Intraluminal acid and gastric mucosal integrity: the importance of blood-borne bicarbonate

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Synnerstad, Ingrid, Mia Johansson, Olof Nylander, and Lena Holm. Intraluminal acid and gastric mucosal integrity: the importance of blood-borne bicarbonate. Am J Physiol Gastrointest Liver Physiol 280: G121–G129, 2001.—The acid-secreting gastric mucosa resists intraluminal acid better than the nonsecreting. Here we investigated pH at the epithelial cell surface, mucosal permeability, and blood flow during intraluminal administration of acid (100 mM) in acid-stimulated and nonstimulated gastric corpus mucosae. Surface pH (H^+-selective microelectrodes), permeability (clearance of ^51Cr-EDTA), and mucosal blood flow (laser-Doppler flowmetry) were studied in Inactin-anesthetized rats. Acid secretion was stimulated with pentagastrin (40 μg·kg⁻¹·h⁻¹) or imipramine (500 μg·kg⁻¹·h⁻¹), or HCO₃⁻ (5 mmol·kg⁻¹·h⁻¹) given intravenously. Surface pH was only slightly reduced by intraluminal acid in acid secretion-stimulated or HCO₃⁻-treated rats but was substantially lowered in nonstimulated rats. Clearance increased threefold and blood flow increased by ~75% in nonstimulated rats. During stimulated acid secretion or intravenous infusion of HCO₃⁻, clearance was unchanged and blood flow increased by only ~30% during intraluminal acid. Increased epithelial transport of HCO₃⁻ buffering the mucus gel is most probably the explanation for the acid-secreting mucosa being less vulnerable to intraluminal acid than the nonsecreting.

MATERIALS AND METHODS

Animal Preparation

Male Sprague-Dawley rats (Møllegaard Breeding Center, Ejby, Denmark), weighing 150–250 g, were kept under standardized conditions of temperature (21–22°C) and illumination (12:12-h light/darkness). They were allowed to adjust to this environment in cages with mesh bottoms for at least 4 days before the experiments began, with free access to tap water and pelleted food (Ewos, Södertälje, Sweden). The rats were deprived of food for 18–20 h before the experiments but had free access to water right up to the beginning of the experiment. They were anesthetized with 120 mg/kg body wt of 5-ethyl-5-(1-methylpropyl)-2-thiobutabarbitral sodium (Inactin) injected intraperitoneally. Tracheotomy was performed to facilitate spontaneous breathing, and body temper-
nature was maintained at 37.5 ± 0.5°C by means of a heating pad controlled by an intrarectal thermistor. For blood withdrawal, a PE-90 cannula, containing heparin dissolved in saline (100 IU/ml), was inserted into the left common carotid artery. The right femoral artery was cannulated for continuous recording of arterial blood pressure, and the right femoral vein was cannulated for continuous infusion of 1.0 ml/h of Ringer solution containing, in mM: 25 NaHCO₃, 120 NaCl, 2.5 KCl, and 0.75 CaCl₂. The left femoral vein was cannulated when needed for drug infusion. The preparation of the gastric mucosa for intravital microscopy has been described previously (12). Briefly, exteriorization of the mucosa through a midline abdominal incision was followed by an incision along the greater curvature in the forestomach. The rat was placed on a Lucite table with a part of the corpus of the stomach loosely draped over a truncated cone in the center of the table, with the mucosal surface facing upwards. A double-bottom “mucosal chamber” with a hole in the bottom was fitted over the mucosa, exposing ~1.2 cm² of the mucosa through the hole, and the junction was sealed with silicone grease. The mucosal chamber did not touch the mucosa, so as not to impair blood flow. The chamber was filled with 5 ml of unbuffered 0.9% saline, kept at 37°C by perfusing the double-bottom chamber with warm water. The saline was replaced at constant intervals of 15 min.

