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SLC26A7 Is a Cl^- Channel Regulated by Intracellular pH*

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Members of the SLC26 transporter family play an essential role in several epithelial functions, as revealed by diseases associated with mutations in members of the family. Several members were shown to function as Cl^- and HCO_3^- transporters that likely play an important role in epithelial Cl^- absorption and HCO_3^- secretion. However, the mechanism of most transporters is not well understood. SLC26A7 is a member of the SLC26 transporter family reported to be expressed in the basolateral membrane of the cortical collecting duct and parietal cells and functions as a coupled Cl^-/HCO_3^- exchanger. In the present work we examined the transport properties of SLC26A7 to determine its transport characteristics and electrogenicity. We found that when expressed in *Xenopus* oocytes or HEK293 cells SLC26A7 functions as a pH_i-regulated Cl^- channel with minimal OH^-/HCO_3^- permeability. Expression of SLC26A7 in oocytes or HEK293 cells generated a Cl^- current with linear *I*/*V* and an instantaneous current that was voltage- and time-independent. Based on measurement of reversal potential the selectivity of SLC26A7 is $NO_3^- \gg Cl^- = Br^- = I^- > SO_4^{2-} = Glu^-$, although I^- partially inhibited the current. Incubating the cells with HCO_3^- or butyrate acidified the cytosol and increased the selectivity of SLC26A7 for Cl^-. Measurement of membrane potential and pH_i showed minimal OH^- and HCO_3^- transport by SLC26A7 when the cells were incubated in Cl^--containing or Cl^--free media. The activity of SLC26A7 was inhibited by all inhibitors of anion transporters tested, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid, diphenylamine-2-carboxylic acid, and glybenclamide. These findings reveal that SLC26A7 functions as a unique Cl^- channel that is regulated by intracellular H^+.

The SLC26 is a relatively new family of Cl^- and HCO_3^- transporters that is coded by at least 10 genes, most of which have several splice variants (1). The founding member of the family is SLC26A3, mutations of which are associated with congenital Cl^- diarrhea (2). Other members of the family include SLC26A2, mutations of which are associated with diastrophic dysplasia (3), pendrin (SLC26A4), mutations of which cause Pendred syndrome (4) and Prestin (SLC26A5), which is involved in hearing (5). SLC26A3 and SLC26A6 appear to play important roles in epithelial Cl^- absorption and HCO_3^- secretion (1). These SLC26 transporters are expressed in the luminal membrane of ductal systems that also express the cystic fibrosis transmembrane conductance regulator (CFTR)[1] (6). All SLC26 transporters examined are activated by CFTR and in turn activate CFTR by increasing its open probability (7).

All members of the family tested so far were shown to function as exchangers with defined substrate specificity. SLC26A1 (8) and SLC26A2 (9) are SO_4^{2-} transporters but can also transport Cl^-. SLC26A3 is a *Buna feed* coupled Cl^-/HCO_3^- exchanger (6, 10), SLC26A4 is an I^- transporter that can also function as a Cl^-/HCO_3^- exchanger (4, 6, 11), and SLC26A6 functions as a Cl^-/oxalate (12) and Cl^-/HCO_3^- exchanger (6, 13). The exact transport mechanism of most SLC26 transporters is not well understood. However, an important recent finding was that the SLC26 transporters are electrogenic (6, 13) with isoform-specific stoichiometry (6).

Another SLC26 transporter that was characterized recently is SLC26A7 (14–16). SLC26A7 shows very restricted distribution, and so far it has been found only in gastric parietal cells (15) and the intercalated cells of the outer medullary collecting duct (16). Unlike other members of the family that are expressed at the luminal membrane of epithelial cells (1), SLC26A7 was found at the basolateral membrane of both parietal cells and intercalated cells (15, 16). It was reported that SLC26A7 functions as a coupled Cl^-/HCO_3^- exchanger (15, 16) and was thus suggested to play an important role in clearing HCO_3^- from the parietal cells during acid secretion (15). Furthermore, SLC26A7 was reported to be markedly activated by cell shrinkage and thus to mediate HCO_3^- efflux into the hypertonic fluid of the collecting duct (16).

As part of our effort to understand the role of the SLC26 transporters in epithelial Cl^- absorption and HCO_3^- secretion and to determine whether SLC26A7 is an electrogenic transporter, we characterized the transport properties of SLC26A7. Surprisingly, we were unable to show that SLC26A7 functions as a Cl^-/HCO_3^- exchanger or even that SLC26A7 transports much of HCO_3^-. In fact, of all the SLC26 transporters examined to date in our laboratory (SLC26A3, -4, -6, and -7), SLC26A7 is the least permeable to HCO_3^-. Rather, when expressed in *Xenopus* oocytes or HEK293 cells SLC26A7 behaves as a Cl^- channel. The selectivity of SLC26A7 was $NO_3^- \gg Cl^- = Br^-$. I^- inhibited the current. Interestingly, SLC26A7 is regulated by pH_i, where H^+_{in} does not increase the current but rather increases the selectivity of SLC26A7 for Cl^-.

EXPERIMENTAL PROCEDURES

Materials—2′,7′-Bis(2-carboxyethyl-5,6-carboxyfluorescein acetoxymethyl ester was from Teff Laboratories, DIDS (4,4′-diisothiocyanostilbene-2,2′-disulfonic acid) was from Molecular Probes, diphenylamine-2-carboxylic acid (DPC) was from Alexis Corp., and glybenclamide was from Sigma. All other chemicals and salts were from Sigma. The expressed sequence tag clone of mouse SLC26A7 was ob-

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[1] The abbreviations used are: CFTR, cystic fibrosis transmembrane conductance regulator; MQAE, *N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide; RP, reversal potential; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; DPC, diphenylamine-2-carboxylic acid; Glyb, glybenclamide.

