Human duodenal mucosal brush border $\text{Na}^+$/H$^+$ exchangers NHE2 and NHE3 alter net bicarbonate movement

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Human duodenal mucosal brush border $\text{Na}^+$/H$^+$ exchangers NHE2 and NHE3 alter net bicarbonate movement. Am J Physiol Gastrointest Liver Physiol 281: G159–G163, 2001.—The proximal duodenal mucosa secretes $\text{HCO}_3^-$ that serves to protect the epithelium from injury. In isolated human duodenal enterocytes in vitro, multiple luminal membrane proteins are involved in acid/base transport. We postulated that one or more isoforms of the $\text{Na}^+$/H$^+$ exchanger (NHE) family is located on the apical surface of human duodenal mucosal epithelial cells and thereby contributes to duodenal mucosal $\text{HCO}_3^-$ transport. Duodenal biopsies were obtained from human volunteers, and the presence of NHE2 and NHE3 was determined by using previously characterized polyclonal antibodies (Ab 597 for NHE2 and Ab 1381 for NHE3). In addition, proximal duodenal mucosal $\text{HCO}_3^-$ transport was measured in humans in vivo in response to luminal perfusion of graded doses of amiloride; $10^{-5}$–$10^{-4}$ M amiloride was used to inhibit NHE2 and $10^{-3}$ M amiloride to inhibit NHE3. Both NHE2 and NHE3 were localized principally to the brush border of duodenal villus cells. Sequential doses of amiloride resulted in significant, step-wise increases in net duodenal $\text{HCO}_3^-$ output. Inhibition of NHE2 with $10^{-5}$ M and $10^{-4}$ M amiloride significantly increased net $\text{HCO}_3^-$ output. Moreover, there was an additional, equivalent increase ($P < 0.05$) in duodenal $\text{HCO}_3^-$ output with $10^{-3}$ M amiloride, which inhibited NHE3. We conclude that 1) NHE2 and NHE3 are localized principally to the brush border of human duodenal villus epithelial cells; 2) sequential inhibition of NHE2 and NHE3 isoforms resulted in step-wise increases in net $\text{HCO}_3^-$ output; 3) NHE2 and NHE3 participate in human duodenal villus cell $\text{HCO}_3^-$ transport; and 4) the contribution of NHE-related transport events should be considered when studying duodenal $\text{HCO}_3^-$ transport processes.

Sodium/hydrogen exchange; sodium/hydrogen exchangers; duodenum; intestine; transport

The duodenal mucosa of all mammalian species secretes $\text{HCO}_3^-$ with a steep proximal-to-distal gradient (3, 10). Surface epithelial $\text{HCO}_3^-$ secretion is involved in preventing mucosal acid peptic damage (8). Moreover, duodenal ulcer patients infected with Helicobacter pylori have diminished basal and stimulated $\text{HCO}_3^-$ secretion that normalizes after H. pylori eradication (11). Duodenal mucosa in vitro obtained from patients with cystic fibrosis has decreased resting and cAMP-stimulated $\text{HCO}_3^-$ secretion (18). Thus duodenal mucosal bicarbonate secretion (DMBS) is important in both health and disease. Similar to other cells that are involved in ion transport, duodenal enterocytes contain apical and basolateral membrane transporters that serve to maintain the intracellular pH near 7.1 (1). To date, the acid/base transporters that have been identified in animal and human duodenal enterocytes include 1) an amiloride-sensitive $\text{Na}^+$/H$^+$ exchanger (NHE) that functions largely as an acid extruder; 2) a stilbene-sensitive NaHCO$_3$ cotransporter that functions as a base loader; and 3) a stilbene-sensitive Cl$^-$/HCO$_3^-$ antiporter that is a base extruder (1, 2). Additionally, the cystic fibrosis transmembrane regulator (CFTR) has also been implicated in $\text{HCO}_3^-$ secretion, in addition to Cl$^-$ conductance (17, 20). There are, however, limited in vivo human studies that integrate mucosal structure with the function of acid/base transporters. Nyberg et al. (16) demonstrated in humans that PGE$_2$-stimulated DMBS was decreased significantly by putative blockade of the Cl$^-$/HCO$_3^-$ exchanger with luminal perfusion of the stilbene derivative DIDS, whereas theophylline-stimulated DMBS (presumably acting by inhibition of cyclic nucleotide phosphodiesterases and thereby increasing cAMP content) was unaltered. These findings suggest that separate luminal transport mechanisms are involved in human PGE$_2$- and cAMP-stimulated duodenal HCO$_3^-$ transport.

