Identification of an apical Cl⁻/HCO₃⁻ exchanger in rat kidney proximal tubule

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Petrovic, Snezana, Liyun Ma, Zhaohui Wang, and Manoocher Soleimani. Identification of an apical Cl⁻/HCO₃⁻ exchanger in rat kidney proximal tubule. Am J Physiol Cell Physiol 285: C608–C617, 2003.—SLC26A6 (or putative anion transporter 1, PAT1) is located on the apical membrane of mouse kidney proximal tubule and mediates Cl⁻/HCO₃⁻ exchange in vitro expression systems. We hypothesized that PAT1 along with a Cl⁻/HCO₃⁻ exchange is present in apical membranes of rat kidney proximal tubules. Northern hybridizations indicated the exclusive expression of SLC26A6 (PAT1 or CFEX) in rat kidney cortex, and immunocytochemical staining localized SLC26A6 on the apical membrane of proximal tubules, with complete prevention of the labeling with the preadsorbed serum. To examine the functional presence of apical Cl⁻/HCO₃⁻ exchanger, proximal tubules were microperfused, loaded with the pH-sensitive dye BCPCF-AM, and examined by digital ratiometric imaging. The pH of the perfusate and bath was kept at 7.4. Buffering capacity was measured, and transport rates were calculated as equivalent base flux. The results showed that in the presence of basolateral DIDS (to inhibit Na⁺/H⁺ exchanger 3), the magnitude of cell acidification in response to addition of luminal Cl⁻ was ~5.0-fold higher in the presence than in the absence of CO₂/HCO₃⁻. The Cl⁻-dependent base transport was inhibited by ~61% in the presence of 0.5 mM luminal DIDS. The presence of physiological concentrations of oxalate in the lumen (200 μM) did not affect the Cl⁻/HCO₃⁻ exchange activity. These results are consistent with the presence of SLC26A6 (PAT1) and Cl⁻/HCO₃⁻ exchanger activity in the apical membrane of rat kidney proximal tubule. We propose that SLC26A6 is likely responsible for the apical Cl⁻/HCO₃⁻ (and Cl⁻/OH⁻) exchanger activities in kidney proximal tubule.

putative anion transporter 1; chloride/formate exchanger; SLC26A6

KIDNEY IS THE MAJOR ORGAN responsible for maintaining electrolyte balance and acid-base homeostasis in mammals. The absorption of Cl⁻ by the proximal tubule constitutes a major component of this process (2–4). Functional studies indicate that the main mechanism of chloride reabsorption in the kidney proximal tubule is via apical Cl⁻/base exchange that works in parallel with the Na⁺/H⁺ exchanger (NHE3) and is essential for chloride reabsorption, fluid balance, and acid-base regulation (1–4). The main functional mode of this exchanger has been the subject of numerous investigations. Both Cl⁻/formate and Cl⁻/OH⁻ exchange have been identified in the apical membranes of kidney proximal tubule (1, 5, 6, 18, 19, 21, 35). However, whereas some studies have suggested that Cl⁻/formate exchange is the main mode of apical Cl⁻/base exchange (18, 19), others have not (21). Similarly, whereas some studies have found Cl⁻/OH⁻ exchange in the apical membrane of kidney proximal tubule (42), others have not (34). In addition to Cl⁻/formate and Cl⁻/OH⁻ exchangers, a Cl⁻/oxalate exchanger has been demonstrated in apical membrane of proximal tubule (4). The controversy with regard to the functional modes of the apical Cl⁻/base exchanger(s) is in part due to the fact that the molecular identities of this (these) transport process(es) remain unknown. None of the anion exchanger family members (AE1, -2, or -3) are expressed on the apical membrane of kidney nephrons.

Recent molecular studies have identified a large, highly conserved family of membrane proteins (designated SLC26A), many of which have been shown to transport anions (7, 13, 15, 16, 23, 32, 37, 40). PAT1 (putative anion transporter 1; or SLC26A6), which is a member of this family (23), was recently shown to be expressed on the apical membrane of mouse kidney proximal tubule (20). Expression studies showed that the mouse ortholog of PAT1 (also called CFEX) mediates Cl⁻/formate exchange when expressed in oocytes (20). We have examined the functional properties of both human and mouse PAT1 (or CFEX). Our expression studies in oocytes indicate that PAT1 mediates Cl⁻/OH⁻ and Cl⁻/HCO₃⁻ exchange (40). Subsequent studies have verified the mediation of Cl⁻/HCO₃⁻ exchange by PAT1 (or SLC26A6) (17, 43). In addition to the mouse kidney proximal tubule, PAT1 is expressed on the apical membrane of the villi of mouse duodenum (40) and on the tubulovesicles of gastric parietal cells (27).

