Sensory pathways and cyclooxygenase regulate mucus gel thickness in rat duodenum

YASUTADA AKIBA, OSAMU FURUKAWA, PAUL H. GUTH, ELI ENGEL, IGOR NASTASKIN, AND JONATHAN D. KAUNITZ

Sensory pathways and cyclooxygenase regulate mucus gel thickness in rat duodenum. Am J Physiol Gastrointest Liver Physiol 280: G470–G474, 2001.—We previously showed that the duodenal hyperemic response to acid occurs through activation of capsaicin-sensitive afferent nerves with subsequent release of vasodilatory substances such as calcitonin gene-related peptide (CGRP) and nitric oxide. We then tested the hypothesis that similar factors regulate duodenal mucus gel thickness. Gel thickness was optically measured using in vivo microscopy in anesthetized rats. Duodenal mucosae were superfused with pH 7.0 buffer with vanilloid receptor agonist capsaicin, bradykinin, or PGE2 injection or were challenged with pH 2.2 solution, with or without the vanilloid antagonist capsazepine, human CGRP(8–37), Nω-nitro-L-arginine methyl ester, and indomethacin. Other rats underwent sensory ablation with high-dose capsaicin pretreatment. Acid, bradykinin, capsaicin, and PGE2 all quickly thickened the gel. Antagonism of vanilloid and CGRP receptors, inhibition of nitric oxide synthesis, and sensory deafferentation delayed gel thickening, suggesting that the capsaicin pathway mediated the initial burst of mucus secretion that thickened the gel. Indomethacin abolished gel thickening due to acid, bradykinin, and capsaicin. Inhibition of gel thickening by indomethacin in response to multiple agonists suggests that cyclooxygenase activity is essential for duodenal gel thickness regulation. Duodenal afferent neural pathways play an important role in the modulation of cyclooxygenase-mediated physiological control of gel thickness.

THE PROXIMAL DUODENAL MUCOSA is unique in that it is the only leaky epithelium exposed to gastric acid. Since the epithelial cell layer is an incomplete barrier to solute diffusion, physiological, nonstructural defense mechanisms such as the mucus gel layer, bicarbonate secretion, and blood flow are of prime importance in the defense from luminal acid. The viscoelastic mucus gel layer is the first line of defense against luminal contents and is thought to play an important role in stabilizing a preepithelial pH gradient, decreasing mucosal injury due to acid (6). Although increased mucus secretion has been observed in vitro in response to numerous secretagogues, the mediators that are involved in the regulation of duodenal mucus gel thickness, and by inference secretion, have not been studied in vivo.

Luminal acid evokes mucosal hyperemia via capsaicin-sensitive afferent nerves in duodenum and in stomach (2, 9). We have observed previously that acid induces a duodenal hyperemic response by stimulating a vanilloid receptor on capsaicin-sensitive afferent nerves, with subsequent release of calcitonin gene-related peptide (CGRP) and nitric oxide (NO) (2). This acid response was, unlike that described in airway and other epithelia, not inhibited by indomethacin pretreatment (2). We have thus postulated a “capsaicin pathway” that mediates the hyperemic response to acid, which includes the acid-sensing vanilloid receptor, capsaicin-sensitive afferent nerves, CGRP, and NO, without involvement of the cyclooxygenase (COX) pathway. The importance of the capsaicin pathway has been underscored by the common clinical and experimental observation that interruption of any one of its components increases the susceptibility of the gastroduodenal mucosa to injury (14, 24) and that administration of a component of the capsaicin pathway (e.g., capsaicin, NO, or CGRP) can attenuate injury due to interruption of the COX pathway (1, 14, 17). We therefore examined the duodenal mucus secretory response to agonists that activate the capsaicin pathway (acid and capsaicin) and potential alternate pathways (bradykinin and prostaglandin) to more fully understand the integrated regulation of duodenal defense mechanisms.

We are able to measure mucus gel thickness continuously and noninvasively in a superfused system in vivo. Gel thickness increases and decreases in parallel with changes of mucus secretion and exudation. Luminal acid and exogenous PGE2 increased gel thickness, and indomethacin pretreatment abolished the acid-induced gel thickness increase, suggesting that regul
loration of mucus gel thickness in response to luminal acid must be mediated via the COX pathway (3). In seeking to further investigate the regulatory mechanisms underlying acid-induced mucus gel thickness increase, we tested the hypothesis that regulation of duodenal mucus gel thickness in response to acid is similar to regulation of the hyperemic response. We hence examined the role of vanilloid receptors, capsaicin-sensitive afferent nerves, CGRP, and NO synthase (capsaicin pathway) and the COX pathway in the control of gel thickness. We also examined the effects of bradykinin, an inflammatory mediator that stimulates afferent nerves and mucus secretion in intestine (8, 22), on gel thickness. We demonstrated that gel thickness increase in response to acid is biphasic, with a rapid phase mediated by the capsaicin pathway, and that COX activation is an obligatory component of duodenal mucus gel thickness increase.