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Epithelial Cell Surface pH

The H⁺ concentration in the mucus gel at the epithelial cell surface was measured with H⁺-selective microelectrodes. Glass tubing (borosilicate tubing with omega dot, OD 1.2 mm, ID 0.9 mm; Frederik Haer, Brunswick, ME) was pulled with a pipette puller (pp-83; Narishige Scientific Instrument Laboratory, Tokyo, Japan) to a tip diameter of 1–3 μm. These pipettes were siliconized at 200°C with tributylchlorosilane according to the procedure described by Tsien and Rink (35) and stored for up to 1 wk at 100°C. Before each experiment, the electrodes were filled up to a distance of 330–550 μm from the tip with a proton cocktail (H⁺ Ionophore II-Cocktail).

The remainder of the electrode was filled with HEPES buffer at pH 7.4, connected by an Ag-AgCl wire to a dual-differential electrometer with a high input impedance (FD 223; Biomedical Center, Uppsala, Sweden) and held in a pipette holder (MEH3SF 1.2; Mark Finlay, WPI, Aston, England). The reference electrode, filled with 3 M KCl and connected by an Ag-AgCl wire to the ground of the electrometer, was inserted into another holder (MEH3SF 3.0; Mark Finlay) so that the tip dipped into the isotonic NaCl solution covering the surface of the mucosa. To eliminate electric disturbances, the experiments were performed in a Faraday cage. The electrodes were calibrated before and after each experiment in solutions at 37°C. The solutions were made isosmolar (300 mosM) with NaCl. Solutions with pH values of 1–3 were obtained by the addition of HCl (155 mM) to an unbuffered NaCl solution (155 mM), and those in the pH range of 4–8 were obtained by the addition of HCl or NaOH to a solution containing 10 mM HEPES and 140 mM NaCl.

Mucosal Permeability

Mucosal permeability was determined by measuring the clearance of ⁵¹Cr-EDTA from blood to lumen (25). The technique appears to provide a highly reproducible measure of mucosal integrity and has the advantage that each animal can serve as its own control (2, 4, 9). After completion of surgery and ~60 min before the start of the experiment, 50–75 μCi was injected as a bolus dose (0.5 ml), followed by a continuous intravenous infusion of ⁵¹Cr-EDTA (10–30 μCi/ml) in the Ringer solution at a rate of 1.0 ml/h. Four 0.2-ml blood samples were drawn during the experiment at a time interval of ~30 min. The first one was taken 60 min after the injection of ⁵¹Cr-EDTA. After each blood sample was withdrawn, the blood volume loss was compensated for by injection of a 10% Ficoll 400 solution in saline. The blood sample was centrifuged, and 50 μl of the plasma was removed for measurements of radioactivity [counts per minute (cpm)]. The gastric mucosa was covered with isotonic saline, which was replaced every 15 min. The luminal solution and the blood plasma were analyzed for ⁵¹Cr activity in a gamma counter (1282 CompuGamma CS; Pharmacia, Uppsala, Sweden). In each experiment, the various blood plasma ⁵¹Cr-EDTA activities were plotted against time and a straight line was drawn between the two nearest values. Each clearance value was calculated by dividing each individual effluent cpm value by a corresponding plasma cpm value. If there was <10% deviation between the different blood plasma counts, a mean plasma cpm per milliliter value was calculated and used for all clearance samples. The part of the stomach that had been exposed in the chamber was cut out and weighed after the experiment. Clearance is expressed as milliliters per minute per 100 g wet tissue weight:

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\text{clearance} = \frac{\text{lumen sample (cpm/ml) \times sample volume (ml) \times 100}}{\text{plasma (cpm/ml) \times tissue weight (g) \times time (min)}}
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Blood Flow Measurements

LDF (Periflux PF 2; Perimed, Stockholm, Sweden) was used for blood flow measurements in all experiments. The nature of the Doppler shift from an illuminated tissue depends on the velocity and number of moving red blood cells (23). The laser light (wavelength 633 nm, helium neon laser) is guided to the tissue by an optical fiber, and the backscattered light is picked up by a pair of fibers with a fiber separation of 0.7 mm. With this technique, blood flow is determined as a voltage output and expressed as percent of baseline values. Blood flow was recorded continuously from the mucosal side of the gastric mucosa, with the probe fixed to a micromanipulator (Leitz) and kept at a distance of 0.5–1 mm above the surface of the mucosa, in the saline solution.
The accuracy of the LDF technique for the gastrointestinal application was evaluated and discussed earlier (1, 11, 20).