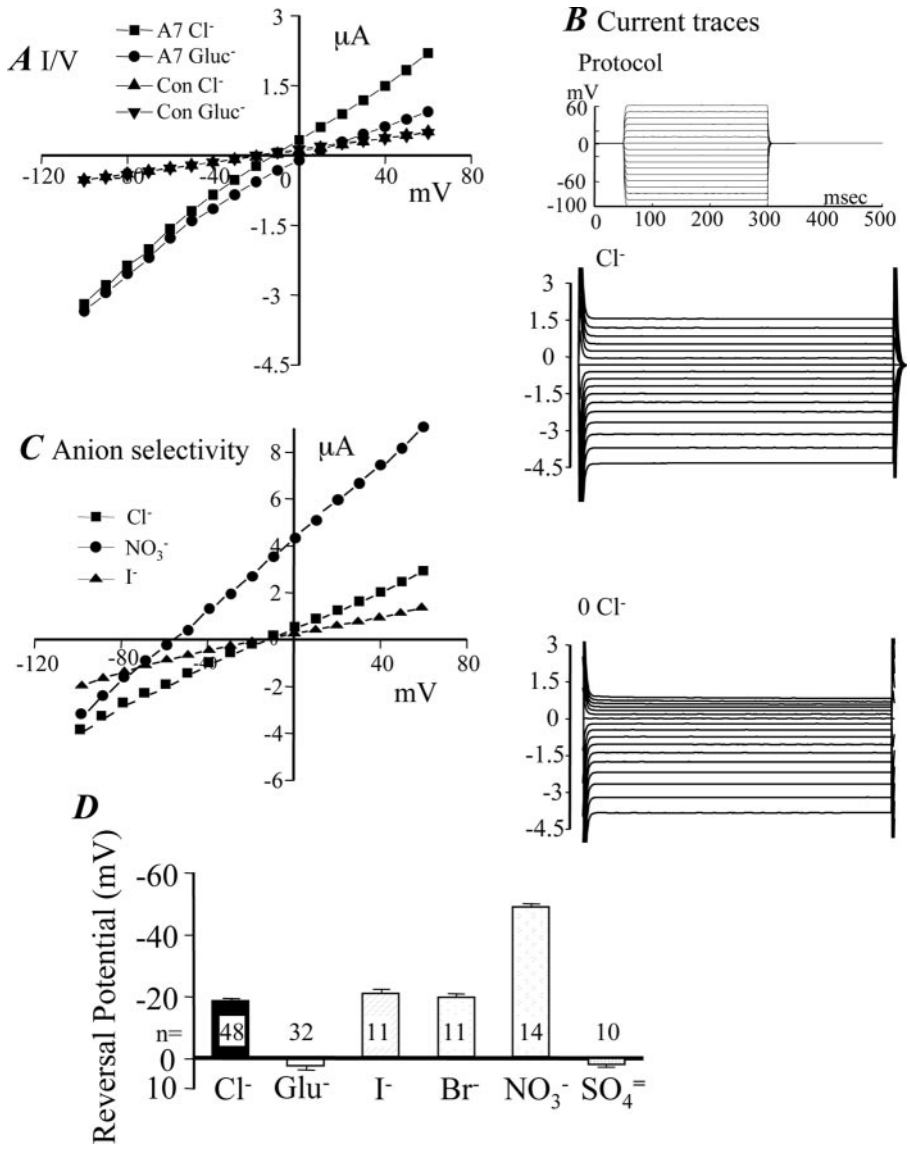

FIG. 1. **Cl^- current in *Xenopus* oocytes expressing SLC26A7.** *A*, control oocytes (▲, ▼) and oocytes expressing SLC26A7 (■, ●) were used to measure the I/V in the presence (■, ▲) and absence (●, ▼) of Cl^-. *B* shows the instantaneous current. *C* shows the current in the presence of Cl^- (■) NO_3^- (●) or I^- (▲). *D* summarizes the reversal potential as measured with the indicated ions. The *boxed numbers* indicate the number of experiments under each condition.

tained from Invitrogen (IMAGE clone number 4020760), completely sequenced in the 5′ and 3′ directions, and subcloned into the MluI and NotI sites of the pCMV.Sport6 plasmid. The standard bath solution for HEK293 cells (solution A) contained (in mM) 145 NaCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES (pH 7.4 with NaOH), and 10 glucose. Cl^--free media were prepared by replacing Cl^- with gluconate. Solutions containing other halids or NO_3^- were prepared by isosmotic replacement of NaCl and KCl with the respective salts. High K^+ solutions were prepared by replacing NaCl with KCl. Where indicated 40 mM Na^+ butyrate replaced 40 mM NaCl. HCO_3^--buffered solutions were prepared by replacing 25 mM NaCl or Na^+-gluconate with 25 mM Na^+-HCO_3^- and reducing HEPES to 5 mM. HCO_3^--buffered solutions were gassed with 5% CO_2 and 95% O_2. The osmolarity of all solutions was adjusted to 310 mosM with the major salt.

Cells—HEK293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin, and streptomycin. For functional studies HEK293 cells were co-transfected with SLC26A7 and a plasmid coding for green fluorescent protein. Green fluorescent protein fluorescence was used to identify the transfected cells. Transient transfection was by Lipofectamine. To prepare cRNA for experiments in *Xenopus* oocytes the plasmid pCMV.Sport6-SLC26A7 was linearized with NotI. The cRNA was transcribed using the mMESSAGE mMACHINE SP6 kit (Ambion, Austin, TX).

Measurement of pH_i and $[Cl^-]_i$ in HEK293 Cells—The procedure for pH_i measurement in HEK293 cells was identical to that described previously (17). HEK293 cells were loaded with 2′,7′-bis(2-carboxyethyl)-5,6-carboxyfluorescein by incubation with 5 μM 2′,7′-bis(2-carboxyethyl)-5,6-carboxyfluorescein acetoxymethyl ester at room temperature for 20 min. Fluorescence was recorded at excitation wavelengths of 490/440 nm, and fluorescence ratios were calibrated using the nigericin-high K^+ clamp as before. $[Cl^-]_i$ was measured with MQAE as before (18). The cells were loaded by incubation with 5 mM MQAE for 1 h at room temperature. MQAE fluorescence was recorded at an excitation wavelength of 360 nm while the cells were perfused with solutions containing 150 mM Cl^- or 150 mM NO_3^-.

Electrophysiology—The whole cell configuration of the patch clamp technique was used to measure the Cl^- current in control and SLC26A7-transfected HEK293 cells as detailed before (19). The pipette solution contained (mM) 140 *N*-methyl-D-glucamine-Cl, 1 $MgCl_2$, 2 EGTA, 5 ATP, and 10 HEPES (pH 7.3 with Tris), and the bath solution was Na^+-free solution A. The current was recorded using an Axopatch 200A patch clamp amplifier and digitized at 2 kHz. The membrane conductance was probed by stepping the membrane potential from a holding potential of 0 mV to membrane potentials between −100 and +60 mV at 10-mV steps for 200 ms with 500-ms intervals between steps. Pipettes had resistance between 5 and 7 megohms when filled with an intracellular solution, and seal resistance was always more than 8 gigohms. Current recording and analysis were performed with the pClamp 6.0.3 software.

For current recording in oocytes the oocytes were obtained by partial ovariectomy of anesthetized female *Xenopus laevis*. Follicles were removed and defolliculated as detailed before (6). Healthy oocytes in stage V to VI were injected with 10–25 ng of cRNA in a final volume of 50 nl. Injected oocytes were incubated at 18 °C in an ND-96 solution composed of (in mM) 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 2.5 pyruvate, 5 HEPES-Na, pH 7.5, and oocytes were used 48–96 h post-injection. Na^+-free media were prepared by replacing Na^+ with *N*-methyl-D-glucamine, and Cl^--free media were prepared by replacing Cl^- with gluconate. To

prepare solutions containing Br^-, I^-, or NO_3^- NaCl and KCl were replaced with the relevant salt that was supplemented with 1.8 mM Ca^{2+}-cyclamate, 1 $MgSO_4$, 2.5 pyruvic acid, 5 HEPES, pH 7.5, with Tris. A two-electrode voltage clamp setup was used to record the current as detailed before (6). Current and voltage were digitized with a Digidata 1322A A/D converter and analyzed by the Clampex 8.1 system.