The present study was based on the hypothesis that one or more of the epithelial, amiloride-sensitive NHE isoforms is located and functional on the human duodenal enterocyte apical surface. Thus immunofluorescence studies for NHE2 and NHE3 were performed on proximal duodenal mucosal biopsies. Furthermore, the effect of graded doses of amiloride that selectively...
inhibit NHE2 and NHE3 were determined. We observed that 1) both NHE2 and NHE3 are located principally on the apical surface of human duodenal villus cells and 2) suppression of NHE2 activity ($10^{-5}$–$10^{-4}$ M amiloride) significantly increased net duodenal HCO$_3^-$ output; moreover, inhibition of NHE3 (by $10^{-3}$ M amiloride) resulted in an additional significant increase in HCO$_3^-$ output.

METHODS

Subjects. Five male subjects aged 32–55 yr participated in these studies. Each was in excellent health, taking no medications, and free of any acute or chronic disease. The experimental protocol was approved by the University of California at San Diego Human Subjects Committee, and each subject gave signed informed consent. Duodenal biopsies and measurement of DMBS were performed on days separated by at least 2 wk.

Duodenal mucosal biopsies and immunofluorescence of NHE2 and 3. Two to three endoscopic biopsies (Radial Jaw, Microvasive; Boston Scientific, Watertown, MA) were obtained in the midportion of the duodenal bulb. Tissues were fixed in 3% neutral buffered formaldehyde, processed on a Technicon, and embedded in paraffin. Immunohistochemistry was performed as described previously (12). After being embedded in paraffin, sections were cut at 4 µm and mounted on gelatin-coated slides. The slides were dewaxed in xylene, rehydrated in ethanol, and rinsed in PBS buffer; endogenous peroxide was blocked by incubation in 0.3% H$_2$O$_2$ in methanol. The slides were blocked in PBS buffer (1% NFDM) with goat serum and incubated overnight at 4°C with primary rabbit polyclonal antibodies Ab 597 (rabbit polyclonal anti-GST-C terminal 87 AA of NHE2) or Ab 1381 (rabbit polyclonal anti-GST-C terminal 85 AA of NHE3) or, as control, secondary antibody alone (goat anti-rabbit IgG; Vector Laboratories, Burlingame, CA). Each of these antibodies has been characterized previously (12, 21). Labeling was visualized by light microscopy and horseradish peroxidase by experts in this technique using the Vectostain ABC kit (Vector Laboratories, Burlingame, CA).

Duodenal mucosal HCO$_3^-$ secretion. Proximal duodenal mucosal net HCO$_3^-$ output (i.e., by the duodenal bulb; DMBS) was measured with well-documented and validated methods described previously (11, 13, 14). In brief, 3 cm of proximal duodenum was isolated by two balloons that straddled the pylorus and a third that was 3 cm beyond. The isolated segment was perfused with 154 mM NaCl at 2 ml/min containing $^{14}$C-labeled polyethylene glycol as a nonabsorbable marker, and the effluent was collected by gravity. HCO$_3^-$ concentration ([HCO$_3^-$]) was determined in duplicate on samples obtained anaerobically. Measurements of pH and Pco$_2$ were obtained (IL 1420, BG3 PCo$_2$ electrode, blood gas analyzer; Instrument Laboratories, Lexington, MA), and [HCO$_3^-$] was calculated by the Henderson-Hasselbalch equation (11, 13, 14). HCO$_3^-$ outputs were calculated as the product of [HCO$_3^-$] times the $^{14}$C polyethylene glycol-corrected volume (>85% of the infusates were recovered). Gastric and distal duodenal markers (phenol red and trypsin, respectively) were also infused continuously to assess for potential contamination of the isolated segment (11, 13, 14).