On the basis of immunolocalization studies indicating the expression of SLC26A6 (PAT1) on the apical membrane of mouse proximal tubules and functional studies in vitro expression systems indicating that...
SLC26A6 mediates Cl⁻/HCO₃⁻ exchange (17, 40, 43), we hypothesized that SLC26A6 (PAT1) is an apical Cl⁻/HCO₃⁻ exchanger in the kidney proximal tubule. Accordingly, we performed immunocytochemical staining and measured apical Cl⁻/base exchanger activity in rat isolated microperfused proximal tubules in the presence or absence of bicarbonate. The results demonstrate that rat proximal tubules express PAT1 along with a robust Cl⁻/HCO₃⁻ exchanger on their apical membrane.

METHODS

Animals

Female Sprague-Dawley rats, weighing 100–150 g, were used for these studies. Animals were allowed free access to water and food. The use of anesthetics (pentobarbital sodium) and the method of euthanasia (pentobarbital overdose) were according to the institutional guidelines and approved protocols.

RNA Isolation and Northern Hybridization

Total cellular RNA was extracted from whole kidney and cortex, outer medulla, and inner medulla cortex by the method of Chomczynski and Sacchi (11), quantitated spectrophotometrically, and stored at −80°C. Total RNA samples (30 μg/lane) were fractionated on a 1.2% agarose-formaldehyde gel and transferred to Magna NT nylon membranes (MSI). Membranes were cross-linked by ultraviolet light and baked for 1 h. Hybridization was performed according to Church and Gilbert (12). The cDNA probes (25 ng) were labeled with 32P-labeled deoxynucleotides by using the Rad-Prime DNA labeling kit (GIBCO-BRL). The membranes were washed, blotted dry, exposed to PhosphorImager cassette at room temperature for 24–72 h, and read by PhosphorImager (Molecular Dynamics, Sunnyvale, CA). For PAT1, an ~200-bp PCR fragment was amplified from a mouse expressed sequence tag (EST) (GenBank accession no. AI747461) and used as a probe.

Immunoblotting and Immunocytochemistry of PAT1

Antibodies. Two polyclonal PAT1-specific antibodies were raised. The first has an amino acid sequence MDLRRRDYHMERPLLQEQHL, and the second has the amino acid sequence CRRDYHMERPLLQEQ, which is a truncated form of the first one and, in addition, has a cysteine residue incorporated for better purification. The specificity of both antibodies has been verified in our previous studies (27, 40).

Electrophoresis and immunoblotting. Semiquantitative immunoblotting experiments were carried out as previously described (40). Briefly, the solubilized membrane proteins from rat kidney cortex were size-fractionated on polyacrylamide minigels (Novex, San Diego, CA), electrophoretically transferred to nitrocellulose membranes, blocked with 5% milk proteins, and then probed with affinity-purified anti-PAT1 immune serum at an IgG concentration of 0.8 μg/ml. The secondary antibody was donkey anti-rabbit IgG conjugated to horseradish peroxidase (Pierce). The sites of antigen-antibody complexation on the nitrocellulose membranes were visualized using a chemiluminescence method as 0.5-μm “optical sections” of the stained cell membrane, using a ×40 objective. The standard argon laser exciter 488-nm filter and the 515/30-nm emission filter was used for the green emitting dye.

Isolation of Proximal Tubules and In Vitro Microperfusion

Rats were killed by intraperitoneal injection of pentobarbital sodium (100 mg/kg body wt). Kidneys were quickly removed and placed in ice-cold dissection medium (Table 1, solution 1). Thin coronal slices (~1 mm) were cut and transferred to the dissection chamber. Proximal straight tubules, comprising the ends of the S2 and S3 segments, were isolated from the cortical part of medullary rays and transferred to the 1-ml temperature-controlled specimen chamber mounted on the inverted Zeiss Axiosvert S-100 microscope (Carl Zeiss, Thornwood, NY). Experiments were done at 37°C. In vitro microperfusion of the isolated tubule segments was performed.

