Identification of an apical Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger in gastric surface mucous and duodenal villus cells

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anion exchanger isoform 4; chloride/bicarbonate exchange; parietal cells; mucous cells; acid injury

ONE OF THE MAJOR MECHANISMS for protection of the surface epithelium of the stomach from injury by gastric acid is the presence of a layer of mucus that contains HCO\(_3\)\(^{-}\) (5, 8, 28), both of which are secreted by mucous cells. As acid diffuses into the mucus gel, it is neutralized by the secreted HCO\(_3\)\(^{-}\), resulting in the formation of a gradient in which the pH at the luminal surface of the epithelium is relatively neutral (5). It has been suggested that the HCO\(_3\)\(^{-}\) secreted from surface cells during gastric acid secretion may originate in part from parietal cells (10, 11). Intracellular HCO\(_3\)\(^{-}\) generated during acid secretion, exits the basolateral membrane of the parietal cell via electroneutral Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange (21, 23) and alkalinizes the blood, which perfuses the basal aspect of surface epithelial cells (10, 11). The available evidence suggests that the HCO\(_3\)\(^{-}\) is taken up by mucous cells, most likely via Na\(^{+}/\)HCO\(_3\)\(^{-}\) cotransport (4, 25), and is then secreted across the luminal membrane of surface mucous cells, thereby providing protection from gastric acid.

The mechanism of HCO\(_3\)\(^{-}\) secretion by surface epithelial cells has been studied by several groups but remains controversial. In frog fundic mucosa, HCO\(_3\)\(^{-}\) secretion was reported to have no effect on the trans-epithelial electrical potential and to be dependent on luminal Cl\(^{-}\), suggesting that electroneutral Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange was involved (9). These results have been disputed (31), however, and there is evidence for a HCO\(_3\)\(^{-}\) conductance pathway (4). Nevertheless, electroneutral Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange remains an attractive possibility because the diffusion of H\(^{+}\) and Cl\(^{-}\) into the mucus layer would both neutralize outwardly transported HCO\(_3\)\(^{-}\) and provide Cl\(^{-}\) for inward transport, thereby maintaining a strong driving force for Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange. This hypothesis has received support by the recent finding of a HCO\(_3\)\(^{-}\) secretory mechanism in rat gastric mucosa that is sensitive to DIDS (23), an inhibitor of some of the electroneutral Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchangers and related transporters.

The identity of the apical anion transporter that mediates HCO\(_3\)\(^{-}\) secretion by surface mucous cells is unclear, but members of either the SLC4 (Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchangers and Na\(^{+}/\)HCO\(_3\)\(^{-}\) cotransporters) or SLC26 (anion exchangers, including Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchangers) families are potential candidates. Recently, a Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger of the SLC4 family, termed anion exchanger (AE) isoform 4, was cloned from rabbit kid-
ney cortical collecting duct (32). It exhibits 48% amino acid identity to the NBC1 Na\(^{+}\)-HCO\(_3\) \(^{-}\) cotransporter and 32% identity to the AE2 Cl\(^{-}\)/HCO\(_3\) \(^{-}\) exchanger (32). Functional studies in oocytes and cultured mammalian cells indicated that AE4 mediates electroneutral Cl\(^{-}\)/HCO\(_3\) \(^{-}\) exchange and does not transport Na\(^{+}\) (32). Because of this functional property and despite its greater similarity to the NBC subfamily, it was named AE4.

On the basis of its localization on the apical membrane of β-intercalated cells in the cortical collecting duct, it was postulated that AE4 mediates HCO\(_3\) \(^{-}\) secretion (32).

In the current studies, we investigated the gastrointestinal distribution of AE4, because little is known about the expression of this transporter outside the kidney. Our results indicate that AE4 mRNA is expressed in both stomach and duodenum and that it functions as a Cl\(^{-}\)/HCO\(_3\) \(^{-}\) exchanger when expressed in oocytes. Immunocytochemical studies demonstrate that AE4 is localized on the apical membranes of gastric surface epithelial cells and villi of the duodenum. We propose that AE4 mediates apical HCO\(_3\) \(^{-}\) secretion in gastric surface epithelium and duodenum, thereby protecting these tissues against acid injury.

