SLC26A9 is expressed in gastric surface epithelial cells, mediates Cl−/HCO3− exchange, and is inhibited by NH4+

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Xu, Jie, Johanna Henriknäs, Sharon Barone, David Witte, Gary E. Shull, John G. Forte, Lena Holm, and Manoocher Soleimani. SLC26A9 is expressed in gastric surface epithelial cells, mediates Cl−/HCO3− exchange, and is inhibited by NH4+. Am J Physiol Cell Physiol 289: C493–C505, 2005. First published March 30, 2005; doi:10.1152/ajpcell.00030.2005.—HCO3− secretion by gastric mucous cells is essential for protection against acidic injury and peptic ulcer. Herein we report the identification of an apical HCO3− transporter in gastric surface epithelial cells. Northern hybridization and RT-PCR demonstrate the expression of this transporter, also known as SLC26A9, in mouse and rat stomach and trachea (but not kidney). In situ hybridization in mouse stomach showed abundant expression of SLC26A9 in surface epithelial cells with apical localization on immunofluorescence labeling. Functional studies in HEK-293 cells demonstrated that SLC26A9 mediates Cl−/HCO3− exchange and is also capable of Cl−-independent HCO3− extrusion. Unlike other anion exchangers or transport proteins reported to date, SLC26A9 activity is inhibited by ammonium (NH4+). The inhibitory effect of NH4+ on gastric HCO3− secretion was also indicated by reduced gastric juxtamucosal pH (pHJm) in rat stomach in vivo. This report is the first to describe the inhibition of HCO3− transport in vitro and the reduction of pHJm in stomach in vivo by NH4+. Given its critical localization on the apical membrane of surface epithelial cells, its ability to transport HCO3−, and its inhibition by NH4+, we propose that SLC26A9 mediates HCO3− secretion in surface epithelial cells and is essential for protection against acidic injury in the stomach. Disease states that are associated with increased ammonia (NH3)/NH4+ generation (e.g., Helicobacter pylori) may impair gastric HCO3− secretion and therefore predispose patients to peptic ulcer by inhibiting SLC26A9.

anion exchange; gastric bicarbonate secretion; peptic ulcer

THE PROTECTION AGAINST INJURY by the acidic gastric juice is largely provided by an adherent layer of mucus-containing HCO3−, which forms a barrier in the lumen of the stomach (9, 13, 37). HCO3− is secreted by surface epithelial cells in response to the acid in stomach lumen. The acid is neutralized by HCO3−, resulting in the formation of a gradient in which the pH at the luminal surface of the epithelium is relatively neutral (9, 31, 37). HCO3− secretion by surface epithelium was reported to be dependent on luminal Cl−, suggesting that Cl−/HCO3− exchange was involved (12). These results have been disputed (42), however, and there is evidence for a HCO3− conductance pathway (7). Both Cl−/HCO3− exchange and the HCO3− conductance pathway remain attractive and relevant possibilities as major HCO3− secretory pathways in neutralizing H+ diffusion into the mucus layer.

The identity of the apical anion transporter that mediates HCO3− secretion by surface mucous cells is unclear. Recently, we reported the expression of anion exchanger isofrom 4 (AE4), a Cl−/HCO3− exchanger of the SLC4 family (44) on the apical membrane of surface epithelial cells (51). However, the functional properties of AE4 are not consonant with the characteristics of apical HCO3− secretory protein in gastric surface epithelial cells, in that AE4 is not inhibited by DIDS (44), whereas acid-stimulated HCO3− secretion in gastric surface epithelial cells is significantly blocked by DIDS (33).

In the search to identify the apical HCO3− transporters in gastric surface epithelial cells, we have examined the expression of members of two groups of HCO3− transporters, namely, the Na+−HCO3− cotransporter (NBC)/anion exchanger (AE) and SLC26 families. The AE family comprises four major isoforms (AE1–AE4), whereas the SLC26 family comprises 10 distinct isoforms (SLC26A1–SLC26A11) (2, 4, 10, 17, 19, 23, 24, 26, 33, 39, 40, 47, 48, 53). Using a combination of RT-PCR with Northern and/or in situ hybridization and immunofluorescence labeling (in which suitable antibody was generated or secured), we examined the expression of selected members of AE, NBC, and SLC26 families (A3, A4, A6, A7, A8, A9 and A11) in mouse, rat, and/or rabbit stomach. In addition to AE4 (51), SLC26A9 is the only other HCO3− transporter that shows localization on apical membranes of gastric surface epithelial cells. Furthermore, SLC26A9 expression in the gastrointestinal tract reveals a unique pattern in that it is limited to the stomach and is absent in the small and large intestines. We have further demonstrated that SLC26A9 mediates Cl−/HCO3− exchange and also may be capable of Cl−-independent HCO3− transport when expressed in mammalian cells. SLC26A9 activity is inhibited by ammonium (NH4+). Last, we have demonstrated that NH4+ inhibits gastric HCO3− secretion in vivo. We propose that disease states such as Helicobacter pylori, which generates ammonia (NH3)/NH4+ through its urease activity, may predispose patients to acidic injury and peptic ulcer by impairing SLC26A9-mediated gastric HCO3− secretion.
EXPERIMENTAL PROCEDURES

RT-PCR of Mouse SLC26A9

For SLC26A9, a mouse expressed sequence tag (EST; GenBank accession no. BB625395) was identified. On the basis of the cDNA sequence of the EST, the following oligonucleotide primers: 5'-GCC CAG AGC TTG TCA ATG TCC CAG (86) and 5'-ACA GCA GTC AGG CAG GCA AGT GTC (656) were designed and used for RT-PCR on RNA isolated from mouse kidney and stomach. No PCR product was obtained from kidney, but an expected product was identified in stomach RNA. The mouse PCR product was purified, and its sequence was verified and used as a probe for Northern hybridization. The primers and the PCR product were used to examine the tissue expression of SLC26A9 in other tissues.

RNA Isolation and Northern Hybridization

Total cellular RNA was extracted from various mouse and rat tissues, including gastrointestinal segments, kidney, liver, heart, brain, and lung using TRI reagent (Molecular Research Center, Cincinnati, OH). RNA (30 μg) was loaded into each lane. Hybridization was performed according to the method described previously by Church and Gilbert (6). The membranes were washed, blotted dry, and exposed to a PhosphorImager screen (Molecular Dynamics, Sunnyvale, CA). A 32P-labeled mouse PCR fragment for SLC26A9 (corresponding to nt 86–656 of mouse SLC26A9 cDNA) was used as the probe for Northern hybridization.