The pH_i of oocytes was measured with single-barreled borosilicate-silanized microelectrodes as described before (20). In brief, ~0.5 μl of proton exchanger resin was introduced into the tip of the microelectrodes, and the electrodes were backfilled with an ND-96 solution and calibrated in standard solutions of pH 6, 7, and 8 before and after each experiment. The electrodes were fitted with a holder with an Ag-AgCl wire attached to a high impedance probe of a two-channel FD-223 electrometer. A second channel was used for measurement of membrane potential using a standard microelectrode. The bath was grounded via a 3 M KCl agar bridge connected to an Ag-AgCl wire. The signal from the voltage microelectrode was subtracted from the voltage of the pH electrode to obtain the pH_i changes. Initial rates of change in pH_i were determined from the slope of the line obtained by fitting pH_i *versus* time to a linear regression line. The slope of the pH electrodes was between 56 and 57 mV (pH unit)$^{-1}$. For measurement of pH_i under voltage clamp conditions, a three-electrode setup was used. Two standard microelectrodes connected to an OC-725C amplifier were used for voltage and current clamps, and one electrode containing the pH resin was attached to a FD-223 electrometer. A common reference electrode was used for both amplifiers. Data were acquired as described above for pH_i measurement.

Statistical Analysis—Results in all experiments are given as the mean ± S.E. of the indicated number of experiments. The results of multiple experiments were analyzed using analysis of variance or by paired or non-paired Student's *t* test as appropriate.

RESULTS AND DISCUSSION

SLC26A7 Is an Electrogenic Transporter—Two recent studies expressed SLC26A7 in *Xenopus* oocytes and measured pH_i to conclude that SLC26A7 is a cell shrinkage-activated Cl^-/HCO_3^- exchanger (15, 16). To test whether SLC26A7 is an electrogenic transporter the protein was expressed in *Xenopus* oocytes, and the resulting current was measured in Cl^--containing and Cl^--free media. Fig. 1*A* displays the nearly linear *I*/*V* curves recorded from control oocytes and oocytes expressing SLC26A7. Expression of SLC26A7 generated a current that at +60 mV averaged 2.7 ± 0.3 μA (n = 18). Removal of external Cl^- reduced the outward current at +60 mV to 1.1 ± 0.2 μA. To further characterize the current mediated by SLC26A7, Fig. 1*B* shows that the instantaneous inward and outward currents are time- and voltage-independent, similar to the properties of the current mediated by CFTR (21).

The ion selectivity of SLC26A7 was measured by ion substitution. Replacing external Na^+ or K^+ with *N*-methyl-D-glucamine had no effect on the current, indicating that SLC26A7 does not conduct these ions (data not shown). Measurement of reversal potentials (RP) as in Fig. 1*C* showed that SLC26A7 has a similar low permeability to gluconate and SO_4^{2-}, identical permeability to Cl^- and Br^- and very high permeability to NO_3^- (Fig. 1*D*). Interestingly, I^- showed anomalous behavior. Replacing external Cl^- with I^- had no effect on the reversal potential, but reduced both the inward and outward currents. Because the inward current is mediated by Cl^- efflux, I^- is an inhibitor of Cl^- transport by SLC26A7. Therefore, the anionic selectivity of SLC26A7 is $NO_3^- > Cl^- = Br^- >$ Glu, and is similar to that reported for CFTR (21), including the anomalous effect of I^- (22).

Regulation of SLC26A7 Selectivity by pH_i—As a first test of HCO_3^- transport by SLC26A7 we recorded the effect of HCO_3^- on the Cl^- current. The current was recorded 5–7 min after the exposure to CO_2/HCO_3^- to allow completion of cellular acidification. The CO_2/HCO_3^- acidifies the oocytes because of the rapid diffusion of CO_2 into the cell and its hydration in the cytosol. In all experiments (n = 14) HCO_3^- had no effect on the current recorded in the presence of Cl^- (Fig. 2*A*). However,

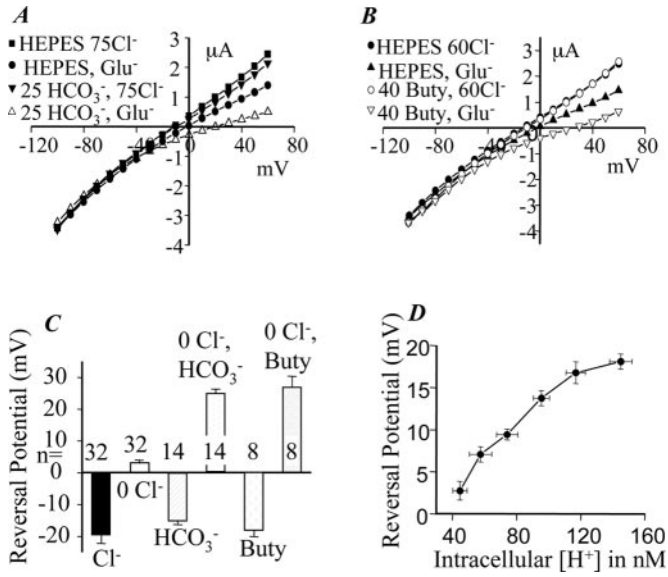

FIG. 2. **Effect of pH_i on the SLC26A7 Cl^- conductance.** *A*, the *I*/V curves were measured in oocytes expressing SLC26A7 and incubated in HEPES- or HCO_3^--buffered media containing 75 or 0 mM Cl^- as indicated on the figure. The *I*/V relations were measured 5–7 min after exposure to HCO_3^--buffered media to allow completion of acidification and stabilization of pH_i (see also Fig. 3). *B*, oocytes incubated in HEPES-buffered media containing 0 or 60 mM Cl^- as indicated and without (●, ▲) or with (○, ▽) 40 mM butyrate (*Buty*). The *I*/V relations were measured 10 min after the exposure to butyrate when pH_i was stable. *C* shows summary of the reversal potential recorded under each condition from the number of experiments indicated next to the columns. Note that HCO_3^- and butyrate had minimal effect on the current and reversal potential in the presence of Cl^- but markedly shifted the reversal potential in the absence of Cl^-. *D*, effect of pH_i on the RP. The oocytes were incubated in solutions containing 40 mM butyrate, and after completion of the acidification and recording of the *I*/V relation at the most acidic pH_i the oocytes were successively incubated in solutions containing 30, 20, 10, 5, and 0 mM butyrate. About 5 min after each change in butyrate concentration pH_i was stable, and the *I*/V relation was recorded. The mean ± S.E. of the RP are plotted as a function of internal H^+ concentration that was calculated from the average pH_i in three separate experiments.