**EXPERIMENTAL PROCEDURES**

**RT-PCR of AE4 in Mouse and Rabbit Gastrointestinal Tract**

A BLAST search of the mouse expressed sequence tag (EST) database against the rabbit AE4 sequence (GenBank accession no. AB038263) identified a mouse EST (GenBank accession no. AW018362) with a high degree of sequence similarity. On the basis of the cDNA sequence of the mouse EST, primers were designed (sense, CAT GCC TGG TGC TCA AGA AAG CTA G; antisense, CAC TCA TGT TAC TGG GCC TGG TGG) and used for RT-PCR experiments. RT-PCR was performed on RNA isolated from stomach, duodenum, and kidney by using the above primers, which amplified a 412-bp fragment corresponding to nucleotides 66–482 of the EST.

For rabbit AE4, primers (sense, GAA ATG GCC CAC TTG CAC C; antisense, AAT TGA CAG ACG CCA TAG G) were designed based on the rabbit AE4 cDNA sequence (GenBank accession no. AB038263). The primers were used for RT-PCR in various tissues, and a PCR fragment corresponding to nucleotides 992–1595 of the rabbit AE4 cDNA was isolated. The reason for using different sets of primers for mouse and rabbit was because of DNA sequence differences in these two species.

**RNA Isolation and Northern Blot Hybridization**

Total cellular RNA was extracted from mouse duodenum, stomach, and kidney and rabbit gastric parietal and mucus cells using TRI reagent. Hybridization was performed according to the method of Church and Gilbert (3). The membranes were washed, blotted dry, and exposed to a PhosphorImager screen (Molecular Dynamics, Sunnyvale, CA). \(^{32}\)P-labeled mouse or rabbit AE4 PCR fragments (see above) were used as hybridization probes.

**Immunocytochemistry of AE4 in Stomach and Kidney**

**Antibodies.** For AE4, a synthetic peptide corresponding to amino acids residues CLMYQPKAPEINISVN of mouse AE4 (using a mouse AE4 EST with Gene Bank accession no. AW018362) was used to generate polyclonal antibodies in two rabbits. This sequence is identical in mouse, rabbit, and human AE4. Antibodies were purified by using a cysteine affinity column. A human AE4 polyclonal antibody was purchased from Alpha Diagnostics (San Antonio, TX). Gastric H-K-ATPase β-subunit antibody was a generous gift from Dr. John Forte (University of California at Berkeley).

**Immunoblotting of AE4 in stomach and duodenum.** Mouse microsomal membranes from the scrapings of gastric epithelium and apical membrane vesicles from the duodenum were isolated according to established methods and as described (22, 34). For mouse, immunoblotting experiments were carried out as previously described (34). Briefly, the solubilized membrane proteins were size fractionated on 8% SDS polyacrylamide minigels (Novex, San Diego, CA) under denaturing conditions, electrophoretically transferred to nitrocellulose membranes, blocked with 5% milk proteins, and then probed with the affinity-purified anti-AE4 immune serum at an IgG concentration of 0.6 μg/ml. The secondary antibody was donkey anti-rabbit IgG conjugated to horseradish peroxidase (Pierce). The antigen-antibody complexes on the nitrocellulose membranes were visualized by chemiluminescence (SuperSignal substrate; Pierce), and the image was captured on light-sensitive imaging film (Kodak).

For human duodenum, apical and basolateral membrane vesicles were isolated from two organ donors by using established methods (24) and in accordance with the institutional protocols approved by the University of Illinois at Chicago. Seventy-five micrograms each of purified brush-border and basolateral membranes were solubilized in Laemmli sample buffer, separated on 7% SDS-PAGE, transferred to Hybond nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ), and visualized with Ponceau stain to ensure the efficiency of the transfer. After being blocked with 5% nonfat dry milk in 1× PBS-0.1% Tween 20, blots were incubated with affinity-purified rabbit anti-human AE4 antibody (Alphapharmacia) diluted (1:150) in 1× PBS-0.1% Tween 20–1% milk overnight at 4°C. After four washes with 1× PBS-0.1% Tween 20, blots were then incubated with horseradish peroxidase goat anti-rabbit IgG secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) diluted in 1× PBS-0.1% Tween 20–1% milk overnight at 4°C. After four washes with 1× PBS-0.1% Tween 20, the bands were visualized by using an enhanced chemiluminescence system (Amersham Biosciences). To determine antigen specificity, in a separate experiment AE4 control peptide (30 μg/ml, sequence near the cytoplasmic COOH terminal of human AE4; Alpha Diagnostics) was preincubated with the antibody solution (5 μg/ml) at 37°C for 2 h before being added to the blot.

