CFTR inhibition augments NHE3 activity during luminal high CO2 exposure in rat duodenal mucosa

Misa Mizumori,1,2,4 Yuri Choi,5 Paul H. Guth,1 Eli Engel,3 Jonathan D. Kaunitz,1,2 and Yasutada Akiba2,4

1Greater Los Angeles Veterans Affairs Healthcare System; 2Department of Medicine, School of Medicine; 3Department of Biostatistics, University of California Los Angeles; 4Brentwood Biomedical Research Institute; and 5Harvard-Westlake School, Los Angeles, California

Submitted 18 January 2008; accepted in final form 12 April 2008

Mizumori M, Choi Y, Guth PH, Engel E, Kaunitz JD, Akiba Y. CFTR inhibition augments NHE3 activity during luminal high CO2 exposure in rat duodenal mucosa. Am J Physiol Gastrointest Liver Physiol 294: G1318–G1327, 2008. First published April 17, 2008; doi:10.1152/ajpgi.00025.2008.—We hypothesized that the function of duodenocyte apical membrane acid-base transporters is essential for H+ absorption from the lumen. We thus examined the effect of inhibition of Na+/H+ exchanger-3 (NHE3), cystic fibrosis transmembrane regulator (CFTR), or apical anion exchangers on transmucosal CO2 diffusion and HCO3− secretion in rat duodenum. Duodena were perfused with a pH 6.4 high CO2 solution or pH 2.2 low CO2 solution with the NHE3 inhibitor, S3226, the anion transporter inhibitor, DIDS, or pretreatment with the potent CFTR inhibitor, CFTRinh-172, with simultaneous measurements of luminal and portal venous (PV) pH and carbon dioxide concentration ([CO2]). Luminal high CO2 solution increased CO2 absorption and HCO3− secretion, accompanied by PV acidification and PV PCO2 increase. During CO2 challenge, CFTRinh-172 induced HCO3− absorption, while inhibiting PV acidification. S3226 reversed CFTRinh-172-associated HCO3− absorption. Luminal pH 2.2 challenge increased H+ and CO2 absorption and acidified the PV, inhibited by CFTRinh-172 and DIDS, but not by S3226. CFTR inhibition and DIDS reverse HCO3− absorption and inhibited PV acidification during CO2 challenge, suggesting that HCO3− secretion helps facilitate CO2/H+ absorption. Furthermore, CFTR inhibition prevented CO2-induced cellular acidification reversed by S3226. Reversal of increased HCO3− loss by NHE3 inhibition and reduced intracellular acidification during CFTR inhibition is consistent with activation or unmasking of NHE3 activity by CFTR inhibition, increasing cell surface H+ available to neutralize luminal HCO3− with consequent CO2 absorption. NHE3, by secreting H+ into the luminal microclimate, facilitates net transmucosal HCO3− absorption with a mechanism similar to proximal tubular HCO3− absorption.

CO2 absorption; bicarbonate secretion; portal venous PCO2

EPITHELIAL HCO3− SECRETION is considered to be the primary defense against duodenal injury from gastric acid. One of the unexplained mysteries of duodenal HCO3− secretion is why it is needed, given that secretion into the lumen from pancreas and liver is more than adequate to fully neutralize the bulk luminal content (13). Epithelial HCO3− secretion has thus been hypothesized to neutralize a distinct “preepithelial layer” adjacent to the apical plasma membrane of the mucosa (10, 24). Moreover, the upper intestine absorbs HCO3− by an electroneutral Na+/H+ dependent mechanism, hypothesized to be Na+/H+ exchange (37). Since the epithelial Na+/H+ exchanger (NHE) 3, which helps facilitate intestinal neutral NaCl absorption, is highly expressed in duodenum (31), we wondered whether duodenal NHE3 additionally functions as an H+ secretor, as it does in the proximal tubule (33, 38), facilitating HCO3− absorption.

The duodenum must also absorb ~450 mmol of gastric H+24 h to decrease luminal hydrogen concentration ([H+]) by 6 log orders over its 15-cm length (12). HCl in the duodenal lumen is neutralized by HCO3− secreted by the pancreas and duodenal epithelium. This generates extremely high luminal CO2 pressures (PCO2 > 400 Torr), which are dissipated by the proximal jejunum (32). Gastric mucosal CO2 and H+ permeability is low, since pyloric obstruction leads to severe metabolic alkalosis due to the inability of the normal stomach to absorb substantial quantities of H+ or CO2 (16). Thus the duodenum is the major site for intestinal H+ and CO2 absorption.

We have demonstrated sequential transmucosal CO2 and H+ movement from duodenal lumen into the portal vein (PV), facilitated by epithelial cytosolic and membrane-bound carbonic anhydrases (CAs) in the rat (27). Cellular acidification by luminal CO2/H+ rapidly induces protective responses such as the hyperemic response, augmented mucus secretion, and HCO3− secretion. Protective mechanisms prevent irreversible cellular acidification (23).

