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Effect of Slc26a6 deletion on apical Cl^-/HCO_3^- exchanger activity and cAMP-stimulated bicarbonate secretion in pancreatic duct

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Ishiguro H, Namkung W, Yamamoto A, Wang Z, Worrell RT, Xu J, Lee MG, Soleimani M. Effect of Slc26a6 deletion on apical Cl^-/HCO_3^- exchanger activity and cAMP-stimulated bicarbonate secretion in pancreatic duct. *Am J Physiol Gastrointest Liver Physiol* 292: G447–G455, 2007. First published August 10, 2006; doi:10.1152/ajpgi.00286.2006.—The role of Slc26a6 (PAT1) on apical Cl^-/HCO_3^- exchange and bicarbonate secretion in pancreatic duct cells was investigated using Slc26a6 null and wild-type (WT) mice. Apical Cl^-/HCO_3^- exchange activity was measured with the pH-sensitive dye BCECF in microperfused interlobular ducts. The HCO_3^--influx mode of apical $[Cl^-]_i/[HCO_3^-]_o$ exchange (where brackets denote concentration and subscripts i and o denote intra- and extracellular, respectively) was dramatically upregulated in Slc26a6 null mice ($P < 0.01$ vs. WT), whereas the HCO_3^--efflux mode of apical $[Cl^-]_o/[HCO_3^-]_i$ exchange was decreased in Slc26a6 null mice ($P < 0.05$ vs. WT), suggesting the unidirectionality of the Slc26a6-mediated HCO_3^- transport. Fluid secretory rate in interlobular ducts were comparable in WT and Slc26a6 null mice ($P > 0.05$). In addition, when pancreatic juice was collected from whole animal in basal and secretin-stimulated conditions, neither juice volume nor its pH showed differences between WT and Slc26a6 null mice. Semiquantitative RT-PCR demonstrated more than fivefold upregulation in Slc26a3 (DRA) expression in Slc26a6 knockout pancreas. In conclusion, these results point to the role of Slc26a6 in HCO_3^- efflux at the apical membrane and also suggest the presence of a robust Slc26a3 compensatory upregulation, which can replace the function of Slc26a6 in pancreatic ducts.

cystic fibrosis transmembrane regulator; cystic fibrosis; sodium bicarbonate exchanger; HCO_3^- secretion; HCO_3^- transporters; downregulated in adenoma; putative anion transporter 1

PANCREATIC DUCT CELLS PRODUCE HCO_3^--rich isotonic fluid secretion, and the excretory duct system of the pancreas serves as a conduit for delivery of an alkaline, bicarbonate-rich fluid containing digestive enzymes to the duodenum in response to the release of secretin (5, 11, 26, 31, 33). Stimulation of the pancreas with secretin, which increases intracellular cAMP, increases both the volume and the HCO_3^- concentration of pancreatic juice (6, 12, 16, 30). Given the fact that more than 90% of the HCO_3^- in the pancreatic juice is derived from the plasma (6, 12, 16, 30), it becomes evident that specialized and high-capacity acid-base transporters are responsible for active HCO_3^- secretion into the pancreatic lumen. Recent studies suggest that basolateral Na^+-HCO_3^- cotransport (NBC1), along with CFTR and apical Cl^-/HCO_3^- exchange is essential for bicarbonate secretion in pancreatic duct. According to these studies, HCO_3^- is taken up across the basolateral membranes of the pancreatic duct via NBC1 and is secreted across the apical membrane via CFTR working in tandem with apical Cl^-/HCO_3^- exchange (14, 15, 28, 40). Acinar cells are thought to secrete Cl^--rich fluid; thus apical Cl^-/HCO_3^- exchange probably provides a major route for HCO_3^- secretion in proximal ducts close to the acini where luminal Cl^- concentration is high.

Identifying the apical Cl^-/HCO_3^- exchanger(s) in the pancreatic duct has been the subject of numerous investigations. Molecular cloning studies have identified two distinct families of anion exchangers: SLC4 and SLC26. The SLC4 family includes four distinct Na-independent Cl^-/HCO_3^- exchangers known as AE1, AE2, AE3, and AE4, with AE1, 2, and 3 exclusively located on the basolateral membrane of epithelial cells (1, 2, 3, 27). AE4 is expressed in the apical membrane of duodenocytes, but its subcellular distribution in the kidney remains controversial (18, 36, 41).

SLC26 isoforms are members of a large, conserved family of anion exchangers, many of which display highly restricted and distinct tissue distribution and share no significant homology to AEs. Overall, ten SLC26 genes or isoforms (SLC26A1–11) have been cloned (8, 23, 24, 25, 32). Several SLC26 isoforms function as Cl^-/HCO_3^- exchangers. These include SLC26A3 (DRA), SLC26A6 (PAT1), SLC26A7 (PAT2), and SLC26A9 (PAT4), with PAT1 and DRA detected on the apical membrane of pancreatic ducts cells (13, 21, 22, 42).

SLC26A6 (PAT1) is a major apical Cl^-/HCO_3^- exchanger in the small intestine and mediates majority of PGE_2-stimulated bicarbonate secretion in the duodenum (37, 38, 39). On the basis of its localization in the apical membrane of the pancreatic duct and its function as a Cl^-/HCO_3^- exchanger, PAT1 has been proposed to be a major contributor to apical HCO_3^- secretion in the pancreatic duct (13). To ascertain its role in apical Cl^-/HCO_3^- exchange and bicarbonate secretion in the pancreatic duct, Slc26a6 null mice and their wild-type (WT) littermates were examined. The results indicated that

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PAT1 null mice display an intriguing pattern of adaptation in apical Cl^-/HCO_3^- exchanger activity in their pancreatic duct cells.

METHODS AND MATERIALS

The following studies were approved by the Ethical Committee on Animal Use for Experiment and Recombinant DNA Experiment Safety Committee of Nagoya University, Japan and by the Committees for the Care and Use of Laboratory Animals at Yonsei University College of Medicine, South Korea and at University of Cincinnati, Cincinnati OH.

Mouse model. Slc26a6 knockout (KO) mice and their WT littermates were used for the experiments. Targeted 129 stem cells were implanted in a C57/BL6 blastocyst, the chimerical mice were bred to C57/BL6, and the progeny were studied (39). The animals used for these studies had been bred for five generations.