Cloning of the Full-Length SLC26A9

Full-length human SLC26A9 cDNA was cloned using RT-PCR from a human trachea poly(A)+ RNA (Clontech, Mountain View, CA) with the following primers: 5'-GGTTGTCCTCCACCACTGCTTTG and 5'-CTTACCAGACTCTCACTCCGTAAGG (GenBank accession no. NM_052934 and Ref. 24). An ~2.64-kb PCR fragment was obtained that contained the full-length coding region of the exchanger (corresponding to nt 79–7290). Amplification of the human SLC26A9 cDNA using PCR was performed according to the Clontech Advantage 2 PCR kit protocol. Each PCR reaction contained 1 μl of cDNA, 5 μl of 10× PCR buffer, 1 μl of 10 mM 2-deoxynucleotide 5'-triphosphate, 10 pmol of each primer, and 1 μl of Advantage 2 polymerase mix in a final volume of 50 μl. Cycling parameters were 94°C, 1 min; 94°C, 30 s; and 68°C, 3 min. Sequence analysis of the PCR products verified the sequences as SLC26A9. Mouse SLC26A9 full-length cDNA was cloned using the following primers: ATG AAC CAG CCC AGG CCC CCG TA (sense), and ACC AAA TGG GTC CTC ACA G (antisense). This cDNA encodes nt 112–2,497 (GenBank accession no. NM_177243) and corresponds to the full-length coding region (nt 112–2,484) plus an additional 13 nt in the 3'-noncoding region. For expression studies in mammalian cells, the full-length SLC26A9 cDNA of mouse or human was subcloned into the pTARGET Vector (Promega, Madison, WI) and used for transfection in human embryonic kidney (HEK)-293 cells.

In Situ Hybridization of SLC26A9 in Mouse Stomach

The distribution of SLC26A9 within the mouse stomach was assessed using in situ hybridization according to established protocols reported previously (3, 25). In brief, mouse stomach was rapidly dissected, fixed in 4% paraformaldehyde, cryoprotected with 30% sucrose in PBS, and frozen in an optimum cutting temperature compound. Cryostat sections (7 μm) were then mounted on silane-coated slides. The mouse SLC26A9 cDNA fragment, encoded by the primers 5'-ACC CAG AGC TTG TCA ATG TCC CAG (86) and 5'-ACA GCA GTC AGG CAG GCA AGT GTC (656), was purified, ligated into a pGEM-T Easy Vector (Promega), and linearized using SacII restriction enzyme. The antisense cRNA was synthesized using SP6 DNA-dependent RNA polymerases (Riboprobe Gemini Core System II transcription kit; Promega). A sense cRNA probe was prepared with T7 polymerase for use as a negative control. The radiolabeled (S35-UTP) probes were hybridized and washed under high-stringency conditions. Hybridization was performed with 0.5–1.0 × 106 counts/min of labeled probe in a final volume of 30 μl/slide. After overnight incubation at 42°C, the sections were treated with 50 μg/ml RNase A and 100 U/ml RNase T1 for 30 min at 37°C and washed to a final stringency in 0.1% saline sodium citrate at 50°C. The slides were dipped in NTB2 emulsion (Eastman Kodak, Rochester, NY), diluted 1:1 with 0.6 M NH4-acetate, and exposed for 2 wk. Thereafter slides were developed with D19 developer (Eastman Kodak) and counterstained with hematoxylin and eosin.

Immunoblot Analysis of SLC26A9

Microsomal proteins were isolated from mouse stomach surface scrapings or HEK-293 cells, resolved by performing SDS-PAGE at 50–100 μg/lane, and transferred onto nitrocellulose membranes. The membranes were blocked with 5% milk proteins and fixed in formaldehyde solution overnight at 4°C. The tissue was frozen on dry ice, and 6-μm sections were cut with a cryostat and stored at −80°C until used. Single and double immunofluorescence labeling were performed as described using either Alexa Fluor 488 (green) or Alexa Fluor 568 (red) goat anti-rabbit antibody as secondary antibodies (29, 44). For single labeling, both 1:5 and 1:20 dilutions of SLC26A9 antibody were used. Double immunofluorescence labeling was performed using gastric H+-K+-ATPase and SLC26A9 antibodies. SLC26A9 antibody was raised against a mouse synthetic peptide with the amino acid sequence CDTETFSLYDSEEAGG (residues 755–778).

Immunofluorescence Labeling of SLC26A9 in Mouse Stomach

Mice were euthanized using an overdose of pentobarbital sodium 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). Stomachs were removed, cut into tissue blocks, and fixed in formaldehyde solution overnight at 4°C. The tissue was frozen on dry ice, and 6-μm sections were cut with a cryostat and stored at −80°C until used. Single and double immunofluorescence labeling were performed as described using either Alexa Fluor 488 (green) or Alexa Fluor 568 (red) goat anti-rabbit antibody as secondary antibodies (29, 44). For single labeling, both 1:5 and 1:20 dilutions of SLC26A9 antibody were used. Double immunofluorescence labeling was performed using gastric H+-K+-ATPase and SLC26A9 antibodies. SLC26A9 antibody was raised against a mouse synthetic peptide with the amino acid sequence CDTEFSLYDSEEAGG (residues 755–778).

Functional Expression of SLC26A9 in HEK-293 Cells

Stable transfection of human and mouse SLC26A9 in HEK-293 cells was obtained as described previously and according to established protocols (40). The tracings presented herein represent experiments with human SLC26A9 cDNA; qualitatively similar results were obtained with murine SLC26A9 cDNA. Intracellular pH (pHi) in cells transfected with SLC26A9 cDNA was measured with the pH-sensitive fluorescent probe 2,7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF; Molecular Probes, Eugene, OR) as described previously (15, 40, 50). Ratiometric fluorescence measurements were performed using a digital imaging system (Photon Technology International, Lawrenceville, NJ). Excitation wavelengths were 450 and 490 nm, and fluorescence emission intensity was recorded at 520 nm. Data analyses were performed using software packages provided with the imaging system. The ratios were obtained, and measured excitation ratios were converted to pHi using a calibration curve that was constructed according to a high-K+/nigericin method at the end of each experiment. It should be mentioned that the absolute calibration curve of pHi using the ionophore nigericin depends on matching the intracellular K+ concentration with the buffer.