Our model of transapical CO2/H+ uptake by the duodenal epithelial cells is based on the Jacobs-Stewart cycle, originally described in 1945 in red cells as a means of transporting large quantities of H+ to the lungs via successive CA-facilitated CO2↔H2CO3 interconversion coupled with transmembrane CO2 diffusion (22). Components of the Jacobs-Stewart cycle include cytosolic and membrane-bound CAs in addition to plasma membrane anion exchanger-facilitated HCO3− secretion. In the Jacobs-Stewart cycle, luminal H+, facilitated by membrane-bound CA, neutralizes HCO3− secreted by a plasma membrane anion exchanger, generating CO2 and H2O. CO2 diffuses through the plasma membrane, where a soluble CA facilitates its hydration into H2CO3, dissociating to H+ and HCO3−. HCO3− is secreted across the plasma membrane by the anion exchanger, completing the cycle. For red cells, cellular H+ is transported to the lungs, where the reverse cycle increases plasma CO2, which is excreted. For epithelial cells, we demonstrated that HCO3− is secreted across the apical membrane, which, coupled with previously demonstrated H+ exit across the basolateral membrane, produces net transepithelial H+ absorption (27). Here, we show that H+ can alternatively exit across the apical membrane, facilitating luminal HCO3−...
absorption. We have demonstrated that all of the Jacobs-Stewart cycle-associated proteins are well expressed in duodenal epithelial cells (3, 40) and that cytosolic and membrane-bound CA activities are essential for luminal H+ uptake (27). We now would like to further test the hypothesis that H+, CO2, and HCO3 absorption pathways across the apical membrane of duodenal epithelial cells follow the model of the Jacobs-Stewart cycle. We thus examined the contribution of apical acid-base transporters towards duodenal transapical H+, CO2, and HCO3 movement.

We assumed that the main sources of H+ and HCO3- necessary for transmucosal CO2 movement are the stomach and pancreas, respectively. Yet, in the kidney, CO2 is generated in the preepithelial microclimate abutting the proximal tubule cell apical membrane by titration of tubular HCO3- with H+ secreted by NHE3 or a proton pump (38). We wondered whether epithelial H+ and HCO3- secretion into the preepithelial microclimate were necessary components for transmucosal acid and base movement, as they are in the proximal tubule and have been hypothesized in the proximal intestine (20, 37). The transport function of NHE3, which plays an important role in lower intestinal Na+ absorption, (33) is unclear in the duodenum. We have previously shown that NHE3 is not involved in CO2/H+ movement between the lumen and cell during high CO2 exposure (27), nor is it involved in the regulation of enterocyte intracellular pH (pHi) at neutral luminal pH (15).

The two major apical transporters that secrete HCO3- into the duodenal lumen are the cystic fibrosis transmembrane conductance regulator (CFTR) and the apical Cl-/HCO3- anion exchanger in the SLC26A family (9, 40). CFTR plays a major role in the HCO3- secretion stimulated by the cAMP pathway (9). Of the SLC26A family, SLC26A3 (downregulated in adenoma (DRA)] and SLC26A6, putative anion transporter 1 (PAT1) are implicated in duodenal HCO3- secretion (21, 39), although their differential axial expression patterns and transport stoichiometries suggest distinct but complementary functions (34).

The intestine switches between secretion and absorption by reciprocal activation and suppression of the coupled ion exchange proteins NHE3 and SLC26Ax. Secretory proteins such as CFTR are also involved through functional coupling, protein-protein interactions via their postsynaptic density protein-Drosophila disc-large tumor suppressor-zonula occcludens-1 protein domains, and also through secretion-induced cell shrinkage (17). In the duodenum, where SLC26A is implicated in HCO3- secretion rather than in Cl- absorption, this mechanism is less clear. We have shown that the selective NHE3 inhibitor, S3226, increases duodenal HCO3- secretion via CFTR activation rather than by decreasing the neutralization of HCO3- by NHE3-secreted H+ (15).

To further study the role of NHE3 in luminal acid absorption, we hypothesized that NHE3 acidifies the enterocyte preepithelial microclimate, neutralizing SLC26Ax-secreted HCO3- to CO2 and H2O and facilitating CO2 absorption across the apical membrane and through the mucosa. We further hypothesized that CFTR inhibition would reciprocally activate NHE3 activity, “unmasking” its role in duodenal HCO3 absorption.

**MATERIALS AND METHODS**

Chemicals and animals. CFTRinh-172 was synthesized by Dr. Samedy Ouk in the Department of Chemistry, University of California at Los Angeles, according to the published chemical structure of CFTRinh-172 (26). It was purified with high-performance liquid chromatography with the structure verified by nuclear magnetic resonance (4). S3226, a selective NHE3 inhibitor (15) was a kind gift of Aventis Pharma Deutschland (Frankfurt am Main, Germany). BCECF- acid and its acetoxy methyl ester (BCECF-AM) and DIDS were obtained from Molecular Probes (Eugene, OR). NaHCO3, HEPES, and all other chemicals were obtained from Sigma Chemical (St. Louis, MO). Krebs solution contained (in mM) 136 NaCl, 2.6 KCl, 1.8 CaCl2, and 10 HEPES at pH 7.0. For neutral pH/high CO2 perfusion, solutions were made as previously described (3, 14, 27) using a 50 mM NaHCO3-105 mM NaCl solution and 20 mM HCO3 solution, generating a pH-9.5 solution (310 mosM) pH 6.4 solution with PCO2 = 260 Torr, calculated using a CO2 aqueous solubility constant of 0.0321 mM/Torr and the first negative log of acidic dissociation constant (pKa) of carbonic acid = 6.1 at 37°C. Solutions were vigorously mixed 1 min before perfusion. We confirmed that the pH of the freshly mixed solution reached steady-state within 10 s, whereas carbon dioxide concentration ([CO2]) reached its equilibrium of carbonic acid, H+, and HCO3 by 1 min and was stable for at least 10 min, as measured by pH and CO2 electrodes (Lazar Research Laboratories, Los Angeles, CA). Each solution was prewarmed at 37°C with the use of a water bath, and the temperature was maintained with a heating pad during the experiment. For low pH/low CO2 perfusion, pH 2.2 saline was used. CFTRinh-172 was dissolved with DMSO at 1 mg/10 μl for intraperitoneal injection. The stock solution was kept in −20°C until use. DMSO (1%) in saline was used as vehicle for intraperitoneal injection. S3226 was dissolved in DMSO, and DIDS was in distilled water and then stored at −20°C until use. DMSO (0.1%) in saline was used for vehicle perfusions. All studies were performed with approval of the Veterans Affairs Institutional Animal Care and Use Committee (VA IACUC). Male Sprague-Dawley rats weighing 200–250 g (Harlan, San Diego, CA) were fasted overnight but had free access to water.

