Adherent surface mucus gel restricts diffusion of macromolecules in rat duodenum in vivo

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Departments of 1Physiology and 2Pathology, Uppsala University, SE-751 23 Uppsala, Sweden; 3Swedish Institute for Infectious Disease Control, SE-105 21 Stockholm, Sweden; 4Department of Physiological Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, United Kingdom

Flemström, Gunnar, Anneli Hälgren, Olof Nylander, Lars Engstrand, Erik Wilander, and Adrian Allen. Adherent surface mucus gel restricts diffusion of macromolecules in rat duodenum in vivo. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G375–G382, 1999.—The aim of this study was to investigate the permeability of the adherent mucus gel layer in rat duodenum in vivo to macromolecules applied in the lumen. Rats were anesthetized with thiobarbital and the duodenum was perfused with isotonic NaCl solution containing large-molecular-size secretagogues. Effects on mucosal HCO3 secretion and blood-to-lumen 51Cr-labeled EDTA clearance were used as indexes that compounds had migrated across the mucus layer. Exposure to a low concentration of papain (10 U/100 ml) for 30 min removed the mucus layer without damage to the epithelium and induced or markedly enhanced HCO3 secretory responses to cholera toxin (molecular mass of 85 kDa) or glucagon (3.5 kDa). Water extracts from a VacA cytotoxin (89 kDa) producing Helicobacter pylori strain, but not from a toxin-negative isogenic mutant, caused a small increase in HCO3 secretion but only after the mucus layer had been removed by papain. The duodenal surface mucus gel thus significantly restricts migration of macromolecules to the duodenal surface. Release of bacterial toxins at the cell-mucus interface may enhance or be a prerequisite for their effects on the gastrointestinal mucosa.

cholera toxin; chromium-labeled EDTA clearance; glucagon; duodenal bicarbonate secretion; Helicobacter pylori; prosta-glandin E2; VacA cytotoxin

THE GASTRODUODENAL MUCOSA is covered by an adherent layer of visco-elastic mucus gel, which provides a physical barrier between the apical cell surfaces and the lumen. The viscous and gel-forming properties of this mucus gel are derived from mucin glycoproteins, which constitute 3–5% of the gel by weight, with the remaining 95% being water together with small amounts of lipids, nucleic acids, and other proteins, including immunoglobulins (1). In stomach, evidence from several sources, both in vivo and in vitro, shows that mucus forms a continuous, stable, and firm gel layer adherent to the surface epithelium (20, 21, 32, 33). HCO3 secretion maintains the pH in the mucus gel adjacent to the gastric (10, 27, 32) and duodenal (13, 27, 29) epithelial surface at a pH value considerably higher than that in the gastric lumen. Furthermore, the adherent mucus layer in the stomach prevents access of the macromolecule pepsin from the lumen to the gastric epithelial surface (4). Transport of acid and pepsin from the lumen to the gastric lumen, in contrast, is not prevented (15) and appears to occur through channels (32) in the mucus gel formed by the secretory hydrostatic pressure (36).

The thickness of the mucus gel layer in the duodenum is, despite the marked differences in epithelial surface topology, similar to that in the stomach. The mean thickness measured with microelectrodes in rat duodenum in vivo amounted to 280 µm (29) and that measured on washed mucosa in vitro amounted to 90 µm (3). By preventing or restricting migration of macromolecules, the mucus layer may provide important mechanisms for protection against toxins ingested with food, luminal proteinases, and other putative mucosa-damaging agents. In the case of toxins produced by bacteria living in the mucus layer, their release at the mucus-cell interface could enhance their deleterious effects on the mucosa. Furthermore, the mucus layer could have implications for oral vaccines by restricting the migration of these macromolecular antigens from the intestinal lumen to the immunocompetent cells at the epithelial surface and thus interfering with the induction of immunity.

Studies in vitro have suggested that the rate of diffusion of solutes through mucus gel progressively decreases with increasing molecular size (1, 7). The access of disaccharide and small peptide substrates to brush-border enzymes in intestinal loops in vitro has been shown to be reduced by the adherent mucus layer (34). Large-molecular-size proteins of several thousand molecular weight do not significantly diffuse through artificial mucus gel layers over several hours (2). However, the permeability of macromolecules through the intestinal mucus layer in vivo in intact animals has not been studied before.

