Dynamic regulation of mucus gel thickness in rat duodenum

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Dynamic regulation of mucus gel thickness in rat duodenum. Am J Physiol Gastrointest Liver Physiol 279: G437–G447, 2000.—We examined the dynamic regulation of mucus gel thickness (MGT) in vivo in rat duodenum in response to luminal acid, cyclooxygenase (COX) inhibition, and exogenous PGE₂. An in vivo microscopic technique was used to measure MGT with fluorescent microspheres in urethan-anesthetized rats. Duodenal mucosa was topically superfused with pH 7.0 or pH 7.2 solutions with or without PGE₂ and indomethacin treatments. Glycoprotein concentration of duodenal loop perfusates was measured with periodic acid/Schiff (PAS) or Alcian blue (AB) staining. MGT and perfusate glycoprotein concentration were stable during a 35-min perfusion with pH 7.0 solution. Acid exposure increased MGT and PAS- and AB-positive perfusate glycoprotein concentrations. Indomethacin pretreatment increased both PAS- and AB-positive perfusate glycoprotein concentrations. Indomethacin augments mucus secretion from goblet cells and Brunner’s glands.

The proximal duodenal mucosa is exposed to frequent pulses of gastric acid. Unlike other acid-exposed organs such as the stomach or esophagus, the duodenum is functionally “leaky” in that the epithelial cell layer is an incomplete barrier to solute diffusion, increasing the importance of nonstructural defense mechanisms such as the mucus gel layer, bicarbonate secretion, and blood flow. The viscoelastic mucus gel layer is the first line of defense against luminal contents and is thought to play an important role in decreasing mucosal injury due to acid. Mucus secretion is increased by luminal acid (19, 33) and the neuro- and inflammatory mediators acetylcholine and interleukin-1 (8), vasoactive intestinal polypeptide (15), secretin (16, 19), and guanylin (10) in duodenum. In the stomach and intestine, mucus secretion and gel thickness are increased by prostaglandins (5, 20, 24, 36), pentagastrin (26, 45), carbachol (30, 31), nitric oxide (7, 30), bradykinin (41) and phorbol esters (29). Of these factors, prostaglandins, products of the cyclooxygenase (COX) pathway, are of paramount clinical importance, because COX inhibition is an important cause of clinical duodenal ulceration (38).

In the gastrointestinal tract, mucus secretion is generally inferred from examination of histological sections stained by the periodic acid/Schiff (PAS) or Alcian blue (AB) techniques (25, 28). Secretion has also been extrapolated from measurements of mucous glycoprotein of luminal perfusates by PAS staining (24, 36) or hexosamine measurement (19); mucus gel thickness (MGT) has been measured in tissue sections by the method developed by Kerss et al (14). In the duodenum, Sababi et al. (33) performed the only study in which MGT was continuously measured in vivo. In that study, the duodenal mucus gel was shown to form a spontaneously secreted, continuous layer. Furthermore, recovery of MGT after mechanical removal was accelerated by luminal acid but inhibited by indomethacin (Indo) and a nitric oxide synthase inhibitor (33). No study, however, has addressed the regulation of MGT in a superfused system in which glycoprotein sloughing (exudation) was measured in the basal steady state or in response to secretory stimuli. Furthermore, because the duodenum secretes at least two mucin subtypes, muc2, which is synthesized in intestinal goblet cells (48), and muc6 from Brunner’s glands (12), it is unknown which mucin subtype contributes to the regulation of MGT.
In this study, we describe a novel optical, noninvasive technique for measuring duodenal MGT and compare MGT with perfusate glycoprotein concentration measured by PAS and AB staining. We demonstrate that MGT is governed by the balance between mucus secretion and release into the superfusate, that spontaneous and stimulated mucus secretion and luminal exudation are regulated by luminal pH and the COX pathway, and that both goblet cells and Brunner’s glands contribute to the changes in MGT and exuded luminal mucus.

MATERIALS AND METHODS

Animals and Chemicals

Male Sprague-Dawley rats weighing ~225–275 g (Harlan Laboratories, San Diego, CA) were fasted overnight but had free access to water. All studies were approved by the Animal Use Committee of the Greater Los Angeles Veterans Affairs Healthcare System.