Measurement of luminal pH and [CO2]. According to a previously reported method (27), a duodenal loop was prepared and perfused, and pH and [CO2] in the perfusate and effluent were simultaneously and continuously measured with flow-through pH and CO2 electrodes. In brief, under isoflurane anesthesia (1.5–2.0%) with the use of a rodent anesthesia inhalation system (Summit Medical Systems, Bend, OR), rats were placed supine on a water recirculating heating block system (Summit Medical) to maintain body temperature at 36–37°C, as monitored by a rectal thermometer. Prewarmed saline was infused via the right femoral vein at 1.08 ml/h using a Harvard infusion pump (Harvard Apparatus, Holliston, MA). Using a pressure transducer (Kent Scientific, Torrington, CT), blood pressure was monitored via a catheter placed in the left femoral artery. The abdomen was incised, both stomach and duodenum were exposed, and the forestomach wall was incised 0.5 cm using a thermal cautery (Geiger Medical Technologies, Monarch Beach, CA). A polyethylene tube (diameter 5 mm) was inserted through the incision until it was 0.5 cm caudal from the pyloric ring where it was secured with a nylon ligature. The distal duodenum was ligated proximal to the ligament of Treitz before the duodenal loop was filled with 1 ml saline prewarmed at 37°C. The distal duodenum was then incised, 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 and cannulated with a polyethylene (PE-10) tube to drain the juice. The resultant closed proximal duodenal loop (perfused length 2 cm) was perfused with prewarmed saline by using a peristaltic pump (Fisher Scientific, Pittsburgh, PA) at 1 ml/min. Input (perfuse) and effluent pH and [CO2] were continuously measured.
with two sets of pH and CO2 electrodes, respectively, placed in series using flow-through cells (Micro Flow-Through pH and CO2 electrodes, Lazar Research Laboratories). These flow-through cells were immersed in a water bath maintained at 37°C by a thermostatically regulated system (Fisher Scientific). CO2 electrodes were calibrated every day with 0.1, 1.0, and 10 mM NaHCO3 in pH 4 citrate buffer, which generated 0.1, 1.0, and 10 mM total CO2 content ([CO2]t), respectively. This system enabled us to calculate the changes in the concentration of luminal CO2, total CO2, HCO3−, and H+ by the following equations derived from the Henderson-Hasselbalch equation

\[
[\text{total CO}_2] = [\text{CO}_2] + [\text{HCO}_3^-] = [\text{CO}_2] \cdot (1 + 10^{pH-pK_a}) \tag{1}
\]

\[
[\text{HCO}_3^-] = [\text{CO}_2] \cdot 10^{pH-pK_a} \tag{2}
\]

where [CO2] (mM) was calculated according to the calibration curve as mentioned above, [HCO3−] is the bicarbonate concentration, and pK_a, the first dissociation constant of carbonic acid, = 6.1 at 37°C. Note that [CO2] directly measured by a CO2 electrode is described as a concentration (mM), distinct from PCO2 (Torr), which is calculated as mentioned above,

\[
[\text{PCO}_2] = [\text{CO}_2]/K_b \tag{3}
\]

where K_b, the Henry solubility constant for sub saturating CO2 concentrations in weak electrolyte solutions, is 0.0321 mM/Torr at 37°C. For example, a high CO2 solution mixed with 1 vol of 50 mM NaHCO3 and 1 vol of 20 mM HCl produces a pH 6.4, [CO2] = 25 mM solution, with [CO2] = 8.3 mM and [HCO3−] = 16.7 mM according to Eq. 1 and 2, and [PCO2] = 260 Torr from Eq. 3.