To examine the Cl−/HCO3− exchanger activity, cells were first perfused with a Cl−- and HCO3−-containing solution of the following composition (in mM): 115 NaCl, 25 NaHCO3, 3 KCl, 1.8 CaCl2, 1
MgCl₂, and 5 HEPES, pH 7.4, gassed with 5% CO₂-95% O₂. Once the baseline pH, was established, the perfusate was then switched to a Cl⁻-free medium of the following composition (in mM): 115 Na⁺-gluconate, 25 NaHCO₃, 3 KCl, 1.8 Ca²⁺-gluconate, 1 Mg²⁺-gluconate, and 5 HEPES, pH 7.4, and gassed with 5% CO₂-95% O₂. This maneuver resulted in cell alkalinization due to reversal of the Cl⁻/HCO₃⁻ exchanger (15, 50). Upon pH stabilization in Cl⁻-free medium, cells were returned to the Cl⁻-containing solution. This procedure should result in the return of pHᵢ to baseline because of activation of the Cl⁻/HCO₃⁻ exchanger in the forward mode. The experiments were repeated in the absence of added HCO₃⁻/CO₂, with Na⁺-gluconate replacing Na⁺-HCO₃ in an equimolar solution. The initial rate of pHᵢ recovery from intracellular alkalization was used as the rate of Cl⁻/OH⁻ (in the absence of HCO₃⁻/CO₂) or Cl⁻/HCO₃⁻ exchanger (in the presence of HCO₃⁻/CO₂) activity (15, 50). The perfusate solution was kept at pH 7.4 throughout the experiment in the presence or absence of added HCO₃⁻/CO₂. For Na⁺-free experiments, the NaCl was replaced with tetrabutylammonium (TMA)-Cl⁻ and Na⁺-HCO₃ was replaced with choline-HCO₃ in equimolar solutions. To switch to Cl⁻-free solution, TMA-Cl⁻ was replaced with TMA-glucate.

To examine Cl⁻-independent HCO₃⁻ transport by SLC26A9, experiments were performed in Cl⁻-free medium. Cells were first perfused with a Cl⁻-free, Na⁺- and HCO₃⁻-containing solution at pH 7.4 and of the following composition (in mM): 115 Na⁺-gluconate, 25 NaHCO₃, 3 K⁺-gluconate, 1.8 Ca²⁺-gluconate, 1 Mg²⁺-gluconate, and 5 HEPES (pH 7.4). After 30 min and upon the stabilization of basal pHᵢ, the solution was switched to a Cl⁻-free, low-HCO₃⁻, acidic perfusate at pH 6.5 and of the following composition (in mM): 137 Na⁺-gluconate, 3 NaHCO₃, 3 K⁺-gluconate, 1.8 Ca²⁺-gluconate, 1 Mg²⁺-gluconate, 5 HEPES, and 1 MES (pH 6.5). All solutions were gassed with 5% CO₂-95% O₂. Amiloride, at 1 mM, was present to inhibit Na⁺/H⁺ exchanger (NHE) activity during the switch from pH 7.4 to the acidic pH 6.5 perfusate. The experiments were repeated with increased perfusate Ca²⁺ concentration (4.0 mM Ca²⁺-gluconate) to adjust for possible chelation of extracellular Ca²⁺ by glucate (see RESULTS). Preliminary experiments were conducted in the absence of Na⁺ in the solutions to verify the absence of significant endogenous Na⁺-HCO₃ cotransporter under the experimental maneuvers.

**Juxtamucosal pH Measurements in Rat Stomach: Effect of NH₄⁺**

**Animal preparation.** All experimental in vivo procedures were approved by the Swedish Laboratory Animal Ethical Committee in Uppsala, Sweden, and were conducted in accordance with the guidelines of the Swedish National Board for Laboratory Animals. Male Sprague-Dawley rats (B&K Universal, Stockholm, Sweden) weighing 197–275 g were kept under standardized conditions of temperature (21–22°C) and illumination (12:12-h light-dark cycle). Animals had free access to tap water and pelleted food (Ewos, Södertälje, Sweden). The rats were deprived of food for 18–20 h before the experiments but had free access to tap water. They were anesthetized with 120 mg/kg body wt concentration of 5-ethyl-5-(1-methylpropyl)-2-thiobutabarbital sodium (Inactin, Research Biochemicals International, Natick, MA), which was injected intraperitoneally. Tracheotomy was performed to facilitate spontaneous breathing, and body temperature was maintained at 37.5 ± 0.5°C using a heating pad controlled by a rectal thermometer probe. A PE-50 cannula containing heparin (12.5 IU·ml⁻¹; Kabivitrum, Stockholm, Sweden) dissolved in isotonic saline was placed into the right femoral artery to monitor blood pressure. The right femoral vein was cannulated for continuous infusion of a modified Ringer solution (in mM: 120 NaCl, 2.5 KCl, 25 NaHCO₃, and 0.75 CaCl₂) at a rate of 1.0 ml·h⁻¹. The preparation of the gastric mucosa for intravital microscopy was described previously (20, 30, 37). Briefly, the mucosa was exteriorized through a midline abdominal incision, followed by an incision along the greater curvature in the forestomach. The rat was placed on a Lucite table with part of the corpus of the stomach loosely draped over a truncated cone in the center of the table, with the mucosal surface facing upward. A mucosal chamber with a hole in the bottom, corresponding to the position of the cone, was fitted over the mucosa, exposing ~1.0 cm² of the gastric mucosa through the hole. To avoid impairment of blood flow, the mucosal chamber did not touch the mucosa and the edges of the hole were sealed with silicon grease. The chamber was filled with 5 ml of 0.9% saline and maintained at 37°C by means of warm water circulating in a jacket in the bottom of the chamber. The saline was replaced at regular intervals of 10–15 min, and pH was measured. The rat was moved to a stereomicroscope stand (Leica MZ12; Leica, Heerbrugg, Switzerland), and the gastric mucosa was transilluminated with light from a 150-W light source guided with the use of fiberoptics.

