

Acute adaptive cellular base uptake in rat duodenal epithelium

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Acute adaptive cellular base uptake in rat duodenal epithelium. *Am J Physiol Gastrointest Liver Physiol* 280: G1083–G1092, 2001.—We studied the role of duodenal cellular ion transport in epithelial defense mechanisms in response to rapid shifts of luminal pH. We used in vivo microscopy to measure duodenal epithelial cell intracellular pH (pH_i), mucus gel thickness, blood flow, and HCO_3^- secretion in anesthetized rats with or without the Na^+/H^+ exchange inhibitor 5-(*N,N*-dimethyl)-amiloride (DMA) or the anion transport inhibitor DIDS. During acid perfusion pH_i decreased, whereas mucus gel thickness and blood flow increased, with pH_i increasing to over baseline (overshoot) and blood flow and gel thickness returning to basal levels during subsequent neutral solution perfusion. During a second brief acid challenge, pH_i decrease was lessened (adaptation). These are best explained by augmented cellular HCO_3^- uptake in response to perfused acid. DIDS, but not DMA, abolished the overshoot and pH_i adaptation and decreased acid-enhanced HCO_3^- secretion. In perfused duodenum, effluent total CO_2 output was not increased by acid perfusion, despite a massive increase of titratable alkalinity, consistent with substantial acid back diffusion and modest CO_2 back diffusion during acid perfusions. Rapid shifts of luminal pH increased duodenal epithelial buffering power, which protected the cells from perfused acid, presumably by activation of $Na^+-HCO_3^-$ cotransport. This adaptation may be a novel, important, and early duodenal protective mechanism against rapid physiological shifts of luminal acidity.

intracellular pH; bicarbonate secretion; mucosal defense; mucus secretion; mucosal blood flow

THE DUODENAL MUCOSA IS REGULARLY exposed to intermittent pulses of gastric acid, with luminal pH varying rapidly between 2 and 7 (23). Without protective mechanisms in place, the duodenal cells, like other cells in the upper gastrointestinal tract, are believed to irreversibly acidify in the presence of acidic luminal contents, injuring the epithelium (4, 21). With the measurement of robust epithelial HCO_3^- secretion and a neutral pH in the juxtamucosal mucus gel despite the

presence of luminal acid, the “bicarbonate hypothesis” was developed, wherein HCO_3^- , secreted by the epithelial cells, completely neutralized luminal acid diffusing through the mucus gel toward the epithelium (9). Correlation of HCO_3^- secretion with mucosal protection from acid-related injury further bolstered this hypothesis (10, 12, 30).

One means of defending the mucosa against rapid shifts of pH is the phenomenon of acute adaptive protection, wherein exposure to a low concentration of acid or other substance decreases injury from a subsequent challenge with a higher concentration of the same or other substance. This phenomenon, although extensively studied in experimental gastroprotection models (13), has been investigated only twice in the duodenum (16, 17). Furthermore, both prior studies addressing this phenomenon used injury, and not alterations of defensive factors, as an endpoint. It is not known, for example, whether duodenal adaptive protective mechanisms primarily result from an enhancement of preepithelial mechanisms such as increased HCO_3^- secretion, from thickening of the overlying mucus gel, from postepithelial mechanisms such as hyperemia, or from an augmentation of intrinsic cellular mechanisms such as cellular buffering power. Furthermore, it was previously assumed that preexposure to a low concentration of injurious substance was required for inducing adaptive changes. An alternative explanation is that rapid shifts of luminal pH per se, and not the “mild-strong” exposure sequence, is of primary importance in inducing acid-related adaptive changes.

HCO_3^- secretion is the most studied duodenal defense mechanism. Despite its obvious appeal as a means of neutralizing gastric acid, most studies have addressed its measurement in the absence of luminal acid, when its acid neutralizing effect is unnecessary. One purpose of this study was therefore to measure HCO_3^- secretion during acid exposure and to determine its relative importance in mucosal defense in general and adaptive protection in particular.

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Our laboratory has recently developed a technique in which the intracellular pH (pH_i) and duodenal blood flow are measured simultaneously in the rat duodenum (3). We demonstrated that a 10-min pulse of strongly acidic perfusate (pH 2.2) increased cellular buffering by a DIDS-sensitive mechanism, suggesting that rapid shifts of perfusate pH enhanced resistance to a subsequent acid challenge and, by extrapolation, injury. We hypothesized that rapid shifts of perfusate pH enhance intrinsic cellular defense mechanisms and further speculated that these pH shifts may underlie the phenomenon of acute adaptive cytoprotection. We have also established a technique for measurement of duodenal mucus gel thickness (MGT) in our system, which we showed was affected by the balance between mucus secretion and exudation (2).

We studied the effects of varying perfusate pH on duodenal mucosal defense mechanisms to test the hypothesis that adaptive changes are produced by rapid shifts of perfusate pH and that a major duodenal protective mechanism is an increase of cellular buffering power induced by the activation of epithelial ion transport.

MATERIALS AND METHODS

Chemicals

2',7'-Bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) acid, BCECF-AM, and DIDS were obtained from Molecular Probes (Eugene, OR). Two-micrometer pink fluorescent microspheres (excitation 575 nm, emission 600 nm) were obtained from Bangs Laboratories (Fishers, IN). 5-(*N,N*-dimethyl)-amiloride (DMA), HEPES, and other chemicals were obtained from Sigma Chemical (St. Louis, MO). Krebs solution contained (in mM) 136 NaCl, 2.6 KCl, 1.8 CaCl_2 , and 10 HEPES at pH 7.0. For acid perfusion, Krebs solution was titrated to pH 6.4, 4.5, 3.5, or 2.2 with 0.2 or 1 N HCl and adjusted to isotonicity (300 mM). Each solution was prewarmed at 37°C using a water bath, and temperature was maintained with a heating pad during the experiment.

Experimental Protocol for In Vivo Microscopic Study

Animal preparation and measurement of pH_i , blood flow, and MGT were performed according to previously published methods (1–3). After loading BCECF, applying fluorescent microspheres on the gel surface, and blood flow stabilization with pH 7.0 Krebs buffer perfusion, time was set as $t = 0$. Perfusate pH was then varied in 10-, 15-, or 30-min time intervals as described below.

Acid perfusion. To examine the effect of sustained luminal acid on pH_i , blood flow, and MGT, the duodenal mucosa was perfused with pH 7.0 Krebs buffer for 15 min, followed with either pH 7.0, 4.5, 3.5, or 2.2 solution for an additional 30 min (single acid challenge period), followed by a 15-min recovery period with pH 7.0 Krebs buffer.