The net changes in concentration were determined as

\[
\Delta = [\text{effluent}] - [\text{perfusate}] \tag{4}
\]

expressed so that positive quantities denote net secretion whereas negative quantities indicate net absorption from the lumen. For example

\[
\Delta[\text{CO}_2] = [\text{CO}_2]_{\text{effluent}} - [\text{CO}_2]_{\text{perfusate}} \tag{5}
\]

\[
\Delta[H^+] = 10^{\text{pH}_{\text{effluent}} - 10^{\text{pH}_{\text{perfusate}}} \tag{6}
\]

were used to calculate the net secretion or absorption of [CO2] and [H+] respectively. Note that, for acid perfusion experiments, since perfusate and effluent pH was very low (~2.2), measured effluent [CO2] ~ [total CO2] = mucosally secreted HCO3−, since perfusate [CO2] and [HCO3−] were ~0. Thus to compare HCO3− secreted into the acidic perfusate with baseline HCO3− secretion into the neutral pH perfusate, we calculated [total CO2] (mM) in the neutral pH solution as shown above, or a pH 2.2 acid saline ([CO2] ~ 0, [HCO3−] ~ 0, low pH/low CO2 solution), from t = 20 min until t = 30 min (challenge period), with or without inclusion of inhibitors as described below. At t = 20 min, the system was gently flushed manually to rapidly change the composition of the perfusate. During the challenge period, the solution was perfused with a syringe pump. At the end of the challenge period (t = 30 min, 10 min after CO2 or acid stress), an aliquot of portal blood was analyzed. Abdominal aortic blood was analyzed as a comparator.

To examine the effect of the inhibition of CFTR on luminal and PV pH and [CO2], the animals were pretreated with CFTRinh-172 (1 mg/kg ip) or vehicle 1 h before anesthesia commenced. This pretreatment is confirmed to inhibit acid-induced HCO3− secretion due to CFTR inhibition in rat duodenum (4). Furthermore, CFTRinh-172 has been recognized as a potent and selective CFTR inhibitor in the gastrointestinal tract of rodents (26, 36), although its efficacy is dependent on tissue type (less potent in airway epithelia) and species (less potent in pig and ferret than in human and mouse) (25). CFTRinh-172 inhibition can mimic CFTR dysfunction in human tracheal epithelia (29). To examine the effect of the inhibition of NHE3 or anion exchanger/Na+−HCO3−cotransporter (NBC), S3226 (10 μM) (15, 27) or DIDS (0.1 mM) (14), respectively, were perfused with the high CO2 solution or acid solution. We confirmed that S3226 or DIDS in these concentrations had no effect on solution pH or [CO2]. Neither inhibitors nor high CO2 or acid perfusate had any measurable change on body temperature, blood pressure, or arterial blood gas analysis.

### pH, and blood flow measurement.

To further examine the effect of CFTR or NHE3 inhibition during CO2 exposure on the epithelial function in rat duodenum, the pH of the upper villous epithelial cells and blood flow were simultaneously measured using BICEF fluorescence ratio imaging and laser Doppler flowmetry as described elsewhere (3, 5). Under isoflurane anesthesia, BICEF-loaded, chambered duodenal mucosa was superfused with pH 7.0 Krebs buffer solution for 10 min (basal period), followed with pH 6.4 saline (PCO2 = 0 Torr) or pH 6.4 high CO2 solution (PCO2 = 260 Torr) with or without S3226 (10 μM) for 10 min (challenge period), then with pH 7.0 Krebs for 15 min (recovery period). Rats were pretreated with vehicle or CFTRinh-172 (1 mg/kg ip) 1 h before the experiment started.

### Statistics.

All data was derived from experimental groups with n = 6 and were expressed as means ± SE. Comparisons between groups were made by one-way ANOVA followed by Fisher’s least significant difference test. P < 0.05 was taken as significant.

### RESULTS

#### Luminal high CO2 exposure.

In the initial series, we defined the basal condition and then examined the contribution of two prominent apical membrane acid-base transporters or transport facilitators, NHE3 and CFTR. There is a small amount of secretion of [CO2] and [HCO3−] into the saline (pH 7.0) perfusate (Δ[CO2] and Δ[HCO3−]), respectively during the basal period (t = 0–20 min), as previously reported (27). The time point t = 30 min, 10 min after changing the solution to the challenge or test solution, was chosen to allow for equilibration of the perfusate with the mucosa and also to coincide with portal blood measurements as previously described (27). Figure 1, A and B, depicts Δ[CO2] and Δ[HCO3−] at t = 30 min, 10 min after perfusion of either pH 6.4 saline ([CO2] ~ 0), or perfusion with pH 6.4 high CO2 saline (PCO2 = 260 Torr). Figure 1, C and D, depicts PV pH and PCO2 at t = 30 min. Positive Δ[CO2] and Δ[HCO3−] in the saline control group revealed net basal HCO3− secretion. Compared with saline controls, Δ[CO2] was decreased to negative values, and Δ[HCO3−] was increased during exposure to high CO2. This is...
consistent with the simultaneous net movement of CO2 from perfusate to mucosa (absorption) and HCO3^- output into the lumen (secretion) (Fig. 1, A and B) or luminal conversion of CO2 to HCO3^- in bulk solution. Furthermore, perfusion of the high CO2 solution lowered PV pH and elevated PV PCO2 (Fig. 1, C and D), consistent with CO2 or H^+ absorption into the PV blood. This demonstrates CO2 movement from lumen to mucosa, rather than luminal conversion of CO2 to HCO3^-.