Table 1. Chemical composition of solutions used for microperfusion

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Values are expressed in mM. Solutions containing bicarbonate were continuously bubbled with 95% O₂-5% CO₂. Solutions had a pH of 7.4 at 37°C and osmolality of 290 ± 3 mosmol/kgH₂O. Solutions without bicarbonate were gassed with 100% O₂. Solutions containing gluconate salts had 4 mM Ca-acetate to account for complexing of Ca²⁺ with gluconate. In addition, solutions bathing the tubule contained 2 mg/dl of 70,000 Dextran (39). In experiments in which the effect of oxalate was tested, Na-oxalate was added to solutions 2 and 3 (bicarbonate-containing solution) and solutions 4 and 5 (bicarbonate-free solutions) at the final concentration of 200 μM. Free oxalate ions were measured by spectrophotometric enzymatic assay (Sigma).

TMA, tetramethylammonium.
formed by using concentric glass pipettes according to the method of Burg (9, 10) as previously described at 5-cm of water pressure (26, 28). Solutions used to perfuse and bath the tubules are listed in Table 1. Solutions were delivered to the specimen chamber in CO2- and O2-impermeable tubing (Cole Palmer, Chicago, IL) by the peristaltic pump (Peristar; WPI, Sarasota, FL) at a rate of 1 ml/min. The chamber was closed by a lid and constantly superperfused with 95% O2-5% CO2 to keep the pH of the bath fluid constant. Chamber pH was frequently checked with a Horiba pH meter (model B 213; Horiba, Kyoto, Japan). Tubules were perfused with 0.15 mg/ml Fast Green dye (Sigma, St. Louis, MO) at the beginning of each experiment to identify the damaged cells (normal cells are impermeable, whereas damaged cells take up this dye). Tubules were carefully inspected and discarded if damaged cells were found in the tubule wall (28, 39).

Intracellular pH Measurements

Intracellular pH was measured by using 5 μM 2′,7′-bis(3-carboxypropyl)-5(6)-carboxyfluorescein acetoxyethyl ester (BCCPF-AM) (27) and 100 μM EIPA, an analog of 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein acetoxyethyl ester (BCECF-AM) with improved spectral characteristics, as previously described (26, 28, 39). Briefly, after 15–20 min of equilibration in the initial bath and luminal solution, the tubule was perfused with 1 μM BCCPF-AM for 2 min and then 10 min were allowed for dye washout. Fluorescence measurements were done on a Zeiss Axiosvert S-100 inverted microscope equipped with an Attofluor RatioVision digital imaging system (Attofluor, Rockville, MD) as previously described (26, 28). An Achroplan ×40/0.8 NA water objective with a 3.6-mm working distance was used. Excitation wavelengths were recorded at 488 and 440 nm, and emission was measured at 520 nm. Digitized images were analyzed using the Attograph software. Only one tubule per animal was examined. Intracellular calibration was performed at the end of each experiment by using the high-K+ -nigericin method (26, 28, 38, 39). Apical Cl-/OH- and Cl-/HCO3 Exchange

Stable basal intracellular pH (pHi) readings were obtained for at least 5 min before any experimental maneuvers. Tubules were perfused with either bicarbonate-containing (Table 1, solution 2) or bicarbonate-free solutions (Table 1, solution 4) in the presence of 300 μM (0.3 mM) DIDS in the bath and 100 μM EIPA in the lumen 10–15 min before the experiments to inhibit the basolateral Na+/H+ cotransporter and luminal Na+/H+ exchanger, respectively. Luminal perfusate was switched to a Cl−-free solution (Table 1, solution 3 for bicarbonate-containing experiments and solution 5 for bicarbonate-free experiments). Apical Cl-/OH-/HCO3− exchanger activity was assessed as the rate of pHi acidification (dpH/dt) calculated from the slope of the initial pH change upon switching the luminal perfusate from Cl−-free to Cl−-containing solution in either bicarbonate-containing (Table 1, solution 3 to solution 2) or bicarbonate-free solutions (Table 1, solution 5 to solution 4). This maneuver causes cell acidification, which is reversed upon subsequent return to the Cl−-free solution. To examine the effect of DIDS on apical Cl-/OH-/HCO3− exchanger, we performed experiments in the absence of luminal EIPA, because EIPA and DIDS precipitate when introduced in the same solution. To test the sensitivity of the exchanger to DIDS, we incubated tubules with 0.5 mM DIDS and 0 mM Cl− in the lumen for 10–15 min and measured apical Cl-/OH-/HCO3− exchanger activity by switching the perfusate to a Cl−-containing solution.