MGT In Vivo

In vivo microscopic preparation. An in vivo microscopic technique was used to visualize epithelial cells of rat duodenal villus tips as previously described (3). Briefly, after urethan (1.25 g/kg) anesthesia, a tracheal cannula was inserted into the chamber to enable rapid changes of superfusate. The exposed mucosa was incubated with 50 μM BCECF-AM for 15 min to load the duodenal epithelial cells before starting the experiment. BCECF localization in the epithelial cells of villus tips has previously been confirmed by examination of cryostat sections (3).

Measurement of MGT. Duodenal MGT measurement was adapted from a technique used to measure MGT in rat stomach (13). After BCECF loading, fluorescent microspheres diluted to 0.05% wt/vol with prewarmed Krebs buffer (pH 7.0) were placed over the mucosa to delineate the luminal surface of the gel layer. After a 5-s incubation, the microsphere-containing solution was removed from the chamber and superfusion with Krebs buffer commenced. Fluorescence of the microscopically observed chambered segment of duodenal mucosa was visualized with a charge-coupled device color video camera (Optometrics Engineering, Goleta, CA) and was captured and stored using an Intel Pentium-based IBM-compatible microcomputer with a FlashPoint framegrabbing videographic card (Integral Technologies, Silver Spring, MD) and image-processing software (Image-Pro Plus v. 1.3, Media Cybernetics, Silver Spring, MD). Optical mucus thickness measurements were made by alternately focusing on the fluorescent cell surface, using a 495-nm excitation and a 515-nm emission filter (green filter set, Chroma, Battleboro, VT), and then focusing on the microspheres layer, visualized with a 575-nm excitation and a 600-nm emission filter (red filter set). The vertical travel of the microscope objective from the plane of the fluorescent cell surface to the plane of the microspheres was measured by using a digital z-axis measuring device (Quick-Check, Metronics, Bedford, NH) connected to the microscope, providing a measure of gel thickness. Gel thickness was measured every 5 min, after capture of the BCECF images of the mucosa. Paired images of mucosa and microspheres were taken a maximum of 10 s apart.

Histological sections were examined to localize the microspheres to the gel surface. After microsphere loading and a 30-min pH 7.0 Krebs superfusion, the observed area of duodenum was excised and mounted in OCT compound (Miles, Elkhart, IN) at −20°C. Frozen cryostat sections (10 μm) were mounted on glass slides (Fisher Scientific, Willard, OH) and counterstained with 10 μM acridine orange. Sections were covered slips using glycerol and observed by a Zeiss MPS microscope with a dual-band filter (Chroma) that enabled simultaneous visualization of the green acridine orange and red microsphere fluorescence.

Effect of luminal acid on MGT. After stabilization of the preparation with a 30-min superfusion of pH 7.0 Krebs buffer, the time was set as time 0. The duodenal mucosa was superfused with pH 7.0 Krebs buffer from time 0 to time 10 (baseline period), pH 7.0 or 2.2 buffer from time 10 to time 20 (challenge period), and pH 7.0 solution from time 20 to time 35 (recovery period).

Experimental design. The following four groups were studied: control (no pretreatment and challenge with pH 7.0); acid (no pretreatment and challenge with pH 2.2), Indo (5 mg/kg ip pretreatment 1 h before anesthesia and challenge with pH 2.2); and PGE₂ (1 mg/kg iv infused at time 10 during continuous pH 7.0 buffer superfusion and challenge with pH 7.0).
MGT and perfusate mucus concentration were measured in each group and condition in separate animals.