The aim of the present investigation was therefore to develop a model for studies of the migration of macromolecules across the duodenal mucus gel in vivo. The concept of our approach was to use some large-molecular-size toxins and secretagogues that should affect duodenal mucosal HCO3 secretion and/or mucosal permeability. Changes in the rate of HCO3 secretion and/or 51Cr-labeled EDTA permeability were used as indexes of the migration across the mucus gel layer to the epithelial surface of macromolecules instilled into...
the intestinal lumen in vivo. The blood-to-lumen clearance of $^{51}$Cr-EDTA used here to estimate permeability is, furthermore, a good estimate of mucosal integrity (25). A method for removal of the adherent mucus gel in the duodenum by mild treatment with papain was established. Macromolecular toxins/secretagogues, and for comparison PGE$_2$, were applied luminally, and experiments were performed in duodenum with an intact mucus gel and after substantial removal of the mucus gel by papain. A preliminary account of some of the results has been published in abstract form (12).

**MATERIALS AND METHODS**

Male F$_1$ hybrids of Lewis × Dark Agouti rats (Animal Department, Biomedical Center, Uppsala, Sweden), weighing 200–300 g, were kept under standardized conditions of temperature (21–22°C) and light (12:12-h light-dark cycle) and given pelleted food (Ewos, Södertälje, Sweden) ad libitum. Before experiments, the animals were fasted overnight in groups of two or more in cages with mesh bottoms but had free access to drinking water. The operative procedures have been described before in detail (14, 25), and a summary and some modifications are provided here. To avoid stress, rats were anesthetized in the Animal Department by their regular keeper with an intraperitoneal injection of 120 mg/kg body wt sodium-5-ethyl-5-(1-methylpropyl)-2-thiobarbituric acid (Inactin). Rats were tracheotomized with a tracheal cannula to facilitate respiration. An external jugular vein and femoral artery and vein were catheterized with PE-50 polyethylene catheters (Becton-Dickinson, Parsippany, NJ). The veins were used for infusion of $^{51}$Cr-EDTA and drug (hexamethonium) injections. For the continuous recording of systemic arterial blood pressure, the arterial catheter, containing heparin (20 IU/ml dissolved in saline), was connected to a pressure transducer (Gould Statham P231D, Oxnard, CA) operating a polygraph (polygraph 7D, Grass, Quincy, MA).

Subsequently, a laparotomy was performed, and the common bile duct was catheterized with a PE-10 polyethylene tube close to its entrance into the duodenum (2–3 mm) to avoid contamination with pancreatico-biliary secretions. A soft silicone tube (Silastic, 1 mm ID; Dow Corning, Midland, MI) was introduced into the esophagus and pushed gently along the esophagus into the stomach and through the pylorus and secured by two ligatures 2–5 mm distal to the pylorus. A PE-320 cannula was inserted into the duodenum 2.5–3.5 cm distal to the pylorus and secured by ligatures. The proximal duodenal cannula was connected to a peristaltic pump (Gilion Minipulse 3, Villiers, Le Bel, France), and continuous perfusion (0.4 ml/min) of the segment with isotonic NaCl solution was started. The effluent was collected in 10-min samples. Surgery was completed by closing the abdominal cavity with sutures, and the wound was covered with plastic foil. After surgery, the animals were allowed to recover for at least 45 min to stabilize cardiovascular, respiratory, and gastrointestinal functions. All experiments had been approved by the Uppsala University Ethical Committee for Experiments with Animals.

Luminal perfusions. The macromolecules added to the duodenal luminal perfusate were chosen on the basis that they were either established as or possible stimuli or inhibitors of duodenal mucosal HCO$_3^-$ secretion. Parenteral administration of the peptide hormone glucagon (molecular mass of 3.5 kDa) increases the HCO$_3^-$ secretion in all species tested except humans (10, 11, 38). Luminally administered cholera toxin [subunit A (molecular mass of 85 kDa)] is a well-known stimulant of electrolyte secretion in more distal small intestine (19). Infection with H. pylori has been proposed to inhibit duodenal mucosal HCO$_3^-$ secretion in patients with duodenal ulcer disease (16). The effects of water extracts of the VacA cytotoxin (molecular mass of 89 kDa) producing reference strain CCUG 17874 and one cytotoxin-negative (VacA−) isogenic mutant were tested in the present study. Furthermore, PGE$_2$ (molecular mass of 335 Da), a smaller and lipophilic molecule and a stimulant of duodenal HCO$_3^-$ secretion in all species tested in vivo and in vitro (10), was perfused luminally in some experiments.

