Localization of Na\(^{+}\)-HCO\(_3^{-}\) cotransporter (NBC-1) variants in rat and human pancreas

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Satoh, Hiroaki, Nobuo Moriyama, Chiaki Hara, Hideomi Yamada, Shoko Horita, Motoei Kunimi, Kazuhisa Tsukamoto, Naoyuki Iso-o, Jun InATOMI, Hayato Kawakami, Akihiko Kudo, Hitoshi Endou, Takashi Igarashi, Atsuo Goto, Toshiro Fujita, and George Seki. Localization of Na\(^{+}\)-HCO\(_3^{-}\) cotransporter (NBC-1) variants in rat and human pancreas. Am J Physiol Cell Physiol 284: C729–C737, 2003. First published November 20, 2002; 10.1152/ajpcell.00166.2002.—Mutations in Na\(^{+}\)-HCO\(_3^{-}\) cotransporter (NBC-1) cause proximal renal tubular acidosis (pRTA) associated with ocular abnormalities. One pRTA patient had increased serum amylase, suggesting possible evidence of pancreatitis. To further delineate a link between NBC-1 inactivation and pancreatic dysfunction, immunohistochemical analysis was performed on rat and human pancreas using antibodies against kidney-type (kNBC-1) and pancreatic-type (pNBC-1) transporters. In rat pancreas, the anti-pNBC-1 antibody labeled acinar cells and both apical and basolateral membranes of medium and large duct cells. In human pancreas, on the other hand, the anti-pNBC-1 antibody did not label acinar cells, although it did label the basolateral membranes of the entire duct system. The labeling by anti-kNBC-1 antibody was detected in only a limited number of rat pancreatic duct cells. To examine the effects of pRTA-related mutations, R342S and R554H, on pNBC-1 function, we performed functional analysis and found that both mutants had reduced transport activities compared with the wild-type pNBC-1. These results indicate that pNBC-1 is the predominant variant that mediates basolateral HCO\(_3^{-}\) uptake into duct cells in both rat and human pancreas. The loss of pNBC-1 function is predicted to have major effects on overall ductal HCO\(_3^{-}\) secretion, which could potentially lead to pancreatic dysfunction. 

Na\(^{+}\)-HCO\(_3^{-}\) cotransporter; pRTA; pancreatic duct cells; pancreatitis

THE NA\(^{+}\)-HCO\(_3^{-}\) CO TRANSPORTER (NBC-1), originally cloned from amphibian kidney (32), has multiple functions. In the renal proximal tubules, it mediates HCO\(_3^{-}\) efflux from cells (7, 36, 42), whereas in other epithelial cells, such as the pancreatic duct cells and the corneal endothelium, it mediates HCO\(_3^{-}\) influx into cells (22, 23, 41). The expression of NBC-1 in nonepithelial cells such as trabecular meshwork cells or neurons has been also demonstrated (35, 40). Up to now, three NBC-1 variants have been identified: kNBC-1 from kidney, pNBC-1 from pancreas, and hNBC-1 from heart. Although kNBC-1 differs from pNBC-1 only at the NH\(_2\) terminus, pNBC-1 is identical to hNBC-1 at the amino acid level and differs only in its 5′-untranslated region (1, 8, 10). These NBC-1 variants originate from the same SLC4A4 gene by alternative splicing (2). It is therefore predictable that mutational inactivation of NBC-1 variants may induce a variety of clinical manifestations, including renal and extrarenal phenotypes. Indeed, we have recently shown that mutations in the common coding region of NBC-1, corresponding to R298S and R510H in kNBC-1 or R342S and R554H in pNBC-1, cause renal proximal tubular acidosis (pRTA) associated with ocular abnormalities such as band keratopathy, glaucoma, and cataracts (19). The expression study in ECV304 cells showed that the kidney-type mutants, R298S and R510H, have reduced transport activities compared with the wild-type kNBC-1 (19). The expression of both kNBC-1 and pNBC-1 in several ocular tissues has been confirmed (6, 40). It remains to be clarified, however, whether the pancreatic-type mutants also have impaired functions, contributing to the clinical manifestations of pRTA patients.