HCO_3^- noticeably shifted the reversal potential in Cl^--free media from +2.75 ± 1.1 mV (n = 32) in HEPES-buffered media to +19.5 ± 1.4 mV (n = 14) in HCO_3^--buffered media (Fig. 2*C*). The effect of HCO_3^- can be mediated by HCO_3^- itself or by the reduction in pH_i caused by exposing the oocytes to a HCO_3^--buffered media equilibrated with CO_2. To distinguish between the two possibilities we measured the effect of butyrate on pH_i (see Fig. 3) and the *I*/V in Cl^--containing and Cl^--free media. Butyrate acidified pH_i similar to HCO_3^-. Fig. 2, *B* and *C*, show that butyrate had the same effect as HCO_3^- by having no effect on the current in Cl^--containing media and shifting the RP in Cl^--free media to +21.9 ± 3.4 mV (n = 8). The pH_i dependence of the shift in RP is shown in Fig. 2*D*. In preliminary experiments we found that the most reliable data could be obtained by acidifying the oocytes with 40 mM butyrate and then reducing the butyrate concentration stepwise from 40 to 30, 20, 10 and 5 mM. Reducing pH_i from a resting level of 7.35 ± 0.02 (n = 3, H^+ concentration of 44.7 nM) to 6.93 ± 0.02 (n = 3, H^+ concentration of 117 nM) was sufficient to cause the maximum shift in RP. Therefore, the findings in Fig. 2 indicate that pH_i regulates SLC26A7 channel selectivity. Acidic pH (increased intracellular H^+ or reduced OH^- ions) increases the selectivity of SLC26A7 for Cl^-. To our knowledge SLC26A7 is the first Cl^- channel the selectivity of which is regulated by intracellular acidification.

SLC26A7 Is a Poor OH^-/HCO_3^- Transporter—The previous reports of HCO_3^- transport by SLC26A7 (15, 16) and the effect

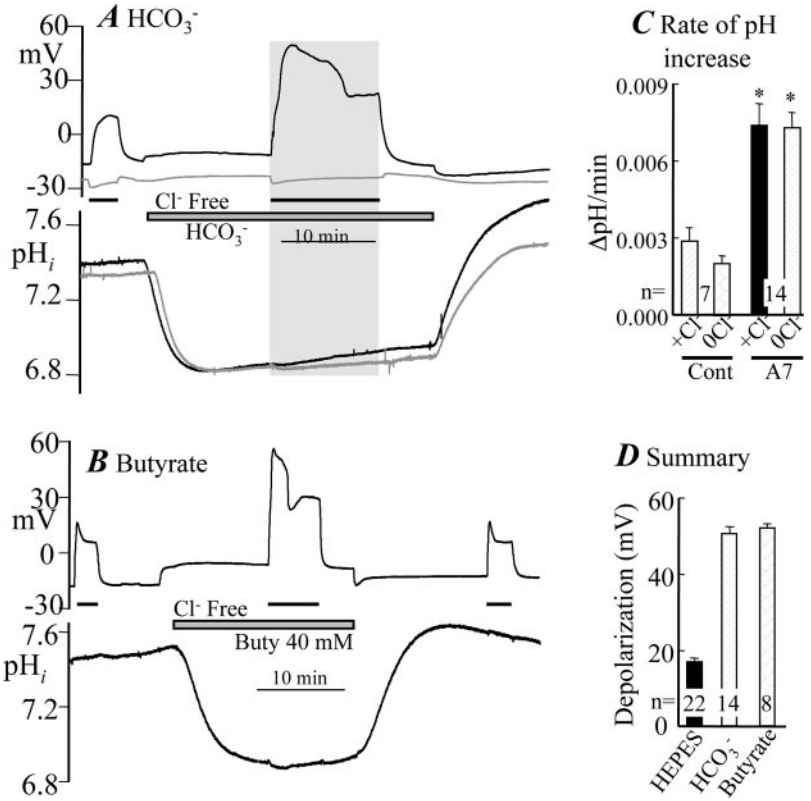

FIG. 3. **Lack of HCO_3^- transport by SLC26A7.** Oocytes were used to measure the membrane potential and pH_i. *A*, control oocytes (*gray traces*) and oocytes expressing SLC26A7 (*black traces*) in HEPES-buffered media were exposed to Cl^--free media that hyperpolarized control and depolarized oocytes expressing SLC26A7. Then the oocytes were incubated in HCO_3^--buffered media, and where indicated the oocytes were incubated in Cl^--free, HCO_3^--buffered media. The *shaded area* marks the period of incubation in Cl^--free medium. Finally, Cl^- was added back to the perfusate, and then HCO_3^- was removed by perfusing the oocytes in HEPES-buffered media. A similar protocol was used in *B* except that the oocytes were incubated in media containing 40 mM butyrate (*Buty*) instead of HCO_3^-. *C* shows the averaged rates of pH_i increase in 7 control (*Cont*) and 14 SLC26A7-expressing (*A7*) oocytes before and after removal of external Cl^-. * indicates difference from the respective control at $p < 0.05$. *D* summarizes the change in membrane potential measured at the indicated number of experiments.

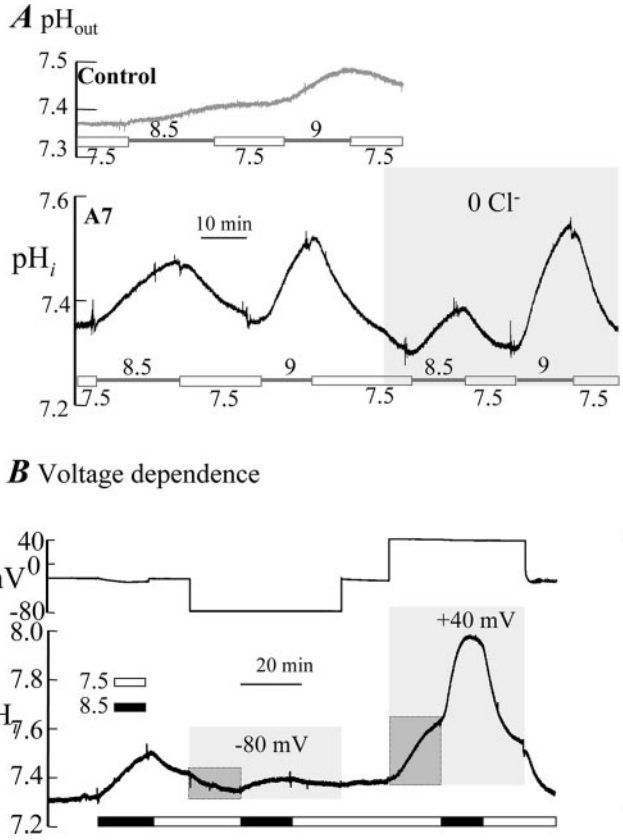

FIG. 4. **Effect of membrane potential and external pH on pH_i.** *A*, pH_i was measured in control oocytes (*upper gray trace*) and oocytes expressing SLC26A7 (*lower black trace*). pH_o was alternated between 7.5, 8.5, and 9.0 as indicated by the bars, first in Cl^--containing and then in Cl^--free, HEPES-buffered media. *B*, pH_i was measured in oocytes expressing SLC26A7 while clamping the membrane potential at −80 or +40 mV as indicated. Note that at constant external Cl^- and pH, hyperpolarization acidified and depolarization alkalinized the cells.