Intrinsic cell buffer capacity (βi) was measured using a NH2Cl prepulse technique according to established protocols (21, 29, 35, 36). Measurement of βi was done in Na+-free solutions (Table 1, solution 6) with 1 mM DIDS on both the bath and luminal side, to keep Na+− and Cl−-dependent acidification mechanisms inactive. In NH4Cl-containing solutions, 20 mM NH4Cl replaced an equimolar concentration of tetramethylammonium (TMA)-Cl. In bicarbonate-free solutions, βi was 31.8 ± 3.9 mM/pH unit (n = 5). In bicarbonate-containing solutions, the total buffering capacity (βT) was estimated as the sum of βi and the bicarbonate buffer capacity (βHCO3−). βHCO3− was calculated as 2.3 × [HCO3−], where [HCO3−] is the intracellular bicarbonate concentration. The buffer capacity in the presence of bicarbonate was 88.9 ± 5 mM (n = 5). Equivalent base flux (EBF) was calculated as dpH/dt × βi and V and expressed in picomoles per millimeter per minute (21), where β is the cell buffer capacity expressed as βi in the experiments in which bicarbonate-free solutions were used and as βT in the experiments in which bicarbonate-containing solutions were used. V designates the cell volume per tubule length, calculated according to the following formula: V = π(d1)2(d2)/4, where d1 and d2 stand for the outer and inner tubule diameter, respectively. Tubule diameters were measured at ×400 magnification using an eyepiece reticle. Average tubule volume was 8.5 ± 0.16 × 10−10 mm3.

Free oxalate concentration in the perfusate was measured by an oxalate oxidase spectrophotometric enzymatic assay according to the manufacturer’s protocol (Sigma).

Cloning and Expression of Mouse PAT1

Full-length mouse PAT1 cDNA was cloned from the duodenum by RT-PCR, according to published reports (20) and as described from our laboratory (40). The following oligonucleotide primers were designed and used for RT-PCR: 5′-CGT CTG CAC TGC TCC TCT CAT TG and 5′-GAG TCC CAG GGC ATC CAT CCA TG (accession no. AY032863). These primers encode nucleotides 45–2498 of the mouse SLC26A6, also known as PAT1 or CFEX (20). Amplification of the mouse PAT1 cDNA by PCR was performed according to the Clontech Advantage 2 PCR kit protocol. The capped PAT1 cRNA was generated using the mMessage mACHINE kit (Ambion) according to the manufacturer’s instructions.

Expression of Mouse PAT1 in Xenopus Oocytes

Xenopus oocytes were injected with mouse PAT1 cRNA, as described previously (40). pHi in oocytes was measured with the pH-sensitive fluorescent probe BCECF-AM (Molecular Probes, Eugene, OR) as described previously (37, 40). Oocytes were loaded with 10 μM BCECF-AM, and cell pHi was monitored by ratiometric fluorescence using the Attofluor digital imaging system (40). Data analyses were performed using the Attograph and Attoview software packages provided with the imaging system. The ratios were obtained from the submembrane region of the oocytes that were visualized with a ×40 water objective. Measured excitation ratios were converted to pHi by using a calibration curve that was constructed with the high-K+/nigericin method at the end of each experiment.

Materials

32P-dCTP was purchased from New England Nuclear (Boston, MA). Nitrocellulose filters and other chemicals were purchased from Sigma. The RadPrime DNA labeling kit was
purchased from GIBCO-BRL. BCPCF-AM was from Molecular Probes. All the other chemicals, including the oxalate oxidase enzymatic assay, were from Sigma. Nigericin was dissolved in ethanol and diluted 1:1,000 for the final concentration of 10 μM. EIPA was dissolved in methanol and diluted 3:1,000 for the final concentration of 100 μM. DIDS was dissolved directly in the solutions used for perfusion.

Statistics

Results are expressed as means ± SE. Statistical significance between experimental groups was determined by Student’s t-test, as required. Significance was asserted if P < 0.05.

RESULTS

Expression of PAT1 in Rat Kidney

Northern hybridization. In the first series of experiments, the mRNA expression of PAT1 was examined in various rat kidney zones by Northern hybridization. As shown in Fig. 1A, PAT1 mRNA is predominantly ex-
pressed in the kidney cortex. There were very faint bands in the outer and inner medulla.

**Immunoblot analysis of PAT1 in rat kidney.** In the next series of experiments, Western blot analysis of PAT1 on microsomal membranes isolated from rat kidney cortex was performed. As shown in Fig. 1B, left, PAT1 immune serum detects an ~90-kDa band in rat kidney cortex. Preadsorption of the immune serum with the synthetic peptide prevented the labeling (Fig. 1B, right), indicating the specificity of the immune serum.