The classic view of HCO\(_3^{-}\) secretion by the pancreatic duct cells has emphasized the roles of a basolateral Na\(^{+}\)/H\(^{+}\) exchanger, intracellular HCO\(_3^{-}\) formation catalyzed by carbonic anhydrase, and an apical Cl\(^{-}\)/HCO\(_3^{-}\) exchanger (4). However, a functional study in guinea pig pancreatic duct cells has shown that the Na\(^{+}\)-HCO\(_3^{-}\) cotransporter contributes ~75% of HCO\(_3^{-}\) uptake during stimulation by secretin (22). In addition, Shumaker et al. (37) have shown that NBC-1 is respons-
sible for secretin-stimulated HCO₃⁻ uptake into human pancreatic duct cells. They have further proposed that the defect in HCO₃⁻ secretion during secretin stimulation in patients with cystic fibrosis (CF) is at least partly due to lack of HCO₃⁻ uptake via NBC-1 (37). If NBC-1 indeed plays a pivotal role in overall ductal HCO₃⁻ secretion, its inactivation could theoretically lead to pancreatic dysfunction as analogous to the situation in CF. Consistent with this speculation, one pRTA patient with a R298S mutation in kNBC-1 had elevated serum amylase concentrations (19, 21). Her serum amylase has remained elevated as high as three times that of the upper normal level for more than 8 yr. The clinical analysis revealed that her elevated amylase is of pancreatic origin, although this female patient, 24 yr old now, has no clinical signs of severe pancreatitis yet (Igarashi T, unpublished observation).

The previous immunological studies have shown the presence of NBC-1 in rat and human pancreatic tissues (27, 38). However, the relative importance of NBC-1 variants in pancreatic HCO₃⁻ secretion has not been definitely determined, because the antibodies used in these studies were raised against the common NBC-1 epitopes and could not discriminate between kNBC-1 and pNBC-1 (27, 38). The aim of the present study was to further delineate a possible link between NBC-1 inactivation and pancreatic dysfunction. To this end, we first performed immunological analysis of human and rat pancreas using the variant specific anti-NBC-1 antibodies (40). Because the results clearly showed that pNBC-1 is the dominant variant in pancreatic HCO₃⁻ secretion, we next performed functional analysis to investigate the impacts of disease-causing mutations on the transport activity of pNBC-1.

**METHODS**

**Immunohistochemistry.** Affinity-purified anti-kNBC-1 and anti-pNBC-1 antibodies were used to determine the localization of NBC-1 variants in pancreas. They are polyclonal antibodies against the NH₂-terminal regions of human kNBC-1 and pNBC-1 (amino acids 4–16) or pNBC-1 (amino acids 2–12). The generation and specificity of these antibodies have been described elsewhere (40). Male Wistar rats were anesthetized with pentobarbital, and the pancreas was quickly removed and immersed in 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4. The specimens were then permeated by sequential incubation in PBS containing 10, 20, and 30% sucrose, embedded into Tissue-Tek OCT (Miles, Naperville, IL), and quickly frozen in liquid nitrogen. Sections 5 μm thick were prepared, air-dried, and immersed in PBS. Paraffin-embedded human pancreatic tissues were obtained with full informed consent from three patients who underwent the total pancreatectomy for the pancreatic carcinoma. The specimens had been immediately immersed in 10% formalin solution and then embedded in paraffin according to the standard method. Only areas that were confirmed to be histologically normal were used for the study. Sections 5 μm thick were cut from paraffin-embedded tissue blocks, rehydrated with xylene and graded alcohols, and immersed in PBS.