Sequential perfusion of acids of different concentrations. Mild (pH 4.5) and strong (pH 2.2) acid concentrations were perfused over the mucosa in 15-min increments. After a 15-min perfusion with pH 7.0 Krebs buffer, we exposed the mucosa to mild \rightarrow strong or strong \rightarrow mild acid concentrations, with exposure at each pH lasting 15 min, followed by a 15-min recovery period at pH 7.0. Thus exposure to mild \rightarrow strong acid would be as follows: pH 7.0 from $t = 0$ –15 min (baseline); pH 4.5 from $t = 15$ –30 min (the 1st acid challenge

period); pH 2.2 from $t = 30$ –45 min (the 2nd acid challenge period); and pH 7.0 from $t = 45$ –60 min (recovery period). Adaptive changes are defined as differences in pH_i , MGT, and blood flow in a group in which perfusate pH is changed at 15-min intervals with respect to groups in which the same perfusate pH was held constant for 30 min.

Repeated acid exposure and effects of ion transport inhibitors. In another experimental series, two pulses of strong acid were used to provoke adaptive responses. Perfusate pH was changed from pH 7.0 for 10 min ($t = 0$ –10 min) to pH 2.2 for 15 min ($t = 10$ –25 min; the 1st acid challenge period), followed by pH 7.0 for 10 min ($t = 25$ –35 min; the 1st recovery period), followed by pH 2.2 for 15 min again ($t = 35$ –50 min; the 2nd acid challenge period), and returned to pH 7.0 for 15 min ($t = 50$ –65 min; the 2nd recovery period). To determine the role of epithelial ion transport in regulation of pH_i , blood flow, and MGT, DMA (0.1 mM), which inhibits Na^+/H^+ exchange, or DIDS (0.5 mM), which inhibits $\text{Na}^+/\text{HCO}_3^-$ cotransport and $\text{HCO}_3^-/\text{Cl}^-$ exchange, was added with the pH 2.2 perfusion during the first acid challenge period. Both inhibitors exert their effects on the epithelial cells primarily on the serosal membrane transporters (3). Adaptive changes are defined as differences in pH_i , MGT, and blood flow during the second acid challenge compared with those during the first acid challenge.

Measurement of Duodenal Loop HCO_3^- Secretion

Preparation of the duodenal loop. In a separate experiment, a duodenal loop was prepared and perfused to measure duodenal HCO_3^- secretion, as modified from previously described methods (26, 27). In urethane-anesthetized rats, the stomach and duodenum were exposed 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 suture. 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, and another polyethylene tube was inserted through the incision and sutured into place. To prevent contamination of the perfusate from bile or 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 by using a peristaltic pump (Cole-Parmer Instrument, Vernon Hills, IL) at 1 ml/min. Input (perfusate) and effluent of the duodenal loop were circulated through a reservoir in which the perfusate was bubbled with 100% O_2 . The pH of the perfusate was kept constant at pH 7.0 with a pH stat (models PHM290 and ABU901; Radiometer Analytical, Lyon, France).

Back titration. HCO_3^- secretion was measured by two complementary methods: back titration and direct CO_2 measurement. For back titration measurements, the amount of 0.01 N HCl added to maintain constant pH was considered equivalent to duodenal HCO_3^- secretion. For pH 7.0 perfusion, a pH stat technique was used with perfusate recirculation. Manual back titration was used for pH 2.2 perfusates. Preliminary in vitro studies indicated an excellent correlation between perfusate HCO_3^- concentration and titratable base measured in the effluent (Fig. 1A). For duodenal HCO_3^- measurement, a 30-min stabilization with pH 7.0 saline ($t = -35$ to -5) was followed by baseline measurements with pH 7.0 saline ($t = -5$ to 10). Acid solution was perfused with a Harvard infusion pump at 1 ml/min. For experiments involving repeated acid exposure, solutions were perfused identi-

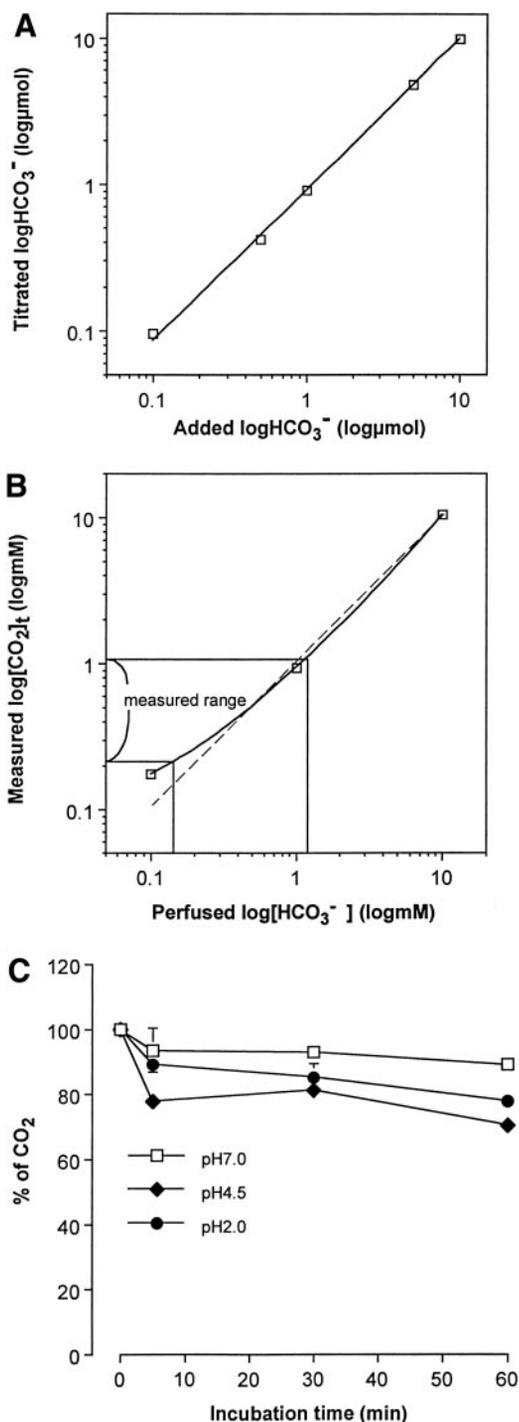


Fig. 1. Calibration of HCO_3^- measurement systems. A: pH stat. Standard HCO_3^- solutions were circulated through the perfusion system tubes. Alkalinity was inferred from the amount of HCl added to maintain starting pH. B: CO_2 electrode. A CO_2 electrode as described in MATERIALS AND METHODS measured total CO_2 content of HCO_3^- solutions. Lines denote the range of measured HCO_3^- concentrations. Note that at the lowest concentration, a 33% overestimate occurred, which disappeared when HCO_3^- concentration exceeded 0.5 mM, compared with the line of identity (dotted). All data are expressed as means of 2–3 experiments. C: stability of CO_2 -containing solutions in an open system. 10 mM NaHCO_3 solutions were prepared, and total dissolved CO_2 concentration ($[\text{CO}_2]_t$) was measured with a CO_2 electrode. At $t = 0$, HCl (0.1 M) was added to titrate to pH 2.0, 4.5, or 7.0. $[\text{CO}_2]_t$ was measured at $t = 5, 30,$ and 60 min.