MATERIALS AND METHODS

Solutions were made, animals were prepared, and mucus gel thickness was measured as previously described (2, 3). The Animal Use Committee of the Greater Los Angeles Veterans Affairs Healthcare System approved all studies.

Experimental Protocol

After stabilization of the preparation with a 30-min superfusion of pH 7.0 Krebs buffer, time was set as t = 0. The duodenal mucosa was superfused with pH 7.0 Krebs buffer from t = 0 until t = 10 min (baseline period), pH 7.0 or 2.2 buffer from t = 10 min until t = 20 min (challenge period) with or without the antagonists or inhibitors described below, and with pH 7.0 solution from t = 20 min until t = 35 min (recuperation period).

Effect of vanilloid receptor antagonism on acid- or bradykinin-induced gel thickness increase. To determine the effects of a vanilloid receptor antagonist on basal gel thickness and on acid-induced gel thickness increase, capsaicpine (0.5 mM) or vehicle was coadministered with pH 7.0 Krebs (pretreatment) for 10 min, followed by exposure to pH 2.2 buffer for 10 min. To test the effect of capsaicine on bradykinin-induced gel thickness increase, bradykinin (0.5 mM) was added to pH 7.0 Krebs solution with or without capsaicine (0.5 mM).

Effect of CGRP-(8–37) and N\textsuperscript{G}-nitro-L-arginine methyl ester on acid-induced gel thickness increase. To inhibit CGRP receptors, CGRP-(8–37) (0.1 mg/kg) was bolus injected intravenously at t = −10 min, the mucosa was superfused with pH 7.0 Krebs from t = −10 min until t = 10 min, and the mucosa was superfused with pH 7.0 buffer from t = 10 min until t = 20 min as described previously (2). To inhibit NO synthase, N\textsuperscript{G}-nitro-L-arginine methyl ester (l-NAME; 0.1 mM) was administered topically with or without l-arginine (50 mM) with pH 7.0 Krebs from t = 0 min until t = 10 min and pH 2.2 buffer from t = 10 min until t = 20 min.

Effect of indomethacin pretreatment and ablation of capsaicin-sensitive afferent nerves on acid-induced gel thickness increase. To abolish the endogenous COX activity, rats were pretreated with indomethacin (5 mg/kg ip) 1 h before anesthesia (3). Ablation of capsaicin-sensitive afferent nerves was performed with high-dose capsaicin (125 mg/kg sc) as described previously (2). Capsaicin-treated rats were used 10–14 days after the injections. Completeness of deafferentation was assessed by the 0.1% NH\textsubscript{4}OH eye drop test. Duodenal mucosa of indomethacin-treated or capsaicin-treated rat was superfused with pH 7.0 or 2.2 solution with or without bradykinin as described above.

Effect of luminal capsaicin and PGE\textsubscript{2} injection on gel thickness. To clarify the interaction between capsaicin pathway and COX pathway, PGE\textsubscript{2} was bolus injected (0.3 mg/kg iv) to nontreated or capsaicin-treated rats. Conversely, capsaicin (0.3 mM) was added to the pH 7.0 perfusate in nontreated or indomethacin-treated rats.

Statistics

All data are expressed as means ± SE. Comparisons between groups were made by one-way ANOVA followed by Fisher’s least significant difference test. P < 0.05 was taken as significant.

RESULTS

Effects of Antagonists and Inhibitors on Acid-Induced Gel Thickness Increase in Duodenum

Gel thickness was constant with pH 7.0 solution administered in the perfusate (Fig. 1A). The pH 2.2 solution administered in the perfusate rapidly increased gel thickness (t = 15 min) to a new level (t = 20 min), which then decreased to basal levels after acid removal (t = 25–35 min) as reported previously (3). CGRP-(8–37) (0.1 mg/kg iv) delayed the acid-induced gel thickness response (Fig. 1A). l-NAME (0.1 mM administered in the perfusate) decreased basal gel thickness and abolished gel thickness increase during acid challenge (Fig. 1B). l-Arginine reversed the effects of l-NAME and partially potentiated the gel thickness response to acid. Capsazepine also delayed acid-induced gel thickness increase but did not affect the basal gel thickness (Fig. 1C). Indomethacin pretreatment (5 mg/kg ip) abolished acid-induced gel thickness increase, as reported previously (3), whereas deafferentation with high-dose capsaicin (125 mg/kg sc) delayed the gel thickness response to acid (Fig. 1D).