of pH_i on SLC26A7 Cl^- selectivity (Fig. 2) prompted us to measure HCO_3^- transport by SLC26A7. In Fig. 3*A* the effect of removal and addition of Cl^- on membrane potential and pH_i was measured in the presence and absence of HCO_3^-. Removal of Cl^- in both HEPES- and HCO_3^--buffered media depolarized the cells. However, in the absence of HCO_3^- removal of Cl^- depolarized the cells by 17 ± 1 mV, whereas in the presence of HCO_3^- removal of Cl^- depolarized the cells by 50 ± 2.5 mV (n = 14) (Fig. 3*D*), consistent with the increased selectivity to Cl^- in HCO_3^--buffered media shown in Fig. 2. The same pattern of depolarization was observed in oocytes incubated with butyrate (Fig. 3*B*) when removal of Cl^- in the absence of butyrate

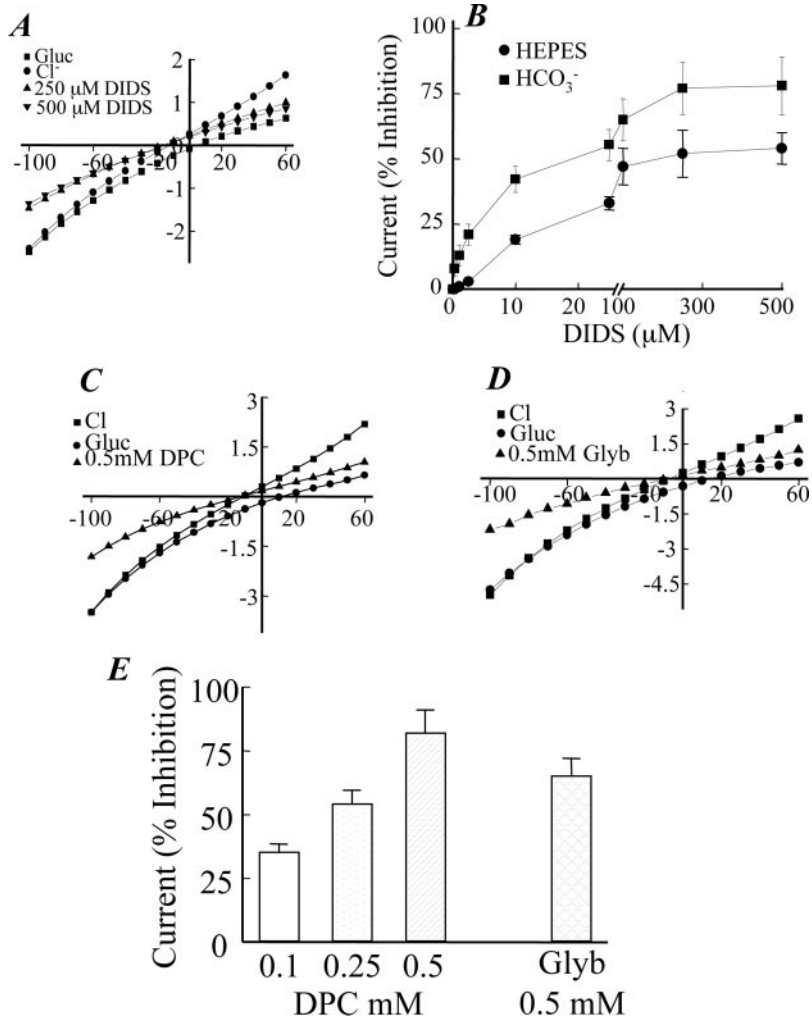

FIG. 5. **Effect of blockers on SLC26A7 activity.** Oocytes in HEPES or HCO_3^--buffered media were used to measure current first in the absence of blockers and then at increasing blockers concentrations. After each increase in blocker concentration the *I*/*V* curves were recorded to verify no change in reversal potential and similar inhibition in all voltages. 100% was taken as the Cl^--dependent current, and inhibition was calculated as percent of this current. *A*, *C*, and *D* show representative *I*/*V* curves, and *B* and *E* show the summary of multiple experiments. *A* and *B* show inhibition by DIDS, *C* and *E* show inhibition by DPC, and *D* and *E* show inhibition by Glyb.

depolarized the oocytes by 16 ± 1.3 and in the presence of butyrate by 52 ± 1.3 mV (n = 8) (Fig. 3*D*).

Surprisingly, SLC26A7 showed minimal OH^- or HCO_3^- transport. Thus, removal of external Cl^- in the presence or absence of HCO_3^- or butyrate resulted in a very slow rate of alkalinization (Fig. 3, *A* and *B*). In fact, in the presence of HCO_3^- the rate of alkalinization before and after removal of Cl^- in oocytes expressing SLC26A7 was the same and was less than 0.01 ΔpH/min (Fig. 3*C*). A small difference was found when the rate of alkalinization was compared between control oocytes and oocytes expressing SLC26A7 (control, 0.0029 ± 0.0005, n = 7; SLC26A7 0.0074 ± 0.0008 ΔpH/min, n = 14, $p <$ 0.05). Previous work used medium buffered with 33 mM HCO_3^-. We repeated the experiments in Fig. 3 in media buffered with 33 mM (n = 7) and 50 mM (n = 5) HCO_3^-. Increasing HCO_3^- did not change the findings of large depolarization and no HCO_3^- influx upon incubation of the oocytes in Cl^--free media.

The results in Fig. 3 suggest that SLC26A7 may have a finite permeability to HCO_3^- and perhaps OH^-. To verify this possibility and explore the role of Cl^- in OH^- transport we measured the effect of increasing pH_o on pH_i in the presence and absence of external Cl^-. The *upper trace* in Fig. 4*A* is the control. The *lower trace* in Fig. 4*A* shows that in oocytes expressing SLC26A7 increasing pH_o from 7.5 to 8.5 and 9.0 increased pH_i accordingly. Importantly, restoring pH_o to 7.5 resulted in recovery of pH_i, and the increase and recovery of pH_i in response to changes in pH_o were minimally affected by removal of external Cl^-. To test whether SLC26A7 OH^- permeability is conductive we measured the effect of the membrane potential on pH_i. The experiments were done in HEPES-buffered media because at the acidic pH_i in HCO_3^--buffered media changes in membrane potential had minimal effect on pH_i (data not shown). The *darkly shaded areas* in Fig. 4*B* show that at constant Cl^-_o and pH_o, clamping the membrane potential at −80 mV acidified the cytosol by 0.07 ± 0.01 pH units, whereas clamping the membrane potential at +40 mV alkalinized the cytosol by 0.22 ± 0.03 pH units (n = 4). Hence, the transport of OH^- by SLC26A7 is purely conductive. The combined results in Figs. 3 and 4 show that SLC26A7 has a very low permeability to OH^- and HCO_3^-, leading us to conclude that SLC26A7 is not a HCO_3^- transporter but rather is a Cl^- channel that poorly conducts OH^- and HCO_3^-.