**Experimental Protocol**

The animals were given at least 1 h to stabilize after the operation before the experiment was started. They were divided into seven groups, three for measurement of the epithelial cell surface pH (groups I–III) and four for assessment of blood flow and mucosal permeability (groups IV–VII).

**Groups I–III.** Before the experiments were started, systemic blood pressure and acid output had been at steady state for 30 min. The H⁺ concentration at the epithelial cell surface was measured continuously, and values were noted every 5 min. In group I (n = 6), the pH-sensitive electrode was inserted into the mucus gel, with the electrode tip at the epithelial cell surface, 15 min after the start of the experiment. Thirty minutes after the experiment began, 100 mM HCl was applied topically to the mucosa in two successive 10-min periods. The topical HCl was then changed to isotonic saline (after one rinse with saline), and the pH was followed for another 30 min. In group II (n = 6), penta gastrin was administered intravenously in a dose of 40 μg·kg⁻¹·h⁻¹ throughout the experiment. Fifteen minutes after the start of pentagastrin infusion, the pH-sensitive electrode was inserted into the mucus gel, with the electrode at the epithelial cell surface. Thirty minutes after the start of pentagastrin infusion, 100 mM HCl was applied topically to the mucosa in two successive 10-min periods. The topical HCl was then changed to isotonic saline (after one rinse with saline), and the pH was followed for another 30 min. In group III (n = 6), NaHCO₃ (5 mmol·kg⁻¹·h⁻¹) was infused intravenously and continuously throughout the experiment. Fifteen minutes after the start of the HCO₃⁻ infusion, the pH-sensitive electrode was inserted into the mucus gel, with the electrode at the epithelial cell surface. Thirty minutes after the start of the HCO₃⁻ infusion, 100 mM HCl was applied topically to the mucosa in two successive 10-min periods. The topical HCl was then changed to isotonic saline (after one rinse with saline), and the pH was followed for another 30 min.

**Groups IV–VII.** In all of these groups, after steady-state values of systemic blood pressure, blood flow (LDF signal), and acid output had been recorded for at least 30 min, 100 mM HCl was applied topically to the mucosa in two successive 10-min periods followed by another 30-min control period. Group IV (n = 6) had no further treatment. In group V (n = 6), impromidine was infused at 500 μg·kg⁻¹·h⁻¹ iv throughout the experiment. Thirty minutes after the start of impromidine infusion, 100 mM HCl was applied topically to the mucosa in two successive 10-min periods. The topical HCl was then changed to isotonic saline (after one rinse with saline), and the pH was followed for another 30 min. Group VI (n = 6), pentagastrin was administered in a dose of 40 μg·kg⁻¹·h⁻¹ iv throughout the experiment. Thirty minutes after the start of pentagastrin infusion, 100 mM HCl was applied topically to the mucosa in two successive 10-min periods. The topical HCl was then changed to isotonic saline (after one rinse with saline), and the pH was followed for another 30 min. Group VII, a continuous intravenous infusion of NaHCO₃ (5 mmol·kg⁻¹·h⁻¹) was given throughout the experiment (n = 6). Thirty minutes after the start of the infusion of HCO₃⁻, 100 mM HCl was applied topically to the mucosa in two successive 10-min periods followed by another 30-min control period. Blood flow was measured continuously by LDF from the epithelial cell surface, as well as the acid secretion, in six untreated rats. Epithelial cell surface pH decreased significantly during application of 100 mM HCl compared with values before the acid period and compared with pentagastrin- and HCO₃⁻-treated groups during acid exposure, but it returned to values not significantly different from the control level 25 min after the acid was washed away. Acid output was significantly increased after the acid application, most probably reflecting acid not totally rinsed away after the exposure. Mucus gel thickness was 223 ± 27 μm during the control period. Mean arterial blood pressure (MAP) was not significantly altered during the experiments (112 ± 4 mmHg during the control period, 108 ± 5 mmHg during topical administration of acid, and 106 ± 5 mmHg after acid exposure).