Perfusate Glycoprotein Concentration Measurement

Preparation and protocol. A duodenal loop was prepared and perfused as modified from previously described methods (43, 44). In urethan-anesthetized rats, the abdomen was opened and the forestomach wall was incised 0.5 cm using a miniature electrocautery. A polyethylene tube (diameter, 5 mm) was inserted through the incision until it was 0.5 cm caudal from the pyloric ring, where it was secured with a nylon ligature. The distal duodenum was ligated proximal to the ligament of Treitz, before the duodenal loop was filled with 1 ml saline prewarmed at 37°C. The distal duodenum was then incised, through which another polyethylene tube was inserted and sutured into place. To prevent the contamination of the perfusate with bile-pancreatic juice, the pancreaticobiliary duct was ligated just proximal to its insertion into the duodenal wall. The resultant closed proximal duodenal loop (perfused length, 2 cm) was perfused with prewarmed saline using a Harvard infusion pump at 1 ml/min. A similar method was used to isolate and perfuse a 2-cm segment of distal duodenum, which extended distally from the common entrance of the bile and pancreatic ducts, a segment that in the rat is devoid of Brunner's glands (1). After a 30-min stabilization with pH 7.0 saline (time 0 to time 10), the perfusate was collected from time 10 to time 20 and for each subsequent 5-min period of perfusion. Solutions were perfused identically to the protocol described previously for MGT measurement; pH 7.0 saline was perfused for 10 min (time 0 to time 10; baseline), followed by either pH 7.0 or pH 2.2 saline for 10 min (time 10 to time 20; challenge period), and pH 7.0 saline for 15 min (time 20 to time 35; recovery period). The duodenum solution was gently flushed with 5 ml of perfusate to rapidly change the perfusate composition at time 10 and time 20. A 1-ml aliquot of sample was lyophilized with a Speed Vac (SC110, Savant Instruments, Holbrook, NY) and resuspended with 50 μl of distilled water (resuspended sample solution). The perfusate was frozen at less than −20°C before measurement of mucus glycoprotein.

Detection of glycoprotein in sample solutions. Mucus concentration in the perfusates was measured with PAS staining or pH 2.5 AB staining of polyvinylidene difluoride (PVDF) membranes as modified from previously described methods (11, 42). Five microliters of the resuspended sample solution were blotted on PVDF membrane (Hybond-P, Amersham International, Little Chalfont, UK) set on wet filter paper. For PAS staining, after a brief distilled water rinse, the membrane was exposed to 0.5% periodic acid solution for 15 min, rinsed with distilled water, and exposed to Schiff's reagent for 30 min followed by two 3-min exposures to 0.6% sodium metabisulphite. For AB staining, after a 15-min incubation with 3% BSA to reduce background, the membrane was stained with 1% AB in 3% acetic acid for 30 min followed by rinsing with 3% acetic acid and distilled water. After a final distilled water rinse, the membrane was dried, and density was digitized by a scanner (ScanJet 6100C, Hewlett Packard, Boise, ID). Image analysis was performed by measurement of dot-blot density as an eight-bit monochrome image (256 shades of gray) with the image-processing software described in Measurement of MGT.

To confirm the molecular mass of the PAS-positive protein in resuspended samples, SDS-PAGE was performed as previously described (47). Samples were boiled for 5 min in sample buffer containing 2.5% (vol/vol) 2-mercaptoethanol and 1% (wt/vol) SDS. Gels were composed of 4% stacking gel/7.5% running gel or 3% stacking gel/4% running gel. High and low molecular markers from Pharmacia (Piscataway, NJ) were used. Gels were stained with Coomassie brilliant blue R-250 (CBB; Bio-Rad) or PAS, as described previously (17, 35, 37).