After measurement of basal HCO$_3^-$ secretion for three 15-min periods, graded doses of amiloride ($10^{-5}$, $10^{-4}$, and $10^{-3}$ M; Sigma, St. Louis, MO) were infused into the test segment in increasing concentrations, each for 30 min, and the effects on net HCO$_3^-$ output were determined. Moreover, additional control experiments were performed on a separate day in three subjects to determine whether there were time-related alterations in HCO$_3^-$ secretion; thus isomolar NaCl was infused alone for the 155-min test period.

Statistics. Data are presented as means ± SE and 95% confidence intervals (CI). Results were analyzed by repeated-measures analysis of variance and the Tukey-Kramer multiple-comparisons test. P values <0.05 were considered significant.

RESULTS

Immunofluorescence of NHE2 and NHE3. Light microscopic sections of the duodenal biopsies were examined by using previously characterized polyclonal antibodies raised in rabbits. These antibodies had been shown to demonstrate the presence of NHE2 and NHE3 in multiple species, for example, in intestine (human [jejunum, ileum, and colon], rat [ileum and colon], chicken [small intestine and colon], and mouse [ileum and colon]) (22–24). As shown in Fig. 1, NHE2 and NHE3 were present principally in the brush border of villus cells from human proximal duodenum; a modest amount of staining extended into the upper crypt region. Neither NHE2 nor NHE3 was present on the basolateral surface of any epithelial cells. In each of the five subjects studied the immunofluorescence patterns were identical. In addition, no staining for either NHE2 or NHE3 was observed in the absence of primary antibody.

Duodenal HCO$_3^-$ secretion. Basal DMBS was 355 ± 19 µmol·cm$^{-1}$·h$^{-1}$ (95% CI: 300–410 µmol·cm$^{-1}$·h$^{-1}$). Amiloride resulted in significant (P < 0.003) concentration-dependent increases in duodenal HCO$_3^-$ output compared with basal output (Fig. 2). The net mean (95% CI) incremental increases above baseline in response to $10^{-5}$, $10^{-4}$, and $10^{-3}$ M amiloride were 47 (11–82), 67 (32–103), and 111 (75–146) µmol·cm$^{-1}$·h$^{-1}$, respectively. In addition, although DMBS in response to each dose of amiloride was significantly greater than basal secretion, the DMBS response to $10^{-3}$ M amiloride was significantly greater than the response to either the $10^{-5}$ M or the $10^{-4}$ M infusion, which were not significantly different from one another. Furthermore, DMBS in the subjects in whom an additional NaCl infusion control test was performed revealed that basal HCO$_3^-$ secretion was 336 (CI 289–383) µmol·cm$^{-1}$·h$^{-1}$ and decreased only modestly to 306 (242–370) µmol·cm$^{-1}$·h$^{-1}$ (P = not significant) during the final 30 min.

In addition, HCO$_3^-$ concentration in the duodenal effluent (the infused was nominally HCO$_3^-$ free) increased significantly from basal concentration in response to $10^{-4}$ M and $10^{-3}$ M amiloride, and the effluent volume also increased modestly (Table 1). The increase in HCO$_3^-$ concentration was caused primarily by an increase in Pco$_2$ (basal: 13.70 ± 0.59 mmHg, $10^{-3}$ M amiloride: 15.75 ± 0.67 mmHg; P < 0.02). Thus, during amiloride perfusion, Pco$_2$, [HCO$_3^-$], and net volume increased. An increase in effluent pH also occurred but did not attain statistical significance (basal: 7.22 ± 0.01, $10^{-3}$ M amiloride: 7.30 ± 0.02; P = 0.08).