Collection and pH measurement of pancreatic juice. SL26A6 gene-disrupted (−/−) and littermate control mice were fasted overnight and then anesthetized with ketamine (3 mg/20 g body wt) and xylazine (0.2 mg/20 g body wt) by intramuscular injection. The body temperature of mice were maintained by placing the mice on a warm pad (37°C) during the experiments. The abdomen was opened, and the lumen of the common pancreaticobiliary duct was cannulated with a modified 31-gauge needle (TSK Striject, Air-Tite, Virginia Beach, VA). After the proximal end of the common duct was ligated to prevent contamination of bile, the pancreatic juice was collected in PE-10 tube for 30 min. Secreted volume was calibrated by measuring the length of the PE-10 tube filled with pancreatic juice. Mice were then treated with secretin (Sigma, 1 CU/kg sc), and the pancreatic juice was further collected in another PE-10 tube for 30 min after a 5-min interval from the secretin treatment. The collected pancreatic juices were transferred to microtubes embedded with mineral oil to prevent evaporation. The pancreatic juice was then equilibrated with 5% CO_2 for 20 min, and the pH of pancreatic juice was measured by using a glass micro-pH combination electrode (Thermo Electron, Beverly, MA).

Isolation of interlobular ducts. Interlobular pancreatic ducts (diameter ~100 μm) were isolated as described previously (12). Mice were killed by cervical dislocation. The pancreas was removed and chopped with scissors into ~1-mm^3 pieces. The tissue was digested with collagenase and hyaluronidase. Interlobular duct segments were microdissected by using sharpened needles under a dissection microscope. Usually 10–15 interlobular duct segments of 300–400 μm in length were isolated from one pancreas. The ducts were cultured overnight, during which time the end of the duct segment sealed spontaneously.

Solutions. The standard HCO_3^--buffered solution contained (in mM) 115 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose, and 25 $NaHCO_3$ and was equilibrated with 95% O_2 + 5% CO_2. Cl^--free HCO_3^--buffered solutions were made by replacing Cl^- with gluconate. High-K^+ (70 mM) HCO_3^--buffered solutions were made by replacing Na^+. All solutions were adjusted to pH 7.4 at 37°C.

Measurement of fluid secretory rate. The fluid secretory rate in isolated ducts was measured with videomicroscopy as described previously (43). The ducts were attached to glass coverslips pretreated with Cell-Tak and were superfused at 37°C on the stage of an inverted microscope. The bright-field images of the ducts were obtained at 5-min intervals by use of a charge-coupled device camera. The initial values for the length (L_0), diameter ($2R_0$), and image area (A_0) of the duct lumen were measured in the first image of the series. The initial volume (V_0) of the duct lumen was calculated, assuming cylindrical geometry, as $\pi R_0^2 L_0$. The luminal surface area of the epithelium (S_o) was taken to be $2\pi R_0 L_0$. In subsequent images of the series, the luminal image area (A) was expressed as relative area (A/A_0). Relative volume (V/V_0) was estimated from relative area using $V/V_0 = (A/A_0)^{3/2}$. The rate of fluid secretion was calculated from the increment in volume and expressed as the secretory rate per unit luminal area of epithelium ($nl \cdot min^{-1} \cdot mm^{-2}$).

Microperfusion of the isolated interlobular ducts. The lumen of the interlobular duct segment was microperfused as described previously (15). Both ends of the duct were cut open with sharpened needles, and one end was cannulated with concentric holding and perfusion pipettes. The bath and luminal solutions were modified separately. The bath was continuously perfused at 37°C.

Measurement of intracellular pH. Intracellular pH (pH_i) in the duct cells was estimated by microfluorometry as described previously (18) using the pH-sensitive fluoroprobe BCECF. After cannulation of the duct for luminal perfusion, the duct cells were loaded with BCECF for 10 min by adding acetoxymethyl ester BCECF-AM (2 μM) to the bathing solution. Small regions of the duct epithelium (10–20 cells) were illuminated alternately at excitation wavelengths of 430 and 480 nm. Values of pH_i were calculated from the fluorescence ratio (F_{480}/F_{430}) measured at 530 nm. The system was calibrated by the high-K^+/nigericin technique (35).

Semiquantitative RT-PCR of apical anion exchangers. RNA isolated from pancreas of WT and Slc26a6 KO mice was examined for the expression of Slc26a3, which is the only other known apical Cl^-/HCO_3^- exchanger detected in the pancreatic duct (13). In addition, the expression of Slc26a4 (PDS, pendrin), Slc26a11, and Slc4a9 (AE4), the other known apical anion exchangers in epithelial tissues, was examined. For Slc26a3, the following oligonucleotide primers from exon 2 were synthesized and utilized for RT-PCR: 5′-GGCAAAATGATCGAAGCCATAGGG (sense) and 5′-GATGGTC-CAGGAATGTC TTGTGATGTC (antisense). The cycling parameters were 94°C, 1 min, then 94°C, 30 s, 68°C, 3 min, 32 cycles. For control in RNA loading, the following oligonucleotide primers from 18S rRNA were used: 5′-CCAGTAAGTGCGGGTCATAAGC (sense) and 5′-GATCCGAGGGCCTCACTAAACC (antisense). This fragment encompasses nucleotides 1645–1747. For Slc26a4 (PDS or pendrin), the primers TCC CGG TGA AAG TGA ATG TC (sense), and CGC AAT GAC CTC ACT CCT AC (antisense) (accession number NM_011867); for Slc26a11, the primers GGT TCT GGA GTG CAC GCA TAT C (sense), and AAC AAA GGC CAG GGC GAC TC (antisense) (accession number NM_178743); and for Slc4a9 (AE4), the primers CAG AGG AGG AGG AGA CCA TC (sense), and GAT GTT GAT TTC TGG AGC CTT G (antisense) (accession number NM_172830) were used.

Materials. BCECF-AM was obtained from Molecular Probes (Eugene, OR), forskolin was from Sigma (St. Louis, MO), and secretin was from Peptide Institute.

Statistics. Data are presented as the means ± SE unless otherwise indicated. Tests for statistically significant differences were made with Student's *t*-test.