Statistics

All data are 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

Imaging of Loaded Mucosa In Vivo and in Microsphere-Loaded Frozen Sections

Examination of mucosa loaded with BCECF and fluorescent microspheres in vivo produced images in which the focal plane included the fluorescent cell surface (Fig. 1A) or the red microspheres adherent to the luminal gel surface (Fig. 1C). Figure 1B depicts the red fluorescence at the tissue plane, in which out-of-focus microspheres are visible, and Fig. 1D depicts green fluorescence at the plane of the gel surface, in which out-of-focus tissue fluorescence is visible. z-Axis travel between these planes is also shown in Fig. 1, A–D (at lower right). The microspheres appear to lie on a plane parallel to that of the mucosal surface. Figure 1F depicts microspheres placed on a glass slide, with a similar appearance to those in Fig. 1D. This further confirms the quasiplanar nature of the microsphere distribution in the z-axis. Before the initial superfusion, the thickness of the gel overlying the duodenal mucosa was 136 ± 4 μm (n = 63, range = 92–236 μm) and stabilized to 90 ± 1 μm (n = 360, range = 54–139 μm) during a 1-h superfusion with Krebs buffer (pH 7.0). During superfusion, the microspheres remained visible in a planar configuration at the luminal gel surface, but the number of visible microspheres slowly decreased over time, indicating gradual removal of the surface layers of the mucus. Figure 2 depicts the temporal spatial distribution of microspheres during neutral and acid superfusions. Although some microspheres disappeared during the course of the experiment, the overall spatial distribution remained constant across time and changes in superfusate composition and MGT, confirming the firm adherence of the microspheres to the gel layer. Microsphere localization was confirmed with frozen sections of tissue loaded in vivo. In the sections, the microspheres were almost exclusively located on the gel surface (Fig. 1E). Interestingly, as shown previously (3), the mucus gel layer was clearly delineated by acridine orange staining. Furthermore, the gel spans the villi, indicating that the mucus gel layer is continuous over the mucosa.

Measurement of Perfusate Glycoprotein Concentration With Dot Blot Using PAS and AB Staining

Figure 3A depicts the dot blot images of solutions of partially purified pig gastric mucus (0.001–10 μg/μl) blotted on PVDF membrane and stained with PAS. The
calibration curve depicted in Fig. 3B is the density of each dot calculated with image analysis as a function of the mucus concentration. An abbreviated calibration curve was included on each dot blot of experimental samples to control for development time and other variables. Because the calibration curve indicates a usable range of 0.03–1 mg/ml, resuspended lyophilized samples were used to increase sensitivity. A similar calibration curve was constructed by AB staining of blotted pig gastric mucus (data not shown).

Figure 4 depicts PAS (Fig. 4A) and AB staining (Fig. 4B) of concentrated perfusates obtained from the four experimental groups. Note that the density in the samples obtained from the control group was stable with minimum density fluctuation, whereas the density of the samples obtained from the acid group increased suddenly at time 15, gradually declining over the remaining time toward baseline. In samples obtained from the Indo group, baseline density (time 0 to time 10) was high, with density decreasing during and after acid exposure. In samples obtained from the PGE2 group, density progressively increased at time 15 and beyond for both stains.

Figure 5 depicts SDS-PAGE stained with CBB (Fig. 5, A and C) and PAS (Fig. 5, B and D) of 100 μg of partially purified gastric mucus and 10 μl of resuspended samples from the control (time 15), acid (time 15), and PGE2 groups (time 30). Fig. 5, A and B (7.5% running gel), and Fig. 5, C and D (4.0% running gel), were cut from the same gel, but stained differently. In Fig. 5A, although CBB stained several proteins with molecular masses of 40–100 kDa, the only PAS-positive material was of extremely high molecular mass, having barely entered the stacking gel. In Fig. 5D, these extreme high-molecular-weight bands are visible in addition to bands near the bottom of the gel, which are low-molecular-weight materials not visible in the corresponding location of the 7.5% gel. It is likely that the very high-mass PAS-positive material is mucus glycoprotein, which has a molecular mass of >600 kDa, consistent with the polymeric form of gastric mucin (molecular mass, 2 × 10⁶ Da [20]), and the low density material is degraded glycoprotein, which is inferred from its molecular mass of <60 kDa and its presence in the sample of partially purified mucin.

Effect of Luminal Acid and Indo Pretreatment on MGT and Perfusate Glycoprotein Concentration