**RESULTS**

**Group I: Control Epithelial Cell Surface pH**

Figure 1 shows the mean values for pH at the epithelial cell surface, as well as the acid secretion, in six untreated rats. Epithelial cell surface pH decreased significantly during application of 100 mM HCl compared with values before the acid period and compared with pentagastrin- and HCO₃⁻-treated groups during acid exposure, but it returned to values not significantly different from the control level 25 min after the acid was washed away. Acid output was significantly increased after the acid application, most probably reflecting acid not totally rinsed away after the exposure. Mucus gel thickness was 223 ± 27 μm during the control period. Mean arterial blood pressure (MAP) was not significantly altered during the experiments (112 ± 4 mmHg during the control period, 108 ± 5 mmHg during topical administration of acid, and 106 ± 5 mmHg after acid exposure).

**Group II: Pentagastrin Epithelial Cell Surface pH**

Figure 2 presents the mean values for pH at the epithelial cell surface, as well as the acid secretion, in six pentagastrin-stimulated rats. During application of 100 mM HCl, there was a slight, but significant, decrease in the epithelial cell surface pH. Acid secretion was significantly higher in the pentagastrin-stimulated animals than in the control group (group I). Mucus gel thickness was 228 ± 35 μm during the control period. MAP was not significantly altered during the experiments (96 ± 4 mmHg during the control period, 106 ± 5 mmHg after acid exposure).
period, 95 ± 4 mmHg during topical administration of acid, and 97 ± 5 mmHg after acid exposure).

**Group III: NaHCO₃ Epithelial Cell Surface pH**

Figure 3 shows the mean values for pH at the epithelial cell surface, as well as the acid secretion, in six rats that received NaHCO₃ intravenously. During application of 100 mM HCl, the epithelial cell surface pH was slightly but significantly reduced. However, after the acid was washed away the cell surface pH returned within 5 min to a level not significantly different from the period before acid exposure. Mucus gel thickness was 198 ± 58 μm during the control period. MAP was slightly but significantly increased during the experiments (93 ± 1 mmHg during the control period, 98 ± 1 mmHg during topical administration of acid, and 101 ± 1 mmHg after acid exposure).

**Group IV: Control LDF and Permeability**

As seen in Fig. 4, the blood flow was increased to a maximum of +75% 5 min after application of acid in the six rats in which acid secretion was not stimulated. The blood flow was still significantly above the control level 20 min after the acid was changed to saline. The clearance from blood to lumen increased to a value three times above the control value of 0.2 ml·min⁻¹·100 g tissue⁻¹ during luminal application of acid (Fig. 4). Acid output, also shown in Fig. 4, was...
significantly increased after the acid exposure, most probably reflecting acid not totally washed away after the exposure.

**Group V: Impromidine LDF and Permeability**

The blood flow was increased to 35% above the control level 5 min after application of 100 mM HCl in the lumen in the impromidine-treated animals (Fig. 5). This blood flow increase was significantly smaller than that in the control experiments (group IV). The blood flow was not, however, altered by impromidine itself (94 ± 13% of control level before impromidine; not shown). The acid secretion was significantly higher in the impromidine-stimulated animals than in the control group (group IV). The clearance during the control period did not differ between this group and group IV, but the clearance was not altered during acid exposure as it was in group IV.

**Group VI: Pentagastrin LDF and Permeability**

As seen in Fig. 6, the blood flow was increased ~25% above the control level 5 and 20 min after application of 100 mM HCl in the lumen in the pentagastrin-treated
animals. The blood flow was not, however, altered by pentagastrin itself (114 ± 26% of the control level; not shown). Acid secretion was significantly higher in the pentagastrin-stimulated animals than in the control group (group IV). As in the other groups, the secretion was significantly increased after acid exposure, again most probably reflecting acid not totally washed away after the exposure. It is also seen in Fig. 6 that the clearance was not altered during acid exposure.