The immunofluorescence detection method was used for the immunohistological analysis. In brief, the rat pancreatic specimens were treated with normal goat serum (Jackson ImmunoResearch Laboratories, West Grove, PA) to block nonspecific protein binding, followed by incubation with anti-kNBC-1 or anti-pNBC-1 antibodies (1:200 dilution in PBS) overnight at 4°C. Antigen deterioration in formalin-fixed, paraffin-embedded preparations is known to result in false negative findings. Therefore, for the paraffin-embedded human specimens, antigen retrieval by autoclave (121°C, 10 min in 0.01 mol/l citrate buffer, pH 6.0), which had been shown to be extremely useful in retrieving the masked antigenicity of proteins in paraffin sections (24), was adopted. After this pretreatment, the specimens were processed similarly as described for the rat pancreas. The rat and human specimens were subsequently incubated with the mixture of Alexa Fluor 488 goat anti-rabbit IgG (H + L), Alexa Fluor 568 phallolidin for labeling of actin, and TO-PRO-3 iodide for labeling of nuclei (all from Molecular Probes, Eugene, OR) for 60 min at room temperature. After being washed with PBS, the specimens were observed with a confocal laser scanning microscope (MRC-1024K, Japan Bio-Rad Laboratories, Tokyo, Japan).

**Transient expression of pNBC-1 and mutants.** The wild-type human kNBC-1 and the kidney-type mutants, R298S and R510H, were cloned into a eukaryotic expression vector pcDNA3.1 (Invitrogen, San Diego, CA) (19) and designated as pkNBC, pR298S, and pR510H, respectively. The pcDNA3.1 containing the wild-type human pNBC-1 was constructed as follows (18). The DNA fragment spanning the pNBC-1 specific region was amplified by PCR from the cDNA of human corneal endothelial cells (41). This fragment was subcloned into the EcoRI sites of pcDNA3.1 in an appropriate orientation and designated as ppNBC-1-pre. The DNA fragment containing the remaining common region of NBC-1 was purified after digestion of pkNBC with ApIIII and subcloned into the ApI/III sites of ppNBC-1-pre. pcDNA3.1 containing the pancreatic-type mutants, R342S and R554H, was similarly constructed using pR298S and pR510H, respectively.

ECV304 cells were maintained in Medium199 (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (Life Technologies). Cells were seeded onto 6-mm round coverslips and transfected with wild-type pNBC-1, R342S, or R554H using LipofectAMINE 2000 (Life Technologies) according to the manufacturer’s instruction. In brief, cells were transfected with an equal amount of DNA in a 1:1 mixture of medium 199 and Opti-MEM I (Life Technologies) containing LipofectAMINE 2000 for 5 h. After the cells were incubated in medium199 supplemented with 10% PBS for 48 h, cell pH (pHᵢ) measurement and Western blot analysis were performed.