Effects of the luminally administered agents on mucosal HCO$_3^-$ secretion and permeability in duodenum with an intact mucus gel (perfused with isotonic NaCl alone) were compared with effects in duodenum after removal of the continuous mucus gel layer by papain.

**Removal of the continuous mucus gel.** Perfusion with papain-containing solution (10 U/100 ml papain, 5 mM L-cysteine, and NaCl to isotonicity; pH 7.3) for 30 min was used to remove the continuous surface mucus gel layer. To avoid effects on epithelial tight junctions, EDTA was not included in the perfusate. The papain was then removed by (10 min of perfusion with isotonic NaCl alone), and perfusion was continued for another 40–120 min with NaCl solution containing purified cholera toxin, water extracts of H. pylori strains, the hormone glucagon, or PGE$_2$.

The absence of a continuous mucus gel layer at the duodenal surface, as well as the absence of any gross mucosal damage, was confirmed at the end of all experiments involving exposure to papain. The duodenal segment under study and the just distal (not perfused) duodenal segment were both removed, and two unfixed specimens of each of these segments were used to measure adherent surface mucus gel thickness by light microscopy (21). The presence of a continuous mucus gel layer was confirmed in all duodena perfused with isotonic NaCl solution alone. For further and detailed morphological examination, the perfused segments from two to three duodena from all experimental groups were fixed in 10% neutral buffered formalin. Two longitudinal specimens were taken, embedded in paraffin, dehydrated, stained with hematoxylin-eosin, and examined for mucosal damage by an experienced pathologist who was unaware of the experimental protocol.

**Measurement of mucosal HCO$_3^-$ secretion.** The rate of secretion was determined by back titration of 1-ml samples of the solution to be infused and the effluent to pH 5.0 with 50 mM HCl during continuous gassing with 100% N$_2$ (to remove CO$_2$), using pH stat equipment (Radiometer, Copenhagen, Denmark). The amount of titratable base in infused solutions containing protein was always <20% of the amount of HCO$_3^-$ secreted by the duodenal mucosa and was deducted from the measured rates. The pH electrode was calibrated with standard buffers before the start of the titrations. The rate of secretion was expressed as microequivalents of HCO$_3^-$ secretion per centimeter of intestine per hour (μeq·cm$^{-1}$·h$^{-1}$).

**Measurement of mucosal permeability.** After completion of surgery, $^{51}$Cr-EDTA was administered intravenously as a bolus of 75 μCi followed by a continuous infusion at a rate of 50 μCi/h. The radioactive isotope was diluted in a Ringer-HCO$_3^-$ solution and infused at a rate of 1 ml/h (Harvard Apparatus, South Natick, MA). Forty-five minutes was permitted for tissue equilibration of the $^{51}$Cr-EDTA. Three blood samples (0.2 ml) were collected during the experiment, and the blood volume loss was compensated for by injection of a 10% Ficoll solution. After centrifugation, 50 μl of the plasma were removed for measurements of radioactivity. The luminal perfusate and the plasma were analyzed for $^{51}$Cr activity in a gamma counter (1282 Compugamma CS, Pharmacia, Uppsala,

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RESULTS

A linear regression analysis of the plasma samples was made to obtain a corresponding plasma value for each effluent sample. The clearance of 51Cr-EDTA from blood to lumen was calculated as described before (16) and was expressed as milliliters per minute per 100 g wet tissue weight (ml·min⁻¹·100 g⁻¹ wet tissue wt).

Macromolecules, drugs, and solutions. Highly purified cholera toxin (no. 101B) was obtained from List Biological Laboratories (Campbell, CA), Glucagon (porcine), PGE₂, hexamethonium chloride, crystallized papain (20–25 U/mg), and L-cysteine (free base) were from Sigma (St. Louis, MO). The anesthetic Inactin was from Research Biochemicals International (Natick, MA), and 51Cr-EDTA was from DuPont NEN (Boston, MA). All agents, except PGE₂, were dissolved in isotonic saline at pH 7.4 on the day of use. PGE₂ was dissolved in ethanol (5 mmol/l), and this stock solution was stored at −20°C. The prostaglandin was added as a small amount (≈15 µl/ml saline) from the stock solution.