Group VII: NaHCO₃ LDF and Permeability

The blood flow increased by ~30% above the control level 10 min after application of 100 mM HCl in the lumen in the rats receiving intravenous NaHCO₃ con-

Fig. 6. The different parameters measured in pentagastrin (40 μg·kg⁻¹·h⁻¹ iv)-stimulated rats (group VI; n = 6) during a control period, during topical application of 100 mM HCl (two 10-min periods), and after the topical HCl was changed to saline. Control values were calculated as mean values during the last 15 min before HCl application. Calculations for significance were performed by ANOVA (*P < 0.05 vs. control). The parameters measured were: gastric mucosal blood flow (LDF; %control), MAP (○), ⁵¹Cr-EDTA clearance (ml·min⁻¹·100 g tissue⁻¹ during 15-min periods; hatched bars), and acid secretion (μeq/min, measured during 15-min periods before and after acid application; black bars).

Fig. 7. The different parameters measured in rats receiving NaHCO₃ (5 mmol·kg⁻¹·h⁻¹ iv) continuously (group VII; n = 6) during a control period, during topical application of 100 mM HCl (two 10-min periods), and after the topical HCl was changed to saline. Control values were calculated as mean values during the last 30 min before HCl application. Calculations for significance were performed by ANOVA (*P < 0.05 vs. control). The parameters measured were: gastric mucosal blood flow (LDF; %control), MAP (○), ⁵¹Cr-EDTA clearance (ml·min⁻¹·100 g tissue⁻¹ during 15-min periods; hatched bars), and acid secretion (μeq/min, measured during 15-min periods before and after acid application; black bars). The last clearance value is not significantly higher than the control values since this high value is due to 1 experiment in which clearance increased to 1.14 ml·min⁻¹·100 g tissue⁻¹.

tinuously (Fig. 7). This blood flow increase was significantly smaller than that found in the control experiments (group IV). The blood flow was not, however, altered by NaHCO₃ in itself (102 ± 9% of control level before NaHCO₃; not shown). Neither was the acid secretion altered by NaHCO₃ (0.04 ± 0.03 μeq/min before and 0.01 ± 0.04 μeq/min during intravenous infusion of NaHCO₃). The clearance during the control period did not differ between this group and group IV, but the clearance was not altered during acid exposure as it was in group IV (Fig. 7).
DISCUSSION

In the present study, we have shown that in the acid-secreting gastric mucosa the epithelial cell surface pH is neutral even when the luminal pH is 1 (100 mM HCl). This is most probably due to transport of HCO\textsubscript{3}\textsuperscript{-} through the epithelial cells to buffer the back-diffusing acid in the mucus at the epithelial cell surface. In the nonstimulated rats with low acid secretion, the pH was significantly reduced to \(\sim 1.6 \pm 0.2\) at the cell surface during application of luminal HCl of pH 1 but returned toward the control level after the exposure. Thus, in the absence of parietal cell production of HCO\textsubscript{3}\textsuperscript{-}, the surface epithelial cells were not able to produce HCO\textsubscript{3}\textsuperscript{-} in a sufficiently large amount to maintain the previously described protective pH gradient in the mucus (29, 30, 31, 36). When HCO\textsubscript{3}\textsuperscript{-} was given intravenously to rats with low spontaneous acid secretion, the surface cell pH was only slightly reduced during application of acid (pH 1). Thus the HCO\textsubscript{3}\textsuperscript{-} infusion experiments confirm the finding in the acid-secreting mucosae that blood-borne HCO\textsubscript{3}\textsuperscript{-} is important for transport into the mucus gel, where it buffers the back-diffusing acid.

Moreover, during stimulation of acid secretion or during HCO\textsubscript{3}\textsuperscript{-} infusion and application of acid of pH 1 in our rat model, no sign of disturbance of the permeability of the epithelial cell lining was seen. However, when acid was applied in the nonstimulated mucosa, H\textsuperscript{+} most probably entered the lamina propria, thereby increasing the permeability of the cell lining. This is in accordance with the observation by Kivilaakso et al. (19) of a reduction in intramural pH during acid application (120 mM) in nonstimulated rabbit mucosa but not in that stimulated with histamine. Furthermore, O’Brien and Silen (27) concluded from experiments in bullfrog fundic mucosa that the secretory status of the mucosa is an important determinant of the tolerance of the tissue to exogenous back-diffusion of H\textsuperscript{+}.