RESULTS

Basal and forskolin-stimulated fluid secretion in sealed interlobular ducts isolated from WT and Slc26a6 −/− mice. Isolated interlobular ducts were superfused with the standard HCO_3^--CO_2-buffered solution at 37°C and were then stimulated by forskolin (1 μM), an activator of adenylate cyclase, in the bath. The rate of basal fluid secretion was 0.18 ± 0.05 $nl \cdot min^{-1} \cdot mm^{-2}$ ($n = 5$) in WT ducts and 0.22 ± 0.05 ($n = 4$) in Slc26a6 −/− ducts (Fig. 1). The rate of forskolin-stimulated fluid secretion was 0.42 ± 0.02 $nl \cdot min^{-1} \cdot mm^{-2}$ in WT ducts and 0.53 ± 0.07 in Slc26a6 −/− ducts. Basal and forskolin-stimulated fluid secretion were not different between WT and Slc26a6 −/− ducts.

Effects of luminal Cl^- removal on pH_i in microperfused interlobular pancreatic ducts isolated from WT mice. To examine the activities of Cl^-/HCO_3^- exchangers in the apical membrane, we have investigated the effects on pH_i of remov-

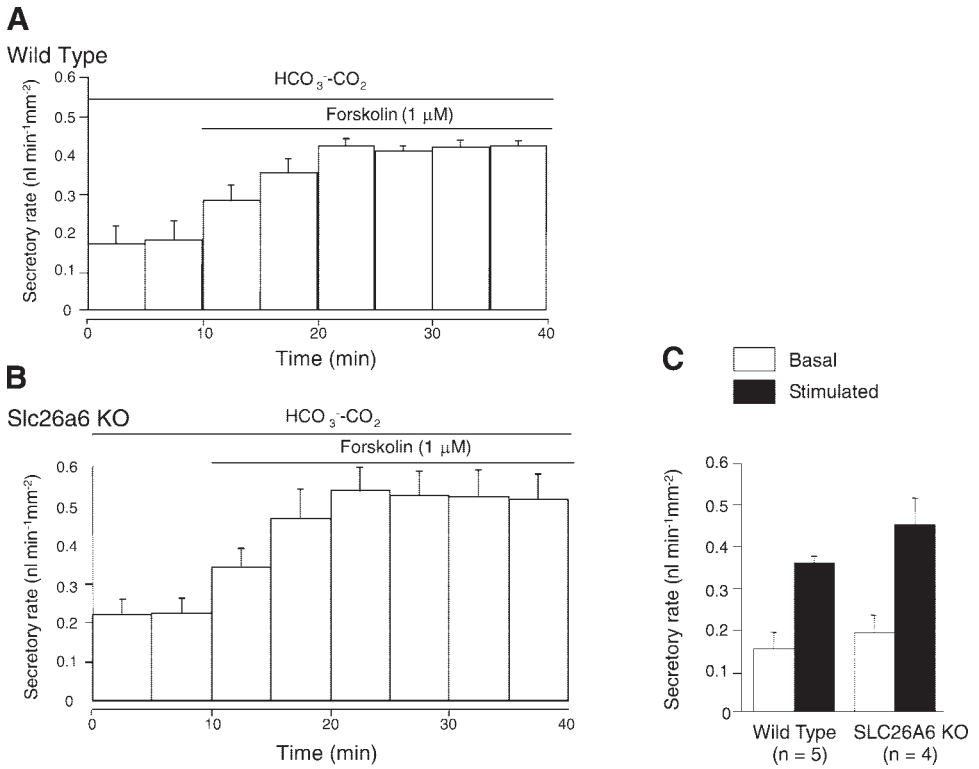

Fig. 1. Basal and forskolin-stimulated fluid secretion in sealed interlobular pancreatic ducts. Sealed interlobular pancreatic ducts isolated from wild-type (WT) and Sls26a6 −/− (KO) mice were superfused with the standard HCO_3^--CO_2-buffered solution, and forskolin (1 μM) was added to the bath. *A* and *B*: time course of fluid secretory rate in WT ($n = 5$) and Slc26a6 −/− ducts ($n = 4$). *C*: averaged values of basal and forskolin-stimulated fluid secretion. Means ± SE are shown.

ing Cl^- from the lumen in the presence of 25 mM HCO_3^--5% CO_2. When both the bath and lumen were perfused with the standard HCO_3^--buffered solution, basal pH_i was 7.328 ± 0.012 ($n = 10$). Removal of Cl^- from the luminal solution by replacement with gluconate (Fig. 2*A*) caused a small increase in pH_i of 0.068 ± 0.018 units ($n = 5$) over a 5-min period in unstimulated condition and of 0.078 ± 0.021 under forskolin (1 μM) stimulation (representative tracings as Fig. 2*A* and aver-