To test the hypothesis that CFTR inhibition augments NHE3 activity, we first examined the effect of CFTR inhibition on PCO2. This is consistent with the previous data that CFTRinh-172 has no effect on the basal HCO3^- activity, we first examined the effect of CFTR inhibition on PCO2. This is consistent with the previous data that CFTRinh-172 has no effect on the basal HCO3^- secretion but inhibits acid-induced HCO3^- secretion in rat duodenum (4). In contrast, CFTRinh-172 converted luminal CO2-induced HCO3^- secretion to HCO3^- loss (Fig. 1, A and B), reversing the transport of luminal CO2 to the PV (Fig. 1, C and D). These results suggest that CFTR inhibition either activates apical Na^+/H^+ exchange, decreasing luminal [HCO3^-] during high CO2 exposure (the high CO2 solution contains 8 mM CO2 and 16 mM HCO3^-), or activates apical anion exchangers to absorb rather than secrete HCO3^-.

To test these possibilities, we examined the effect of NHE3 inhibition during high CO2 exposure. Although a selective NHE3 inhibitor, S3226 (10 μM), itself has no effect on CO2/H^+ movement (27), S3226 perfusion during high CO2 exposure reversed CFTRinh-172-induced HCO3^- absorption and CO2 loss (Fig. 1, A and B). This suggests that NHE3 is activated by CFTR inhibition during high CO2 exposure, secreting H^+ into the lumen to neutralize HCO3^-.

We then examined the contribution of apical anion exchangers to net CO2 and HCO3^- movement. Luminal DIDS (0.1 mM), the stilbene anion transport inhibitor, had no additive effect on CFTRinh-172-induced HCO3^- loss and PV changes during CO2 exposure (Figs. 2, A–D), suggesting that a DIDS-sensitive anion exchanger is not involved in the effect of CFTR inhibition. Furthermore, DIDS and CFTRinh-172 had similar effects during CO2 exposure. DIDS did not alter CO2 absorption, but rather increased HCO3^- loss and inhibited PV acidification and PCO2 increase (Fig. 3, A–D). Nevertheless, these DIDS-related effects were not affected by S3226 (10 μM), suggesting that its effects on CO2/HCO3^- movement occurred by a different mechanism than by CFTR inhibition. The differential expression pattern of SLC26A3 (DRA) and SLC26A6 (PAT1) along the intestinal longitudinal axis (duodenum to colon) and crypt-villous axis (35, 39) and their differential sensitivity to DIDS (SLC26A3 is DIDS insensitive, whereas SLC26A6 is DIDS sensitive) (7, 30) suggest that DIDS may preferentially inhibit duodenal SLC26A6. Since luminal DIDS ≥0.5 mM likely inhibits the basolateral NBC as previously reported (2), we also examined the effect of DIDS (0.5 mM) on CO2/HCO3^- movement. Since the effect of 0.5 mM DIDS was the same as the effect of 0.1 mM DIDS (data not shown), DIDS presumably inhibits the apical SLC26A (presumably A6) anion exchanger and the basolateral NBC at the concentration used.

Taken together, during high CO2 exposure, CFTR and apical anion exchangers are involved in HCO3^- secretion and PV acidification, but NHE3 is not. In contrast, CFTR inhibition activates NHE3 to secrete H^+, which facilitates CO2 absorption, presumably by neutralizing HCO3^- in the microclimate.

**Luminal acid exposure.** Since the duodenal lumen can be acidic in addition to being hypercarbic, we next measured Δ[total CO2] and Δ[H^+] during acid challenge. Note that, since [CO2] and [HCO3^-] in the perfusate are ~0, and with pH < 4, [total CO2] ∼ [CO2], the positive Δ[total CO2] is equivalent to HCO3^- output or secretion. As shown in Fig. 4, A and B, we measured net changes in total CO2 concentration (Δ[total CO2]) and [H^+] (Δ[H^+]) at t = 30 min. Since the pH 7.0 saline and pH 2.2 saline (acid solution) perfusates contained [CO2] ∼ 0, the positive Δ[total CO2] measured during perfusion of pH 7.0 saline or pH 2.2 saline was consistent with HCO3^- secretion during the basal condition or during exposure to luminal acid,
respectively. Nevertheless, $\Delta$[total CO$_2$] was diminished, and $\Delta[H^+]$ was negative during acid exposure compared with perfusion of pH 7.0 saline (Fig. 4, A and B). This is similar to our previous reports (1, 27) and consistent with partial CO$_2$ loss or diminution of HCO$_3$ secretion and H$^+$ loss during acid perfusion. Note that, in most publications in which duodenal HCO$_3$ secretion is measured, HCO$_3$ secretion is increased at least 10 min after the termination of acid perfusion, where it is measured as titratable alkalinity of a HCO$_3$-free perfusate, unlike what we report here (see Ref. 1). Perfusion with pH 2.2 saline was accompanied by PV acidification and PCO$_2$ increase (Fig. 4, C and D), corresponding to transmucosal CO$_2$ or H$^+$ absorption.

CFTRinh-172 had no effect on HCO$_3$ or CO$_2$ movement during the basal period, again showing that CFTRinh-172 has no effect on basal HCO$_3$ secretion. Nonetheless, CFTRinh-172 diminished the decrease of total CO$_2$ output and the increase of H$^+$ loss during acid perfusion (Fig. 4, A and B) and diminished PV acidification (Fig. 4, C and D), suggesting that CFTR is positively involved in the transmucosal movement of CO$_2$/H$^+$ during acid exposure. Despite having no significant effect on total CO$_2$ output (Fig. 5A), DIDS reduced H$^+$ loss (Fig. 5B) and inhibited PV acidification during acid exposure (Fig. 5, C and D). In contrast, S3226, which had no effect on the acid-induced H$^+$ loss or PV acidification (Fig. 5, B–D), increased total CO$_2$ output, similar to the previously observed augmented HCO$_3$ secretion during NHE inhibition (15).