One explanation for the better resistance to luminal acid in an acid-secreting than in a nonsecreting mucosa is that HCO\textsubscript{3}\textsuperscript{-} is transported across the basolateral membrane of the parietal cells to the lamina propria simultaneously with acid secretion into the gland lumen. The microvasculature of the gastric mucosa is organized so that the HCO\textsubscript{3}\textsuperscript{-} will be carried from the parietal cells to the surface cells (5, 6). Thus, during acid secretion, HCO\textsubscript{3}\textsuperscript{-} not only alkalinizes the lamina propria but is also available for transport across the surface epithelial cells into the mucus gel and, as we have shown here, increases the efficiency of the first line of defense. The epithelial cell surface pH is slightly less reduced during luminal application of acid in the acid-secreting stomachs (pentagastrin stimulated) than in the HCO\textsubscript{3}\textsuperscript{-}-treated rats. If the amount of HCO\textsubscript{3}\textsuperscript{-} added to the circulation, either by intravenous infusion or by the parietal cells during acid secretion, is estimated, it is obvious that the intravenous infusion might be 2–3 times higher than the parietal cell production. However, the concentration or delivery of HCO\textsubscript{3}\textsuperscript{-} to the surface epithelial cells might still be higher during acid secretion since the HCO\textsubscript{3}\textsuperscript{-} is produced in the epithelial cells and since the systemic HCO\textsubscript{3}\textsuperscript{-} is regulated and eliminated by the kidneys. Furthermore, pentagastrin (gastrin) might also have other protective properties, enhancing the possibility of maintaining a neutral epithelial cell surface pH (32, 33).

Since we invariably found a neutral or slightly alkaline pH at the epithelial cell surface during acid secretion, the acid must have penetrated the mucus layer from the site of production to the lumen of the stomach without acidifying the epithelial cell surface. In earlier studies, we presented evidence for the existence of “channels” in the mucus for acid and pepsin transport from the gastric pit to the lumen of the stomach (10, 16). A low pH at the epithelial surface of acid-secreting stomachs was never observed in the present study, suggesting that acid is penetrating the mucus within structures not penetrated by our microelectrodes (tip diameter of 1–3 \(\mu\)m). The nature of these channels is at present being studied in our laboratory, and we have demonstrated slender and firm structures (16).

O’Brien and Bushell (26) reported that increasing the HCO\textsubscript{3}\textsuperscript{-} concentration on the serosal side in isolated amphibian gastric mucosa reduced the back-diffusion of acid that had been induced by passage of electrical current from the secretory to the nutrient side in combination with HCl. The back-diffusion could not be prevented solely by increasing the pH to 8.2 on the nutrient side with a buffer lacking HCO\textsubscript{3}\textsuperscript{-}. This indicates the importance of secreting the HCO\textsubscript{3}\textsuperscript{-} through the surface epithelial cells into the mucus, since this involves active transport processes existing only for HCO\textsubscript{3}\textsuperscript{-}. Furthermore, Kivilaakso (18) found that intravenous infusion of NaHCO\textsubscript{3} causing high-HCO\textsubscript{3}\textsuperscript{-} metabolic alkalosis significantly decreased the incidence of acid-induced mucosal injury. Interestingly, low-HCO\textsubscript{3}\textsuperscript{-} respiratory alkalosis of a similar degree was not able to protect the mucosa against acid. These results indicate that it is the presence of HCO\textsubscript{3}\textsuperscript{-} rather than the alkalization per se that protects the mucosa against acid.

Intraluminal application of acid with a pH of 1.7 has been shown to induce intracellular (surface cell) acidification both in the nonstimulated and in the pentagastrin-stimulated rat mucosa (24). The acidification was stronger in the nonstimulated mucosa, but distinct acidification was nevertheless observed in the pentagastrin-stimulated mucosa, suggesting that the pH gradient in the mucus might already have been destroyed at a pH of 1.7 in that rat model. Another study by the same group suggested that acid diffuses from its site of secretion toward the lumen, since they found an inverted pH gradient, with pH 5 in the lumen and pH 3.5 at the cell surface during pentagastrin stimulation (3). They have measured the gastric surface pH in rats using an inverted confocal microscope and a pH-sensitive dye (CI-NERF). In pilot experiments, we tried to mimic their experimental conditions with a high superfusion rate of Krebs saline (pH 5) in pentagastrin-stimulated rats (n = 2). During those conditions, we still measured a neutral pH (7.6 \pm 0.1) at the epithelial cell surface. Hence, the superfusion of buffered luminal...
solutions at a high rate is not responsible for the differences in results between the study of Chu et al. and ours (3).