pHᵢ measurement. Cell-coated coverslips were incubated with HCO₃⁻-free, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)-buffered Ringer solution containing 10 μmol/l 2’,7’-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-AM for 30–60 min at room temperature. The coverslip was transferred into a chamber mounted on an inverted microscope (IMT-2, Olympus, Tokyo, Japan). We noticed that ECV304 cells have a significant activity of Na⁺/H⁻ cotransporter (NHE3), which could interfere with the assessment of NBC-1 activities. Therefore, the coverslip was superfused for 30 min at ~5 ml/min with Cl⁻-free HCO₃⁻ solution containing (in mmol/l) 144 Na⁺, 5 K⁺, 5 Ca²⁺, 1 Mg²⁺, 132 gluconate⁻, 2 H₂PO₄⁻, 1 SO₄²⁻, 25 HCO₃⁻, and 5.5 glucose⁻ to reduce intracellular Cl⁻. Our preliminary experiments confirmed that this treatment was sufficient to minimize the influence of Na⁺-dependent Cl⁻/HCO₃⁻ exchange activity. pHᵢ was measured with a microscopic fluorescence photometry system (OST-10, Olympus) as previously described (18, 41). The intracellular pH was alternately excited...
at two wavelengths (440 and 490 nm), and emission was measured at a wavelength of 530 nm. Autofluorescence of cells was measured at the beginning of an experimental day, and these values were subtracted from the raw data. We used 10 μmol/l nigericin to acidify the cells. This method has been shown to induce a more stable and predictable acidification than the NH4Cl-pulse technique, especially in Cl−-free solution (18, 40). We have confirmed that Na+/H+ exchange activity assayed by the nigericin acidification was identical to that assayed by the NH4Cl-pulse technique (18, 40). Therefore, the problem of proton leak that might be caused by residual nigericin has been shown to be minimal, if any, in our system. After cell acidification, the coverslip was superimposed for 3–4 min with Na+/free, Cl−-free HCO3− solution (Na+ was replaced with N-methyl-D-glucamine). Thereafter, pH recovery was induced by Cl−-free HCO3− solution containing 1 mmol/l amiloride that completely inhibits an endogenous Na+/H+ exchange activity as described (19). In some experiments, Cl−-free HCO3− solution containing both 1 mmol/l amiloride and 0.3 mmol/l 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) was used to confirm that the Na+/H+ exchange activity in this condition is due to NBC-1 activity (8, 19). The calibration curve for pH recovery was made according to the method by Thomas et al. (39). In brief, cells were exposed to HEPES-buffered solution containing 120 mmol/l K+ and 10 μmol/l nigericin. Solution pH was adjusted at the different levels (from 6.4 to 7.8) with 1 N NaOH, whereas Na+ concentration in solution was kept constant at 20 mmol/l as previously reported (28). Throughout the paper, the results are expressed as mean values ± SE. ANOVA with Bonferroni’s adjustment was used for multiple comparisons of data, and a P value of <0.05 was considered statistically significant. BCECF-AM was obtained from Dojindo (Kyushu, Japan), and DIDS, amiloride, and nigericin were from Sigma Chemical (St. Louis, MO). All other chemicals were from Wako Pure Chemicals Industries (Osaka, Japan) unless otherwise specified.

Western blot analysis. Pancreases and kidneys were removed from Wistar rats and immediately homogenized in ice-cold buffer containing 280 mmol/l sucrose and 0.2 mmol/l Pefabloc SC (Boehringer Mannheim, Mannheim, Germany). The plasma membrane fraction was obtained by differential and discontinuous sucrose gradient procedures as described (18). Protein samples were boiled for 5 min in sample buffer, separated by SDS-PAGE on 7% acrylamide minigels, and blotted onto a nitrocellulose membrane. After incubation in blocking buffer, the membrane was treated with the diluted anti-NBC-1 antibodies (1:200) and then with horseradish peroxidase-conjugated anti-rabbit IgG (Bio-Rad, Richmond, CA) as the secondary antibody. The signal was detected by an ECL Plus system (Amersham, Aylesbury, UK).

Two days after transfection, ECV304 cells were lysed and the plasma membrane fraction was obtained. An equal amount of protein samples (50 μg/lane) was processed for Western blot analysis, as described above, using the antibody against NBC-1 COOH-terminal region (18) as the primary antibody.

Reverse transcription-polymerase chain reaction. For reverse transcription-polymerase chain reaction (RT-PCR) analysis, total RNA was isolated from freshly isolated rat kidney and pancreas using the guanidinium isothiocyanate and phenol-chloroform extraction method described by Chomczynski and Sacchi (12). Primers were designed from the rat kNBC-1 sequence (9) as follows: 5'-GAT GTC CAC TGA AAA TGT GGA-3' (sense primer) and 5'-AGC ATG ACA GCC CTG CTC TGA-3' (antisense primer). These primers were set to amplify the kNBC-1 specific region and a part of the NBC-1 common region. The conditions for PCR were as follows: 30 cycles of 30 s at 94°C, 30 s at 60°C, and 45 s at 72°C, with an initial 9-min denaturing step and a final 5-min elongation step.