cally to the protocol described in *Repeated acid exposure and effects of ion transport inhibitors* as follows: pH 7.0 saline was perfused for 10 min ($t = 0-10$; baseline), followed by pH 2.2 saline for 15 min ($t = 10-25$; 1st acid challenge period), followed by pH 7.0 saline for 10 min ($t = 25-35$; 1st recovery period), followed by pH 2.2 saline for 15 min ($t = 35-50$; 2nd acid challenge period), and then pH 7.0 saline for 15 min ($t = 50-65$; 2nd recovery period). O_2 gas-bubbled pH 7.0 saline was recirculated with a peristaltic pump, whereas pH 2.2 saline was perfused via syringe pump. The duodenal loop solution was gently flushed with 5 ml of perfusate to rapidly change the perfusate composition at $t = 10, 25, 35,$ and 50 min. Samples from acid exposure periods were collected in tubes every 5 min and analyzed for HCO_3^- secretion by back titration to pH 2.2 with 0.1 N HCl.

CO_2 measurement. To determine the relative contributions of HCO_3^- secretion and H^+ back diffusion during acid exposure, total dissolved CO_2 was measured with a CO_2 electrode (FK1501CO2; Radiometer America, Westlake, OH) connected to a pH meter (PHM 62; Radiometer, Copenhagen, Denmark). The duodenal loops were prepared and perfused as described in *Preparation of the duodenal loop*. Every 5 min, effluent pH was measured immediately after collection. One-half milliliter of 1 M citrate buffer (pH 4.5) was then added to the sample (5 ml) to convert free HCO_3^- to CO_2 , followed by measurement of electrode potential (mV) with the CO_2 electrode. Total dissolved CO_2 concentration ($[\text{CO}_2]_t$) was calculated according to a calibration curve using freshly prepared 0.1, 1, and 10 mM NaHCO_3 solutions as standards, which generate 0.1, 1, and 10 mM $[\text{CO}_2]_t$, respectively, at pH 4.8–5.0. Since the calibration curve had a slope ~ 56 mV/log $\Delta[\text{CO}_2]_t$ within the range of 0.1–10 mM at 20–25°C, all samples were analyzed at 25°C. $[\text{CO}_2]_t$ and pH in the perfusate provide CO_2 concentration ($[\text{CO}_2]$) and HCO_3^- concentration ($[\text{HCO}_3^-]$) from the Henderson-Hasselbach formula

$$\text{pH} = \text{pK}_a + \log([\text{HCO}_3^-]/[\text{CO}_2]) \quad (1)$$

$$[\text{CO}_2]_t = [\text{CO}_2] + [\text{HCO}_3^-] \quad (2)$$

Thus

$$[\text{CO}_2] = [\text{CO}_2]_t / (1 + 10^{\text{pH}-\text{pK}_a}) \quad (3)$$

$$[\text{HCO}_3^-] = [\text{CO}_2]_t \cdot 10^{\text{pH}-\text{pK}_a} / (1 + 10^{\text{pH}-\text{pK}_a}) \quad (4)$$

According to Henry's law

$$\text{PCO}_2 = [\text{CO}_2]/K_h \quad (5)$$

Substituting Eq. 3 into Eq. 5, PCO_2 can be calculated from $[\text{CO}_2]_t$ and pH

$$\text{PCO}_2 = [\text{CO}_2]_t / (1 + 10^{\text{pH}-\text{pK}_a}) / K_h \quad (6)$$

where pK_a , the first dissociation constant for carbonic acid in saline, is 6.2 at 25°C (25), and K_h , the Henry solubility constant for subsaturating CO_2 concentrations in weak electrolyte solutions, is 0.047 mM/mmHg at 25°C (15). To examine the accuracy of CO_2 measurements through the perfusion system, $[\text{CO}_2]_t$ of the perfusate was measured in O_2 -bubbled saline containing 0.1, 1, or 10 mM NaHCO_3 that was perfused through the system using the pump and tubing used for duodenal perfusions. Neither addition nor loss of CO_2 was observed when the afferent $[\text{HCO}_3^-]$ was above ~ 0.5 mM, but there was a 33% overestimation at the lowest measured CO_2 concentration (0.21 mM), with no difference between measured and predicted CO_2 at the highest measured concentration (1.06 mM; Fig. 1B). Further experiments were done to test the stability of dissolved CO_2 over time in an open system. Ten-millimolar HCO_3^- solutions were prepared, their

$[\text{CO}_2]_t$ was measured, they were titrated to pH 2.0, 4.5, or 7.0 at $t = 0$, and they were kept at 25°C in the same containers used to collect the effluent. Aliquots were removed at $t = 5$ min, 30 min, and 60 min. Figure 1C reveals that there was an initial decrease of $[\text{CO}_2]_t$ in the first 5 min, probably reflecting liberation of CO_2 during acid titration, but $[\text{CO}_2]_t$ was stable for 55 min thereafter.

Prostaglandin injection was used to augment HCO_3^- secretion in the absence of acid as a positive control for duodenal HCO_3^- secretion. PGE_2 (Oxford Biochemical, Oxford, MI) was administered with a single intravenous injection (0.3 mg/kg) as previously described (26). Baseline HCO_3^- output, measured by pH stat, and total CO_2 output, measured by the CO_2 electrode during pH 7.0 saline perfusion, were 0.10 ± 0.02 and $0.20 \pm 0.01 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-1}$, respectively. PGE_2 increased HCO_3^- and CO_2 output in parallel (0.39 ± 0.11 and 0.72 ± 0.17 , respectively; $P < 0.05$), confirming that titratable alkalinity and measured CO_2 output increased concordantly. Furthermore, to measure CO_2 and H^+ back diffusion into the mucosa, solutions of nominal pH 2.2, 6.4, and 7.0 and varying $[\text{CO}_2]_t$ were perfused through the duodenal loop (Table 1). Net loss of H^+ and CO_2 was calculated from changes of H^+ concentration and $[\text{CO}_2]_t$ between perfusate and effluent, with PCO_2 (mmHg) calculated from pH and $[\text{CO}_2]_t$ of the solutions according to Eq. 6.