For production of water extracts of H. pylori, strains were grown on Columbia agar plates containing 10% FCS under microaerophilic conditions for 4 days. The cytotoxin-negative (VacA−) isogenic mutant (18) was kindly provided by Dr. L. Janzon, Astra, Södertälje, Sweden. The colonies were harvested and diluted in sterile water (10⁶ colony forming units/ml). The solution was stored at room temperature for 30 min and then centrifuged at 12,250 g for 15 min. The supernatant was transferred to a new tube and centrifuged at 25,400 g for 20 min. Finally, the solution was sterile filtered through a 0.2-µm filter (Sterile Acrodisc, Gelman Sciences) and stored in batches at −20°C until use. The protein concentration was 2 mg/ml.

Statistical analyses. All values are expressed as means ± SE, with n representing the number of experiments. Rates of duodenal HCO₃⁻ secretion in control animals perfused with isotonic NaCl alone remained stable over the time periods used in the present experiments (up to 240 min). This was confirmed in animals with an intact surface mucus gel as well as after removal of the mucus gel by papain. Statistical analyses of the effects of luminal secretagogues were performed by ANOVA followed by a Fisher’s protected least-significant difference test, comparing rates of HCO₃⁻ secretion before and after addition of a potential secretagogue (repeated measures). Only one concentration of a secretagogue was tested in the same animal, and two-way ANOVA was used (always stated in the text) to compare effects in groups of animals exposed to different perfusate concentrations of cholera toxin, glucagon, and PGE₂. A P value of <0.05 was considered significant. The mean arterial blood pressure was continuously recorded, and HCO₃⁻ secretion and blood-to-lumen clearance of 51Cr-EDTA were measured at 10-min intervals.

RESULTS

Removal of the surface mucus gel. The concentration of papain in the luminal perfusate (10 U/100 ml) and time of exposure (30 min) were kept low to avoid damage of the mucosa. Unfixed specimens of papain-exposed duodena were examined at the end of each experiment by light microscopy, enabling detection of the mucus gel layer (21). The continuity of the duodenal surface mucus gel was always broken after papain, with most of the surface being free of visible mucus. However, patches (20–50 µm length) of a thin (<20 µm) layer of adherent mucus could be observed after some of the experiments. No areas free of adherent mucus gel were observed in adjacent (just distal) nonperfused segments of duodenum from the same animals.

In contrast to papain-perfused duodenal segments, a continuous mucus layer, of minimum thickness >20 µm, was always observed covering perfused but not papain-exposed mucosa. Light microscopy of unfixed specimens does not allow detailed examination of mucosal cells. However, no damage to the villi or other pathological changes could be observed on examination of fixed specimens of duodenum from all papain-exposed groups (observed blind to the experimental protocol). Furthermore, after treatment with papain, mucosal HCO₃⁻ secretion (Fig. 1) and mucosal permeability measured as blood-to-lumen clearance of 51Cr-EDTA (Fig. 2) were unaffected compared with measurements in non-papain-treated duodenum. Duodenum in
these non-papain-treated controls was perfused with isotonic NaCl alone. The rate of HCO₃⁻ secretion remained stable over the time periods used in the present experiments and was 4.6 ± 0.5 at the start of experiments and 5.3 ± 1.1 µeq·cm⁻¹·h⁻¹ after 240 min (n = 7, not shown). Blood-to-lumen clearance of ⁵¹Cr-EDTA showed a slow continuous decline, from 0.30 ± 0.06 to 0.18 ± 0.03 ml·min⁻¹·100 g⁻¹.

Effects of cholera toxin. Cholera toxin is a well-known stimulus of NaCl and water secretion in more distal small intestine (19, 22). Effects on the HCO₃⁻ secretion by the duodenal mucosa are shown in Fig. 1. In duodenum with an intact mucus gel, there was a significant rise in secretion after exposure to the higher (8 µg/ml) concentration of cholera toxin (P < 0.05 at 150 min, compared with basal rates in the same animals). However, a marked (greater than twofold) response was observed only in three of nine duodena. Exposure to a lower (2 µg/ml) concentration of cholera toxin did not significantly increase (0.10 > P > 0.05) the HCO₃⁻ secretion.