RESULTS

Western blot analysis. To determine the dominant NBC-1 variant in pancreas, we first performed Western blot analysis. For human pancreas, we could obtain only paraffin-embedded specimens, which were not suitable for Western blot analysis. Therefore, Western blot was performed only on rat pancreas. As shown in Fig. 1, the anti-pNBC-1 antibody recognized a ~145-kDa band on immunoblots of rat pancreas. On the other hand, the anti-kNBC-1 antibody did not recognize a significant band in rat pancreas, although this antibody did recognize a prominent band at ~130 kDa in rat kidney. These results suggest that pNBC-1 is the dominant variant in pancreas. A recent study also reported that the apparent molecular weight of pNBC-1 expressed in pancreas is slightly larger than that of kNBC-1 expressed in kidney (6). The molecular weight of pNBC-1 predicted from its cDNA is ~121 kDa (1), whereas that of kNBC-1 is ~116 kDa (8), suggesting that NBC-1 proteins are posttranslationally modified in slightly different ways in pancreas and kidney. Consistent with this view, the antibody against the NBC-1 COOH-terminal region that recognizes both NBC-1 and pNBC-1 (18) yielded a ~130-kDa band in rat kidney but a ~145-kDa band in rat pancreas (data not shown).

Localization of NBC-1 variants in rat and human pancreas. We next examined localization of NBC-1 variants in pancreas with the immunofluorescence detection method using confocal microscopy. Consistent with the results of Western blot analysis, the expression of pNBC-1 appeared dominant in both rat and human pancreas. However, there was a significant species difference in the patterns of pNBC-1 expression. The anti-pNBC-1 and anti-kNBC-1 antibodies did not label the islet cells in both rat and human pancreas.

In rat pancreas, the anti-pNBC-1 antibody labeled the acinar cells and the duct cells (Fig. 2, a and b). The nonimmunized rabbit IgG gave no fluorescence signals.
Furthermore, the labeling by the anti-pNBC-1 antibody was diminished in the presence of antigen peptide (Fig. 2c), confirming the specificity of immunoreactions. In the acinar cells, the labeling appeared basolaterally dominant (Fig. 2a). In the medium and large ducts, such as the interlobular and the main ducts, the labeling was observed on both apical and basolateral membranes (Fig. 2, a and b), although the apical staining was less intense than the basolateral staining. In the intralobular ducts, the labeling was not so prominent and most of the intercalated ducts were not labeled. The anti-kNBC-1 antibody did not label the acinar cells, although it did label a limited number of the medium and large ducts (Fig. 2d). This occasional ductal labeling by anti-kNBC-1 antibody appeared rather apically dominant and was diminished in the presence of antigen peptide.

In human pancreas, in contrast, the anti-pNBC-1 antibody did not label the acinar cells. Instead, this antibody quite intensively labeled the small intercalated cells (Fig. 3a). Higher magnification (Fig. 3c) and Nomarski-differential interference microscopic image (Fig. 3d) show that this labeling was dominant in the basolateral membranes of small ducts but was not observed in acinar cells. The labeling was diminished in the presence of antigen peptide (Fig. 3d). The anti-
pNBC-1 antibody also labeled the medium and large ducts, such as the intralobular, the interlobular, and the main ducts, and the labeling of these ducts appeared to be confined to the basolateral membranes (Fig. 3e). The labeling by anti-kNBC-1 antibody was not observed in human pancreas.

Detection of kNBC-1 mRNA in rat pancreas. The anti-kNBC-1 antibody did not yield a clear band in Western blot analysis, although it did label a limited number of ducts in rat pancreas. This suggests that a small amount of kNBC-1 mRNA is expressed in rat pancreas. To test for this view, we performed RT-PCR analysis. As shown in Fig. 4, RT-PCR on the kNBC-1 mRNA with (+) or without (−) the reverse transcription (RT) step. K, rat kidney; P, rat pancreas.
specific region yielded a band of the expected size (800 bp) from rat pancreas as well as from rat kidney.