**Immunofluorescence labeling of PAT1.** To examine the cell distribution and subcellular localization of PAT1 in rat kidney, we performed immunocytochemical staining. As shown in Fig. 1C, PAT1 immune serum labeled the apical membrane of rat proximal tubules (top). The labeling was specific, because no staining was detected with the preadsorbed immune serum (Fig. 1C, bottom). Occasionally, faint labelings on basolateral membranes were detected, but these were not consistent. No labeling was detected in the medulla (not shown). The localization of PAT1 on the apical membrane of rat kidney proximal tubule is in agreement with recent results in mouse kidney (20).

**Cl⁻/HCO₃⁻ and Cl⁻/OH⁻ Exchange in Rat Straight Proximal Tubules**

PAT1 expression in oocytes causes mediation of Cl⁻/HCO₃⁻ exchange, as examined using the pH-sensitive dye BCPCF-AM (28). Because immunofluorescence studies localized PAT1 to the apical membrane of rat proximal tubule (Fig. 1), we next tested whether an apical Cl⁻/HCO₃⁻ exchanger is present in the proximal tubule. Tubules were first bathed and perfused with solutions containing 25 mM HCO₃⁻ at pH 7.4. DIDS and EIPA were introduced to the bath and perfusate to inhibit basolateral Na⁺/H⁺ cotransport and luminal Na⁺/H⁺ exchange, respectively, and Cl⁻ was removed from the lumen. Representative pHᵢ tracings are shown in Fig. 2 demonstrating the induction of cell acidification in response to luminal Cl⁻ addition in the presence (A) or absence of CO₂/HCO₃⁻ (B). In the presence of CO₂/HCO₃⁻, addition of Cl⁻ in the lumen decreased the pHᵢ from 7.46 ± 0.01 to 7.08 ± 0.02 at a rate of 0.39 ± 0.01 pH unit/min. In the absence of CO₂/HCO₃⁻, addition of Cl⁻ resulted in pHᵢ reduction from 7.39 ± 0.03 to 7.16 ± 0.05 at the rate of 0.2 ± 0.05 pH unit/min. When expressed in EBP, the rate of Cl⁻/base exchange was significantly higher in the presence of CO₂/HCO₃⁻ (Fig. 3), with the equivalent base transport of 30.9 ± 6.3 pmol·mm⁻¹·min⁻¹ (n = 5) in the presence and 5.7 ± 1.1 pmol·mm⁻¹·min⁻¹ (n = 5) in the absence of CO₂/HCO₃⁻ (P < 0.05). These results demonstrate that rats express an apical Cl⁻/HCO₃⁻ exchanger in the proximal tubule, with Cl⁻/OH⁻ exchange being a minor portion of the observed Cl⁻/base exchange.

Metabolic production of CO₂ can result in the generation of cytosolic HCO₃⁻ levels that are probably greater than those of hydroxyl (OH⁻). As such, it is plausible that even in the absence of exogenous CO₂/HCO₃⁻, the intracellular base species that is transported in exchange for luminal Cl⁻ (Fig. 2B) is actually HCO₃⁻ and not OH⁻. To determine whether OH⁻ is indeed the base species transported in Fig. 2B, we assayed the apical Cl⁻/base exchanger activity in isolated microperfused kidney proximal tubules in the reverse mode and in the absence of CO₂/HCO₃⁻. This means that the cell pH was assayed in response to the removal (rather than the addition) of luminal Cl⁻. This maneuver results in the movement of luminal (extracellular) OH⁻ into the cell via Cl⁻/OH⁻ exchange and, as a result, bypasses the contribution of the endogenous CO₂ production to the anion exchanger activity. As shown in Fig. 3B, removal of luminal Cl⁻ resulted in intracellular alkalization, which returned to baseline upon switching back to the Cl⁻-containing perfusate. The results of multiple experiments demonstrated that removal of luminal Cl⁻ caused significant intracellular alkalization in isolated microperfused proximal tubule cells (with the rate of 0.095 ± 0.03, P < 0.05 vs. baseline pHᵢ; n = 5). Taken together, the results of experiments in Figs. 2 and 3 demonstrate the presence of Cl⁻/OH⁻ exchange and Cl⁻/HCO₃⁻ exchange in the apical membrane of kidney proximal tubule.
In the next series of experiments, we tested the effect of DIDS on the apical Cl⁻/HCO₃⁻/H¹⁺ exchange. In the experiments shown in Figs. 2 and 3, DIDS was present at 0.3 mM in the bath to inhibit the basolateral Na⁺/HCO₃⁻ cotransporter. To examine the effect of luminal DIDS on the apical Cl⁻/HCO₃⁻/base exchanger, we added 0.5 mM DIDS to the lumen. No EIPA was added to the lumen in these experiments. The reason for removing EIPA from the lumen in these series of experiments is that DIDS and EIPA coprecipitate when added to the same solution. Under these circumstances, the activity of the luminal Cl⁻/HCO₃⁻ exchanger measured upon addition of luminal Cl⁻ decreased from 0.23 ± 0.03 pH unit/min in the absence of luminal DIDS to 0.03 pH ± 0.01 pH unit/min in the presence of 0.5 mM DIDS in the lumen (P < 0.05; n = 3). Figure 4B summarizes the results of these experiments and demonstrates that DIDS inhibited the apical Cl⁻/OH⁻ exchange by 50% (P < 0.05).