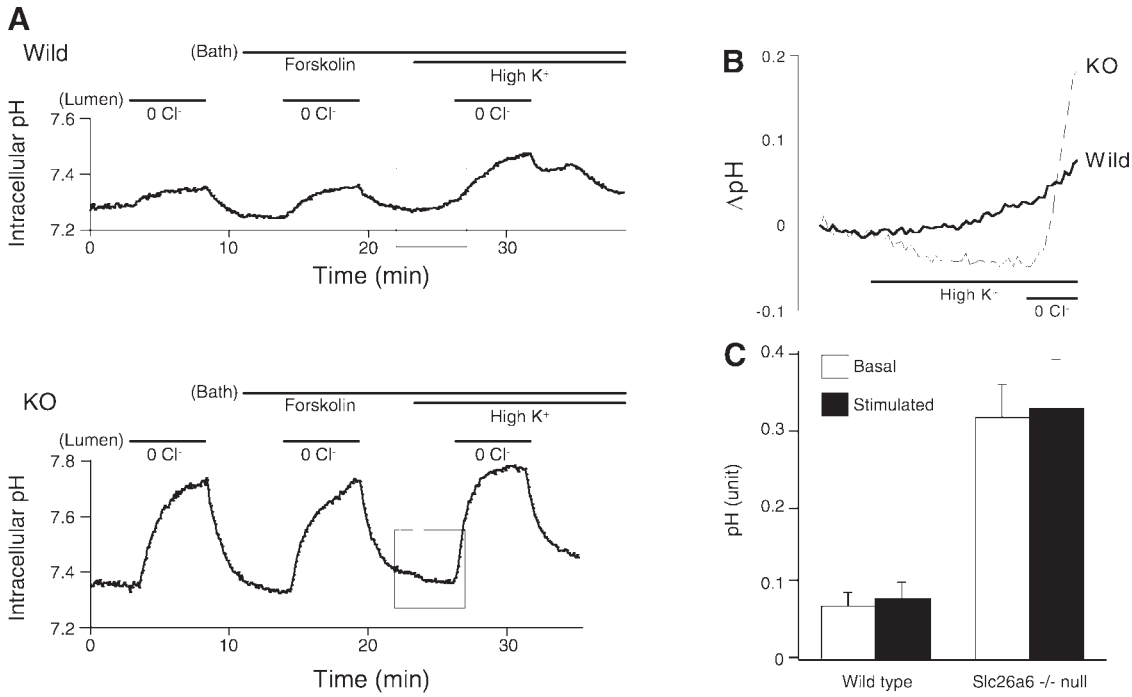

Fig. 2. Effects of luminal Cl^- removal on intracellular pH in microperfused interlobular pancreatic ducts. *A*: representative tracing. Initially, the bath and lumen of the duct segments were separately perfused with the standard HCO_3^--buffered solution containing 124 mM Cl^-. At the time indicated, the luminal perfusate was switched to Cl^--free HCO_3^--buffered solution (Cl^- replaced with glucuronate), forskolin (1 μM) was applied to the bath, and the bath perfusate was switched to high-K^+ (70 mM) solutions. Experiments are each representative of 5 experiments. *B*: effects of high K^+ are shown in magnified scale. *C*: summation of results. The averaged responses of intracellular pH upon removal of luminal Cl^- (means ± SE, $n = 5$).

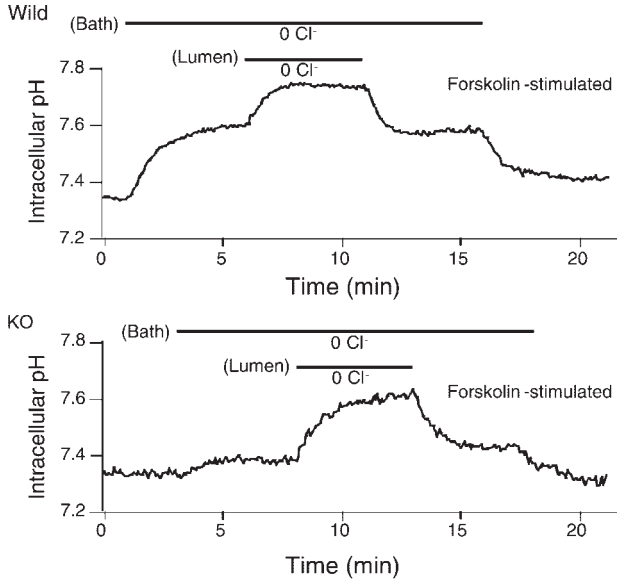

Fig. 3. Effects of luminal and bath Cl^- removal on intracellular pH in microperfused interlobular pancreatic ducts. Initially, the bath and lumen of the duct segments were separately perfused with the standard HCO_3^--buffered solution and stimulated by forskolin (1 μM). Thereafter, the luminal perfusate and/or the bath solution was switched to Cl^--free HCO_3^--buffered solution. Experiments are each representative of 5 experiments.

aged responses as Fig. 2*C*). Forskolin stimulation did not cause significant changes in basal pH_i, suggesting that both basolateral HCO_3^- uptake and apical HCO_3^- efflux were activated.

When the cells were depolarized by raising K^+ concentration in the bath (70 mM), basal pH_i increased by 0.076 ± 0.022 ($n = 5$) in 3 min under forskolin stimulation (Fig. 2*A*). This small but significant pH_i change is clearly visualized when magnified (Fig. 2*B*). Removal of luminal Cl^- under high K^+ condition caused further increase of pH_i.

Effects of luminal Cl^- removal on pH_i in microperfused interlobular pancreatic ducts isolated from Slc26a6 −/− mice. Basal pH_i of Slc26a6 −/− ducts in the presence of 25 mM HCO_3^--5% CO_2 was 7.322 ± 0.015 ($n = 10$), which was not different from that of WT ducts. Surprisingly, removal of luminal Cl^- (Fig. 2) caused a much larger ($P < 0.01$) increase of pH_i in Slc26a6 −/− ducts (0.313 ± 0.042 units in 5 min in unstimulated condition and 0.325 ± 0.062 under forskolin stimulation, $n = 5$) than in WT ducts (representative tracings as Fig. 2*A* and averaged responses as Fig. 2*C*). Forskolin stimulation did not cause significant changes in basal pH_i.

Imposition of high K^+ gradient in the bath caused a small decrease in basal pH_i by 0.053 ± 0.005 ($n = 5$) in 3 min under forskolin stimulation, which is also in contrast to WT ducts (Fig. 2, *A* and *B*, in magnified scale). Removal of luminal Cl^- under high-K^+ conditions caused further increase of pH_i.

Effects of luminal and bath Cl^- removal on pH_i in microperfused interlobular pancreatic ducts isolated from WT and Slc26a6 −/− mice. In the next set of experiments, we examined the effect of bath chloride removal on intracellular pH. In WT animals, the increase of pH_i by luminal Cl^- removal was much smaller than that by Cl^- removal from the bath (0.335 ± 0.054 in 5 min, $n = 5$, Fig. 3). This is similar to the findings in interlobular ducts from guinea pig pancreas (16). In Slc26a6 null animals, the increase in pH_i by Cl^- removal from the bath (0.130 ± 0.064 in 5 min, $n = 5$, Fig. 3) was smaller ($P < 0.05$) than that in WT ducts. These data suggest that another apical Cl^-/HCO_3^- exchanger is upregulated and basolateral Cl^-/HCO_3^- exchanger is downregulated in Slc26a6 −/− ducts.

Effects of restoring luminal Cl^- on pH_i in microperfused interlobular pancreatic ducts. To examine the activities of apical Cl^-/HCO_3^- exchangers working for HCO_3^- efflux, intracellular Cl^- was depleted as much as possible and Cl^- was restored to the lumen (Fig. 4). Both the bath and lumen were perfused with Cl^--free HCO_3^--buffered solution in the presence of forskolin (1 μM) for ~30 min before measurement. Initial pH_i was not different between WT (7.93 ± 0.02, $n = 6$) and Slc26a6 −/− ducts (7.90 ± 0.02, $n = 6$). After a 3-min period, the luminal solution was switched to standard HCO_3^--buffered solution containing 124 mM Cl^- in the presence of forskolin. The rate of pH_i decrease on restoring luminal Cl^- was 0.334 ± 0.053 pH units/min in WT ducts and 0.196 ± 0.028 pH units/min ($n = 6$) in Slc26a6 −/− ducts. The activities of apical Cl^-/HCO_3^- exchange working for HCO_3^- efflux were significantly ($P < 0.05$) smaller in Slc26a6 −/− ducts.

