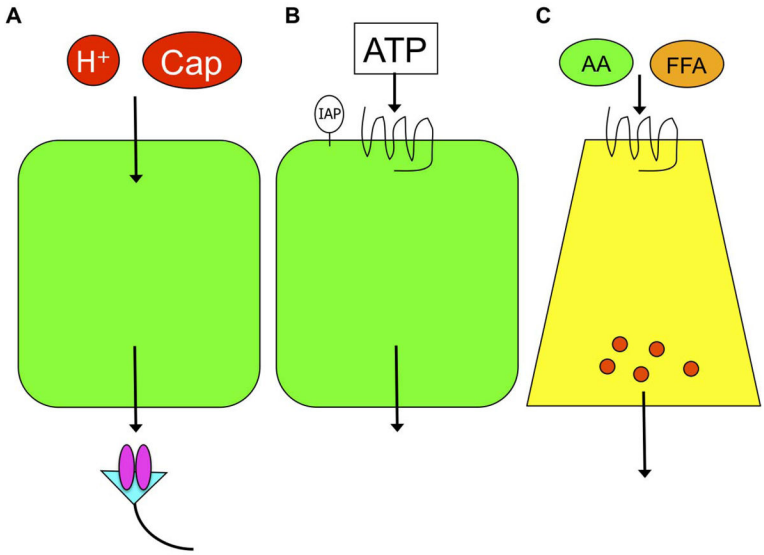

Fig. 1. Luminal chemosensing patterns in the duodenum

The scheme shows three patterns of luminal chemosensing, with resulting local mucosal protective responses as well as remote effects via neuronal or hormonal pathway. Luminal acid (H^+) or capsaicin (Cap) is transported through the epithelial layer and activates the receptors on afferent nerves (**A**). Luminal ATP with inhibition of hydrolysis by intestinal alkaline phosphatase (IAP), activates the apical P2Y receptor, exerting epithelial responses (**B**). Luminal nutrients such as amino acids (AA) or free fatty acids (FFA) activate the corresponding receptors on enteroendocrine cells, releasing gut hormones (**C**). Adapted from ref 1.

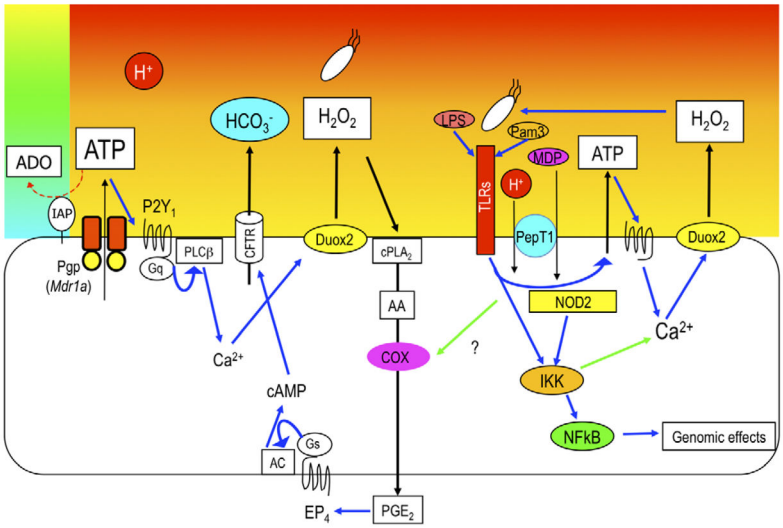

Fig. 2. Proposed mechanisms of luminal ATP-P2Y signaling during acid exposure

Acid exposure increases luminal ATP concentration. ATP stimulates P2Y receptors on the apical membrane of epithelial cells, and then increases intracellular Ca^{2+} concentration. Ca^{2+} activates dual oxidase 2 (Duox2), which generates H_2O_2 in the lumen. H_2O_2 activates cytosolic phospholipase A2 ($cPLA_2$), followed by cyclooxygenase (COX)-mediated prostaglandin E2 (PGE_2) synthesis. PGE_2 stimulates EP4 receptor, followed by cystic fibrosis transmembrane conductance regulator (CFTR) stimulation, increasing protective HCO_3^- secretion. On the other hand, toll-like receptor (TLR) ligand, Pam3 or lipopolysaccharide (LPS), and nucleotide-binding oligomerization domain 2 (NOD2) ligand muramyl dipeptide (MDP) coordinately increases H_2O_2 production with enhanced ATP release and IkB kinase (IKK) activation. This bacterial sensing pathway also increases PGE_2 production and stimulates HCO_3^- secretion.