**pH measurement in stomach lumen: effect of NH₄⁺** The H⁺ concentration in the mucus gel at the epithelial cell surface, the juxtamucosal pH (pHᵢₓm), was measured with H⁺-selective microelectrodes as described previously (20, 30, 37, 41). Glass tubing (1.2-mm optical density, 0.9-mm ID Omega Dot; Frederik Haer, Brunswick, ME) was pulled with a pipette puller (pp-83; Narishige Scientific Instrument Laboratories, Tokyo, Japan) to a tip diameter of 1–3 µm. The microelectrodes were siliconized at 200°C with tributylchlorosilane and stored at 100°C. Before the experiments, the electrodes were filled to a distance ~300 µm from the tip with a proton cocktail (H⁺ Ionophore II-Cocktail; Fluka, Buchs, Switzerland). The remaining part of the electrode was filled with HEPES buffer at pH 7.4, connected by an Ag-AgCl wire to a dual differential electrometer with a high-input impedance (Biomedical Center, Uppsala, Sweden), and inserted into a pipette holder (MEH3SF 1.2, Mark Finlay; WPI, Aston, UK). The reference electrode was filled with 3 M KCl, connected by an Ag-AgCl wire to the ground of the electrometer, and placed into the saline covering the gastric mucosa. To eliminate electrical disturbances, the experiments were performed in a Faraday cage. The electrodes were calibrated before and after the experiments in solutions with pH 2–8 at 37°C. The solutions were made isomolar (310 mosmol) with NaCl. The solutions with pH 2 and 3 were obtained by adding 155 mM HCl to an unbuffered NaCl solution (155 mM), and those with pH 4–8 were obtained by adding HCl or NaOH to a solution containing 10 mM HEPES and 140 mM NaCl. The microelectrodes were inserted into the mucus gel at a 30–40° angle relative to the mucosa by means of a micromanipulator (Leitz, Wetzlar, Germany). We have performed pH measurements with the H⁺-sensitive open microelectrode as well as with a conventional glass electrode and recorded the same pH with both electrodes in NH₄⁺/HCl pH 2 as well as NH₄⁺-saline solution. Furthermore, we recorded no difference in pH between NH₄⁺/HCl pH 2 and pH 2 alone using the H⁺-sensitive microelectrode.

**Materials**

[³²P]dCTP was purchased from New England Nuclear (Boston, MA). Nitrocellulose filters and other chemicals were purchased from Sigma Chemical (St. Louis, MO). The RadPrime DNA labeling kit was purchased from GIBCO-BRL (Grand Island, NY). BCECF was obtained from Molecular Probes (Eugene, OR). The mMessage mMachine kit was purchased from Ambion (Austin, TX). The human multiple tissue blots were purchased from Clontech.

**Statistical Analyses**

Values are expressed as means ± SE. Statistical analysis in pHᵢ studies in HEK-293 cells was performed using ANOVA. Differences in pHᵢₓm in stomachs within the same animal group and between animal groups were evaluated statistically using ANOVA (Mann-Whitney U-test). P < 0.05 was considered statistically significant.
RESULTS

SLC26A9 Tissue Expression

To examine the distribution of SLC26A9 mRNA, RT-PCR was performed on RNA isolated from various mouse tissues using mouse-specific primers (see EXPERIMENTAL PROCEDURES). As demonstrated in Fig. 1A, left, a band of expected size (~570 bp) was identified in RNA isolated from stomach when specific primers were used (see EXPERIMENTAL PROCEDURES). Sequencing of the purified band verified its identity as SLC26A9. No PCR fragment was identified in heart, brain, or liver. To better examine SLC26A9 mRNA expression in mouse tissues, Northern hybridizations were performed on RNA isolated from various tissues and segments of gastrointestinal tract using a SLC26A9-specific cDNA probe. As indicated, SLC26A9 mRNA appeared as a 5-kb band and was abundantly expressed in the stomach, with lower levels observed in the lung (Fig. 1B). No expression was detected in the kidney. The two bands shown in Fig. 1B likely represent splice variants of SLC26A9. As shown, SLC26A9 mRNA expression in the gastrointestinal tract was limited to the stomach and was absent in the small and large intestines (Fig. 1B).

In Situ Hybridization of SLC26A9 in Mouse Stomach

To determine the cellular expression of SLC26A9 in the stomach, in situ hybridizations were performed using sense (negative control) and antisense (specific labeling) cRNA probes as described in EXPERIMENTAL PROCEDURES. As shown in Fig. 2, left, SLC26A9 is abundantly expressed in surface epithelial cells and the deep cells in the gastric gland. The labeling with the sense cRNA (control) was completely negative (Fig. 2, right), indicating the specificity of the antisense labeling. In separate experiments, we detected abundant expression of SLC26A9 in RNA isolated from rabbit and rat stomachs (data not shown).

Immunoblotting: Single- and Double-Immunofluorescent Labeling of SLC26A9 in Mouse Stomach

To examine the expression of SLC26A9 in the stomach, Western blot analysis was performed with gastric microsomes as described in EXPERIMENTAL PROCEDURES. As shown in Fig. 3A (left), SLC26A9 immune serum detected an ~80- to ~82-kDa band in mouse stomach. The labeling of the ~80-kDa band was blocked completely by preadsorbed immune serum (Fig. 3A, right).

To determine the cellular distribution and subcellular localization of SLC26A9, immunofluorescent staining with the
Fig. 3. Immunoblotting and immunofluorescence labeling of SLC26A9 in the stomach. A: immunoblotting of SLC26A9 in microsomal membranes from mouse stomach. Western blot analysis using purified SLC26A9 immune serum on microsomal membranes from mouse surface cell scrapings revealed a band of ~80 kDa (lane 1; arrow), which was completely blocked with the preadsorbed immune serum (lane 2). B: immunofluorescence staining of SLC26A9 in stomach. Results indicate that SLC26A9 was localized predominantly on the apical membrane of surface epithelial cells (top left; 1:20 dilution). Preadsorbed immune serum did not detect any labeling (bottom left). Labeling with higher antibody concentrations (1:5 dilution) detected specific labeling in surface epithelial cells as well as cells in the body of gastric glands (top right and bottom right). Arrows in B indicate specific labeling. C: immunofluorescence double labeling with SLC26A9 (red staining; left) and gastric H^+-K^+-ATPase (green staining; right). Bottom image is a merged image showing labeling with SLC26A9 (red) and H^+-K^+-ATPase (green). The distribution of gastric H^+-K^+-ATPase β-subunit was distinct from SLC26A9 when dual images were acquired.
purified immune serum was performed in mouse stomach. As shown in Fig. 3B (top left), SLC26A9 is predominantly localized on the apical membrane of surface epithelial cells. In addition, and when higher antibody concentrations were used (1:5 dilution), specific labeling was observed on the membranes of cells in the glandular portion of the stomach (Fig. 3B, top right and bottom right). There was no labeling with the preabsorbed immune serum (Fig. 3B, bottom left).