DISCUSSION

The results of this study indicate that NHE2 and NHE3 are localized principally to the apical membrane of human proximal duodenal villus cells and that the
sequential inhibition of each NHE by amiloride results in stepwise and significant increases in net duodenal $\text{HCO}_3^-$ output. These findings indicate that NHE2 and NHE3 affect luminal duodenal pH by altering $\text{HCO}_3^-$ secretion and that human duodenal villus cell function contributes to net duodenal $\text{HCO}_3^-$ transport. Thus, similar to the mediation of renal proximal tubule $\text{HCO}_3^-$ absorption by apical membrane $\text{Na}^+/\text{H}^+$ exchange, duodenal $\text{HCO}_3^-$ transport is also influenced by apical NHEs (19). This process is likely caused by proton transport into the lumen via $\text{Na}^+/\text{H}^+$ exchange, neutralizing $\text{HCO}_3^-$ and resulting in $\text{H}_2\text{O}$ and $\text{CO}_2$ production, the latter being partially permeable across the cell membrane. The intracellular $\text{CO}_2$ is then catalyzed by carbonic anhydrases to produce intracellular $\text{HCO}_3^-$ and $\text{H}^+$, which in turn are available for transport by their respective membrane acid/base antiporters. A potential alternative process would be inhibition of apically located NHEs resulting in intracellular acidification (due to decreased $\text{Na}^+/\text{H}^+$ exchange), thereby activating basolateral $\text{NaHCO}_3$ cotransport resulting in increased $\text{HCO}_3^-$ entry and transport into the lumen. However, it is also possible that the basolateral “housekeeping” NHE1 would transport $\text{H}^+$ out of the cell before activation of $\text{NaHCO}_3$ cotransport.

In health there is a sharp pH gradient from the non-acid-secreting antral portion of the stomach, where the luminal pH may be as low as 0.85, and the most proximal duodenum, where the luminal pH is near neutral, except for brief ($\sim30$ s) transient periods when the pH decreases to $\sim2–3$ (5, 9). The mechanisms that contribute to duodenal neutrality are $\text{HCO}_3^-$ secretion by the proximal duodenal mucosa and pancreaticobiliary $\text{HCO}_3^-$ (25, 26). This study indicates that intraduodenal $\text{HCO}_3^-$ concentration is affected by $\text{Na}^+/\text{H}^+$ exchange that can result in neutralization of...
luminal HCO$_3^-$. It is possible that under physiological conditions the exchangers may change direction. That is, when the lumen is very acidic, as occurs in the bulb, NHEs may take up protons and transport HCO$_3^-$ and Na$^+$ into the lumen. However, it has been demonstrated that a pH gradient is present in the in vivo duodenum and that the juxtamucosal pH is ~7 (3).

The cellular events that result in net HCO$_3^-$ secretion by the intestine, as well as other organs, have been the subject of intense study (11). In the intestine, the key transporters involved in HCO$_3^-$ transport across the apical membrane are 1) the Cl$^-$/HCO$_3^-$ exchanger, 2) CFTR, and, as this study demonstrates, 3) NHE2 and NHE3. CFTR conducts Cl$^-$ that can be exchanged after secretion into the lumen across the apical membrane for intracellular HCO$_3^-$ by the brush border Cl$^-$/HCO$_3^-$ exchanger. In addition, there is accumulating evidence that CFTR also has a HCO$_3^-$ conductance. There are fewer HCO$_3^-$ transporters across the basolateral membrane; HCO$_3^-$ enters the cell principally via the basolateral NaHCO$_3$ cotransporter (12, 22). However, the location of an anion exchanger in the duodenum is in question. A recent report by Alper et al. (4) describes in mice that AE2 immunostaining of enterocytes was restricted to a basolateral distribution. Further work is needed to delineate membrane transporters in human duodenum.