Figure 6 depicts MGT (Fig. 6A) and perfusate glycoprotein concentration measured by PAS (Fig. 6B) and AB staining (Fig. 6C) for the control, acid, and Indo groups. In the control group, MGT and PAS- and AB-positive perfusate glycoprotein concentrations were
stable, not affected by solution changes at time 10 and time 20. In the acid group, MGT rapidly increased after mucosal exposure to pH 2.2, with rapid restoration of MGT toward baseline during the recovery period. Acid exposure also increased PAS-positive perfusate glycoprotein concentration from time 15 until time 22, followed by recovery to the baseline, whereas AB-positive glycoprotein transiently increased at time 15 only. Rapid parallel increases of MGT and perfusate glycoprotein concentration are consistent with simultaneous augmentation of mucus secretion and exudation rates, with the rate of increase of secretion exceeding the rate of exudation until a new steady state is reached. The rapid decrease of MGT corresponds to a decrease of secretion rate while the rate of exudation remains elevated, followed by a decrease in the exudation rate until a new steady state is reached. Rapid parallel increases of PAS- and AB-positive glycoprotein concentration are consistent with an increase of goblet cell-type mucus release, whereas sustained increase of only PAS-positive glycoprotein concentration may correspond to Brunner’s gland-type mucus exudation. In the Indo group, baseline MGT was the same as in the control group whereas PAS-positive perfusate glycoprotein concentration was over fourfold higher and AB-positive was sixfold higher in the Indo group than in the control group. During the acid-challenge period, MGT decreased and remained below baseline during the recovery period. No further increase in perfusate glycoprotein concentration was observed during acid exposure, with PAS-positive perfusate glycoprotein concentration remaining higher than in the control group until time 20, whereas AB-positive glycoprotein concentration remained constant despite changes of superfusate composition and an increase of mucus gel thickness (MGT) at time 15. Elapsed time is shown (each time is given as h:min:s:1/10–1/100 s) with time 0 defined as the start of the baseline superfusion period.
of perfused acid. Delayed increase of AB-positive glycoprotein with rapid increase of PAS-positive glycoprotein suggests that PGE₂-stimulated mucus secretion augments Brunner’s gland secretion before that of goblet cells.

**DISCUSSION**

Described herein is a new method for the study of the dynamic regulation of rat duodenal MGT in response to luminal acid, PGE₂, and COX inhibition in the duodenum. This is the first study in which steady-state duodenal MGT was measured in vivo in parallel to measurement of perfusate mucus concentration, the first to demonstrate a rapid response of duodenal MGT to exogenous prostaglandins, and the first to document a spontaneous, rapid decrease of MGT. Furthermore, this is the first study in which the relative contributions of Brunner’s glands and goblet cells to the regulation of MGT were examined. With this system, we were able to formulate a preliminary understanding of how duodenal MGT is regulated.

The dynamic regulation of MGT in the gastrointestinal tract is one of the least-studied aspects of mucosal barrier function, perhaps due to the difficulties inherent in obtaining this measure in a living preparation. To measure MGT, we initially attempted to delineate the luminal gel surface with several different types of particles, including carbon. We found that polystyrene microspheres reliably and faithfully marked the luminal surface of the adherent gel. The spheres were chosen to be of a size large enough to be visualized but small enough not to interfere with the underlying cellular fluorescence; to have emission at a longer wavelength than BCECF fluorescence, which enabled microsphere identification on the basis of color; and to have density and surface characteristics that enable the microspheres to adhere to the luminal gel surface. MGT presumably represents only the contribution of the “firmly adherent gel,” e.g., the component that resists hydromechanical shear forces, as opposed to the “loosely adherent” overlying layer, as originally described by Sababi et al. (33) and Sellers et al. (36). Shear forces were provided by continuous superfusion at a rate chosen to mimic physiological transit of luminal contents. Our studies are strongly consistent with removal of the loosely adherent layer during the stabilization period in which the superfusion rate provides the mechanical forces necessary to remove this layer, exposing the stable, adherent layer. MGT measured by this method (presuperfusion, 136 μm; steady state, 90 μm) is similar to that measured by other methods, i.e., 82 ± 7 μm in rats (24), 162 ± 45 μm in humans (34), and 236 ± 56 μm (before mechanical removal) and 74 ± 6 μm (recovery period after mechanical removal) in rats in vivo (33), but smaller than ~450 μm or 833 ± 72 μm in rats in vivo (9, 23), the latter reflecting the contribution of the unstirred layer (4).