Volume and pH of pancreatic juice. In the last series of experiments, we examined the pancreatic juice output and its pH. WT and Slc26a6 null animals were anesthetized and pure pancreatic juice was collected in basal and secretin-stimulated conditions. As illustrated in Fig. 5, pancreatic fluid and bicarbonate secretions were comparable in WT and Slc26a6 null animals. Neither juice volume nor its pH showed significant

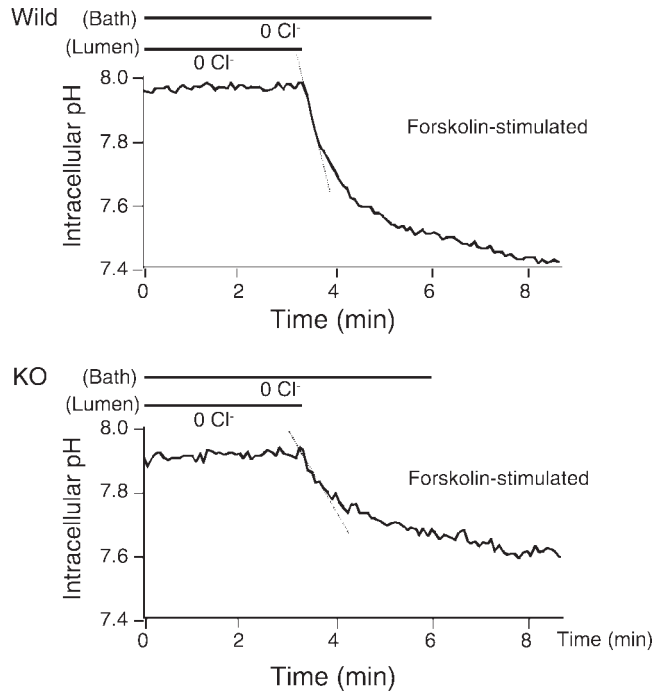

Fig. 4. Effects of restoring luminal Cl^- on intracellular pH in microperfused interlobular pancreatic ducts. Before measurement, intracellular Cl^- was depleted as much as possible by perfusing both the bath and lumen with Cl^--free HCO_3^--buffered solution in the presence of forskolin (1 μM). At the time indicated, Cl^- was restored to the lumen by switching the luminal perfusate to the standard HCO_3^--buffered solution containing 124 mM Cl^-. The initial rate of pH_i decline (the line was overlaid on the trace) was calculated as a measure of exchange activity of intracellular HCO_3^- for luminal Cl^-. Experiments are each representative of 6 experiments.

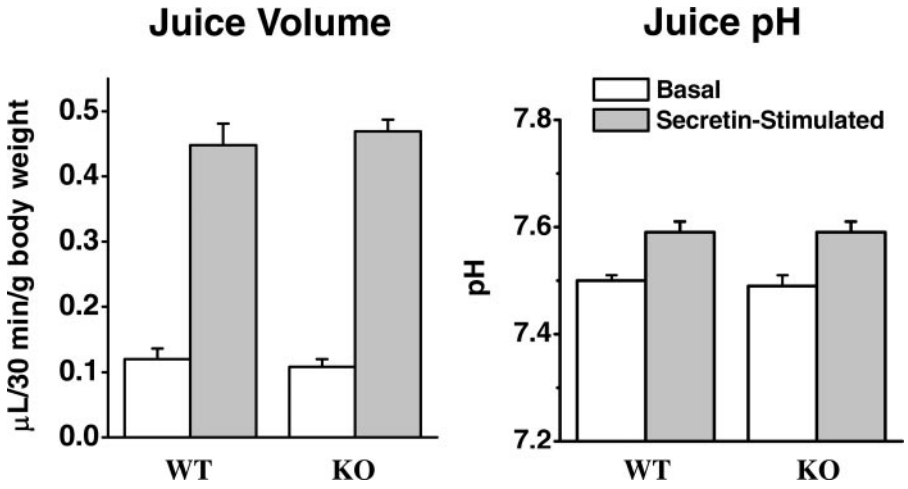

Fig. 5. pH measurement and volume of pure pancreatic juice collected in vivo under secretin stimulation. Pancreatic juice volume and pH were comparable in WT and Slc26a6 null mice.

differences between WT and Slc26a6 null mice in both basal and secretin-stimulated secretions.

Expression of Slc26a3 (DRA) in pancreas of Slc26a6 KO mice. To determine the identity of the Cl^-/HCO_3^- exchanger, upregulated in the pancreatic ducts of Slc26a6 KO mice, semiquantitative RT-PCR was performed on RNA isolated from WT and KO mice pancreata to determine the expression of Slc26a3, Slc26a4 (pendrin), Slc25a11, and Slc4a9 (AE4), four known apical anion exchangers in epithelial tissues. Figure 6*A* is an ethidium bromide staining of an agarose gel and shows that the expression of DRA is significantly increased in pancreas of Slc26a6 null mice. The expression of 18S rRNA is shown as control for the loading and amplification. Sequencing verified the identity of the amplified bands. Summation of the results showed a more than fivefold increase in Slc26a3 expression in Slc26a6 KO mice (Fig. 6*B*, $n = 4$). Examination of the expression of other known apical Cl^-/HCO_3^- exchangers (METHODS AND MATERIALS) in the pancreas was as follows. Slc26a4 (pendrin) expression in the pancreas was absent in WT and KO mice (Fig. 6*C*, *top*). AE4 expression was absent in both WT and KO mice (Fig. 6*C*, *middle*). Positive control for AE4 is shown in the kidney (Fig. 6*C*, *middle*). Expression of Slc26a11 was very low in WT mice and did not change in Slc26a6 KO mice (Fig. 6*C*, *bottom*). Positive controls for Slc26a4 (PDS), Slc4a9 (AE4), and Slc26a11 were run simultaneously and are shown in their respective panels (*top*, *middle*, and *bottom*, Fig. 6*C*), indicating the accuracy of PCR reactions.