A recent study indicated that the presence of physiological concentrations of oxalate inhibits the Cl⁻/HCO₃⁻ exchange mode of PAT1 in oocytes injected with PAT1 cRNA (17). To examine whether oxalate can inhibit the apical Cl⁻/HCO₃⁻ and Cl⁻/OH⁻ exchangers in rat kidney proximal tubule, we determined the rate of cell acidification in response to luminal Cl⁻ addition in isolated microperfused proximal tubule in the presence of 200 μM of oxalate added to the lumen. Experiments were performed in the presence or absence of bicarbonate at pH 7.4, with DIDS in the bath and EIPA in the lumen. Representative pH tracings for bicarbonate-containing solutions are shown in Fig. 5A and demonstrate comparable cell acidification in response to luminal Cl⁻ addition in the presence or absence of oxalate in the lumen. In the presence of bicarbonate in the bath and lumen and oxalate in the lumen (Fig. 5Aa), addition of Cl⁻ to the lumen decreased the pH from 7.41 ± 0.01 to 6.88 ± 0.02 at a rate of 0.37 ± 0.03 pH unit/min.

In the next series of experiments, we tested the effect of DIDS on the apical Cl⁻/HCO₃⁻/OH⁻ exchanger. In the experiments shown in Figs. 2 and 3, DIDS was present at 0.3 mM in the bath to inhibit the basolateral Na⁺/HCO₃⁻ cotransporter. To examine the effect of luminal DIDS on the apical Cl⁻/base exchanger, we added 0.5 mM DIDS to the lumen. No EIPA was added to the lumen in these experiments. The reason for removing EIPA from the lumen in these series of experiments is that DIDS and EIPA coprecipitate when added to the same solution. Under these circumstances, the activity of the luminal Cl⁻/HCO₃⁻ exchanger measured upon addition of luminal Cl⁻ decreased from 0.23 ± 0.03 pH unit/min in the absence of luminal DIDS to 0.02 ± 0.01 pH unit/min in the presence of 0.5 mM DIDS in the lumen (P < 0.01; n = 3). Figure 4A summarizes the results of these experiments and demonstrates that DIDS inhibited the apical Cl⁻/HCO₃⁻ exchange by 61% (P < 0.05).

Next, we examined the effect of DIDS on the activity of the apical Cl⁻/OH⁻ exchange in a manner similar to the above experiments. The activity of the luminal Cl⁻/OH⁻ exchanger measured upon addition of luminal Cl⁻ decreased from 0.16 ± 0.03 pH unit/min in the absence of luminal DIDS to 0.08 pH ± 0.01 pH unit/min in the presence of 0.5 mM DIDS in the lumen (P < 0.05; n = 3). Figure 4B summarizes the results of these experiments and demonstrates that DIDS inhibited the apical Cl⁻/OH⁻ exchange by 50% (P < 0.05).
the pH from 7.43 ± 0.02 to 7.04 ± 0.04 at a rate of 0.31 ± 0.03 pH unit/min (n = 4). Representative pH tracings are shown in Fig. 6Aa. When expressed in EBF, the results translated into a rate of 8.9 ± 0.9 pmol·mm⁻¹·min⁻¹. Compared with the rate of cell acidification in the absence of oxalate (0.2 ± 0.05 pH unit/min and an EBF of 5.7 ± 1.1 pmol·mm⁻¹·min⁻¹) (Fig. 6Ab), the results indicate the presence of a minor, albeit nonsignificant, stimulatory effect of oxalate on the apical Cl⁻/OH⁻ exchanger (Fig. 6B). Taken together, the results of the above experiments indicate that the presence of physiological concentrations of luminal oxalate does not inhibit the apical Cl⁻/HCO₃⁻ and Cl⁻/OH⁻ exchangers in rat kidney proximal tu-

**Fig. 6.** Effect of oxalate on apical Cl⁻/OH⁻ exchanger activity in isolated, microperfused rat kidney proximal tubules. In oxalate-containing solutions, 200 μM oxalate was present in the perfusate. A: representative tracings of apical Cl⁻/HCO₃⁻ exchanger activity in the presence (a) or absence of oxalate (b). B: summary of EBF results. Data are means ± SE.
bule (and indeed it may have a minor stimulatory effect on Cl−/OH− exchanger).