Physiological secretion of mucus is controlled in three distinct phases. In the spontaneous or basal state (phase I), mucus is slowly secreted by exocytosis, with
the rate of synthesis presumably matching the secretory rate (40). In phase I, goblet cells are loaded with apical mucus granules. In the steady state, MGT is governed by the opposing forces of mucus secretion and exudation. In response to secretory stimuli such as cholinergic agonists, prostaglandins, or guanylin, a rapid burst of secretion occurs by compound exocytosis within 2 min of the inciting signal, depleting the cells of preformed granules [phase II (10, 28, 39)] accompanied by membrane cavitation. In our studies, this presumed phase II secretory burst after acid exposure or PGE$_2$ was accompanied by a rapid increase of MGT, followed by stabilization and a gradual decline toward baseline. Increased mucus secretion relative to exudation increases MGT whereas increased exudation relative to secretion decreases net MGT. The rapid increase of MGT in response to acid or PGE$_2$ must hence reflect a marked increase of secretion relative to a smaller increase in rate of release into the perfusate. De novo mucus synthesis, occurring over hours, is far too slow to produce these changes (39). Enhanced exudation accompanied the sudden increase of MGT whereas increased exudation relative to secretion decreases net MGT. The rapid increase of MGT in response to acid or PGE$_2$ must hence reflect a marked increase of secretion relative to a smaller increase in rate of release into the perfusate.

Enhanced mucus exudation accompanied the sudden increase of MGT in response to acid or PGE$_2$, as has been observed previously in the stomach (36), suggesting that a sudden increase of gel thickness per se, rather than acid exposure, increased mucus exudation. As an alternative possibility, we had considered that mucosal bicarbonate secretion, which is also enhanced by luminal acid and prostaglandins (43), may expand the gel layer by rapid generation of intragel CO$_2$ produced in response to an acid-bicarbonate mixture. Increased mucus exudation in response to PGE$_2$ and Indo in the absence of acid does not support this possibility. On the basis of our observations, the most likely explanation for the rapid shifts of MGT is that neurochemical stimuli such as luminal acid or prostaglandins induce phase II mucus secretion, which rapidly increases MGT. This sudden thick gel layer is somewhat less stable than normal, raising its susceptibility to shear forces and thus increasing the rate of mucus release into the superfusate. These forces decrease MGT, which then reaches a new steady state as the increased rates of secretion and exudation come into balance. As the rate of secretion diminishes, MGT decreases toward baseline. To restore the mucus granule population, the synthesis rate increases in the ensuing hours (phase III).

The methods used to measure fluid mucus concentration have not been standardized. We chose the PAS and AB blotting methods for their sensitivity and simplicity (11, 22), bearing in mind that a reliable and reproducible means of quantitating fluid mucin content was more desirable than an exhaustive reckoning of all glycoprotein species. To minimize contributions from free carbohydrates, several modifications to the staining techniques were made, such as the use of PVDF membranes, which only bind to glycoproteins (42). The dot-blot system hence only detected PAS- and
AB-positive glycoproteins. Furthermore, PAS staining of SDS gels showed that PAS-positive material in the perfusate had either a molecular mass of >600 kDa or <60 kDa, consistent with previous studies (35, 37) in which PAS-stained PAGE of crude or gel-purified mucins revealed bands that barely penetrated the gel. Mucins are the only very high-molecular-mass (>600 kDa) glycoproteins that are likely to be present in an intestinal perfusate. The lack of PAS-positive material with molecular mass between the very high and low molecular mass materials is consistent with a lack of contamination by nonmucin glycoproteins. The low molecular mass materials are likely to be degraded mucin glycoproteins that are compressed into a narrow band as an artifact of running the 4% gel. Our data are also supported by a previous study (19), in which perfusate hexosamine concentration was increased in perfused rabbit duodenal loops in response to acid exposure.