Sensitivity to Blockers—Common blockers used to probe anion transporters are DIDS, DPC, and glybenclamide (Glyb). The effect of these blockers on the SLC26A7 current is depicted in Fig. 5. Fig. 5, *A* and *B*, shows the complex inhibition by DIDS. In HEPES-buffered media the inhibition saturated at 100 μM DIDS but was partial. Increasing DIDS to 500 μM did not change the extent of the inhibition, which plateaued at 56 ± 8% (n = 5). HCO_3^- increased the apparent affinity to DIDS from about 17 ± 4 to 7.1 ± 2.3 μM and the maximal inhibition to 74 ± 13% (n = 5) (Fig. 5*B*). This finding further indicates that pH_i and HCO_3^- modify the properties of SLC26A7 as was found in Fig. 2 for the selectivity to Cl^-. DPC also inhibited the current mediated by SLC26A7 (Fig. 5*C*) in a dose-dependent manner (Fig. 5*E*), with 78 ± 14% (n = 3) inhibition at 0.5 mM DPC. Finally, SLC26A7 current was inhibited by Glyb (Fig. 5*D*) with 65 ± 9% (n = 3) inhibition by 0.5 mM Glyb. The inhibitor

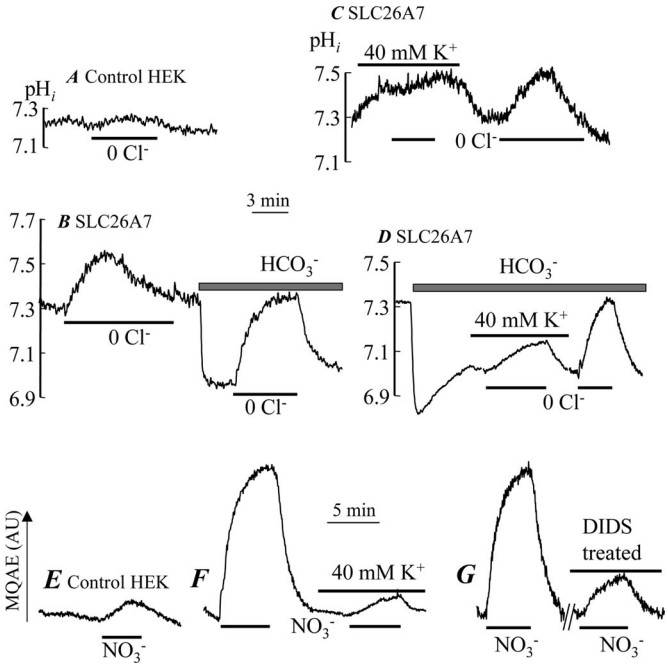

FIG. 6. **OH^-/HCO_3^- permeability and Cl^- fluxes by SLC26A7 in HEK293 cells.** Control HEK293 cells (*A*) and HEK293 cells transfected with SLC26A7 (*B–D*) loaded with 2′,7′-bis(2-carboxyethyl-5,6-carboxyfluorescein were used to measure pH_i in HEPES- or HCO_3^--buffered media as indicated by the *thick bars*. The cells were incubated in Cl^--free media as indicated by the *solid bars*. *C* and *D*, the cells were incubated in media containing 40 mM KCl and then in the control solution A during incubation in Cl^--free media. Control (*E*) and SLC26A7-transfected HEK293 cells (*F* and *G*) loaded with MQAE were used to measure Cl^- channel activity by incubating the cells in media in which Cl^- was replaced by NO_3^-. *F*, where indicated the media contained 40 mM KCl. *G*, after the control period the cells with preincubated with 0.25 mM DIDS for 5 min, and then DIDS was removed because it interfered with MQAE fluorescence.

profile found for the current mediated by SLC26A7 emphasizes the need to interpret such studies with much caution because these blockers were shown to inhibit other Cl^- transporters. Of particular interest is the inhibition by Glyb. Often, inhibition of a Cl^- current by Glyb is taken as evidence that the current is mediated by CFTR. Inhibition of the SLC26A7 current by Glyb indicates that inhibition by Glyb cannot be taken as evidence of current mediation by CFTR.

Properties of SLC26A7 Expressed in HEK293 Cells—The difference between the current work leading to the conclusion that SLC26A7 is a pH_i-regulated Cl^- channel and that reported before concluding that SLC26A7 is a coupled Cl^-/HCO_3^- exchanger (15, 16) raises the possibility that expression in oocytes may lead to activation of differential endogenous transporters that result in the different findings. It was therefore important to express the mammalian clone in a mammalian cell and examine its transport properties. HEK293 cells are suitable for such studies because they have low endogenous Cl^-/OH^- exchange activity (Fig. 6*A*). Expression of SLC26A7 in HEK293 cells and incubation of the cells in HEPES- or HCO_3^--buffered, Cl^--free media resulted in an increase in pH_i as if SLC26A7 mediates Cl^-/OH^- and Cl^-/HCO_3^- exchange, respectively (Fig. 6*B*). However, this is not the case because in HEPES-buffered media the increase in pH_i was transient, spontaneously returning to basal level ($n > 20$). In addition, the effect of removing external Cl^- on pH_i was blocked by depolarizing the cells with 40 mM external K^+ (Fig. 6*C*). Although the increase in pH_i due to Cl^- removal of cells incubated in HCO_3^--buffered media was more stable, it was also reduced by cell depolarization (Fig. 6*D*). All the pH_i changes recorded under the conditions of Fig. 6 were inhibited by about 60–75% by 0.25 mM DIDS (data not shown).

Two assays were used to show that SLC26A7 expressed in HEK293 cells functions as a Cl^- channel. First, we measured $[Cl^-]_i$ with MQAE and followed the rate of Cl^-/NO_3^- exchange as was used before to assay CFTR channel activity (6, 18). Fig. 6*E* is the control, and Fig. 6*F* shows the high rate of Cl^-/NO_3^- exchange mediated by SLC26A7. Importantly, the SLC26A7-mediated Cl^-/NO_3^- exchange was inhibited 78 ± 6% ($n = 3$) by depolarizing the cells with 40 mM external K^+. The SLC26A7-mediated Cl^-/NO_3^- exchange was inhibited by preincubation of the cells with 0.25 mM DIDS (Fig. 6*G*).