Figure 3C shows double-immunofluorescence labeling with SLC26A9 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 SLC26A9 is expressed primarily in surface epithelial cells. The SLC26A9-expressing cells in the glandular portion of the stomach were not distinctly visualized in double-labeling studies, likely as a result of strong labeling with H^+-K^+-ATPase antibody. It is therefore difficult to make any definitive conclusion regarding the type of SLC26A9-expressing cells in the gastric gland.

**Functional Identity of SLC26A9**

Cl^−/OH^− exchange. On the basis of structural similarity with downregulated in adenoma (DRA), pendrin, and putative anion transporter 1 and the fact that these three transporters mediate Cl^−/base exchange (10, 26, 39, 40, 49), we tested whether SLC26A9 can function in Cl^−/base exchange mode. In the absence of CO_2/HCO_3^−, the rate of pH_i alteration in response to Cl^− removal or its readadministration was very low in SLC26A9-expressing cells as well as in wild-type cells, indicating that the affinity of SLC26A9 for Cl^−/OH^− exchange was negligible (Fig. 4, A and B). The perfusate solution was kept at pH 7.4 in the presence or absence of Cl^− (see experimental procedures).

Cl^−/HCO_3^− exchange. The representative tracings shown in Fig. 4, C and D, demonstrate that in the presence of CO_2/HCO_3^− in the perfusate, the rate of cell alkalization (in response to exposure to the Cl^−-free solution) or the subsequent pH_i recovery (upon switching back to the Cl^−-containing solution) was significantly higher in SLC26A9-expressing cells than in wild-type cells. The rate of cell alkalization was 0.15 ± 0.01 pH/min in SLC26A9-expressing cells but only 0.02 ± 0.002 pH/min in wild-type cells (n = 8; P < 0.01). The SLC26A9-mediated intracellular alkalization was inhibited by >90% in the presence of 0.5 mM DIDS, a known inhibitor of anion-HCO_3^− cotransporters (Fig. 4, E and F). Mouse SLC26A9, similar to its human ortholog, showed intracellular alkalization upon removal of perfusate Cl^− that was significantly higher than that in nontransfected cells (n = 4; P < 0.05).

To determine whether intracellular alkalization in response to Cl^− removal in Fig. 4, C or E, is dependent on extracellular Na^+, the experiments were repeated in the absence of Na^+ (see experimental procedures). A representative pH_i tracing in Fig. 4G demonstrates that in the absence of Na^+, switching to the Cl^−-free solution resulted in intracellular alkalization, which returned toward baseline upon switching back to the Cl^−-containing solution. The rate of cell alkalization was 0.13 ± 0.02 pH/min in SLC26A9-expressing cells but only 0.03 ± 0.003 pH/min in wild-type cells (n = 5; P < 0.03).

Northern hybridizations in wild-type HEK-293 cells and cells stably transfected with SLC26A9 demonstrated the absence of SLC26A9 mRNA in wild-type HEK-293 cells (Fig. 4H). Clones 2 and 6 (Fig. 4H), which showed abundant expression of SLC26A9, were expanded and used for functional studies. Western blot analysis of microsomes from SLC26A9-expressing cells (Fig. 4H, left) allowed us to detect specific labeling in an ~80-kDa area that was absent in nontransfected cells (Fig. 4I, right). The tracings shown herein represent clone 6 functional activity. Taken together, these results are consistent with SLC26A9 functioning as a DIDS-sensitive Cl^−/HCO_3^− exchanger. The experiments with Na^+-free perfusate were not paired with those performed in the presence of Na^+ (experiments performed on separate days). Hence, direct comparison of basal pH_i, as well as the rates of Cl^−/HCO_3^− exchanger activity in the presence and absence of Na^+, was not possible. The results indicate, however, that SLC26A9 can mediate Na^+-independent Cl^−/HCO_3^− exchange. The question of whether the presence of Na^+ has any modulating effect on Cl^−/HCO_3^− exchange activity via SLC26A9 warrants additional experiments.

In the next series of experiments, we examined whether SLC26A9 can function as an acid-stimulated [acidic extracellular pH (pH_e)], Cl^−-independent, HCO_3^−-transporting pathway. Accordingly, HEK-293 transfected and wild-type cells were perfused with a Cl^−-, HCO_3^−-, or HCO_3^−/free perfusate at pH 7.4 for 30 min (see experimental procedures) and then switched to a Cl^−-free acidic perfusate at pH 6.5 (see experimental procedures). Amiloride, at 1 mM, was present to inhibit NHE, and both solutions were gassed with 5% CO_2 (see experimental procedures). The experiments were first performed in the absence of CO_2/HCO_3^− to determine the
**APICAL HCO₃⁻ TRANSPORTER IN GASTRIC MUCOUS CELLS**

**A** SLC26A9  
100% O₂, pH 7.4, HCO₃⁻ 0 mM

**B** Wild type (non-transfected)  
100% O₂, pH 7.4, HCO₃⁻ 0 mM

**C** SLC26A9  
HCO₃⁻ 24 mM/CO₂ 5%

**D** Wild type  
HCO₃⁻ 24 mM/CO₂ 5%

**E** SLC26A9  
HCO₃⁻ 24 mM/5% CO₂

**F** SLC26A9 With DIDS  
HCO₃⁻ 24 mM/CO₂ 5%

**G** SLC26A9-mediated Cl⁻/HCO₃⁻ exchanger activity is not affected by removal of perfusate sodium. [Na⁺] was 0 mM throughout the experiment.

**H** Northern hybridization of SLC26A9 in wild type and A9 transfected cells

**I** Western blot analysis of SLC26A9 in wild type and A9 transfected HEK 293 cells

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effect of acidic pH on pH in wild-type and A9-transfected cells. As demonstrated (Fig. 5, A and B), switching to the acidic perfusate resulted in a comparable degree of pH decline in wild-type and A9-transfected cells. The summary of six separate experiments demonstrated that the rate of intracellular acidosis was 0.06 ± 0.006 pH/min in wild-type cells and 0.05 ± 0.007 pH/min in A9-transfected cells (n = 5 for each set; P > 0.05). We next repeated the same procedure in the presence of CO2/HCO3-. As indicated, in the presence of CO2/HCO3-, switching to the acidic solution resulted in a significant degree of acidification in SLC26A9-transfected cells, whereas nontransfected cells showed very mild acidification in response to the same procedure (Fig. 5, C and D). The rate of pHi acidification was 0.21 ± 0.02 pH/min in A9-transfected cells but only 0.08 ± 0.006 pH/min in control cells (n = 8 for each set; P < 0.01). The above results show that the rate of intracellular acidification in wild-type cells upon exposure to acidic perfusate is not significantly different in the absence or presence of HCO3- (compare Fig. 5B with Fig. 5D and summation results) and may represent proton influx (likely via a proton channel). The A9-transfected cells, on the other hand, showed a significantly higher acidification rate in the presence of CO2/HCO3- (compare Fig. 5C with Fig. 5A), consistent with Cl--independent HCO3- transport by SLC26A9.