These results suggest that, during luminal acid perfusion, HCO$_3$ secretion and transmucosal CO$_2$ and H$^+$ movement are mediated by CFTR and DIDS-sensitive transporters but not by NHE3.
Effect of CFTR inhibition on epithelial cell pH during luminal CO₂ exposure. Since CO₂ acidifies cells, we next measured pH under the same conditions to measure CO₂ movement into the enterocytes. During CO₂ challenge, epithelial cell pH fell, followed by recovery and overshoot (reaching over the baseline) after CO₂ removal (Fig. 6A) and was accompanied by a hyperemic response (Fig. 6B) as previously described (3). Coperfusion of S3226 with the high CO₂ solution had no effect on CO₂-induced intracellular acidification and CO₂-induced hyperemia (Fig. 6, A and B), consistent with a prior report (27). CFTRinh-172 pretreatment reduced CO₂-induced acidification during the challenge period and eliminated the overshoot during the recovery period (Fig. 7A), confirming our early observations that CFTR dysfunction reduces acid-induced cellular acidification (18). Furthermore, CFTRinh-172 prevented CO₂-induced hyperemia (Fig. 7B), probably due to the inhibition of intracellular acidification, which is necessary to trigger a hyperemic response (3). With CFTR inhibition, S3226 reversed the effect of CFTRinh-172 during the CO₂ challenge period, followed by a delayed pH recovery (Fig. 7A) and a delayed hyperemic response to CO₂ perfusion (Fig. 7B). CFTR inhibition during CO₂ challenge might thus activate apical NHE3 to secrete H⁺, consistent with the reciprocal activation of NHE3 during CFTR inhibition. The small magnitude of cellular acidification in this instance appeared insufficient to induce a hyperemic response. Simultaneous inhibition of CFTR and NHE3 during CO₂ challenge enabled CO₂ diffusion into the cells, which acidified cells

**Fig. 4.** Effect of CFTRinh-172 on luminal acid challenge in rat duodenum. A and B: luminal changes. A: net changes in total CO₂ concentration (Δ[total CO₂]). B: net changes in H⁺ concentration (Δ[H⁺]). C and D: PV changes. C: pH; D: PCO₂. All data were obtained 10 min after completion of the challenge period (t = 30 min). Each data point is expressed as mean ± SE (n = 6). *P < 0.05 vs. pH 7.0 saline group, †P < 0.05 vs. pH 2.2 saline group.

**Fig. 5.** Effect of DIDS and S3226 on luminal acid challenge in rat duodenum. A and B: luminal changes. A: Δ[total CO₂]; B: Δ[H⁺]. C and D: PV changes. C: pH; D: PCO₂. All data were obtained 10 min after completion of the challenge period (t = 30 min). Each data point is expressed as mean ± SE (n = 6). *P < 0.05 vs. pH 7.0 saline group, †P < 0.05 vs. pH 2.2 saline group.


similarly to the CO₂ alone group. This observation, compared with the observed lack of CO₂ loss and PV acidification in this condition (shown in Fig. 1), suggests that simultaneous CFTR and NHE3 inhibition affected basolateral acid-base transporters, as was previously suggested (28).

**DISCUSSION**

We demonstrated that CFTR inhibition induced NHE3-dependent HCO₃⁻ absorption from the lumen during CO₂ challenge but inhibited CO₂/H⁺ absorption and PV acidification during acid challenge. Furthermore, DIDS augmented HCO₃⁻ absorption during CO₂ exposure independent of NHE3 activation, and inhibited CO₂/H⁺ absorption and PV acidification during acid exposure. NHE3 inhibition alone had no effect on transmucosal CO₂/H⁺ movement during acid or CO₂ exposure, as previously demonstrated (27). This is the first study indicating that CFTR inhibition activates NHE3-related transport activity during CO₂ exposure in vivo in rat duodenum. Since NHE3 inhibition activates CFTR in vivo (15), our results are consistent with the reciprocal activation of CFTR and NHE3 in rat duodenum. Moreover, since CFTR inhibition and DIDS both inhibit stimulated duodenal HCO₃⁻ secretion (2, 4), our study suggests that HCO₃⁻ secretion is essential for the absorption of CO₂/H⁺ from the duodenal lumen into the PV.