After removal of the mucus gel layer by exposure to papain, there was a greater than twofold rise in secretion in response to 8 µg/ml of cholera toxin in all nine duodena tested (P < 0.05 at 110 min). In addition, there was a significant response to the lower concentration of cholera toxin (P < 0.05 at 140 min). The response to 8 µg/ml also occurred more rapidly and was greater (P < 0.05, comparison between groups) than that in papain-pretreated duodenum exposed to 2 µg/ml of cholera toxin or duodenum with an intact mucus gel layer. The slight and continuous decline in blood-to-lumen ⁵¹Cr-EDTA clearance (Fig. 2) was similar in all groups exposed to cholera toxin or to papain alone. Some experiments were performed to elucidate the site and mechanism of action of cholera toxin in stimulating mucosal HCO₃⁻ secretion. The ganglion-blocking agent hexamethonium (10 mg/kg iv) decreased the basal secretion of HCO₃⁻ but did not prevent the increase in secretion (P < 0.05 at 150 min) in response to cholera toxin (8 µg/ml) in papain-pretreated duodenum (Fig. 3), suggesting that the response does not depend on reflexes in the enteric nervous system but reflects an action on enteroctye receptors.

Effects of Helicobacter pylori extracts. There is a clear association between the presence of peptic ulcer disease and infection with H. pylori in humans (5). Furthermore, basal HCO₃⁻ secretion and the ability of the duodenal mucosa to respond to luminal acid with a rise in mucosal HCO₃⁻ secretion are reduced in patients with duodenal ulcers (17, 24), and secretion was recently reported to normalize, in part, after eradication of the bacterium (16).

It was therefore interesting to study the effects of H. pylori in the present preparation. Effects of extracts of the reference strain CCUG 17874 are shown in Fig. 4. Extracts of this toxin-producing (VacA⁺) variant did not inhibit but caused a small continuous rise in mucosal HCO₃⁻ secretion (P < 0.05 at 150 min, compared with basal rates in the same animals). This rise in HCO₃⁻ secretion was observed only after removal of the surface mucus gel by papain. No significant effects (P > 0.10) were observed with extracts of the isogenic mutant (VacA⁻), which does not produce cytotoxin, before (not shown) or after (Fig. 4) papain treatment.

Effects of glucagon. Glucagon stimulates duodenal mucosal HCO₃⁻ secretion when administered parenterally in animals in vivo or to the serosal (blood) side of duodenal mucosa in vitro (10, 11). Lumen administration of a high concentration (15 µM) of this peptide induced a small but significant (P < 0.05 at 90 min, 1.1 µeq·cm⁻¹·h⁻¹) response.
compared with basal rates in the same animals) increase in secretion (Fig. 5), which was reversible on removal of the peptide from the luminal perfusate (not shown). A lower concentration of glucagon (0.15 µM) did not affect the HCO₃⁻ secretion. When papain had been used to remove the continuous mucus gel (Fig. 5), the response to 15 µM glucagon was greatly enhanced (P < 0.05, comparison with intact mucus gel), but a significant response to the lower glucagon concentration of 0.15 µM did not occur (0.10 > P > 0.05, compared with basal rates in the same animals). However, six of nine mucosae now showed a small response to the 100-fold lower concentration of the peptide. No changes in ⁵¹Cr-EDTA clearance (not shown) occurred in these experiments.

Effects of PGE₂. This lipophilic molecule is important in the physiological control of the duodenal secretion, and exogenous E-type prostaglandins stimulate secretion in all species tested (10). Luminal perfusion with 20 µM PGE₂ caused a rise in HCO₃⁻ secretion (P < 0.05 at 60 min, compared with basal rates in the same animal) in duodenum with an intact mucus gel (Fig. 6) similar in size to that observed before with this concentration of the prostaglandin (10, 11). Perfusion with a higher concentration (75 µM) induced a greater response (P < 0.05, compared with 20 µM). Removal of the mucus gel by papain resulted in a more rapidly occurring and greater (P < 0.05, compared with intact mucus gel) response to the lower concentration of the prostaglandin. No changes in mucosal permeability, measured as ⁵¹Cr-EDTA clearance, were observed.