**Immunofluorescence Labeling Studies**

Mice were euthanized with an overdose of sodium pentobarbital and perfused through the left ventricle with 0.9% saline followed by cold 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). Rabbıts were euthanized with an overdose of sodium pentobarbital via earlobe vein and perfused through the left ventricle similar to mice. Stomachs and duodenas were removed, cut in tissue blocks, and left in the fixative solution overnight at 4°C. For cryosections, tissue blocks were removed from the fixative solution and soaked in 30% sucrose overnight. The tissue was frozen on dry ice, and 5-μm sections were cut with a cryostat and stored at −80°C until use.
Single and double immunofluorescence labeling were performed as described recently (22). For double labeling with H-K-ATPase, gastric H-K-ATPase β-subunit antibody was diluted at 1:250. Goat anti-rabbit IgG conjugated with Oregon green 488 and goat anti-mouse IgG conjugated with Alexa Fluor 568 dye (Molecular Probes, Eugene, OR) were used at 1:150 dilution for AE4 and H-K-ATPase, respectively. Sections were examined and images were acquired on a Nikon PCM 2000 laser confocal scanning microscope as 0.5 μm optical sections of the stained cells.

Cloning of AE4 cDNA

Full-length human AE4 cDNA was cloned from a human kidney cDNA library by PCR using the following primers: 5'-GAC TGT ACT GTG TCT GAG ATT CTG TGC AAG and 5'-CTG CCG GCT TAC TGG AGA TAT TAT TTA GGG, which were designed based on sequences from the human genome database. PCR amplification of the human AE4 cDNA was performed by using the Clontech Advantage 2 PCR kit protocol. Each PCR reaction contained 5 μl cDNA, 5 μl 10× PCR buffer, 1 μl 10 mM dNTPs, 10 pmol of each primer, and 1 μl Advantage 2 polymerase mix in a final volume of 50 μl. Cycling parameters were 95°C, 1 min; 95°C, 30 s; and 68°C, 4 min. An ~3.2-kb PCR fragment was obtained that contained the full-length coding region of the exchanger. The product was gel purified and subcloned into the pGEM-T Easy vector for expression studies.

Expression of AE4 cRNA in Xenopus Oocytes

The capped AE4 cRNA was generated by using the mMESSAGE mMACHINE kit (Ambion) according to the manufacturer’s instructions. Xenopus oocytes were injected with 50 nl of the human AE4 cRNA (0.2–1.3 μg/μl) by using a Drummond 510 microdispenser via a sterile glass pipette.

Intracellular pH Studies

Intracellular pH (pHi) in oocytes was measured with the pH-sensitive fluorescent probe BCECF (Molecular Probes) as described (34). Briefly, oocytes were loaded with 10 μM BCECF-AM for 20 min at room temperature in the same medium in which they were kept after cRNA injection. Oocytes were then transferred to the 1-ml perfusion chamber, placed on the nylon mesh with vegetable pole facing the fluorescent beam, and perfused at rate of 3 ml/min with the following solution (in mM): 63 NaCl, 33 NaHCO3, 2 KCl, 1.8 CaCl2, 1 MgCl2, and 5 HEPES. Another 20–30 min were allowed for the equilibration and dye ester hydrolysis, which yields the fluorescent probe. Ratios of fluorescence measurements were performed by using an Attolaser digital imaging system (Atto, Rockville, MD). Excitation wavelengths were 450 and 490 nm, and fluorescence emission intensity was recorded at 520 nm. Ratios were calculated and stored. Data analyses were performed by using the Attogrid and Attoview software packages provided with the imaging system. Background signal (always <1%) was recorded and automatically subtracted from the subsequent fluorescence measurements. The ratios were obtained from the submembrane region of the oocytes that were visualized with a ×10 objective. Measured excitation ratios were converted to pHi by using a calibration curve that was constructed with a high K+/nigericin method at the end of each experiment (34).