DISCUSSION

The present studies examined the role of Slc26a6 (PAT1) in apical Cl^-/HCO_3^- exchange and fluid secretion in interlobular pancreatic duct cells and pancreatic fluid and HCO_3^- secretion in vivo by using Slc26a6 null mice. Our results demonstrated the unexpected, and seemingly paradoxical, upregulation of apical Cl^-/HCO_3^- exchanger activity ($[Cl^-]_i/[HCO_3^-]_o$ exchange, where brackets denote concentration and subscripts i and o denote intra- and extracellular, respectively) in interlobular ducts of Slc26a6 null animals (Fig. 2). To examine the exchange activity of intracellular HCO_3^- for luminal Cl^- across the apical membrane, the interlobular duct cells were depleted of Cl^- (Fig. 4). This time, however, the apical Cl^-/HCO_3^- exchanger activity ($[Cl^-]_o/[HCO_3^-]_i$ exchange) was actually decreased in Slc26a6 null mice. Absence of Slc26a6 did not affect basal and cAMP-stimulated fluid secretion in isolated interlobular ducts superfused with the standard HCO_3^-buffered solution (Fig. 1). Similarly, the volume and pH of pure pancreatic juice collected in vivo at both basal and secretin-stimulated conditions were comparable in WT and Slc26a6 null mice (Fig. 5). Slc26a3 (DRA) is strongly upregulated in Slc26a6 KO mouse pancreas (Fig. 6).

As noted, the increase in pH_i (mostly due to apical HCO_3^- influx) on removal of luminal Cl^- was much larger in Slc26a6 $-/-$ ducts (Fig. 2). These data are consistent with either the inhibitory effect of Slc26a6 on HCO_3^- influx in WT animals or the activation of a distinct apical anion exchanger in Slc26a6 null mice. Assuming that Slc26a6 is an electrogenic Cl^-/HCO_3^- exchanger with $n HCO_3^-$ exchanged per Cl^-, where n is >1, as proposed (28), WT animals may have difficulty taking up HCO_3^- in native hyperpolarized epithelia in response to Cl^- removal from the luminal perfusate. This could explain the small effect of luminal Cl^- removal on intracellular pH in WT ducts (Fig. 2) and in guinea pig ducts (18). Enhanced intracellular alkalinization upon removal of luminal Cl^- in Slc26a6 null mice (Fig. 2) suggests that another Cl^-/HCO_3^- exchanger is upregulated in the apical membrane. Anion exchanger with stoichiometry of $>2{:}1$ $Cl^-{:}HCO_3^-$ (compatible with Slc26a3; DRA, Refs. 22, 28) would easily uptake HCO_3^- when luminal Cl^- is removed. However, the intracellular alkalinization may also occur by apical HCO_3^- influx through CFTR and basolateral HCO_3^- influx via Na^+-$n HCO_3^-$ cotransport, both of which may be activated by depolarization. The former possibility is unlikely because intracellular HCO_3^- concentration would not exceed luminal HCO_3^- concentration unless the cells were highly depolarized to approximately zero. In fact, removal of luminal Cl^- caused pH_i increase to ~7.7 in Slc26a6 null ducts (Fig. 2*A*). The extent of intracellular alkalinization upon removal of perfusate chloride may be determined by the electrogenicity of the chloride/base exchanger, the magnitude of membrane potential, and intracellular Cl^- concentration. It is worth mentioning that these two last parameters may differ between WT and Slc26a6 $-/-$ ducts and also may be altered by cAMP stimulation. As indicated the activity of basolateral Cl^--HCO_3^- exchange is reduced in Slc26a6 null ducts (Fig. 3). Downregu-