DISCUSSION

The gastrointestinal mucosa provides a dynamic barrier between the host and contents of the lumen, allowing the passage of certain molecules into the body while restricting the entry of others. This function is present throughout the consecutive regions of the gastrointestinal tract, including the duodenum, but is faced with very different challenges in the different regions. It protects the epithelium against proteolytic enzymes and ingested bacteriotoxins and the body against antigenic challenges (31). However, for ion-transporting enterocytes or immunoactive cells (or receptors on neurons and/or paracrine cells mediating responses) to respond to changes in composition of the luminal bulk solution, they must first be able to recognize the stimulant. The layer of adherent mucus gel is an important part of the gastrointestinal mucosal barrier, but its permeability characteristics have been only broadly defined. Studies measuring surface pH gradients and epithelial HCO₃⁻ transport in intact mucosa indicate that both H⁺ and HCO₃⁻ rapidly diffuse through the adherent mucus gel layer on top of gastric and duodenal mucosa in vivo (10, 13, 29). Studies of mucus gel in vitro have demonstrated that H⁺ diffuse 4- to 14-fold slower (26, 37) and HCO₃⁻ diffuse 11-fold slower (23) in mucus than in an equivalent unstirred layer of saline. These rates are still very fast, and the diffusion of H⁺ and other monovalent ions through the mucus gel layer is not thought to be significantly retarded in physiological terms (2, 8). This is confirmed in the present studies in which treatment with papain, shown to remove the mucus layer, did not change the rate of HCO₃⁻ secretion. In contrast to smaller molecular solutes, studies of mucus gel in vitro have suggested that the rate of diffusion of larger molecules across the gel progressively decreases with increasing molecular size (1, 7).

In the present study, after removal of the duodenal mucus barrier by papain, there was a significantly greater rise in HCO₃⁻ secretion in response to luminal perfusion with the probe macromolecules cholera toxin (molecular mass of 85 kDa), glucagon (3.5 kDa), and H. pylori cytotoxin (89 kDa). The combined results demonstrate that the gel layer at the duodenal surface provides a significant barrier between the lumen and the underlying epithelium for these large-molecular-weight HCO₃⁻ secretagogues. The same concentrations...
of toxins or glucagon did not affect mucosal blood-to-lumen \(^{51}\text{Cr}-\text{EDTA}\) clearance, indicating, furthermore, that the increase in HCO\(_3\) secretion reflects an increase in transepithelial transport of HCO\(_3\) and not an increase in mucosal permeability.

In the case of cholera toxin and glucagon at higher concentrations, some stimulation of HCO\(_3\) secretion occurred without prior removal of the mucus gel layer and with the mucus barrier visually intact on unfixed sections. Some macromolecules thus seem to penetrate across the mucus gel to the epithelial surface provided that their luminal concentration is sufficiently high. This would suggest that the overall cover of the mucus layer in the duodenum is not totally impermeable to large-molecular-weight proteins and that such molecules can gain limited access to the epithelium. The mucus gel layer in rat duodenum in vivo can be locally almost completely removed using suction with a small catheter coupled to a syringe (29). Shear forces of the digestive processes, or perfusion of the duodenal lumen as used in the present study, could cause thinning, leading to small discontinuities in the mucus gel not apparent in unfixed sections of washed mucosa. Small discontinuities in the gel layer in vivo could also be lost by annealing of the mucus gel on sectioning of the mucosa.

However, there was clearly an increased stimulation of HCO\(_3\) secretion after removal of the surface mucus gel in response to a low concentration (20 \(\mu\)M) of PGE\(_2\) (molecular mass of 335 Da). This is interesting because it demonstrates that the duodenal mucus gel in vivo can also retard access to the mucosa of relatively small molecules and, furthermore, strongly suggests that such a barrier can be maintained by the gel layer at the duodenal surface despite luminal perfusion with fluid. If there are discontinuities in the mucus layer, they seem few and small. Further studies are necessary to distinguish whether the barrier effect of mucus against low-dose PGE\(_2\) is a function of its retarding diffusion per se or whether it is because of binding of the prostaglandin to the mucin or other components of the mucus gel.

To avoid cellular damage, the concentration of papain in the luminal perfusate and time of exposure were kept at the minimum necessary to remove most of the mucus layer, and it should be emphasized that the papain-perfused duodenal mucosa showed clear (and enhanced) responses to the various secretagogues tested in the present study. The morphological examination of fixed sections of the duodenum provides further strong evidence that treatment with papain, as used in the present study, does not damage the mucosa. The absence of an effect of papain on the blood-to-lumen clearance of \(^{51}\text{Cr}-\text{EDTA}\) should also be noted. The latter provides another reliable estimate of mucosal integrity (25).