To test for Cl-/HCO3− exchange activity, the chamber was first perfused with solution containing 63 mM NaCl and 33 mM NaHCO3. The oocytes were then switched to Cl−-free medium (63 mM Na-glucuronate and 33 mM NaHCO3). All other Cl−-containing chemicals (KCl, etc.) were replaced with glucuronate salts. This maneuver results in cell alkalinization due to reversal of Cl−/HCO3− exchange (16). On pHi stabilization in Cl−-free medium, oocytes were switched back to the Cl−-containing solution. This should result in cell acidification back to baseline due to activation of Cl−/HCO3− exchange. The initial rate of cell pHi recovery was used as the rate of Cl−/HCO3− exchange activity (34). In additional studies, experiments were repeated with Na+−-free solutions, with

Fig. 1. Expression of anion exchanger (AE) isoform 4 mRNA in the gastrointestinal tract. A: mouse kidney and stomach RNA were analyzed by RT-PCR in the presence or absence of reverse transcriptase (RT) by using mouse AE4-specific primers, and the products were fractionated by agarose gel electrophoresis. Ethidium bromide staining shows amplification of the expected PCR fragment (∼420 bp) in both tissues. B: RNA from rabbit mucous and parietal cells was analyzed by RT-PCR in the presence (+) or absence (−) of RT by using rabbit AE4-specific primers, and the products were fractionated by agarose gel electrophoresis. Ethidium bromide staining shows amplification of the expected PCR fragment (∼600 bp) in both cell types. C: Northern blot analysis of AE4 mRNA from rabbit mucous and parietal cells reveals AE4 expression in both cell types.
tetramethylammonium chloride replacing NaCl and choline bicarbonate replacing NaHCO₃.

Rabbit Gastric Cell Purification

Rabbit gastric mucous and parietal cells were isolated as described (26) and were processed for total RNA isolation. The use of rabbit in these studies was for the purpose of determination of the gastric epithelial cell origin of AE4, because reasonably purified mucous and parietal cells have been successfully isolated from rabbit stomach.

Materials

³²P-dCTP was purchased from New England Nuclear (Boston, MA). Nitrocellulose filters were purchased from Sigma (St. Louis, MO). RadPrime DNA labeling kit was purchased from GIBCO-BRL. All other chemicals were purchased from Sigma unless otherwise stated.

Statistical Analyses

Values are expressed as means ± SE. The significance of differences between mean values were examined by using ANOVA. \( P < 0.05 \) was considered statistically significant.

RESULTS

AE4 mRNA Expression in the Upper Gastrointestinal Tract

To examine the distribution of AE4 mRNA in stomach, RT-PCR was performed by using RNA isolated from mouse stomach and was compared with that of kidney by using mouse-specific primers (see EXPERIMENTAL PROCEDURES). Figure 1A shows ethidium bromide staining of an agarose gel, which revealed that the expected PCR fragment of 412 bp is amplified from mouse stomach and kidney. The sequence of the gel-
puriﬁed PCR product from kidney and stomach was determined, verifying the product as AE4. The above results demonstrate that in addition to the kidney, AE4 is expressed in the stomach.

Expression of AE4 mRNA in Gastric Mucous and Parietal Cells

To determine the cellular distribution of AE4 mRNA in the stomach, RNA was isolated from parietal and mucous cells of rabbit stomach and was analyzed by RT-PCR using rabbit-speciﬁc primers. Figure 1B shows an ethidium bromide-stained agarose gel, demonstrating that the expected PCR fragment (~600 bp), which was veriﬁed by sequence analysis, was ampliﬁed from both mucous and parietal cells. The results of these RT-PCR experiments were veriﬁed by Northern blot analysis, which demonstrated that AE4 mRNA is expressed in both gastric mucous and parietal cells (Fig. 1C). The use of rabbit in these series of studies was for the purpose of determination of the epithelial cell origin of AE4. As is known, rabbit is the only mammalian species that has successfully been used for the isolation of reasonably puriﬁed mucous and parietal cells.