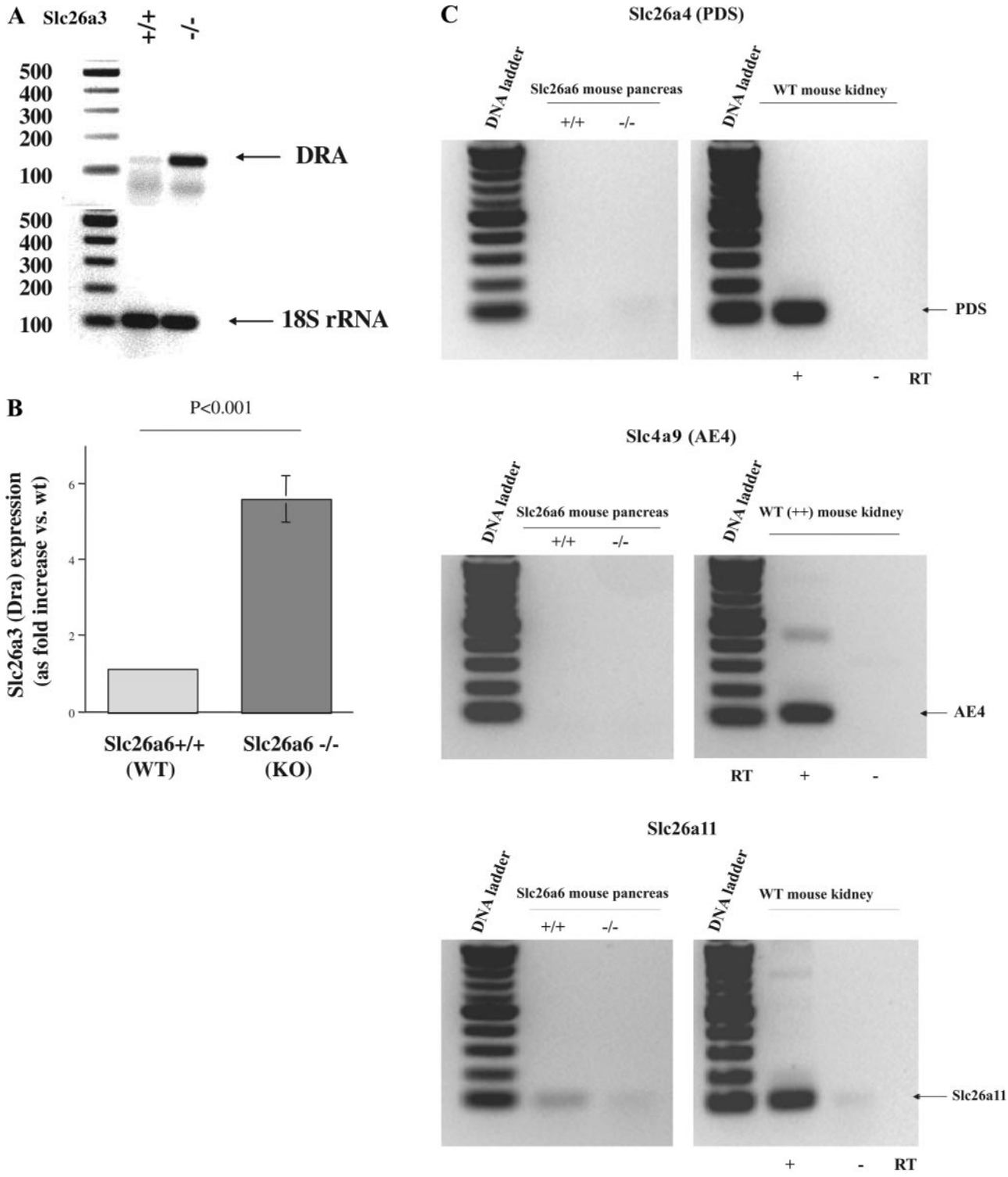

Fig. 6. Expression of Slc26 bicarbonate transporters in the pancreas of Slc26a6 KO mouse. *A*: ethidium bromide staining of agarose gel depicting Slc26a3 RT-PCR. *B*: summation of results. *C*: ethidium bromide staining of agarose gels depicting the expression of Slc26a4 (PDS; pendrin), Slc4a9 (AE4), and Slc26a11. Positive controls for Slc26a4, AE4, and Slc26a11 are shown in the kidney (*right* panels in Fig. 6*C*).

lation of basolateral Cl^-/HCO_3^- exchanger (possibly AE2) would be beneficial for HCO_3^- secretion (analogous to a compensatory regulation) because it works as a base extruder.

Effects of depolarization (by high-K^+ in the bath) on pH_i were opposite in WT and Slc26a6 null ducts (Fig. 2); depolarization caused a small increase of pH_i in WT but caused a decrease in Slc26a6 null ducts, which is compatible to the electrogenic property of Slc26a6. The increase in intracellular HCO_3^- concentration in WT ducts can be explained by combination of the depolarization-induced inhibition of Slc26a6-

mediated exchange of luminal $1Cl^-$ for intracellular $nHCO_3^-$ and an apical HCO_3^- conductance, and by depolarization-induced activation of basolateral Na^+-$nHCO_3^-$ cotransport. On the contrary, the reduction in intracellular HCO_3^- concentration in Slc26a6 null ducts can be explained by the activation of apical Cl^-/HCO_3^- exchanger of opposite stoichiometry ($nCl^-/1HCO_3^-$ exchange), which seems to be upregulated in Slc26a6 null ducts as shown in Fig. 2*A*.

Although there were significant changes in the apical Cl^-/HCO_3^- exchange activity (Figs. 2 and 4), the overall pancreatic fluid and bicarbonate secretions (both at the level of interlobular duct cells and in vivo) were not different between WT and Slc26a6 null mice (Figs. 1 and 5). A possible explanation for this discrepancy is the presence of compensatory mechanisms that overcome the loss of Slc26a6. For example, there would be multiple HCO_3^- exit mechanisms on the luminal membrane of pancreatic duct cells and abolition of only one of these would not make significant changes in the overall fluid secretions. Indeed, this was the case in the salivary glands, where basolateral K^+ channels in acinar cells play a major role in fluid secretion to maintain the membrane potential for Cl^- exit through luminal Cl^- channels. Salivary gland acinar cells express both intermediate (IK, also known as Sk4) and large (BK, also known as Slo) conductance Ca^{2+}-activated K^+ channels. Deletion of both K^+ channels, but not the single deletion of each K^+ channel, markedly decreased fluid secretion (26a). Therefore, a future study using the mice deleted with multiple luminal HCO_3^- transporters would be helpful further in identifying the role of Slc26a6 in pancreatic secretions.

There are few reliable data on in vivo fluid and HCO_3^- secretion from mouse pancreas (4) owing to the very small volume of secreted fluid. Fluid and HCO_3^- secretion was usually measured by collection of a mixture of bile-pancreatic juice (34) or duodenal aspiration (10). HCO_3^- concentration in a mixture of bile-pancreatic juice was 20–30 mM and did not significantly increase after stimulation (34). pH of duodenal aspiration after secretin stimulation was ~8.1 in WT and ~6.6 in cystic fibrosis mice (10). The present study for the first time successfully examined the volume and pH of pure pancreatic juice from mouse. Secretin stimulation increased juice pH from 7.50 to 7.59 in littermate controls, which represents ~25% increase of HCO_3^- concentrations from 30.0 mmol/l in basal secretion to 37.1 mmol/l in secretin-stimulated secretion. However, secretin stimulation increased fluid secretion approximately by fourfold, from 0.12 to 0.45 $\mu l \cdot min^{-1} \cdot g$ body wt^{-1}. These results imply that secretin may induce pancreatic secretion by augmenting the secretion of an ion besides HCO_3^- in mice. Importantly, neither juice volume nor HCO_3^- secretion were affected by the deletion of Slc26a6 (Fig. 5). Fluid secretory rate under stimulation with 1 μM of forskolin (maximal stimulation with cAMP) in interlobular ducts isolated from WT mice was ~0.4 $nl \cdot min^{-1} \cdot mm^{-2}$, which is much smaller than secretory rate in guinea pig ducts (~2.0 $nl \cdot min^{-1} \cdot mm^{-2}$, Ref. 38) and that in rat ducts (~1.2 $nl \cdot min^{-1} \cdot mm^{-2}$, Ref. 20). Another group recently examined fluid secretion in mouse pancreatic ducts (8) and found that interlobular ducts secrete fluid in the absence of HCO_3^-, which depends on basolateral Na^+-K^+-$2Cl^-$ cotransport. Thus the mouse pancreatic duct system probably secretes a mixture of $NaHCO_3$ and NaCl, which is consistent with our data of juice pH (~7.6) (Fig. 5). These data indicate that the capacity of HCO_3^- and fluid transport of mouse pancreatic duct cells is significantly smaller than other species and that the mouse duct cells cannot raise luminal HCO_3^- concentration to higher than 50 mM. The contribution of Slc26a6 in ductal HCO_3^- secretion may be