Statistics

All data from six rats in each group are expressed as means \pm SE. Comparisons between groups were made by one-way ANOVA followed by Fischer's least significant difference test. Comparisons of two time points were assessed by paired, one-tailed t -test. P values of <0.05 were taken as significant.

RESULTS

Effect of Sustained Acid Perfusion

Blood flow and pH_i were stabilized with a 1-h perfusion of pH 7.0 Krebs buffer solution as previously described (3). MGT was also stable during this period. Figure 2 depicts pH_i , blood flow, and MGT at baseline ($t = 0$) and 5 min after acid exposure ($t = 20$). Acid exposure rapidly and significantly decreased pH_i to a new steady state during acid perfusion (Fig. 2A), with return to baseline after acid removal (Fig. 3). Steady-state pH_i was perfusate pH dependent (Fig. 2A). Blood flow and MGT rapidly increased during perfusion with pH 3.5 or pH 2.2 but not during pH 4.5 perfusion (Fig. 2, B and C).

Effect of Rapid Shifts of Luminal Acid

In the mild \rightarrow strong group, pH_i during the second acid challenge period was higher than in the constant pH 2.2 group (Fig. 3A). pH_i recovery to baseline levels

Table 1. Nominal pH, added NaHCO_3 , $[\text{CO}_2]_t$, and PCO_2 of perfusates used in Fig. 8

Nominal pH	NaHCO_3 , mM	$[\text{CO}_2]_t$, mM	PCO_2 , mmHg
7.0	10	10	29
2.2	10	10	212
6.4	25	25	206

$[\text{CO}_2]_t$, total CO_2 concentration.

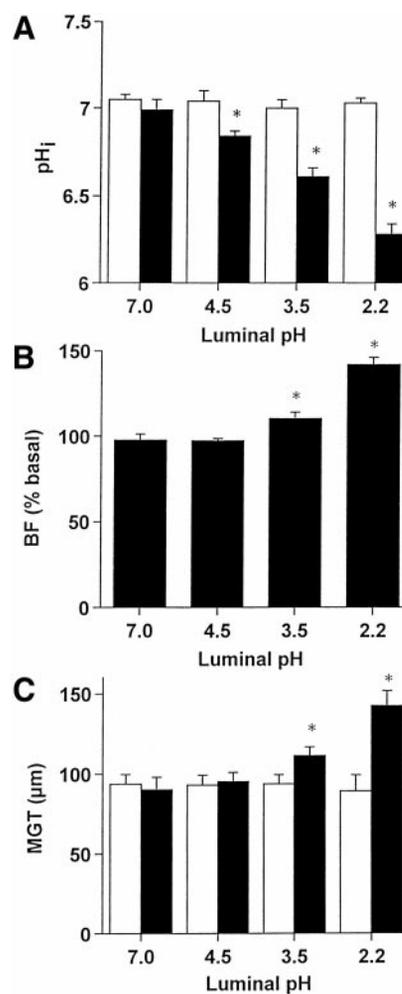


Fig. 2. Effect of sustained acid perfusion on intracellular pH (pH_i), blood flow, and mucosal gel thickness (MGT). pH_i was measured at baseline ($t = 0$) and 5 min after acid perfusion ($t = 20$). pH_i decreased rapidly and significantly at $t = 20$, the magnitude relative to perfusate pH (A). Blood flow (BF; B) and MGT (C) increased during pH 3.5 or 2.2 perfusion but not perfusion at pH 4.5. * $P < 0.05$ vs. pH 7.0 Krebs group. Values are means \pm SE from 6 rats.

and subsequent alkalization were also observed after acid removal. No adaptive change was seen in blood flow and MGT during the second acid challenge period compared with the corresponding constant pH group (data not shown).

In the strong \rightarrow mild acid group, pH_i gradually increased after the perfusate was changed to pH 4.5, and alkalization to above the predicted levels occurred during the second acid challenge period (Fig. 3B). No adaptive changes occurred in blood flow and MGT in the strong \rightarrow mild acid group (data not shown).

Effect of Repeated Acid Exposure With or Without DMA or DIDS

Since both the mild \rightarrow strong and strong \rightarrow mild perfusion sequences produced adaptive pH_i changes, we hypothesized that rapid shifts of perfusate pH, but not a mild \rightarrow strong sequence per se, produced adaptation. To test this hypothesis, we exposed the mucosa

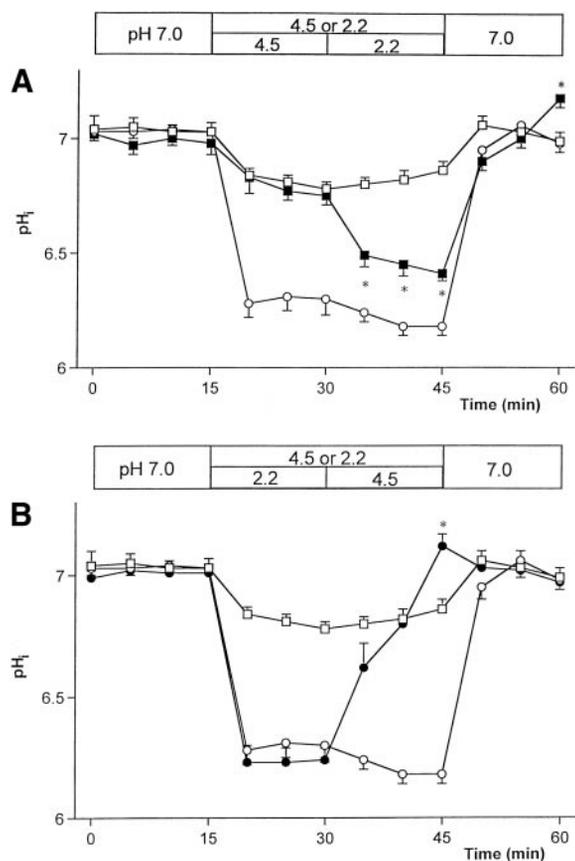


Fig. 3. Effect of mild \rightarrow strong or strong \rightarrow mild acid exposure on pH_i . In the mild \rightarrow strong group (\blacksquare), pH_i during the 2nd acid challenge period was higher than in the constant pH 2.2 group, followed by pH_i recovery to baseline and subsequent overshoot at 60 min (A). In the strong \rightarrow mild group (\bullet), pH_i during the 2nd acid challenge period gradually increased; an overshoot is observed at 45 min (B). Constant pH 4.5 (pH 4.5–4.5; \square) and constant pH 2.2 (pH 2.2–2.2; \circ) perfusion groups are shown for comparison. $*P < 0.05$ vs. the corresponding constant pH group. Values are means \pm SE from 6 rats.