Measurement of Free Oxalate in Oxalate-Containing Experiments

To determine the concentration of free oxalate in the lumen under experimental conditions, we measured free oxalate in the perfusate by spectrophotometric enzymatic assay (see METHODS). The results indicated that there was no precipitation or chelation of oxalate by other ions (i.e., Ca2+ or Mg2+) in perfusate solutions at the physiological concentration of oxalate (200 μM) used in our experiments.

The above experiments demonstrate the localization of SLC26A6 as well as Cl−/OH− and Cl−/HCO3− exchange on the apical membranes of rat kidney proximal tubule. In the last series of these experiments, we tested whether SLC26A6 (PAT1) mediates both Cl−/OH− and Cl−/HCO3− exchange in oocytes. Accordingly, oocytes were injected with PAT1 cRNA, and their pHi was monitored in response to sequential removal and addition of Cl− according to published methods from our laboratory (40).

The representative pHi tracings in Fig. 7A demonstrate that in the absence of CO2/HCO3− in the perfusate, switching to the Cl−-free solution resulted in intracellular alkalinization in PAT1-expressing oocytes, which returned to baseline upon switching back to the Cl−-containing solution. In the presence of CO2/HCO3−, switching to the Cl−-free solution resulted in an intracellular alkalinization that was faster than in the absence of CO2/HCO3− in PAT1-expressing oocytes (Fig. 7B). Switching back to the Cl−-containing solution caused the pHi to return to normal. Control (water injected) oocytes did not demonstrate any pHi alteration in response to exposure to the Cl−-free medium in the absence of CO2/HCO3− (Fig. 7C). The rate of Cl−/base exchanger activity was 0.045 ± 0.001 pH unit/min (n = 3) in the absence of CO2/HCO3− (mediated via Cl−/OH− exchange) and 0.128 ± 0.002 pH unit/min (n = 4) in the presence of CO2/HCO3− (mediated via Cl−/HCO3− exchange) in oocytes injected with mouse PAT1 cRNA (P < 0.02 for absence vs. presence of bicarbonate). Taken together, these results indicate that PAT1 mediates both Cl−/OH− and Cl−/HCO3− exchange.

DISCUSSION

Elucidating the identity of the apical Cl−/base exchangers in kidney proximal tubule has been the subject of many investigations. However, the results of these studies are conflicting. Whereas some investigations have reported that Cl−/formate exchange is the main Cl−/base exchanger in kidney proximal tubule, others have not. Similarly, whereas some have suggested that Cl−/OH− exchange is the main apical Cl−/base exchanger, others have not. The reasons for these conflicting reports could be several, including the use of different species (rat vs. rabbit) or methods of tissue isolation (perfused tubule vs. membrane vesicles) in...
various studies. One major factor that has contributed to the ambiguity with regard to the modes of apical Cl\(^{-}\)/base exchange in kidney proximal tubule has been the absence of any information on the molecular identity of this (these) exchanger(s). That is changing very rapidly.

A new family of anion exchangers has been identified that is referred to as SLC26A. Several members of this family function as an apical Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchanger in distinct epithelial tissues. One member of this family, SLC26A3, or DRA (downregulated in adenoma), is an apical Cl\(^{-}\)/HCO\(_3\) exchanger in the colon and pancreatic ducts (16, 24). Another is SLC26A4, or pendrin, which is an apical Cl\(^{-}\)/HCO\(_3\) exchanger (36, 37) in the kidney cortical collecting duct (30, 36). Whereas the mRNA expression of pendrin is detected in both proximal tubule and cortical collecting ducts (37), immunocytochemical and immunohistochemical staining localizes this exchanger only to cortical collecting ducts (28, 36, 41).