Effect of Capsazepine, Capsaicin Treatment, and Indomethacin Treatment on Bradykinin-Induced Gel Thickness Increase in Duodenum

Bradykinin (0.5 mM) rapidly increased gel thickness, which then decreased to basal thickness after bradykinin removal, similar to the response of gel thickness to acid. Nevertheless, in contrast to acid, capsaicin had no effect on the increased gel thickness induced by bradykinin (Fig. 2A). Indomethacin treatment abolished, whereas capsaicin treatment delayed, bradykinin-induced gel thickening (Fig. 2B).

Interactive Effects of PGE\textsubscript{2} and Capsaicin on Gel Thickness Response in Duodenum

PGE\textsubscript{2} injection (0.3 mg/kg iv) gradually increased gel thickness, as reported previously (Fig. 3A). Capsaicin treatment attenuated the acute response to PGE\textsubscript{2} (t = 25–30 min). Capsaicin (0.3 mM) rapidly increased gel thickness, which remained elevated (Fig. 3B). Indomethacin treatment abolished the effect of capsaicin on gel thickness.
DISCUSSION

Using our recently reported technique to measure gel thickness in vivo, we studied the regulation of the gel thickness response to luminal acid, bradykinin, capsaicin, PGE$_2$ injection, COX inhibition, and deafferentation of capsaicin-sensitive afferent nerves in rat duodenum. This is the first study to demonstrate that the rapid mucus secretory response to acid involves vanilloid receptor, capsaicin-sensitive afferent nerves, CGRP, and NO synthase. Furthermore, capsaicin, bradykinin, and PGE$_2$ mimic the effect of perfused acid on gel thickness. Moreover, COX inhibition abolishes the response to all of these stimuli, consistent with the essential role of COX stimulation for duodenal mucus gel thickness increase. Furthermore, this is the first study in which the relative contributions of capsaicin and COX pathways to the regulation of duodenal gel thickness were examined.

There have been many studies addressing the involvement of the capsaicin pathway in preventing injury to the gastroduodenal mucosa. Whittle and co-workers (24), for example, found that sensory denervation, NO synthase inhibition, and COX inhibition had additive effects on gastric damage. The effect of the capsaicin pathway on blood flow has been most commonly studied (9), with only a few studies addressing the role of the capsaicin pathway in the regulation of gastroduodenal mucus secretion. Oral capsaicin increased luminal hexose content in rat intestine (13). CGRP increased mucin synthesis in gastric corpus, which was blocked by NO synthase inhibition (12). Indomethacin and L-NAME decreased the rate of mucus gel thickness restoration in rats measured in vivo (20). Isosorbide dinitrate, an NO donor, increased immunologically detected mucin release from rat gastric mucosal cells in primary culture (5) and increased gel thickness in rat stomach (4). Our finding that capsaicin increased gel thickness and that L-NAME decreased basal gel thickness before acid administered in the perfusate also confirms that the capsaicin pathway has an important role in basal and acid-induced mucus gel thickness increase.

Unlike the acid response, capsazepine had no effect on the response to bradykinin, although bradykinin and acid both increased gel thickness over a similar time course. This suggests that the bradykinin receptor is distinct from the vanilloid receptor. Since the bradykinin-induced gel thickness increase was delayed...
by capsaicin treatment and abolished by indomethacin treatment, the bradykinin response, like that to acid, requires the presence of intact capsaicin-sensitive afferent nerves and the COX pathway. Our observation is supported by previous studies in which bradykinin stimulated CGRP release in guinea pig atria (8) and rat trachea (11). In these tissues, the bradykinin effect on CGRP release is mediated by prostaglandins (8, 11).

The effect of bradykinin on mucus gel thickness has been well studied in airway epithelia (16, 18) but has been examined on only one prior occasion in the gastrointestinal tract (22). Since bradykinin is released in the site of intestinal mucosal inflammation (7), the bradykinin-induced gel thickening may underlie the acute inflammatory response.