The above experiments did not adjust for a possible chelating effect of gluconate on extracellular Ca2+. To address this concern, the above experiments were repeated with increased extracellular Ca2+ (4 mM). As demonstrated (Fig. 5, E and F), in the presence of CO2/HCO3- and increased extracellular Ca2+, switching to the acidic solution (pH 6.5) resulted in more rapid intracellular acidosis in A9-transfected cells than in wild-type cells. The rate of pHi acidification was 0.19 ± 0.02 pH/min in A9-transfected cells but only 0.07 ± 0.008 pH/min in control cells (n = 5 for each set; P < 0.01). These results indicate that SLC26A9 may function as a Cl--independent, HCO3--extruding pathway under certain experimental conditions. The Cl--independent HCO3- extrusion mediated via SLC26A9 was inhibited by only 55% in the presence of 0.5 mM DIDS (with the rate of cell acidification reduced to 0.10 ± 0.01 pH/min in the presence of DIDS in A9-transfected cells). The electrogenicity of Cl--independent HCO3- extrusion mediated via SLC26A9 could not be determined unequivocally, because there was significant depolarization in wild-type cells in response to exposure to acidic perfusate under the experimental conditions. The use of an oocyte expression system to assess the electrogenic of SLC26A9 also proved challenging, owing to profound depolarization in control (water-injected) oocytes upon exposure to the acidic perfusate in the presence of CO2/HCO3-.

Inhibition of SLC26A9 activity by NH4+

The results of the above experiments indicate that SLC26A9 is a Cl--/HCO3- exchanger but also may have the capability to mediate acid-stimulated HCO3- conductance. In the next series of experiments, we tested the effect of NH4+ on Cl-/HCO3- exchange and Cl--independent HCO3- extrusion in SLC26A9-transfected cells. Accordingly, the above experiments were repeated with 4 mM NH4+ acetate present throughout the experiments. To adjust for a possible effect of acetate, 4 mM Na+-acetate was present in NH4+-free experiments. As shown in Fig. 6, A and B, the cell alkalinization generated in response to switching to the Cl--free perfusate (Cl-/HCO3- exchange mode) was significantly inhibited in the presence of 4 mM NH4+. A summary of the results indicated that the rate of Cl-/HCO3- exchanger activity was 0.18 ± 0.016 pH/min in A9-expressing cells in the absence of NH4+, but only 0.07 ± 0.005 pH/min in the presence of NH4+. Similarly, the cell acidification that was generated upon switching to the acidic solution in the absence of Cl- throughout the experiment was also inhibited in the presence of 4 mM NH4+ (acidification rate decreasing to 0.12 ± 0.015 pH/min in the presence of NH4+, n = 5 for each set; P < 0.04 vs. no NH4+ above). The inhibitory effect of NH4+ was specific to these pathways and did not affect the endogenous NHE activity elicited in response to Na+ removal. In the absence of CO2/HCO3-, switching to the Na+-free perfusate in A9-transfected cells resulted in intracellular acidification (NHE activity) with a rate of 0.11 ± 0.02 pH/min in the absence of NH4+ and 0.13 ± 0.02 in the presence of NH4+ (n = 4 for each set; P > 0.05).

Juxtamucosal pH Experiments in Stomach: Effect of NH4+

To determine whether the inhibition of SLC26A9-mediated HCO3- transport by NH4+ is relevant to the pathophysiology of acidic injury or peptic ulcer, the effect of NH4+ on gastric pH (pHjm) in the mucus at the gastric surface epithelial cells was examined in vivo. Toward this end, rats were anesthetized, the gastric mucosa was exteriorized as described, and pHjm was measured in response to HCl instillation in the absence or presence of NH4+ (see EXPERIMENTAL PROCEDURES). The animals were divided into two groups: control animals (n = 5) and NH4+-exposed animals (n = 6). The electrode was placed into the mucus gel at the surface of epithelial cells, avoiding gastric pits, and the position was continuously verified using the stereomicroscope. pHjm was measured at several places on the surface throughout the experiments. Figure 7A shows the mean values for pHjm in control (n = 5) and NH4+-exposed animals (n = 6). pHjm was neutral during the control period in both groups. When HCl at pH 2 was applied for the first time, pHjm decreased slightly but significantly, from 7.5 ± 0.1 to 6.8 ± 0.3 in the control animals and from 7.5 ± 0.1 to 5.2 ± 0.4 in the NH4+-exposed group. pHjm returned to control levels when the acid was washed away. Luminal exposure to 4 mM NH4Cl did not affect pHjm. During the second instillation of acidic solution (pH 2.0), pHjm decreased to 3.7 ± 0.7 in NH4+-containing solution, which was significantly lower (P < 0.05) than in the control animals (pHjm 5.6 ± 0.8).

Mean Arterial Blood Pressure and Acid Secretion Did Not Differ Significantly Between the Groups

The acid secretion before any treatment, during saline/NH4+, and after second HCl exposure was 0.27 ± 0.13, 0.09 ± 0.03, and 0.06 ± 0.03 µeq·min⁻¹·cm⁻², respectively, in the control group and 0.20 ± 0.08, 0.23 ± 0.20, and 0.16 ± 0.09 µeq·min⁻¹·cm⁻², respectively, in the NH4+-treated group (see Fig. 7). pHjm returned to normal levels when the NH4+-containing acid solution was washed away, indicating that the inhibition by NH4+ was reversible. Figure 7B shows abundant mRNA expression of SLC26A9 in three samples isolated from the stomachs of
Fig. 5. Functional expression of SLC26A9 in cultured cells (Cl⁻-independent experiments). A and B: pH recordings in response to exposure to Cl⁻-free, acidic perfusate in the absence of CO₂/HCO₃⁻. Representative tracings show Cl⁻-independent base exit (or proton uptake) in HEK-293 cells expressing SLC26A9. Cells were loaded with BCECF and perfused with Cl⁻-free solutions throughout the experiments. Solutions were gassed with 100% O₂. As indicated, switching from a Cl⁻-free, normal pH perfusate (pH 7.4) to a Cl⁻-free, acidic perfusate (pH 6.5) resulted in intracellular acidification in both wild-type cells and cells expressing SLC26A9. Amiloride at 1 mM was present throughout the experiments.