SLC26A6, or PAT1 (or CFEX), is an apical Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchanger in the duodenum (40). The duodenum and proximal tubule share remarkable similarity with each other with respect to their role in fluid and electrolyte absorption. Both are responsible for the bulk of fluid and electrolyte absorption in their respective organs, which is accomplished via identical apical membrane protein repertoire. These include Na\(^{+}\)/H\(^{+}\) exchanger (NHE3) and Na\(^{+}\)-glucose cotransporter (SGLT2) to absorb Na\(^{+}\) and apical Cl\(^{-}\)/HCO\(_3\) exchanger (PAT1) to absorb Cl\(^{-}\) (8, 14, 25, 33, 40). The secretion of bicarbonate in exchange for Cl\(^{-}\) (via Cl\(^{-}\)/HCO\(_3\) exchange) does not conflict with the role of the duodenum and kidney proximal tubule in HCO\(_3\)\(^{-}\) absorption via the apical Na\(^{+}\)/H\(^{+}\) exchanger NHE3. The role of this latter exchanger in HCO\(_3\)\(^{-}\) absorption in both tissues has been well documented in NHE3 null mice, which develop an alkaline luminal fluid in their duodenum and show reduced HCO\(_3\)\(^{-}\) absorption in the kidney proximal tubule (31). Taken together, the results of the above studies demonstrate the coordinated regulation of Na\(^{+}\) and HCO\(_3\)\(^{-}\) absorption via apical Na\(^{+}\)/H\(^{+}\) exchanger as well as Cl\(^{-}\) absorption and HCO\(_3\)\(^{-}\) secretion via the apical Cl\(^{-}\)/HCO\(_3\) exchanger in a model epithelial cell. For the apical NHE3 and PAT1 (SLC26A6) to mediate net HCO\(_3\)\(^{-}\) and Cl\(^{-}\) absorption in the duodenum, the stoichiometry of NHE3 and PAT1 may not be one to one. In other words, whereas the activation of the apical Cl\(^{-}\)/HCO\(_3\) exchanger results in the absorption of Cl\(^{-}\) and secretion of HCO\(_3\)\(^{-}\), the small intestine may demonstrate a net absorption of HCO\(_3\)\(^{-}\) secondary to the activation of the apical Na\(^{+}\)/H\(^{+}\) exchanger. This has become very clear in NHE3 null mice (31). Similar to the duodenum, the parallel arrangement of apical Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchanger and Na\(^{+}\)/H\(^{+}\) exchanger should accomplish the reabsorption of Cl\(^{-}\) and HCO\(_3\)\(^{-}\) via PAT1 and NHE3, respectively, in the kidney proximal tubule.

Two recent studies confirmed that PAT1 indeed mediates Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchange (17, 43). Both studies, in addition, indicated that PAT1 can also mediate oxalate exchange in an oocyte expression system (17, 43). One of these studies (17) found that the presence of oxalate can inhibit the mediation of Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchange by PAT1. In our studies, physiological concentrations of oxalate in the lumen did not inhibit the apical Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchange in isolated microperfused proximal tubule, strongly suggesting that Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchange is a major mechanism for the reabsorption of Cl\(^{-}\) under physiological conditions. Our functional studies demonstrate that PAT1 mediates Cl\(^{-}\)/OH\(^{-}\) and Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchange in an in vitro expression system (Fig. 7). Our microperfusion studies demonstrate the presence of both Cl\(^{-}\)/OH\(^{-}\) and Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchangers on the apical membranes of rat kidney proximal tubule (Figs. 2 and 3). On the basis of immunocytochemical studies demonstrating the expression of PAT1 on the apical membranes of rat kidney proximal tubule, we suggest that PAT1 is mediating the apical Cl\(^{-}\)/OH\(^{-}\) and Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchanges in rat kidney proximal tubule.

The electrogenicity of SLC26A6 (20, 43) does not conflict with the historical observations indicating that reabsorption of NaCl in the proximal tubule was electroneutral and did not alter the transepithelial membrane potential. The presence of apical K\(^{+}\) channels in kidney proximal tubule likely offsets any alterations in membrane potential otherwise resulting from the operation of electrogenic apical Cl\(^{-}\)/base exchanger.

In conclusion, SLC26A6 (also referred to as PAT1 or CFEX) is located on the apical membrane of rat kidney proximal tubule. Functional studies in isolated microperfused rat proximal tubule demonstrated the presence of a robust apical Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchange that is not inhibited by oxalate. We propose that SLC26A6 (PAT1) is likely responsible for the apical Cl\(^{-}\)/HCO\(_3\)\(^{-}\} and Cl\(^{-}\)/OH\(^{-}\) exchanger in kidney proximal tubule.

DISCLOSURES

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