The gastric mucosal blood flow increased during acid instillation in the corpus mucosa in the present study. This increase was significantly attenuated during stimulation of acid secretion with pentagastrin or imidazole (12). Infusion of NaHCO₃ compared with the response to exposure to acid under resting conditions, again indicating a better resistance to acid in the acid-secreting stomach. Together, these results indicate that acid will diffuse into the mucosa if it has a concentration high enough to overcome the endogenous HCO₃⁻ secretion that otherwise will neutralize the acid within the mucus gel. In our rat preparation, 100 mM HCl would most likely enter the mucosa to signal for an increase in blood flow. This blood flow increase was significantly greater when the pH at the epithelial cell surface was substantially reduced than when it was only slightly reduced.

A blood flow increase was also observed in the gastric mucosa of urethane-anesthetized rats during application of 200 mM HCl (22) and was shown to be mediated by capsaicin-sensitive afferent neurons. We have studies (17) showing that the blood flow increase induced by acid in the lumen was not abolished by indomethacin pretreatment, indicating that prostaglandins are not involved in this hyperemic response. However, inhibition of the endogenous production of nitric oxide (NO) by pretreatment with Nω-nitro-L-arginine prevented the blood flow increase induced by topical application of acid. These findings are in agreement with results of a study by Lippe et al. (21), who showed in anesthetized rats that endothelium-derived NO plays an important role in gastric mucosal vasodilation caused by back-diffusion of acid, whereas prostacyclins are not involved. Holzer et al. (14) have demonstrated that back-diffused acid activates capsaicin-sensitive c-fibers. These, in turn, release calcitonin gene-related peptide, a potent vasodilator in the submucosa (7, 13). There is also strong evidence for the involvement of NO in the hyperemic response to activation of these c-fibers, because calcitonin gene-related peptide probably releases NO (15, 37). Thus the hyperemia induced by topically applied HCl in our studies most probably depends on NO release, possibly through activation of capsaicin-sensitive c-fibers.

Disruption of the gastric mucosal barrier has been shown to lead to an increase in gastric mucosal blood flow, which is thought to be a defensive mechanism that minimizes further injury. Intraluminal acid in combination with various agents such as (acetylsalicylic acid), ethanol, sodium taurocholate, or electric current have been used as barrier breakers (27, 28, 30). Guttu et al. (8) found that if 2 M NaCl was used as a barrier breaker and the stomachs of anesthetized cats were perfused with HCl of pH 1 before and after application of the barrier breaker, the blood flow increased approximately threefold after NaCl application and the damaged mucosa was restored to a high degree. When the blood flow increase was prevented, there was much less mucosal restitution, but increasing the blood concentration of HCO₃⁻ completely counteracted this effect. This is in conformity with our finding in the present study that high blood concentrations of HCO₃⁻, achieved either by endogenous acid secretion or by intravenous infusion of HCO₃⁻, abolished the increase in ⁵¹Cr-EDTA clearance seen in the resting state during luminal application of acid. Furthermore, an increased blood flow was shown to enhance mucosal restitution and is hence beneficial to the damaged mucosa (8). In the present study, the blood flow was increased to a significantly greater extent in the non-secreting rats, in which the mucosal permeability had increased on intraluminal application of HCl.

In this study we have shown that stimulation of acid secretion protects the corpus mucosa against luminal acid down to pH 1. The results suggest that the defense mechanisms include HCO₃⁻ delivery by the blood from the parietal cells to the surface mucus cells for transport into the mucus, where there is a preepithelial buffer layer. In the non-acid-secreting gastric mucosa, acid will most probably diffuse from the lumen into the mucosa and thereby induce an increase in the permeability of the mucosal lining, as well as a substantial increase in blood flow.

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