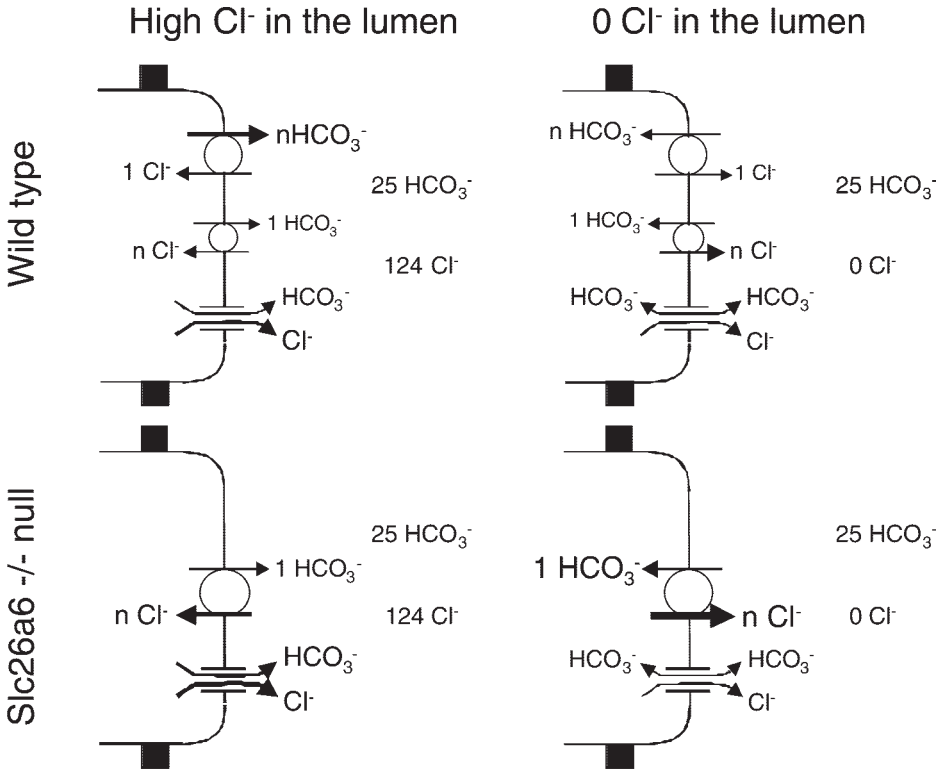

Fig. 7. Schematic diagram demonstrating apical and basolateral bicarbonate transporters in interlobular pancreatic duct.

small in species such as mice that do not have a very high HCO_3^- concentration in their pancreatic juice. In pancreatic duct cells of human or guinea pig, which face pancreatic juice containing high HCO_3^- (up to ~140 mM at maximal stimulation) and low Cl^-, Slc26a6 should play an important role to prevent HCO_3^- absorption.

Investigation into the electrogenicity of Slc26a6-mediated Cl^-/HCO_3^- exchange has been less than conclusive (7, 22, 28). However, an intriguing aspect is the interaction of Slc26 anion exchangers with CFTR (22). Recent studies demonstrated that CFTR interacts with Slc26a6 and Slc26a3 in a synergistic way (22). It was speculated that this interaction might play an important role in CFTR-dependent generation of HCO_3^--rich pancreatic juice. The authors further suggested that expression of $2HCO_3^-$-$1Cl^-$ exchanger (i.e., Slc26a6) in the proximal duct and $1HCO_3^-$-$2Cl^-$ exchanger (i.e., Slc26a3) in the distal duct could explain HCO_3^--rich (140 mM) fluid secretion (22).

The results of our studies are in agreement with a model in which two apical Cl^-/HCO_3^- exchangers with opposite stoichiometry exist in the apical membrane of the same cell. This model has been depicted in Fig. 7 and explains the present data. Figure 7, *left* simulates HCO_3^- and Cl^- transport across the apical membrane with high Cl^- in the lumen. It resembles in vivo condition of mouse pancreatic duct and also the experiments shown as Fig. 4 in which Cl^- was restored to the lumen. Figure 7, *right* simulates apical anion transport with 0 Cl^- in the lumen. It resembles in vivo condition of human and guinea pig pancreatic duct and also the experiments shown as Fig. 2 in which Cl^- was removed from the lumen. Our assumption is that *1*) both $n HCO_3^-$-$1Cl^-$ exchanger (i.e., Slc26a6) and $1HCO_3^-$-nCl^- exchanger (i.e., Slc26a3) are localized in the apical membrane, and *2*) the former is the dominant exchanger in WT ducts, whereas the latter is robustly upregulated in Slc26a6 null ducts. Our studies in Fig. 6 support the notion that Slc26a3 is the dominant apical exchanger in Slc26a6 KO mouse.

In WT ducts, luminal Cl^--dependent HCO_3^- secretion is mediated by Slc26a6 and some HCO_3^- is secreted also via CFTR (Fig. 7). Thus HCO_3^- efflux upon restoration of luminal Cl^- is smaller in Slc26a6 $-/-$ null ducts even in the presence of enhanced expression of Dra, presumably working in $1HCO_3^-$-nCl^- exchange mode (because it needs more Cl^- to export HCO_3^-). Cells of Slc26a6 $-/-$ null ducts will be relatively hyperpolarized because of the electrogenicity of the $1HCO_3^-$-nCl^- exchanger (Dra upregulation), and thus anion conductive pathway (CFTR) may mediate more HCO_3^- secretion. Thus overall HCO_3^- secretion is maintained in Slc26a6 $-/-$ null ducts and basal pH_i is not altered.

When luminal Cl^- is removed, both anion exchangers (Fig. 7) work in an opposite direction (for HCO_3^- uptake). In WT ducts, cells uptake less HCO_3^- because $n HCO_3^-$-$1Cl^-$ exchanger (Slc26a6) is dominant. The $n HCO_3^-$-$1Cl^-$ exchanger does not work easily for HCO_3^- uptake when cell-negative membrane potential is maintained. In Slc26a6 $-/-$ null ducts, the upregulated $1HCO_3^-$-nCl^- exchanger, mediated via Dra, easily works for HCO_3^- uptake. Thus, the magnitude of enhanced intracellular alkalinization upon removal of luminal Cl^- is larger in Slc26a6 $-/-$ null ducts.

In conclusion, the apical Cl^-/HCO_3^- exchanger activity is dramatically upregulated and cAMP-stimulated fluid secretion remains unchanged in interlobular pancreatic ducts in Slc26a6 null mice. In vivo pancreas, secretin-stimulated bicarbonate secretion, and juice volume remain unchanged in Slc26a6 KO animals. Slc26a3 expression in the pancreas is heavily upregulated in Slc26a6 null mice. We propose that deletion of Slc26a6 in pancreatic duct results in the compensatory upregulation of Slc26a3, which helps maintain bicarbonate secretion and pancreatic juice volume.

GRANTS

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