These findings implicate NHE2 and NHE3 in the overall regulation of human proximal DMBS. The inhibitory constant values of NHE2 and NHE3 to amiloride are ~1 and 90 μM, respectively (23, 24). Thus 10$^{-5}$ M and 10$^{-4}$ M amiloride inhibited NHE2 but had minimal if any effect on NHE3 in the presence of 154 mM Na$^+$, whereas 10$^{-3}$ M amiloride inhibited NHE3 (27). The amiloride dose-dependent increases in net HCO$_3^-$ secretion (due to inhibition of H$^+$ transport caused by NHE2 and NHE3) and apical location of both NHE isoforms indicate that both NHE2 and NHE3 can contribute to overall duodenal HCO$_3^-$ output. Although it would be prudent to use more specific NHE3 and NHE2 inhibitors, such as S3226 and HOE694, their use in humans has not been approved. Amiloride-sensitive Na$^+$ uptake is a widely used definition of Na$^+$/H$^+$ exchange in settings in which no other recognized amiloride-sensitive Na$^+$ uptake processes are present. Whereas the epithelial Na$^+$ channel is in the colon, this transporter has not been localized in duodenum. Therefore, the amiloride-sensitive Na$^+$ uptake processes appear to be limited to NHEs.

The relative amounts of NHE-induced CO$_2$ absorption and HCO$_3^-$ secretion can be estimated from these studies in which basal net HCO$_3^-$ secretion (which is made up of both basal CO$_2$ absorption and HCO$_3^-$ secretion) was 355 μmol·cm$^{-1}$·h$^{-1}$. Assuming that 10$^{-3}$ M amiloride blocks both apical NHEs and CO$_2$ absorption, it can be estimated that basal CO$_2$ absorption accounts for ~111 μmol·cm$^{-1}$·h$^{-1}$ of HCO$_3^-$, because this represents the net increase in HCO$_3^-$ secretion above basal levels caused by the 10$^{-3}$ M amiloride. Furthermore, it can be estimated that basal HCO$_3^-$ secretion is ~466 μmol·cm$^{-1}$·h$^{-1}$, calculated from the net basal HCO$_3^-$ transport rate of 355 μmol·cm$^{-1}$·h$^{-1}$ plus that estimated to be due to CO$_2$ absorption, 111 μmol·cm$^{-1}$·h$^{-1}$. Thus the contribution of CO$_2$ absorption by NHEs is estimated to be ~24% of the basal HCO$_3^-$ secretory rate. Moreover, these studies suggest that NHE2 and NHE3 contribute approximately
equally to basal HCO$_3^-$ secretion. Additional studies are required to determine whether the increase in HCO$_3^-$ transport was caused by increased HCO$_3^-$ conductance and/or by Cl$^-$/HCO$_3^-$ exchange.

Thus the results suggest that both NHE2 and NHE3 contribute to human duodenal intestinal Na$^+$ absorption. Although this was observed in rabbit ileum, in which both apical NHE2 and NHE3 contributed approximately equally to basal Na$^+$ absorption (27), in other species one or another NHE may predominate in a given intestinal segment. For instance, in chicken small intestine and colon, NHE2 and not NHE3 accounts for the majority of basal Na$^+$ absorption (6). Although the contribution of apical NHE2 versus NHE3 in human intestinal Na$^+$ or proton-induced CO$_2$ absorption has not been compared in other intestinal segments, both are present in the brush border of villus cells in human jejenum, ileum, and colon, although in the colon NHE2 message is considerably larger (7).

Given the importance of understanding the regulation of duodenal HCO$_3^-$ secretion in health and disease, it now becomes important to recognize that regulation of both HCO$_3^-$ secretory and absorptive processes must be considered and examined separately. Moreover, in carrying out studies of HCO$_3^-$ secretion, experiments must be done in a way that the HCO$_3^-$ absorptive and secretory processes can be measured separately.

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