to two pulses of strong acid. Two 15-min acid pulses separated by a 10-min recovery period produced an overshoot during the recovery periods and an adaptive response during the second acid challenge, in which the fall of pH_i during the second acid challenge was attenuated (Fig. 4). DIDS but not DMA exposure during the first acid challenge inhibited pH_i recovery during the first recovery period and abolished the pH_i adaptive response during the second acid challenge. Blood flow increased during the first and second acid challenges (Fig. 5); DIDS had no effect on the blood flow response during the first or second acid challenges. DMA abolished the hyperemic response during both acid challenge periods, similar to our previous description of a single acid challenge (3). MGT increased during the first acid challenge and further increased during the second acid challenge (Fig. 6). DIDS reduced the MGT response during the second acid challenge, whereas DMA had no effect.

Spontaneous HCO_3^- secretion was measured during pH 7.0 saline perfusion (Fig. 7A). Acid exposure increased titratable alkalinity to extreme levels during

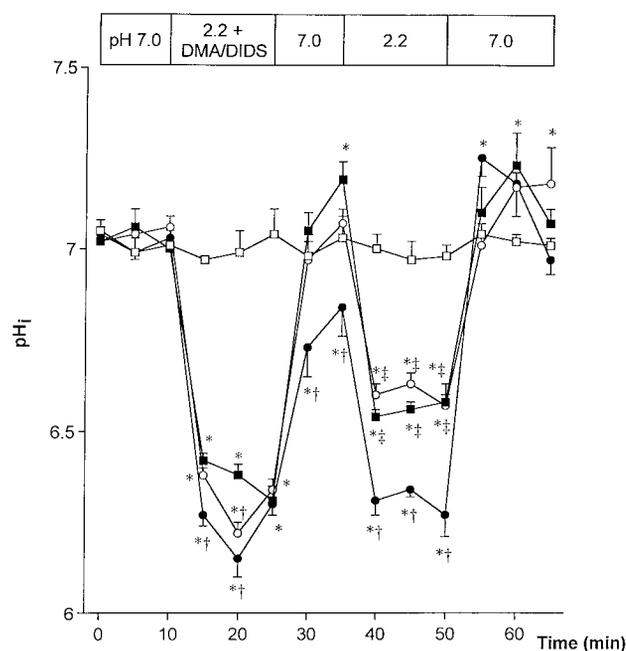


Fig. 4. Effect of repeated acid exposure with or without 5-(*N,N*-dimethyl)-amiloride (DMA) or DIDS on pH_i . Two acid pulses separated by a 10-min recovery period (\blacksquare) produced an overshoot after the 1st and 2nd acid challenges at 35 and 55 min compared with the pH 7.0 Krebs group (\square). An adaptive response of pH_i during the 2nd acid challenge at 40–50 min was observed. Although DMA (\circ) and DIDS (\bullet) with pH 2.2 further decreased pH_i during the 1st acid challenge at 20 min, DIDS but not DMA abolished the overshoot after the 1st acid challenge and pH_i adaptation during the 2nd acid challenge. $*P < 0.05$ vs. pH 7.0 Krebs group; $\dagger P < 0.05$ vs. repeated pH 2.2 alone group by ANOVA; $\ddagger P < 0.05$ vs. the corresponding time during the 1st acid challenge period by paired *t*-test.

the first and second acid challenges. During the recovery periods, acid loss in the pH 2.2 saline group was higher than in the pH 7.0 saline group, consistent with post-acid augmented HCO_3^- secretion. This stimulated secretion was reduced by DIDS but not by DMA. Although DIDS had no effect on acid loss during the acid

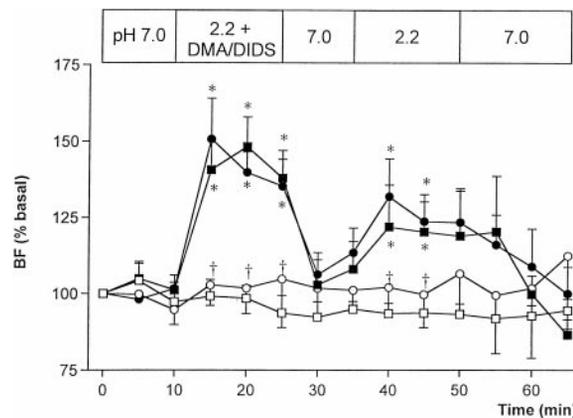


Fig. 5. Effect of repeated acid exposure with or without DMA or DIDS on blood flow. Blood flow increased during the 1st and 2nd acid challenge periods (\blacksquare). DMA (\circ), but not DIDS (\bullet), abolished the hyperemic response during both acid challenge periods. $*P < 0.05$ vs. pH 7.0 Krebs group; $\dagger P < 0.05$ vs. repeated pH 2.2 alone group by ANOVA; $\ddagger P < 0.05$ vs. the corresponding time during the 1st acid challenge period by paired *t*-test. Values are means \pm SE from 6 rats.