In the second assay we characterized the current generated by expressing SLC26A7 in HEK293 cells. Fig. 7*A* shows that expression of SLC26A7 in HEK293 cells resulted in a large Cl^- current that averaged 673 ± 114 pA ($n = 5$). In symmetrical Cl^- concentrations the current followed linear *I*/V with reversal potential of 2 ± 3 mV. As was found in oocytes (Fig. 1), the instantaneous current in HEK293 cells was time- and voltage-independent (Fig. 7, *B* and *C*). Interestingly, at 150 mM intracellular Cl^- the current in HEK293 cells maintained in HEPES-buffered media was highly Cl^- selective, as evident from the shift in the reversal potential to 58 ± 5 mV ($n = 5$) and the a lack of outward current in Cl^--free medium (Fig. 7*A*). The lack of outward current made it difficult to demonstrate the effect of pH_i on SLC26A7 selectivity in HEK293 cells. Therefore, we measured the SLC26A7-mediated current in cells infused with the more physiological Cl^- concentration of 30 mM. Fig. 7, *D* and *E*, show that the RP of the SLC26A7 current in HEK293 cells infused with a pipette solution containing 30 mM Cl^-, pH of 7.5, and incubated in Cl^--free media is about 43 ± 3 mV. Exposing these cells to HCO_3^--buffered media for 1 min, a time sufficient for completion of the acidification and before substantial recovery of pH_i (Fig. 6), shifted the RP to 57 ± 2 mV. When the cells were infused with a pipette solution buffered with HEPES to a pH of 6.5, the RP was 65 ± 3.5 mV, and exposing these cells to a HCO_3^--buffered media had no further effect on the RP, which averaged 53 ± 4.4 mV. These findings indicate that in HEK293 cells SLC26A7 Cl^- channel activity is also regulated by pH_i and that HCO_3^- likely does not regulate the channel independent of pH_i because it did not affect the SLC26A7 current in cells maintained at a pH_i of 6.5.

In conclusion, measurement of membrane current and voltage and pH_i in *Xenopus* oocytes and HEK293 cells expressing SLC26A7 reveal that SLC26A7 functions as a Cl^- channel that is regulated by pH_i. Several protocols were used to evaluate OH^-/HCO_3^- transport by SLC26A7, and all reported very low permeability. In fact, OH^-/HCO_3^- transport was found only when pH_i was above 7 in either oocytes (Fig. 4) or HEK293 cells (Fig. 6). Even under these conditions the OH^-/HCO_3^- permeability was conductive as evident from the effect of the membrane potential on pH_i and inhibition of the pH_i changes by membrane depolarization (Figs. 4 and 6). We, therefore, have to conclude that SLC26A7 does not function as a Cl^-/HCO_3^- exchanger as suggested before (15, 16) but rather as a pH_i-regulated Cl^- channel. We have no immediate explanation for the difference between our findings and those reported before, although expression in *Xenopus* oocytes sometimes leads to activation of native transporters. Previous work did not measure membrane current or the behavior of SLC26A7 in HEK293 cells. Such measurements may clarify the disparity between the findings.

The finding that SLC26A7 functions as a channel highlights the remarkable diverse functions and substrate selectivity of the members of the SLC26 transporters family. For example, SLC26A3 (DRA), SLC26A4 (Pendrin), and SLC26A6 function as Cl^-/HCO_3^- exchangers (6, 7, 10–13, 23, 24) that are activated

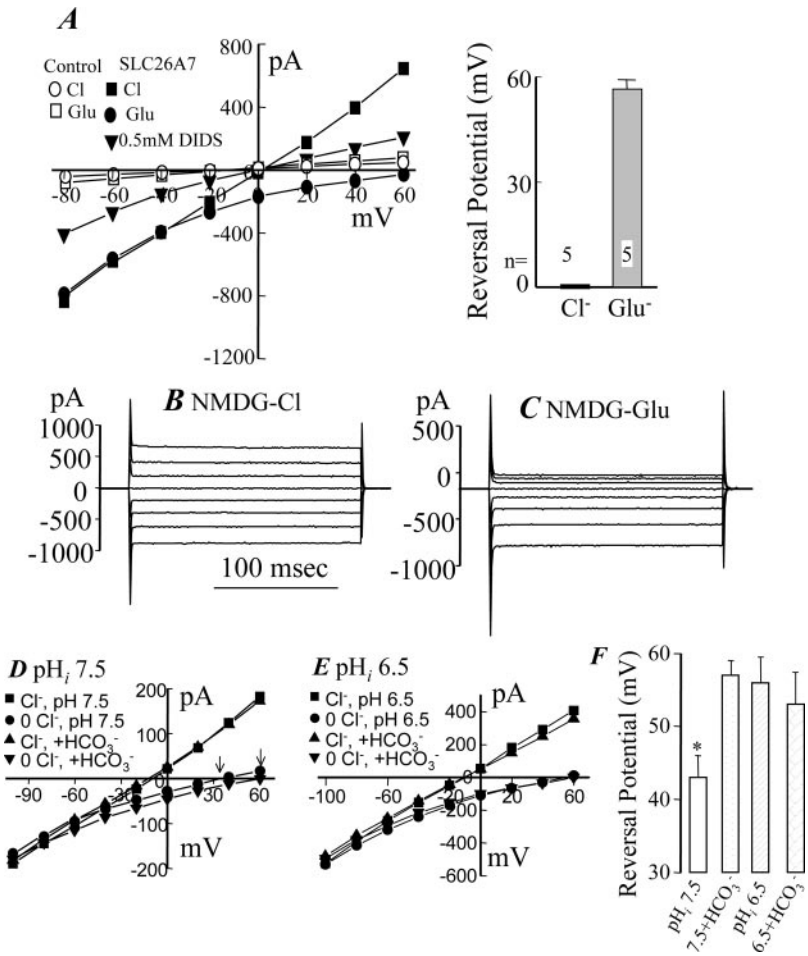

FIG. 7. **SLC26A7-mediated Cl⁻ current in HEK293 cells.** *A*, control HEK293 cells (○, □) and HEK293 cells expressing SLC26A7 (■, ●, and ▼) were used to measure Cl⁻ current in the presence and absence of medium Cl⁻ as indicated on the figure. The columns summarize the reversal potential measures in five experiments. *B* and *C* show the instantaneous current in the presence (*B*) and absence (*C*) of external Cl⁻. *D* and *E*, the pipette solution contained 30 mM Cl⁻ and was buffered with HEPES to a pH of 7.5 (*D*) or 6.5 (*E*). The cells were incubated in a medium containing either 125 or 0 mM Cl⁻ (150 mM *N*-methyl-D-glucamine (*NMDG*)) and then exposed to HCO_3^--buffered media containing either 125 or 0 Cl⁻. The RPs of three experiments are summarized in *F*, which shows the mean ± S.E. The *asterisk* indicates that all values are statistically different from the RP measured at a pH_i of 7.5 in the absence of HCO_3^- at $p < 0.05$.

by CFTR (6). However, although SLC26A3 functions only as a Cl^-/HCO_3^- exchanger (10), SLC26A4 also transports I^- (4) and SLC26A6 also transports oxalate (12). Although all transporters examined so far function as electrogenic transporters, SLC26A3 may function as a $2Cl^-/HCO_3^-$ exchanger, SLC26A6 as a $2HCO_3^-/Cl^-$ exchanger, and SLC26A7 as a Cl^- channel with limited HCO_3^- permeability that is modulated by pH_i. A comparative structure-function study of representative members of the family with diverse transport modes should be very informative.