Functional analysis of pNBC-1 mutants. These immunohistological findings indicate that pNBC-1 is the dominant variant in both rat and human pancreas. Because one patient with a NBC-1 missense mutation presented high serum amylase levels, we decided to examine whether disease-causing mutations (19) affect the transport activity of pNBC-1. To accomplish this task, we transiently expressed the wild-type pNBC-1 or the R342S or R554H mutants in ECV304 cells and compared the rates of DIDS-sensitive pHi recovery from acid-load in Cl⁻-free HCO₃⁻ solution (Fig. 6). On the other hand, the cells transfected with the wild-type pNBC-1 were acidified by nigericin, the cells did not show any pHᵢ recovery (Fig. 5A). In this case, readdition of Na⁺/H⁺ solution indeed re induced a modest pHᵢ recovery in both cells transfected with the wild-type pNBC-1 and vector alone. Thus the rates of pHᵢ recovery were 0.002 ± 0.002 pH/min for pNBC-1 (n = 8) and 0.001 ± 0.002 pH/min for vector (n = 8), and both values were not significantly different from zero. In Cl⁻-free HCO₃⁻ solution, readdition of Na⁺ induced a modest pHᵢ recovery in cells transfected with R342S (Fig. 5C) or R554H, which was again completely inhibited by DIDS (n = 6 for each). Figure 6 shows the DIDS-sensitive transport activities thus analyzed at the identical pHᵢ level: 6.86 ± 0.02 for wild-type (n = 22), 6.85 ± 0.03 for R342S (n = 10), and 6.88 ± 0.02 for R554H (n = 11). As shown, the activities of R342S or R554H mutants were significantly reduced (P < 0.005) and were 49.8 ± 3.0% and 48.1 ± 3.3% of the activity of wild-type pNBC-1, respectively. As shown in Fig. 7, the protein expression of pNBC-1 was comparable among the wild-type and mutants. Densitometry analysis of three independent experiments did not reveal significant differences in the level of protein expression.

DISCUSSION

To clarify the relative importance of NBC-1 variants in pancreatic HCO₃⁻ secretion, we performed immunohistological analysis using anti-kNBC-1 and anti-
pNBC-1 antibodies (40). The results clearly demonstrate that pNBC-1 is the dominant variant in HCO₃⁻ secretion in both rat and human pancreas. The anti-kNBC-1 antibody labeled the apical membranes of some ducts cells in rat pancreas, but this labeling was limited to only a small portion of duct cells. Moreover, the anti-kNBC-1 antibody, unlike the anti-pNBC-1 antibody, did not recognize a clear band by Western blot analysis on rat pancreas. We could not obtain the fresh human pancreatic tissues suitable for Western blot analysis, but the labeling by anti-kNBC-1 antibody was not detected in paraffin-embedded human pancreatic tissues. These results might reflect a species-dependent difference in the kNBC-1 expression in pancreas. Consistent with this view, a previous Northern blot analysis detected pNBC-1 mRNA but not kNBC-1 mRNA in human pancreas (1). Nevertheless, our RT-PCR analysis indicated that a small amount of kNBC-1 mRNA is really expressed in rat pancreas.

It should be pointed out that another NBC-1-related isoform (rb2NBC) has been cloned from rat brain (5). This isoform has a unique COOH terminus, but its NH₂ terminus is identical to that of pNBC-1. It could be possible, therefore, that the anti-pNBC-1 antibody used in the present study also recognizes this isoform. However, our preliminary Western blot analysis on rat pancreas using the antibody against the unique COOH-terminal region of rb2NBC failed to yield a clear band, suggesting that pNBC-1 is really the dominant variant in pancreas.

In rat pancreas, the anti-pNBC-1 antibody labeled both acinar cells and duct cells. The labeling in acinar cells was quite intense and appeared rather basolateral dominant. Whereas the antibody labeled both apical and basolateral membranes in the medium and large ducts, most of the small, intercalated ducts were not labeled. In human pancreas, on the other hand, the anti-pNBC-1 antibody did not label the acinar cells, although it did intensively label the basolateral membranes of entire duct system, including the smallest intercalated cells. In view of the high proteolytic activity of acinar cells, we cannot exclude the possibility that a small amount of pNBC-1 is also expressed in human acinar cells but is not detected by the methods employed in the present study. However, the previous immunohistological studies using the antibodies against the common NBC-1 epitopes have also reported a very similar species difference in NBC-1 expression in pancreas. Thus, in rat pancreas, Thévenod et al. (38) have reported that NBC-1 is expressed in the basolateral membranes of acinar cells and in both apical and basolateral membranes of duct cells. In human pancreas, by contrast, Marino et al. (27) have reported that NBC-1 is expressed in the basolateral membranes of duct cells but not in the acinar cells. These results and observations may suggest different fluid and electrolyte secretion mechanisms in rat and human pancreas.