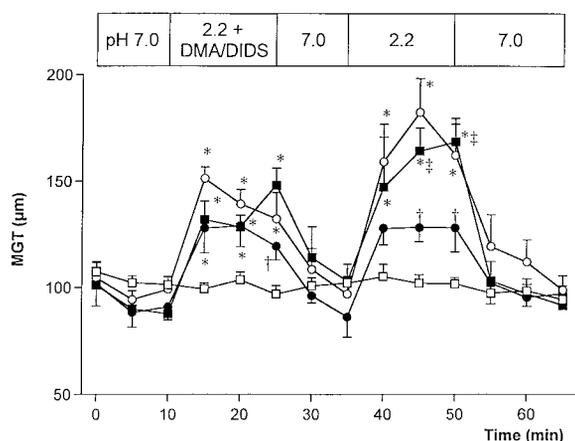


Fig. 6. Effect of repeated acid exposure with or without DMA or DIDS on MGT. Acid exposure increased MGT during the 1st acid challenge and further increased during the 2nd acid challenge (■). Although DMA (○) has no effect on MGT, DIDS (●) reduced MGT response during the 2nd acid challenge. * $P < 0.05$ vs. pH 7.0 Krebs group; † $P < 0.05$ vs. repeated pH 2.2 alone group by ANOVA; ‡ $P < 0.05$ vs. the corresponding time during the 1st acid challenge period by paired t -test. Values are means \pm SE from 6 rats.

challenges, DMA reduced acid loss during the second acid challenge. Figure 7B depicts total CO_2 output in the perfusate of pH 7.0 saline and repeated acid exposure groups. Total CO_2 output stabilized during pH 7.0 saline perfusion but decreased during both acid challenges and progressively increased during both recovery periods. Decreased total CO_2 output compared with the extreme increase of titratable alkalinity during acid exposures suggests that loss of luminal acidity during acid perfusion may be due to H^+ back diffusion rather than HCO_3^- secretion or CO_2 loss. In contrast, increased total CO_2 output during the recovery periods was consistent with post-acid augmented HCO_3^- secretion.

H^+ and CO_2 Back Diffusion in Duodenal Loops

Figure 8 depicts pH and $[\text{CO}_2]_t$ and calculated PCO_2 in the perfusates and effluents. $[\text{CO}_2]_t$ loss was 20.6–27.5% and H^+ loss was 12.3–27.5% as measured in the perfusate and effluent, respectively. These data are similar to those of Feitelberg et al. (6), in which PCO_2 in fixed pH perfusates (pH 5) collected from human prox-

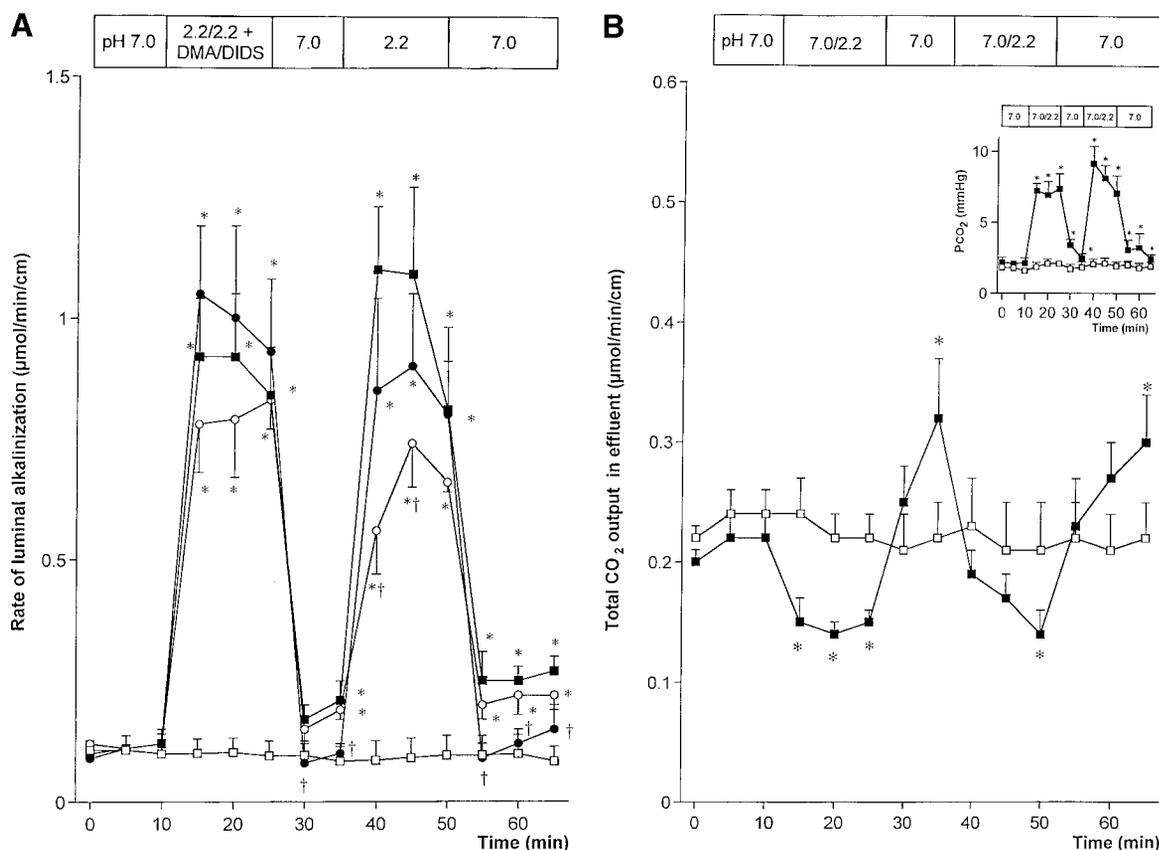


Fig. 7. Effect of repeated acid exposure with or without DMA or DIDS on effluent alkalization (acid disappearance) and $[\text{CO}_2]_t$. A: Back titration. Acid increased titratable alkalinity during both challenges and during both recovery periods (■). Although DMA (○) and DIDS (●) had no effect on acid loss during the 1st challenge period, DMA reduced acid loss during the 2nd acid challenge and DIDS, but not DMA, reduced HCO_3^- secretion during the recovery periods. B: CO_2 measurements. Separate experiments indicated that total CO_2 output was somewhat higher during neutral perfusion than was estimated by back diffusion. During the 1st and the last point of the 2nd acid challenge periods, CO_2 output was suppressed, indicating that the 90–95% acid loss during this period resulted mostly from H^+ back diffusion. Inset: PCO_2 was calculated from $[\text{CO}_2]_t$ and pH (see eq. 6). * $P < 0.05$ vs. pH 7.0 Krebs group; † $P < 0.05$ vs. repeated pH 2.2 alone group by ANOVA. Values are means \pm SE from 6 rats.