SLC26A7 is expressed in the basolateral membrane of the acid-secreting cells in the cortical collecting duct and the gastric parietal cells. How might a pH_i-modulated Cl^- channel function in such cells? A clue may be suggested by the behavior of SLC26A7 as a selective Cl^- channel at acidic pH_i (Fig. 2) and the reduced Cl^- selectivity and increased OH^-/HCO_3^- permeability at a pH_i above 7 (Figs. 4 and 6). It is possible that at rest SLC26A7 functions as a selective Cl^- channel. Stimulation of acid secretion leads to an increase in pH_i of the parietal cell (25). This will result in an increased OH^-/HCO_3^- permeability of SLC26A7 that may provide a pathway to clear the excess base equivalents while allowing Cl^- entry into the cells that is needed for acid secretion. The same mechanism may function at the cortical collecting duct. Although the net effect is Cl^-/HCO_3^- exchange, the regulation of SLC26A7 by pH_i may function as a sensor to activate the exchange only at the time of acid secretion. It remains to be discovered why a channel is preferable to an exchanger in fulfilling this function. However, it is worth noting that the Cl^- exit pathway at the apical membrane is a Cl^- channel (26). A Cl^- entry pathway at the basolateral membrane will allow for electrical coupling between the Cl^- exit and Cl^- entry pathways during stimulated acid secretion.

REFERENCES

1. Mount, D. B., and Romero, M. F. (2004) *Pfluegers Arch. Eur. J. Physiol.* **447,** 710–721
2. Hoglund, P., Haila, S., Socha, J., Tomaszewski, L., Saarialho-Kere, U., Karjalainen-Lindsberg, M. L., Airola, K., Holmberg, C., de la Chapelle, A., and Kere, J. (1996) *Nat. Genet.* **14,** 316–319
3. Hastbacka, J., de la Chapelle, A., Mahtani, M. M., Clines, G., Reeve-Daly, M. P., Daly, M., Hamilton, B. A., Kusumi, K., Trivedi, B., Weaver, A., Coloma, A., Lovett, M., Buckler, A., Kaitila, I., and Lander, E. S. (1994) *Cell* **78,** 1073–1087
4. Everett, L. A., Glaser, B., Beck, J. C., Idol, J. R., Buchs, A., Heyman, M., Adawi, F., Hazani, E., Nassir, E., Baxevanis, A., Sheffield, V. C., and Green, E. D. (1997) *Nat. Genet.* **17,** 411–422
5. Zheng, J., Shen, W., He, D. Z., Long, K. B., Madison, L. D., and Dallos, P. (2000) *Nature* **405,** 149–155
6. Ko, S. B. H., Shcheynikov, N., Choi, J. Y., Luo, X., Ishibashi, K., Thomas, P. J., Kim, J. Y., Lee, M. G., Naruse, S. and Muallem, S. (2002) *EMBO J.* **21,** 5562–5572
7. Ko, S. B. H., Zeng, W., Dorwart, M. R., Luo, X., Kim, K. H., Millen, L., Goto, H., Naruse, S., Soyombo, A., Thomas, P. J., and Muallem, S. (2004) *Nat. Cell Biol.* **6,** 343–350
8. Lee, A., Beck, L., and Markovich, D. (2003) *DNA Cell Biol.* **22,** 19–31
9. Satoh, H., Susaki, M., Shukunami, C., Iyama, K., Negoro, T., and Hiraki, Y. (1998) *J. Biol. Chem.* **273,** 12307–12315
10. Melvin, J. E., Park, K., Richardson, L., Schultheis, P. J., and Shull, G. E. (1999) *J. Biol. Chem.* **274,** 22855–22861
11. Royaux, I. E., Wall, S. M., Karniski, L. P., Everett, L. A., Suzuki, K., Knepper, M. A., and Green, E. D. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 4221–4226
12. Knauf, F., Yang, C. L., Thomson, R. B., Mentone, S. A., Giebisch, G., and Aronson, P. S. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 9425–9430
13. Xie, Q., Welch, R., Mercado, A., Romero, M. F., and Mount, D. B. (2002) *Am. J. Physiol.* **283,** F826–F838
14. Lohi, H., Kujala, M., Makela, S., Lehtonen, E., Kestila, M., Saarialho-Kere, U., Markovich, D., and Kere, J. (2002) *J. Biol. Chem.* **277,** 14246–14254
15. Petrovic, S., Ju, X., Barone, S., Seidler, U., Alper, S. L., Lohi, H., Kere, J., and Soleimani, M. (2003) *Am. J. Physiol.* **284,** G1093–G1103
16. Petrovic, S., Barone, S., Xu, J., Conforti, L., Ma, L., Kujala, M., Kere, J., and Soleimani, M. (2004) *Am. J. Physiol.* **286,** F161–F169
17. Lee, M. G., Choi, J. Y., Luo, X., Strickland, E., Thomas, P. J., and Muallem, S. (1999) *J. Biol. Chem.* **274,** 14670–14677
18. Choi, J. Y., Muallem, D., Kiselyov, K., Lee, M. G., Thomas, P. J., and Muallem,

S. (2001) *Nature* **410,** 94–97
19. Zeng, W., Lee, M. G., and Muallem, S. (1997) *J. Biol. Chem.* **272,** 32956–32965
20. Shcheynikov, N., Kim, K. H., Kim, K.-M., Dorwart, M. R., Ko, S. B. H., Goto, H., Naruse, S., Thomas, P. J., and Muallem, S. (2004) *J. Biol. Chem.* **279,** 21857–21865
21. Dawson, D. C., Smith, S. S., and Mansoura, M. K. (1999) *Physiol. Rev.* **79,** Suppl 1, 47–75
22. Tabcharani, J. A., Linsdell, P., and Hanrahan, J. W. (1997) *J. Gen Physiol.* **110,** 341–354
23. Soleimani, M., Greeley, T., Petrovic, S., Wang, Z., Amlal, H., Kopp, P., and Burnham, C. E. (2001) *Am. J. Physiol.* **280,** F356–F364
24. Wang, Z., Petrovic, S., Mann, E., and Soleimani, M. (2002) *Am. J. Physiol.* **282,** G573–G579
25. Muallem, S., Blissard, D., Cragoe, E. J., Jr., and Sachs, G. (1988) *J. Biol. Chem.* **263,** 14703–14711
26. Hersey, S. J., and Sachs, G. (1995) *Physiol. Rev.* **75,** 155–189