Immunoblotting and Immunofluorescence Labeling of AE4 in Stomach and Duodenum

To determine the protein expression of AE4, microsomal membrane proteins from scrapings of the gastric epithelium and apical membrane vesicles from the duodenum were isolated from mouse and were subjected to immunoblot analysis. Figure 2A shows an immunoblot analysis of microsomal membranes from mouse stomach, which demonstrates that AE4 appears as a ~120-kDa protein. The reaction is speciﬁc because the preadsorbed immune serum failed to detect the band (Fig. 2A). To determine the cellular distribution and subcellular localization of AE4, immunocytochemical staining was performed on sections of mouse stomach. As shown in Fig. 2B, AE4 is expressed on the apical membrane of surface epithelial cells (left). The preadsorbed immune serum did not detect any labeling (right). Figure 2C shows double immunofluorescence labeling with AE4 and gastric H-K-ATPase β-subunit in mouse stomach. It demonstrates that the H-K-ATPase is restricted to parietal cells, as expected, whereas AE4 is expressed primarily in surface epithelial cells, with lower levels of expression in gastric parietal cells. The staining in parietal cells was variable, with intracellular labeling being more frequent than the apical labeling. No basolateral labeling in parietal cells was observed.

In the next series of studies, we examined the localization of AE4 in rabbit stomach by immunofluorescence labeling. As shown in Fig. 3A, AE4 is located on the apical membrane of surface epithelial cells in rabbit stomach. As a control and to verify that the AE4 antibody indeed recognizes the AE4 protein, double immunofluorescence labeling with AE4 and peanut lectin-binding protein was performed in rabbit kidney. As indicated in Fig. 3B, AE4 and peanut lectin-binding protein, which labels only the apical membrane of β-intercalated cells, colocalize to the same membrane domain. These results indicate that the AE4 immune serum recognizes only the AE4 protein and does not cross-react with other proteins. It further veriﬁes the results of molecular studies in which AE4 was cloned from β-intercalated cells of rabbit kidney and was shown to be expressed only in those same cells (32).

Fig. 4. Immunoblot localization of AE4 in the duodenum. A: immunoblot analysis of AE4 in human duodenum from 2 donors reveals an ~110-kDa protein in brush-border membrane (BBM) vesicles (left), but no bands were detected in basolateral membrane (BLM) vesicles. Preadsorbed immune serum did not detect any bands (right). B: immunoblot analysis of AE4 in mouse duodenum using immune serum reveals an ~120-kDa protein in apical membrane vesicles (left). The labeling of the ~120-kDa band was prevented by preadsorbed immune serum (right).
The experiments shown in Fig. 4 examine the expression and subcellular localization of AE4 in mouse, rabbit, and human duodenum. In human, AE4 appears as an ~110-kDa band in apical membranes of the duodenum but not in basolateral membranes (Fig. 4A, left). Preadsorbed immune serum did not react with any proteins (Fig. 4A, right). In the mouse, AE4 appears as an ~120-kDa protein in apical membrane vesicles from the duodenum (Fig. 4B, left). No labeling was observed with the preadsorbed immune serum (Fig. 4B, right).

The results of the above experiments indicate that AE4 is localized to the apical membrane proteins of the duodenum in both mouse and human. To determine the cellular distribution and subcellular localization of AE4 in the duodenum in more detail, immunofluorescence labeling was performed in mouse and rabbit duodenum. In mouse duodenum, AE4 is expressed

Fig. 5. Immunocytochemical staining of AE4 in mouse and rabbit duodenum. A: immunocytochemical staining of AE4 in mouse duodenum indicates that AE4 is expressed on the apical membrane of the villi with lower expression levels on the apical membrane of crypt cells (left). This reaction was specific, because the labeling was prevented by immune preadsorption (right). B: immunocytochemical staining of AE4 in rabbit duodenum. The preadsorbed immune serum shows nonspecific labeling on the basolateral membrane of villi in the duodenum (right). The AE4 immune serum (left) specifically labels the apical membrane of the villi of the duodenum (arrows).

AE4 Immune serum  Preadsorbed serum
exclusively on apical membranes of epithelial cells in the villi (Fig. 5A, left). No labeling was detected with the preadsorbed immune serum (Fig. 5A, right). In rabbit, the preadsorbed immune serum showed non-specific labeling in the basolateral membrane of villi in the duodenum (Fig. 5B, right). Compared with the preadsorbed serum, the AE4 immune serum specifically labeled the apical membranes of villi in the duodenum (Fig. 5B, left).