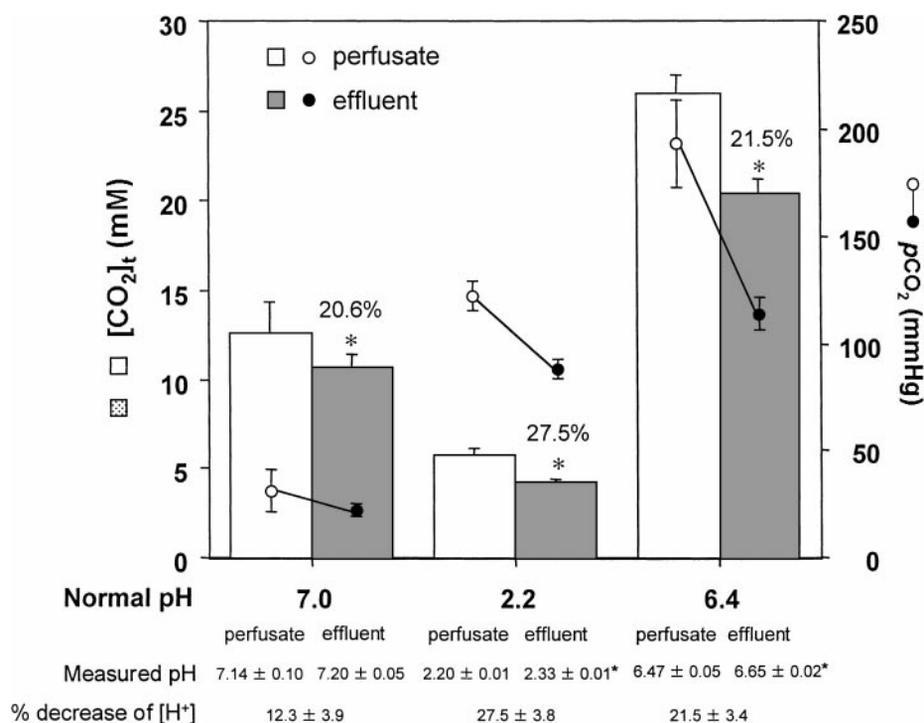


Fig. 8. Changes of H^+ concentration ($[\text{H}^+]$) and $[\text{CO}_2]_t$ in perfusates and effluents of different pH and $[\text{CO}_2]_t$ solutions. Measured pH, measured $[\text{CO}_2]_t$, and calculated PCO_2 of perfusates and effluents for solutions of nominal pH 7.0, 2.2, and 6.4 are shown. The numbers over the closed columns indicate %net loss of $[\text{CO}_2]_t$. * $P < 0.05$ vs. perfusate in pH and $[\text{CO}_2]_t$ by ANOVA. Values are means \pm SE from 3 rats.

imal duodenal segments decreased 23 and 27% in solutions bubbled with 10 and 20% CO_2 , respectively.

DISCUSSION

Simultaneous and parallel measurements of rat duodenal defenses in vivo provided a useful means of examining the response of the mucosa to luminal acid. Alteration of perfusate pH or a brief acid challenge induced cellular base uptake, protecting the epithelial cells from acidification during subsequent acid exposure. Responses of MGT and blood flow to the acid challenges showed little such adaptation to the second acid challenge or to a change of perfusate pH. Adaptive pH_i changes were abolished by DIDS, which had no effect on blood flow but did prevent the increase of MGT during the second acid challenge. Furthermore, DIDS inhibited the post-acid challenge increases of HCO_3^- secretion. Conversely, DMA inhibited the blood flow increase during the first and second acid challenges but had no effect on MGT or on HCO_3^- secretion following acid challenge. Measurement of HCO_3^- secretion by back titration and total CO_2 measurement revealed that with pH 2.2 perfusate, most acid disappearance was due to H^+ back diffusion and not alkaline secretion. Furthermore, DMA decreased the rate of acid back diffusion, presumably by inhibiting the hyperemic response to acid (3).

Augmented HCO_3^- secretion following acid perfusion is currently accepted as the most important duodenal defense mechanism (7, 30). The mechanism by which HCO_3^- secretion protects the mucosa has generally been assumed to be complete neutralization of luminal acid diffusing toward the mucosa within the mucus gel. Since duodenal epithelial cells readily acidify in the presence of even moderate concentrations of perfused

acid, it is doubtful that complete neutralization of back-diffusing acid occurs in the preepithelial mucus gel. If acid is able to enter the cells, perhaps an alternate mucosal protective mechanism exists: intracellular buffering or pH_i regulation. If pH_i regulation is important, then HCO_3^- secretion may not need to increase in the presence of luminal acid. This prospect prompted our measurement of HCO_3^- secretion during the same repeated acid exposure that produced pH_i adaptation. We hence endeavored to ascertain the relative contributions of acid back diffusion, of CO_2 back diffusion, and of HCO_3^- secretion toward the disappearance of luminal acid in duodenal perfusions. With the use of back titration, the amount of titratable alkalinity increased 10-fold during acid perfusion, in agreement with Flemström and Kivilaakso (8), although the relative increase was greater than that found by Nylander and colleagues (18, 19). To further study this increase of titratable alkalinity during acid perfusion, we measured $[\text{CO}_2]_t$. Measurement of $[\text{CO}_2]_t$ is a more direct means of measuring HCO_3^- secretion than is back titration since it has the advantage of not being affected by secretion of other alkali or loss of H^+ due to back diffusion. The concordant increase of effluent $[\text{CO}_2]_t$ and titratable alkalinity in PGE_2 -treated rats confirms the validity of these measurements in the absence of luminal acid. As long as total PCO_2 is < 10 mmHg at 25°C (the highest $[\text{CO}_2]_t$ was < 2 mM in this study), the aqueous solubility of CO_2 is such that little loss of CO_2 to the atmosphere occurs over the measurement period, as we demonstrated in preliminary studies. The rapid perfusion technique has been applied successfully by Olbe and co-workers (5), who found that the accuracy of clinical gastric HCO_3^- secretion measurements with a CO_2 electrode was improved by in-