C and D: pH recordings in response to exposure to Cl⁻-free, acidic external pH (pHe) perfusate in the presence of CO₂/HCO₃⁻ (Cl⁻-independent, acidic pHₐ-stimulated HCO₃⁻ transport). Representative tracings demonstrating Cl⁻-independent HCO₃⁻ extrusion in HEK-293 cells expressing SLC26A9. Cells were loaded with BCECF and perfused with Cl⁻-free solutions throughout the experiments. Solutions were gassed with 95% O₂-5% CO₂. As indicated, switching from a Cl⁻-free, normal pH perfusate (pH 7.4) to a Cl⁻-free, acidic perfusate (pH 6.5) resulted in rapid intracellular acidification in cells expressing SLC26A9. Control (nontransfected) cells showed less reduction in the rate of intracellular acidification in response to switching to the acidic perfusate (see RESULTS). Amiloride at 1 mM was present throughout the experiment.

E and F: Cl⁻-independent, acidic pHₐ-stimulated HCO₃⁻ transport (in the presence of increased extracellular Ca²⁺). Representative tracings show Cl⁻-independent HCO₃⁻ extrusion in HEK-293 cells expressing SLC26A9. Cells were loaded with BCECF and perfused with Cl⁻-free solutions throughout the experiment. Ca²⁺-gluconate at 4 mM was present throughout the experiments. Solutions were gassed with 95% O₂-5% CO₂. As indicated, switching from a Cl⁻-free, normal pH perfusate (pH 7.4) to a Cl⁻-free, acidic perfusate (pH 6.5) resulted in rapid intracellular acidification in cells expressing SLC26A9. Control (nontransfected) cells showed less reduction in the rate of intracellular acidification upon switching to the acidic perfusate. Amiloride at 1 mM was present throughout the experiment.
Effect of NH4+ on Cl-/HCO3- exchanger activity mediated by SLC26A9

A: HCO3- 24 mM/CO2 5% NH4+ 0 mM

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B: HCO3- 24 mM/CO2 5% NH4+ 4 mM

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three different rats, indicating that rats and mice have similar SLC26A9 expression profiles in the stomach.

In the last series of experiments, we examined the expression of SLC26A9 in the stomachs of adult NHE2-null mice. Adult NHE2-null mice develop achlorhydria because of loss of their gastric parietal cells (38). The purpose of these experiments was to better examine the nature of gastric gland cells that express SLC26A9 and ascertain the impact of achlorhydria on A9 expression in surface epithelial cells. Northern analysis experiments demonstrated that the expression of SLC26A9 was almost abolished in the stomachs of NHE2-null mice (Fig. 8A). Immunofluorescence staining (Fig. 8B) confirmed the results of Northern analysis and further revealed that SLC26A9 labeling in both surface epithelial cells and cells in gastric glands is completely eliminated. Taken together, these results suggest that the cells in gastric glands that express SLC26A9 are of a parietal cell type. The results further demonstrate that deletion of NHE2 reduces the expression of SLC26A9 in surface epithelial cells.

DISCUSSION

The acidic solution that is secreted from gastric parietal cells can achieve a pH as low as 1.5 (1, 11, 14). To protect itself against the corrosive effects of luminal acid, the gastric surface epithelium secretes a HCO3-/H11002-rich fluid into the mucus gel layer (9, 30, 31, 41). Recent studies from our laboratories identified AE4 as a Cl-/H11002/HCO3-/H11002 exchanger expressed on the apical membrane of gastric surface epithelial cells and villus cells in the duodenum (51). AE4 is not inhibited by DIDS (44), whereas acid-stimulated HCO3-/H11002 secretion in gastric surface epithelial cells is significantly inhibited by DIDS (30, 41). This suggests that HCO3- transporters other than AE4 play a major role in HCO3- secretion in the stomach.

SLC26A9 displays a unique expression pattern in the gastrointestinal tract in that abundant mRNA levels are detected in the stomach, with no detectable levels in the small and large intestines (Fig. 1). Lower levels of SLC26A9 mRNA were detected in the lung (Fig. 1). No detectable levels of SLC26A9 were identified in any other tissues, including kidney (Fig. 1). The expression of SLC26A9 in the stomach is limited to the apical domain of surface epithelial cells and certain cells in the stomach.

Fig. 6. Inhibition of SLC26A9 activity by NH4+. A and B: effect of NH4+ on Cl-/HCO3- exchanger activity. The experiments in A and B were repeated with 4 mM NH4+-acetate present throughout the experiments. As shown, the intracellular alkalization generated in response to switching to the Cl--free perfusate (Cl-/HCO3- exchange mode) was significantly inhibited in the presence of 4 mM NH4+.

Fig. 7. Effect of NH4+ on juxtamucosal pH (pHjm). A: effect of NH4+ on pHjm in rat stomach was neutral during the control period in both groups. When HCl at pH 2 was applied for the first time, pHjm decreased slightly, but significantly, from 7.5 ± 0.1 to 6.8 ± 0.3 in the control animals, and from 7.5 ± 0.1 to 6.5 ± 0.4 in the NH4+ group. Juxtamucosal pH returned to control levels when the acid was washed away. During the second instillation of acidic solution (pH 2.0), pHjm decreased to 3.7 ± 0.7 in NH4+-containing solution, which was significantly lower than in the control animals (5.6 ± 0.8). B: expression of SLC26A9 in rat stomach. Effect of NH4+ on gastric juxtamucosal pH. B: Northern hybridization of SLC26A9 in rat stomach. SLC26A9 is abundantly expressed in rat stomach (RNA from three separate stomachs was used).
gastric gland (Figs. 1–3). Expression studies in HEK-293 cells demonstrated that SLC26A9 mediates Cl\(^-\)/HCO\(_3\)^- exchange as well as Cl\(^-\)-independent HCO\(_3\)^- transport (Figs. 4 and 5).