To explain the movement of CO₂ into and out of the duodenal mucosa, we have postulated a model (Fig. 8) in which the erythrocyte-based Jacobs-Stewart cycle (22) is present across the apical brush-border membrane of duodenal epithelial cells (3, 27). Luminal H⁺ reacts with HCO₃⁻ secreted by the epithelium, generating CO₂. CO₂ traverses the apical membrane into the cytoplasm and is hydrated to H₂CO₃ by cytosolic CA, acidifying the cell while dissociating into H⁺ and HCO₃⁻. H⁺ is extruded via the basolateral NHE1 into the subepithelium, activating acid sensors such as the transient receptor potential vanilloid-1 to increase blood flow. Subepithelial H⁺ (or CO₂) diffuses into the PV, acidifying the portal blood. Plasma HCO₃⁻ is transported to the subepithelium, where it is taken up by the basolateral NBC into the enterocyte cytoplasm, its amount dependent on blood flow. After uptake, intracellular HCO₃⁻ exits via apical ion transporters such as CFTR and SLC26A family (Fig. 8A). Membrane-bound apical CA facilitates the interconversion of luminal CO₂ and HCO₃⁻. The Jacobs-Stewart cycle-based model predicts that the inhibition of epithelial HCO₃⁻ secretion inhibits CO₂ diffusion from lumen to PV by depleting the microclimate of titratable HCO₃⁻. CFTR inhibition reversed HCO₃⁻ secretion to net HCO₃⁻ absorption during exposure to luminal high CO₂, with impairment of PV acidification. This HCO₃⁻ absorption during acid inhibition can be attributed to the activity of an apical anion exchanger, presumably SLC26A6, which might absorb HCO₃⁻ due to its 2 HCO₃⁻:1 Cl⁻ stoichiometry under favorable gradients. The other major possibility is that H⁺ secreted by NHE3 is neutralized by luminal HCO₃⁻ to CO₂, which then diffuses into the cells, measured as net HCO₃⁻ absorption. In both cases, there is no actual transmucosal H⁺ movement and thus no measurable PV acidification. Since NHE3 inhibitor reversed, but DIDS had no effect on CFTR inhibition-related enhanced HCO₃⁻ absorption, the most likely of these possibilities is that HCO₃⁻ is absorbed across the apical membrane by titration to CO₂ by H⁺ secreted by NHE3 (Fig. 8B). In the

![Fig. 6. Effect of NHE3 inhibition on CO₂-induced intracellular acidification and hyperemia in rat duodenal epithelium. A: intracellular pH (pHi). Each data point is expressed as mean ± SE (n = 6). *p < 0.05 vs. pH 6.4 saline group. B: blood flow. Each data point is expressed as mean ± SE (n = 6). *p < 0.05 vs. pH 6.4 saline group.](http://apjg.physiology.org/)

![Fig. 7. Effect of CFTR inhibition on CO₂-induced intracellular acidification and hyperemia in rat duodenal epithelium. A: pH. Data are expressed as mean ± SE (n = 6). *p < 0.05 vs. high CO₂ group. †p < 0.05 vs. high CO₂ group. ‡p < 0.05 vs. high CO₂ group. B: blood flow. *p < 0.05 vs. pH 6.4 saline group. †p < 0.05 vs. high CO₂ group. ‡p < 0.05 vs. CFTRinh-172 + CO₂ group.](http://apjg.physiology.org/)
absence of CFTR inhibition, DIDS increased HCO$_3^-$ absorption, which was unaffected by NHE3 inhibition, suggesting that inhibition of HCO$_3^-$ secretion itself is not sufficient to activate NHE3. Another possibility is that DIDS inhibits basolateral NBC activity, lowering intracellular [HCO$_3^-$], increasing the gradient for HCO$_3^-$ uptake from lumen to cell (2). Furthermore, although S3226 increased HCO$_3^-$ secretion via CFTR activation (15), S3226 has no effect on CO$_2$/HCO$_3^-$ movement during CO$_2$ exposure (27), suggesting that the presence of luminal HCO$_3^-$ or CO$_2$ may mask S3226-induced HCO$_3^-$ secretion. Therefore, DIDS most likely impairs basolateral HCO$_3^-$ uptake due to NBC inhibition, diminishing the effect of S3226-induced CFTR activation on HCO$_3^-$ secretion.

During acid challenge, impairment of HCO$_3^-$ secretion by CFTR inhibition or by DIDS reduced H$^+$ loss from the lumen and reduced transmucosal H$^+$ absorption, supporting our hypothesis that HCO$_3^-$ secretion is essential for net H$^+$ absorption through the duodenal mucosa. This also confirms the presence of a transapical Jacobs-Stewart cycle (Fig. 8, C and D).

Another means of demonstrating a CFTR-NHE3 interaction is to examine the effect of CFTR or NHE3 inhibition on epithelial cell pH$_i$. In the pH$_i$ study, CO$_2$-induced intracellular acidification was reduced by CFTRinh-172 pretreatment and was increased by NHE3 inhibition, suggesting that CFTR inhibition activated NHE3 function, supporting our hypothesis. Nevertheless, CO$_2$-induced intracellular acidification was not associated with PV acidification or with PV CO$_2$ increase when CFTR and NHE3 were inhibited simultaneously. Furthermore, delayed recovery of pH$_i$ and a delayed hyperemic response to luminal CO$_2$ during dual inhibition of CFTR and NHE3 suggest...