creasing the perfusion rate, which ensured that CO_2 concentrations would remain well below the Henry solubility limit. Furthermore, the 20–30% loss of $[\text{CO}_2]_t$ and ~12–28% loss of H^+ between perfusate and effluent of duodenal perfusions is in close agreement with published measurements of CO_2 loss from duodenally perfused HCO_3^- solutions (6), confirming that the duodenum has a measurable, finite permeability to CO_2 and H^+ . The rapid increase of titratable alkalinity measured during acid perfusion was not accompanied by a commensurate increase of effluent $[\text{CO}_2]_t$, strongly consistent with our hypothesis that HCO_3^- secretion is not augmented during acid perfusion but rather that luminal acid disappears via H^+ back diffusion. To explain these data, we hypothesized that, during luminal acid perfusion, either: 1) HCO_3^- secretion increases ~1,000% within 5 min, HCO_3^- is converted by acid to CO_2 gas, >90% of the CO_2 is lost due to back diffusion, and HCO_3^- secretion decreases to near baseline within 5 min, or 2) HCO_3^- secretion is unchanged, ~25% of H^+ is lost, and a further ~25% CO_2 is lost due to conversion to CO_2 gas with back diffusion. Since the data depicted in Fig. 8 and the work of others indicate that CO_2 loss, even in solutions with high P_{CO_2} , in a rapidly perfused system is ~25% and that increases and decreases of the rate of HCO_3^- secretion generally develop over a 30- to 60-min time period, the latter H^+ back diffusion model fits most closely with the data. Furthermore, we demonstrated that, even in an open system, CO_2 loss cannot account for the discrepancy between $[\text{CO}_2]_t$ measurements and acid disappearance. These measurements add confidence to our contention that HCO_3^- secretion was not augmented, although H^+ back diffusion was substantially increased during luminal acid perfusion.

Importantly, since the only time that HCO_3^- secretion is necessary for mucosal protection is during acid exposure, its lack of increase by acid diminishes its importance as a mucosal defense mechanism. During acid stress, it would seem more logical for the cell to retain its protective HCO_3^- buffer rather than release it into the lumen, as would be predicted by models implicating HCO_3^- secretion during acid exposure as a major defensive factor. From these studies, we could conclude that most of the luminal acid loss during acid challenge is from H^+ back diffusion, suggesting that HCO_3^- secretion of itself cannot defend pH_i against acid challenge and may merely serve as an easily measurable sequelum to prior cellular base uptake.

The pH_i response to repeated acid challenge differed between the first and second challenges. During the first challenge, cells acidified more in the presence of DIDS and DMA. Following acid challenge, DIDS attenuated pH_i recovery and overshoot, coincident with its inhibition of cellular base uptake. During the second acid challenge, DIDS also abolished the adaptive pH_i response, although an early overshoot was observed. These observations are in full agreement with those of Paimela et al. (20), who noted that addition of the DIDS-related stilbene derivative SITS or removal of HCO_3^- from the serosal bathing solution of isolated

Necturus duodenal mucosa increased cellular acidification during luminal acid challenge. Our interpretation differs from theirs, however, in that we believe that the increased susceptibility to acidification more likely represents suppression of cellular base uptake than it does inhibition of HCO_3^- secretion. In the absence of perfused acid, augmented HCO_3^- secretion during the first and second recovery periods was attenuated by DIDS but not by DMA, indicating that DIDS-inhibitable HCO_3^- secretion is correlated with DIDS-inhibitable cellular HCO_3^- uptake.

As shown previously (1, 3), the duodenum has a predictable and robust hyperemic response to perfused acid, even though there is no evidence of augmentation of this response to repeated acid challenges. Although the hyperemic response has been unquestionably implicated in the reduction of gastric injury susceptibility (11), the protective role of blood flow in the duodenum is controversial (14, 24, 30). One explanation for the lower amount of acid back diffusion in DMA-treated rats during the second acid challenge is that suppression of acid-related hyperemia by DMA reduced acid back diffusion during the second acid challenge. The most comprehensive study of the relationship between duodenal blood flow and injury susceptibility was published by Lugea et al. (16, 17), who found that a 1-ml intraduodenal bolus of 100 mM HCl (pH 1) significantly reduced damage due to a bolus of 400 mM HCl (pH 0.4) given 30 min later. Although they measured a significant increase of duodenal blood flow in response to a bolus of 100 mM HCl, they did not measure blood flow during the mild \rightarrow strong acid sequence. It is thus difficult to ascertain the contribution of blood flow to the observed adaptive protection from injury observed in that study. The suppression of the large increase of titratable alkalinity by DMA, which also suppressed acid-related hyperemia (3), underscores the putative role of blood flow in removing mucosal (luminal) acid via back diffusion. In other words, if the function of acid-related hyperemia is to carry away back-diffusing acid, one would predict that suppression of the hyperemic response would decrease the amount of acid back diffusion, as was observed in this study.

MGT also increased during acid perfusion, as we have previously demonstrated. Augmentation of MGT during acid perfusion indirectly supports its defensive role against acid. It is likely that blood flow, MGT increase, and cellular base uptake, all of which are increased by luminal acid, act in concert to increase duodenal resistance to acid.

One of the most interesting aspects of this study was the demonstration that rapid shifts of perfusate pH per se, and not necessarily the mild \rightarrow strong exposure sequence, induced the adaptive responses observed. To our knowledge, this possibility has heretofore never been explored, although our data, in which adaptive responses of pH_i were observed after reversing the mild \rightarrow strong sequence or exposing the mucosa to repeated pulses of strong acid, convincingly suggests that, at least in terms of HCO_3^- uptake, the rapid shifts

of perfusate pH are all that are necessary. These shifts simulate the physiological state of the duodenum, wherein the mucosa is exposed alternately to peristaltically conveyed waves of gastric acid and bursts of pancreatic HCO_3^- after meal ingestion. Measurements of duodenal pH indicate shifts of 4–5 pH units over <1 min (23). Although we have not proven that the observed resistance to acidification truly reflects protection from injury, numerous studies of gastric epithelium have confirmed that resistance to mucosal acidification during acid challenge consistently correlates with decreased injury susceptibility in standard models (22, 28, 29).

In summary, we found that acute adaptive responses occurred when perfusate pH was shifted every 10–15 min, regardless of the sequence of acid concentrations used. Furthermore, the only consistent acute adaptive alteration of a defense mechanism was presumably increased cellular HCO_3^- uptake, which produced intracellular alkalinization and resistance to acidification from perfused acid. Significant back diffusion of perfused acid correlated with increased mucosal blood flow. We propose that intracellular HCO_3^- uptake via a DIDS-sensitive basolateral $\text{Na}^+-\text{HCO}_3^-$ cotransporter, which temporally precedes HCO_3^- secretion in response to rapid shifts of luminal pH, is an early acute duodenal response to luminal acid. Furthermore, we postulate that HCO_3^- secretion may not be the primary mucosal defense mechanism. The reason why HCO_3^- secretion correlates well with duodenal acid resistance may signify its role in restoring epithelial pH_i after acid-induced base uptake. We conclude that cellular HCO_3^- uptake is likely to be an important duodenal defense mechanism that is induced by physiological postprandial rapid shifts of luminal pH and precedes active HCO_3^- secretion.

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