Gel thickening in response to superfused acid differs from the acid-induced hyperemic response measured in rat duodenum. The hyperemic response was abolished by inhibition of the COX pathway but was not affected by inhibition of the COX pathway (2), whereas the gel thickness response was delayed by inhibition of capsaicin pathway but abolished by inhibition of the COX pathway. These differential responses to inhibition of the COX and capsaicin pathways suggest that hyperemia and mucus gel thickness increase may have disparate roles in mucosal protection or else may have other, unrelated functions.

Regulation of duodenal bicarbonate secretion (23) and gastric mucosal injury susceptibility (10) are dependent on interactions between the capsaicin and COX pathways. In our prior study (3), we found that there was a rapid but transient increase of Alcian blue-positive glycoprotein, which represents goblet cell secretion, in the effluent from acid-exposed duodenum, in contrast to a more sustained increase in periodic acid-Schiff-positive glycoprotein, which can be secreted by either goblet cells or Brunner’s glands. Mucus secretion has three distinct phases: phase I, in which there is slow turnover, phase II, in which there is rapid secretion due to degranulation, and phase III, in which increased mucin synthesis repletes the mucus granules. The rapid response to acid, PGE2, bradykinin, and capsaicin represents phase II secretion from goblet cells (3). This conclusion was based on the observation that in duodenal perfusions with acid, increased amounts of Alcian blue-positive effluent glycoproteins, which originate in goblet cells, were short lived compared with sustained increases of periodic acid-Schiff-positive material, which may represent Brunner’s gland secretion. The delayed response of gel thickness

Fig. 2. Effects of capsazepine, deafferentation, and cyclooxygenase (COX) inhibition on bradykinin-induced gel thickness increase in duodenum. A: bradykinin (BK) rapidly increases gel thickness (MGT). Capsazepine (BK+CPZ) has no effect on bradykinin-induced gel thickness response. B: capsaicin treatment (cap-t+BK) delays, whereas indomethacin treatment (indo-t+BK) abolishes, bradykinin-induced gel thickness increase. *P < 0.05 vs. pH 7.0 Krebs administered in the perfusate; †P < 0.05 vs. bradykinin administered in the perfusate. Values are means ± SE from 6 rats.

Fig. 3. Interactive effect of PGE2 and capsaicin on gel thickness increase in duodenum. A: PGE2 (0.3 mg/kg iv) increases gel thickness (MGT). Capsaicin treatment (cap-t+PGE2) attenuates the PGE2 effect on gel thickness. *P < 0.05 vs. pH 7.0 Krebs administered in the perfusate; †P < 0.05 vs. PGE2 group in nontreated rats. B: capsacin (cap; 0.3 mM) increases gel thickness, whereas capsaicin effect is abolished by indomethacin treatment (indo-t+cap). *P < 0.05 vs. pH 7.0 Krebs administered in the perfusate; †P < 0.05 vs. capsaicin group in nontreated rats. Values are means ± SE from 6 rats.
increase that occurs in the presence of inhibition of the capsaicin pathway may represent the sustained secretion of Brunner’s glands unmasked by inhibition of goblet cell secretion, although proof of this concept is dependent on the measurement of the individual goblet cell and Brunner’s gland secretions. On the other hand, inhibition of the COX pathway abolished both phases of gel thickness response, suggesting that COX exerts a fundamental, “downstream” control of mucus secretion. Since COX inhibitors such as indomethacin have effects on mucus synthesis, composition, and exudation (3, 15, 21), in addition to having effects on motility (19), the use of a selective prostaglandin receptor antagonist will probably clarify the role of prostaglandins in the regulation of the rapid increase of mucus gel thickness increase and its interaction with capsaicin pathway.

In summary, duodenal mucus gel thickness increase in response to luminal acid involves two pathways: the capsaicin pathway and COX activation, which plays an obligatory role in the increase of gel thickness. The capsaicin pathway is a major luminal acid-sensing pathway in duodenum and may play an additional role by responding to inflammatory mediators, whereas COX may be a final common mechanism for duodenal mucus gel thickness regulation. Duodenal acid-sensing pathways, including vanilloid receptor and afferent nerves, play an important role in the regulation of COX-mediated physiological control of mucus gel thickness in rat duodenum.

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Current address of Y. Akiba: Division of Gastroenterology and Hepatology, Dept. of Internal Medicine, School of Medicine, Keio University, 35, Shinanomachi, Shinjuku-ku, Tokyo 150, Japan.

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