In the stomach, the adherent mucus gel over the surface of the mucosa appears firmer (1, 20, 32) than that in the duodenum (29). The barrier to migration of peptide macromolecules of the gastric mucus layer in vivo has been exemplified in humans by the uptake by the gastric mucosa of cationized ferritin only after removal of the mucus layer (35). In the rat, damage from excess luminal pepsin (molecular mass of 34.7 kDa) occurred only when the enzyme itself had digested away the adherent gastric mucus gel layer over a period of hours (4). Further studies seem necessary for an evaluation of a possible difference between the stomach and duodenum in mucus gel continuity and permeability. The greater gross surface area in the duodenum together with the inherent capability of the duodenum to secrete HCO\(_3\); at high rates (10, 11) may make this tissue particularly suitable for the present approach.

Cholera toxin is a well-established stimulant of NaCl and water secretion in the more distal small intestine, and it has been demonstrated that cholera toxin-specific receptors on the enterocytes (22), as well as reflexes within the enteric nervous system involving nicotinic transmission (19), mediate the secretory response. The onset of action is, however, often remarkably slow and continues after removal of the toxin (19). Our experiments offer one explanation for this in terms of the barrier effect of the mucus layer. Thus removal of the mucus layer with papain increased both rapidity of onset and magnitude of stimulation of cholera toxin-induced HCO\(_3\) secretion (Fig. 1). Pretreatment with the nicotinic antagonist hexamethonium did not affect the response to cholera toxin, strongly suggesting that the stimulation of the duodenal secretion reflects an action on enterocyte receptors and thus migration of the (cholera toxin) probe macromolecule to the epithelial surface.

Glucagon and PGE\(_2\), like cholera toxin used as probe molecules in the present study, are well-established stimulants of duodenal mucosal HCO\(_3\) secretion (10, 11). PGE\(_2\) has been shown to increase in intracellular cAMP production in a mixture of guinea pig duodenal crypt and villus enterocytes (28), but the cell type responding to glucagon (villus or crypt) and the mode of intracellular signaling have not been clarified. However, villus as well as crypt duodenal enterocytes respond to vasoactive intestinal polypeptide and dopamine with an increase in intracellular cAMP production (30) and to carbachol and cholecystokinin octapeptide with a rise in intracellular Ca\(^{2+}\) (6). Migration of luminally instilled glucagon and PGE\(_2\) across the mucous gel to the lumen-facing villus tip region may be sufficient for eliciting an HCO\(_3\) secretory response.

The HCO\(_3\) secretion and in particular the ability of duodenal mucosa to respond to luminal acid with a rise in HCO\(_3\) secretion protect acid-exposed mucosa in animals (10), and the HCO\(_3\) secretion is decreased in patients with acute and chronic duodenal ulcer disease (17, 24). Interestingly, it was recently reported that eradication of H. pylori infection in part restores the HCO\(_3\) secretion in such patients (16). Acid exposure to water extracts containing H. pylori cytotoxin, however, did not inhibit but caused a small increase in duodenal HCO\(_3\) secretion in the present study. This occurred only after removal of the mucus layer by papain and was not seen with extracts from an isogenic mutant.
Further studies are required for an evaluation of the mucosal access to even small-sized luminal HCO$_3^-$.

It should be noted that the stimulation of secretion by chola toxin observed in the present study very probably reflects an action mediated by enterocyte receptors at the epithelial surface. It is possible that inhibition of secretion reflects effects of H. pylori toxins and/or the process of chronic inflammation on neurohumoral pathways (9, 10, 14, 16) mediating the physiological control of the HCO$_3^-$ secretion.

In summary, this study shows that the adherent duodenal mucus gel layer can significantly hinder mucosal access to even small-sized luminal HCO$_3^-$ secretagogues and severely limit the access of larger-sized toxins. Furthermore, the release of H. pylori cytotoxin and other bacteriotoxins at the cell-mucus interface may thus greatly enhance their effects on mucus-covered gastrointestinal mucus. The useful experimental model described here also emphasizes that access through the mucus is an important consideration when studying the effects of luminal secretagogues and other agents on the underlying epithelium.

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