Functional Expression of AE4

Two recent studies indicated that AE4 is a Cl⁻/HCO₃⁻ exchanger (16, 32). The purpose of the next series of experiments was to examine and verify the functional identity of AE4 by using the oocyte expression system. In the first series of experiments, oocytes were injected with human AE4 cRNA and were loaded with BCECF in the presence of a Cl⁻/HCO₃⁻-containing solution (see EXPERIMENTAL PROCEDURES), and intracellular pH was monitored. The representative pHi tracings in Fig. 6A demonstrate that switching to a Cl⁻-free solution resulted in a rapid intracellular alkalinization in oocytes expressing AE4. Switching back to the Cl⁻-containing solution caused a return of pHᵢ to baseline. No pHᵢ alteration in response to exposure to the Cl⁻-free medium was observed in control (water-injected) oocytes (Fig. 6A). In the absence of HCO₃⁻ in the medium, the rate of cell alkalinization in response to Cl⁻ removal was minimal. These results are consistent with AE4 functioning as a Cl⁻/HCO₃⁻ exchanger. A summary of multiple experiments is shown in Fig. 6B and demonstrates that the rate of Cl⁻/HCO₃⁻ exchange activity was 0.14 ± 0.01 in oocytes expressing AE4 (n = 5). The baseline pHᵢ in CO₂/HCO₃⁻-containing solutions was 7.14 ± 0.03 in water-injected oocytes and 7.17 ± 0.03 in AE4-injected oocytes (P > 0.05; n = 5). The Cl⁻/OH⁻ exchange activity in control oocytes was not significantly different from zero (Fig. 6, A and B). The absence of Na⁺ in the medium did not prevent cell alkalinization in response to Cl⁻ removal, indicating that AE4-mediated Cl⁻/HCO₃⁻ exchange is independent of Na⁺. In the absence of HCO₃⁻, the rate of Cl⁻/OH⁻ exchange activity (mediated by Cl⁻/OH⁻ exchange) was minimal, indicating the low affinity of AE4 for OH⁻. AE4 did not function in a Na⁺-HCO₃⁻ cotransport mode (data not shown).

DISCUSSION

The lumen of the stomach is exposed to an acidic solution secreted from gastric parietal cells, which can achieve a pH as low as 1.5 (1, 7, 12). To protect itself against the corrosive effects of luminal acid, the gastric surface epithelium secretes a HCO₃⁻-rich fluid into the mucus gel layer (5). Despite the essential role of HCO₃⁻ secretion in mucus protection, the molecular identity and functional characteristics of the transporter(s) responsible for this process have remained unclear. The results of the present study show that in addition to its expression in kidney, AE4 mRNA is expressed in the stomach and in the duodenum, which is also expressed to gastric acid. Furthermore, immunohistochemical staining demonstrated that expression of AE4 protein in the stomach occurs predominantly in the apical membrane of surface epithelial cells and that its expression in the duodenum is restricted to the apical membranes of villus cells. Expression of AE4 in parietal cells was mostly limited to the cytoplasmic region, with faint and occasional apical labeling. Functional expression of AE4 in oocytes confirmed previous studies showing that this transporter mediates Cl⁻/HCO₃⁻ exchange (32). These observations suggest that AE4 is responsible, at least in part, for HCO₃⁻ secretion into the mucus layer of the gastric epithelium and across
the apical membrane of villus cells in the duodenum, consistent with the hypothesis that it plays an important role in mucosal protection in both organs.