Histological staining of duodenum indicates that the two major mucus subtypes are identified by PAS and AB staining: PAS-positive Brunner’s gland mucus and mixed AB- and PAS-positive goblet cell mucus. The correspondence between histological staining and core mucin polypeptide subtype (e.g., muc2, muc3, and muc6) has not yet been firmly established (12, 25). Acid-induced augmentation of mixed PAS- and AB-positive glycoproteins suggests that increased mucus secretion and exudation originates mainly from goblet cells. PGE2-associated increases in PAS-positive glycoproteins followed by increases
in AB-positive glycoproteins suggest that PGE\textsubscript{2} increases mucus secretion and exudation from both Brunner’s glands and goblet cells. The short duration of increased AB-positive perfusate glycoprotein in response to acid and PGE\textsubscript{2} suggests that goblet cells are rapidly depleted of mucus granules (10, 39). These studies indicate that proximal duodenal mucus secretion, and by implication the adherent mucus gel, is composed of mixed Brunner’s gland and goblet cell secretions. A similar paradigm is likely present with gastric mucus, the adherent gel being composed of a laminar array of foveolar (MUC5AC) and deep gland (MUC6)-derived mucus (27). The limitation of the staining technique is that only relative changes can be measured because measurement of absolute amounts of mucus secretion requires comparison against purified rat goblet cell or Brunner’s gland-derived mucus, which would be further complicated by the heterogeneous nature of goblet cell mucins and mucus (12). Nevertheless, the relative changes in perfusate glycoprotein concentration provide valuable data regarding the interplay between MGT, mucus secretion, and mucus exudation in the presence of acid, COX inhibition, and PGE\textsubscript{2}. Furthermore, the similar relative amounts of PAS- and AB-positive mucus derived from distal duodenum provide strong evidence in favor of goblet cell-derived mucus being an important component of exuded mucus from proximal and distal duodenum.

Fig. 7. Effects of PGE\textsubscript{2} on MGT and perfusate glycoprotein concentration. PGE\textsubscript{2} (1 mg/kg iv) progressively increased MGT (A) and PAS- (B) and AB-positive (C) perfusate glycoprotein concentration. *P < 0.05 vs. control group. Values are expressed as means ± SE from 6 rats.
Perhaps the most difficult data to interpret were those obtained from the Indo group. At baseline, MGT was normal, although perfusate glycoprotein concentration was markedly elevated. To explain these data, we initially hypothesized that Indo pretreatment increased exudation of nonmucin glycoproteins into the perfusate, a contention not supported by our data, in which the only visible glycoproteins by PAGE were typical of mucins. The few studies of the effects of COX inhibition and exogenous prostaglandins on duodenal mucus secretion, MGT, and exudation also shed little light on this observation. For example, neither intragastric 16,16-dimethyl PGE₂ nor Indo changed rat duodenal MGT, as measured by examination of thick sections (24). In dogs, intravenous Indo did not increase baseline duodenal perfusate glycoprotein release, although Indo inhibited a marked increase of mucin release by the prostaglandin precursor arachidonic acid (18). Indo reduced surface hydrophobicity of rat duodena, which may reflect altered mucus composition (21). Furthermore, Indo decreased mucin synthesis in gastric explants (41). Nevertheless, acute Indo administration does not alter MGT in stomach (6, 24, 46). Our observations are in substantial agreement with the findings of Sababi et al. (33), in which Indo inhibited the rate of MGT restoration after mechanical removal (33), which would correspond to the blunted increase of MGT during acid superfusion. Taking these data into account, it is possible to integrate our observations and those of others under a unifying hypothesis. In the steady state, Indo accelerates spontaneous phase I mucus secretory rate while inhibiting basal synthesis, gradually depleting the cells of granules. The mucus secreted in this fashion, perhaps due to altered composition, is less stable than normal and thus sheds into the lumen more readily. Combined increased exudation and secretion did not alter steady-state phase I MGT, at least within 1 h of Indo administration. With acid perfusion, there is no further secretion due to depletion of the remaining mucus granules, inhibiting the usual increase of MGT. In the recovery period, MGT stabilizes at a lower level as the mucus stores are exhausted. Over time, MGT presumably will decrease below baseline, because phase III synthesis is presumably inhibited. Another explanation is the effect of Indo on motility, since Indo increases gastroduodenal motility (32), which may affect the exudation rate.

In summary, the thickness of the duodenal mucus gel layer represents the balance between the opposing forces of mucus secretion and the sloughing of the luminal portion of the gel surface by mechanical forces. The gel in its steady state is intrinsically stable relative to a gel that has suddenly increased thickness or one that has formed after exposure to systemic Indo. The significant role played by prostaglandins and COX inhibition on mucus dynamics may explain how COX inhibitors weaken the preepithelial component of the duodenal mucosal barrier to acid, increasing susceptibility to injury.

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REFERENCES