Fig. 8. Mechanisms of CO$_2$/H$^+$ movement across the apical membrane of duodenal epithelium. A and B: high CO$_2$ exposure. A: luminal CO$_2$ diffuses into and acidifies epithelial cells due to cytosolic carbonic anhydrase (CA) activity. The generated H$^+$ exits via basolateral NHE1 into the subepithelium, acidifying the PV blood. HCO$_3^-$, generated by cellular CA and entering via the basolateral NBC, exits via CFTR/SLC26A into the preepithelial layer, creating an alkaline microclimate, followed by conversion to CO$_2$ by extracellular CA. Generated CO$_2$ rediffuses into the mucosa, completing the Jacob-Stewart cycle across the apical membrane. Finally, effluent pH increases due to the presence of secreted HCO$_3^-$ and absorbed CO$_2$. In this condition, apical NHE3 is inactive or its activity is masked (see Ref. 27). B: under CFTR inhibition or dysfunction, CO$_2$ diffusion is followed by H$^+$ secretion via activated or unmasked NHE3 activity, creating an acidic microclimate in the preepithelial layer, damping intracellular acidification. Secreted H$^+$ reacts with luminal HCO$_3^-$, producing CO$_2$, which rediffuses into the cell. Since luminal HCO$_3^-$ is consumed and CO$_2$/H$^+$ does not enter the submucosal space or the PV, HCO$_3^-$ is absorbed, decreasing effluent [CO$_2$] and [HCO$_3^-$], with unchanged PV blood gas. The destination of generated, cytosolic HCO$_3^-$ remains unknown. C and D: acid exposure. C: HCO$_3^-$ secreted via CFTR/SLC26A reacts with luminal H$^+$, generating CO$_2$, which diffuses into and acidifies epithelial cells. Generated H$^+$ exits via NHE1, acidifying the PV. Cytosolic HCO$_3^-$ exits via CFTR/SLC26A, regenerating luminal CO$_2$ again, completing the Jacobs-Stewart cycle. Finally, effluent pH increases due to net H$^+$ absorption (CO$_2$ absorption combined with HCO$_3^-$ secretion). Note that in an acidic solution effluent [total CO$_2$] = measured [CO$_2$], which corresponds to secreted HCO$_3^-$ minus absorbed CO$_2$. D: under CFTR inhibition, reduced HCO$_3^-$ generates less CO$_2$, reducing CO$_2$ diffusion into the epithelial cells, which are less acidified (2, 18). In this condition, apical NHE3 is inactive or masked due to high luminal [H$^+$].

G1325 CFTR INHIBITION AND NHE3 ACTIVATION AJP-Gastrointest Liver Physiol • VOL 294 • JUNE 2008 • www.ajpgi.org
gest that disruption of apical ion transporters affects the function of basolateral acid-base transporters such as NHE1. NHE1 is the major pH regulator, implicated in the mechanism of acid and CO₂-induced hyperemia (3, 5) and in CO₂/H+ movement from the lumen to PV (27). Luminal CO₂ similarly acidified the cells during inhibition of CFTR and NHE3 but did not increase blood flow, despite intracellular acidification, also suggesting disruption of basolateral H+ extrusion or submucosal chemosensing.

When luminal pH is neutral, NHE3 inhibition activates CFTR to secrete HCO₃⁻, whereas, during CO₂ challenge, CFTR inhibition activates NHE3 to secrete H⁺, facilitating CO₂ absorption. These findings indicate reciprocal activation of NHE3 and CFTR. CFTR and NHE3 interact via a shared regulatory complex consisting of NHE regulatory factors, ezrin, and PKA (11, 41). It is unclear, however, whether CFTR and NHE3 are coexpressed in the same cells. CFTR is expressed much less than NHE3 since CFTR is located predominantly in the crypts and basal parts of the villi (6), whereas NHE3 is most strongly expressed in the villous cells (19).

Traditionally, the duodenum is considered to be a net HCO₃⁻ secretor, although most prior measurements were made using HCO₃⁻-free perfusates. This created an exaggerated serosal-to-lumen HCO₃⁻ gradient, rather than the reduced outward gradient formed when physiological [HCO₃⁻] (~16 mM) and [CO₂] (~8 mM) are present in the perfusate (20, 32, 37). In the presence of a more physiological plasma-to-lumen HCO₃⁻ gradient, we demonstrated that net HCO₃⁻ absorption can occur. This absorption is facilitated by NHE3-mediated H⁺ secretion only when CFTR is inhibited, again indicating that CFTR inhibition upregulates NHE3 activity. The HCO₃⁻ absorptive mechanism of the renal proximal tubule is also dependent on NHE3-dependent H⁺ secretion (8). We speculate that this reciprocal functional activation of CFTR and NHE3 regulates microclimate pH of the duodenal epithelium and may explain defective cellular acidification associated with CFTR dysfunction (2, 18).

We previously hypothesized that defective cellular acidification associated with CFTR dysfunction explains the observed scarcity of duodenal ulceration in CF patients, despite the presence of impaired pancreatic and duodenal HCO₃⁻ secretion (18). We had previously speculated that the impaired apical HCO₃⁻ secretion, coupled with intact basolateral HCO₃⁻ uptake, traps HCO₃⁻ in the cytosol and raises pHi. Augmented NHE3 function could prevent further acidification in the presence of a luminal acid load.

In conclusion, CFTR and NHE3 are reciprocally regulated; CFTR inhibition unmasks NHE3 activity particularly during luminal CO₂ rather than during H⁺ stress. Epithelial HCO₃⁻ secretion is essential for transapical epithelial CO₂ and H⁺ absorption in rat duodenal mucosa by the Jacobs-Stewart cycle.

ACKNOWLEDGMENTS

We thank Rebecca Cho and Jenifer Kugler for their assistance with manuscript preparation.

GRANTS

This work was supported by a Department of Veterans Affairs Merit Review Award, National Institutes of Health-National Institute of Diabetes and Digestive and Kidney Diseases R01 DK54221 (J. Kaunitz), and the animal core of National Institutes of Health-National Institute of Diabetes and Digestive and Kidney Diseases P30 DK0413 (J. Rozengurt).

REFERENCES