There are conflicting studies on the nature of HCO₃⁻-secreting transporter(s) in gastric surface epithelial cells. Although a Cl⁻/HCO₃⁻ exchanger has been proposed to be the primary mechanism for HCO₃⁻ secretion in frog gastric mucosa (9), other investigators have proposed that a HCO₃⁻-conductive pathway is responsible for this activity (32). AE4 functions as a Cl⁻/HCO₃⁻ exchanger in heterologous expression systems (Refs. 16 and 32 and the current study), indicating that at least a portion of the HCO₃⁻ secretion in surface epithelial cells is likely to be mediated by this transporter operating in a Cl⁻/HCO₃⁻ exchange mode. A recent preliminary study (21a) suggested that AE4 may also function as an electroneutral Na-HCO₃ co-transporter. Our functional expression studies in oocytes did not reveal Na-HCO₃ cotransport activity by AE4, confirming recent results indicating that AE4 only functions in a Cl⁻/HCO₃⁻ exchange mode (32). These results not withstanding, either Cl⁻/HCO₃⁻ exchange or Na-HCO₃ cotransport activity via AE4 would function in a HCO₃⁻-secreting mode, because luminal acidity precludes the functioning of AE4 (or any HCO₃⁻ transporter) in a net HCO₃⁻-absorbing mode. As an apical Cl⁻/HCO₃⁻ exchanger, AE4 can neutralize the gastric acidity according to the following scheme. Following secretion into the gastric lumen via coordinated action of gastric H-K-ATPase and apical Cl⁻ channel in parietal cells, the resulting HCl dissociates into H⁺ and Cl⁻. The secreted Cl⁻ will be exchanged with intracellular HCO₃⁻ via the Cl⁻/HCO₃⁻ exchanger AE4 located on the apical membrane of mucous cells. The net effect of these processes is the secretion of HCl by parietal cells and the secretion of HCO₃⁻ from mucous cells for protection against injury by the acid. In the case of proton pump inhibitors, inhibition of acid secretion is associated with decreased generation of HCO₃⁻, which in turn leads to reduced HCO₃⁻ exit across the basolateral membrane of parietal cells, eventually reducing the uptake of HCO₃⁻ by mucous cells (presumably via basolateral NBC). The reason that gastric luminal pH does not climb very high in response to proton pump inhibitor (PPI) might be due to decreased intracellular HCO₃⁻ concentration in mucous cells secondary to decreased HCO₃⁻ generation in parietal cells.

Recent studies (2, 6, 13, 14, 15, 17, 18, 19, 20, 24, 27, 29, 30, 33, 35) have identified a family of anion exchangers, referred to as the SLC26A family, that include at least 10 distinct genes. Three well-known members of this family are SLC26A3 [or downregulated in adenoma (DRA)], SLC26A4 (or pendrin) and SLC26A6 [putative anion transporter 1 (PAT1)] or chloride format exchange (CFEX)] (6, 18, 29), which are located apically in a limited and distinct number of epithelia and function as Cl⁻/OH⁻/HCO₃⁻ exchangers (20, 30, 34). DRA is predominantly expressed in the large intestine with lower levels in the small intestine (20), whereas PAT1 is predominantly expressed in the small intestine with very low expression levels in the large intestine (34). Pendrin is located on the apical membrane of thyroid follicular cells and kidney collecting ducts (27, 30). None of these exchangers are expressed on the apical membrane of surface epithelial cells in the stomach. In mouse stomach, immunocytochemical staining localized PAT1 to the tubulovesicle membranes of gastric parietal cells (22), whereas it failed to detect the expression of pendrin despite detectable mRNA levels (data not shown).

In addition to the stomach, AE4 is expressed in the duodenum. Immunoblotting or immunocytochemical staining demonstrates that AE4 is expressed on the apical membrane of mouse, rabbit, and human duodenum. This observation is intriguing because it confirms that more than one apical Cl⁻/HCO₃⁻ exchanger is expressed in the duodenum. Recently, PAT1 (SLC26A6) was identified as a major Cl⁻/HCO₃⁻ exchanger in the apical membrane of the duodenum (34), along with the DRA (SLC26A3) Cl⁻/HCO₃⁻ exchanger (20). Our studies in the duodenum indicate that the expression levels of PAT1 are the highest, followed by DRA and AE4 (Ref. 34 and unpublished data). It would be difficult to conclude that, based on the expression level studies in the duodenum, one anion exchanger (i.e., PAT1) might be the major transporter mediating HCO₃⁻ secretion (or Cl⁻ absorption) and the others are not. The presence of more than one apical HCO₃⁻ transporter in the duodenum suggests possible differential regulation of these transporters in physiological or pathophysiological states.

In conclusion, AE4 is expressed in the upper gastrointestinal tract, with expression in the small intestine and stomach. Immunohistochemical staining or immunoblotting studies localized AE4 to the apical membranes of surface epithelial cells in the stomach and to the apical domain of villus cells in the duodenum. Functional studies demonstrated that AE4 operates in an electroneutral Cl⁻/HCO₃⁻ exchange mode. We propose that AE4 is an apical HCO₃⁻ transporter in surface epithelial cells of the stomach and villus cells of the duodenum and that it may play an important role in protecting the gastric and duodenal epithelium against injury by the acid secreted from gastric parietal cells.

DISCLOSURES

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