Studies of the nature of HCO\(_3\)^- secreting transporters in gastric surface epithelial cells have produced conflicting results. While a Cl\(^-\)/HCO\(_3\)^- exchanger has been proposed to be the primary mechanism for HCO\(_3\)^- secretion in frog gastric mucosa (12), a HCO\(_3\)^- conductive pathway has been proposed to be responsible for this activity (7). SLC26A9 can function as a Cl\(^-\)/HCO\(_3\)^- exchanger and also may conduct HCO\(_3\)^- in heterologous expression systems (Fig. 4), indicating that at least a portion of the HCO\(_3\)^- secretion in surface epithelial cells is likely to be mediated via this transporter operating in Cl\(^-\)/HCO\(_3\)^- exchange or in a Cl\(^-\)-independent, HCO\(_3\)^- extruding mode. As an apical HCO\(_3\)^- transporter, SLC26A9 can neutralize gastric acidity by secreting HCO\(_3\)^-. AE4, which is expressed on the apical membrane of surface epithelial cells and mediates Cl\(^-\)/HCO\(_3\)^- exchange (44), could either contribute to HCO\(_3\)^- secretion in a similar manner, albeit at a lower rate, or demonstrate differential regulation vs. SLC26A9 in response to different signal transduction pathways.

SLC26A9-mediated Cl\(^-\)/HCO\(_3\)^- exchange and Cl\(^-\)-independent HCO\(_3\)^- transport were inhibited significantly by 4 mM NH\(_4\)^+ (Fig. 6). Interestingly, an equimolar concentration of NH\(_4\)^+ (4 mM) in HCl pH 2 perfusate reduced the pH on the apical side of the gastric surface epithelial cells in vivo (Fig. 7), which is the site of abundant SLC26A9 expression. This report is the first to suggest an inhibition of HCO\(_3\)^- transport in heterologous expression systems and the stomach by NH\(_4\)^+.

Previous reports demonstrated that other HCO\(_3\)^- transporters, including members of the SLC26 family, are activated by NH\(_4\)^+ (5, 21). One such transporter is SLC26A3 (DRA), which is expressed predominantly on the apical membranes of the colon (22), the site of an abundant concentration of NH\(_4\)^+ in vivo (32). The activation of DRA by NH\(_4\)^+ (5) may point to the regulatory effect of the physiological milieu of the colon on
NaCl absorption beyond the known interaction of DRA and NHE3, the apical NHE.

The reduction of pH$_{jm}$ by NH$_3^+$ (Fig. 7) strongly suggests that disease states associated with increased NH$_4^+$ production in the stomach may have a detrimental effect on HCO$_3^-$ secretion and predispose patients to acidic injury. H. pylori, which resides in the gastric mucus layer, generates NH$_3$/NH$_4^+$ in the stomach through the action of its urease enzyme (18, 35, 36, 45). Several studies have measured the concentration of NH$_3$ in gastric juice of individuals with H. pylori infection. These studies demonstrated that the concentration of NH$_3$ was in the range of 3 to 7 mM in individuals with H. pylori infection and decreased to very low levels (<0.5 mM) with the eradication of H. pylori, indicating that H. pylori was responsible for the generation of NH$_3$ (27, 46). Given a negative log dissociation constant (pK) of ~9.0 for NH$_3$/NH$_4^+$ dissociation and assuming a gastric juice pH in the same range as the pK (which is very unlikely because it is usually lower) or <9 (which is more likely), one arrives at a gastric juice NH$_4^+$ concentration in the range of 7–30 mM in individuals with H. pylori infection, well above the concentration of NH$_4^+$ used for the in vitro and in vivo studies in our present study. This clearly suggests that H. pylori is capable of inhibiting gastric HCO$_3^-$ secretion through NH$_3$/NH$_4^+$ generation.

Several studies have demonstrated the harmful effect of NH$_3$/NH$_4^+$ on the gastric mucosa (28, 43, 52). It should be noted that the concentration of NH$_3^+$ that was used in those studies (28, 43, 52) was at least 5–10 times higher than the concentration of NH$_4^+$ used in our experiments. Furthermore, those experiments were invariably conducted at higher pH, thereby resulting in an elevated concentration of NH$_3$, which has been presumed to be toxic (28, 43, 52). Because of a much lower concentration of NH$_4^+$ and a much lower pH, we think that the concentration of NH$_3$ in our experiments is negligible. Last, the inhibitory effect of NH$_4^+$ on HCO$_3^-$ secretion in the stomach was transient (Fig. 7), and the pH$_{jm}$ returned to normal levels upon removal of NH$_4^+$, indicating that the possibility of a toxic effect of NH$_4^+$ on the integrity of the gastric mucosa is unlikely. It has been observed that NH$_4^+$ at 10 mM inhibits acid secretion (16). Those studies, however, applied 10 mM NH$_4^+$ basolaterally and not lumina!ly (16). In our study, we have applied 4 mM NH$_4^+$ on only the apical side and thus the concentration of NH$_4^+$ on the basolateral side (if any) must be very low. Therefore, we think that the effect of 4 mM NH$_4^+$ on gastric pH$_{jm}$ is independent of acid secretion. Indeed, the acid secretion remained constant before any treatment, during saline/NH$_4^+$, and after second HCl exposure (see RESULTS).

In addition to the stomach, SLC26A9 is also expressed in trachea, where it is located on the apical membrane of tracheal epithelial cells (24). The expression of SLC26A9 in the lung (Fig. 1) originates predominantly from the tracheal-bronchial tree and not from the alveolar cells. In support of this conclusion, we have found strong expression of SLC26A9 in mRNA from human trachea (data not shown), in accordance with previously published reports (24). Both Cl$^-$/HCO$_3^-$ exchange and Cl$^-$-independent HCO$_3^-$ conductance have been identified on the apical membranes of tracheal epithelial cells (8, 50). Given its apical localization, mediation of Cl$^-$/HCO$_3^-$ exchange, and Cl$^-$-independent HCO$_3^-$ transport, we suggest that SLC26A9 plays an important role in HCO$_3^-$ secretion in tracheal epithelial cells.

In conclusion, SLC26A9 is abundantly expressed in the stomach. In situ hybridization and immunocytochemical staining localized SLC26A9 to the apical membranes of surface epithelial cells and the membranes of parietal cells in the stomach. Functional studies demonstrated that SLC26A9 operates in Cl$^-$/HCO$_3^-$ exchange and also may function as a Cl$^-$-independent HCO$_3^-$-extruding pathway. We propose that SLC26A9 is an apical HCO$_3^-$ transporter in gastric surface epithelial cells and that it may play an important role in protecting the gastric epithelium against injury by the acid secreted from gastric parietal cells.

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