A previous functional study has shown the presence of Na⁺-HCO₃⁻ cotransport activity in rat acinar cells, which has been interpreted to be important for intracellular pH regulation (29). The cotransport activity has also been detected in the basolateral membranes of rat pancreatic ducts (43), but this finding has not been confirmed by others (31). Although Na⁺-HCO₃⁻ cotransport activity has not been investigated in human acinar cells, the transport activity of NBC-1 has been confirmed in the human ductal pancreatic adenocarcinoma cell lines (37). Although the physiological significance of apical NBC-1 expression in rat pancreatic duct cells is not obvious at present, a similar apical NBC-1 expression has also been reported in rat submandibular and parotid glands (33). In any case, the pure basolateral localization of pNBC-1 along the entire duct system of human pancreas might be more advantageous for net HCO₃⁻ secretion, considering the primary role of NBC-1 as HCO₃⁻ uptake into duct cells (22, 37). In this regard, it would be interesting to note that the HCO₃⁻ concentration in secretin-stimulated pancreatic juice is much higher in human than in rat (4).

We have previously shown that missense mutations identified in pRTA patients reduce the transport activity of kNBC-1. The present study confirmed that these mutations also impair pNBC-1 function, which could potentially explain the pancreatic phenotype associated with pRTA (19, 21). We cannot exclude the possibility, however, that these mutations might also affect the targeting and/or recycling of the NBC-1 protein in human tissues. Recently, Shumaker et al. (37) have shown that both CF transmembrane conductance regulator (CFTR) and NBC-1 cooperatively function to accomplish HCO₃⁻ uptake into the human ductal pancreatic adenocarcinoma cell lines. According to their model, activation of CFTR by cAMP depolarizes the membrane potentials, which in turn stimulates the basolateral HCO₃⁻ uptake through the electrogenic Na⁺-HCO₃⁻ cotransporter, NBC-1. The HCO₃⁻ is then secreted at the apical membranes via an apical Cl⁻/HCO₃⁻ exchanger and possibly a bicarbonate conductive pathway. Pancreatic dysfunction associated with CF is thought to result primarily from impairment of secretin-stimulated ducal Cl⁻ and HCO₃⁻ secretion (17, 25). In particular, the reduction in intraductal pH will not only facilitate precipitation of proteins secreted from acinar cells but also disrupt vesicular trafficking in the apical domain of acinar cells, leading ultimately to pancreatic fibrosis and insufficiency (15, 34). Usually, typical CF patients with pancreatic insufficiency tend to have severe CFTR mutations in both alleles, among which delta F508 is most frequent, but other mutations such as G542X are also found (26). However, a subset of patients with idiopathic chronic pancreatitis has only mild CFTR mutations without other clinical features of CF (13, 14, 30). A recent elegant study by Choi et al. (11) suggests that the defect in CFTR-dependent HCO₃⁻ transport, but not the inactivation of CFTR Cl⁻ channel activity itself, may actually be responsible for the pancreatic phenotype in these cases. A study in CFTR knockout mice (16) also supports the view that acidic pH changes in luminal secretions play quite an important role in the pathogenesis of pancre-
atric dysfunction observed in CF. Because only a few NBC-1 mutations have been identified so far (3, 19, 20), a definite conclusion cannot be drawn at present as to a possible link between NBC-1 inactivation and pancreatic dysfunction. In addition, clear evidence of pancreatitis is not yet reported in another pRTA patient with S427L mutation in kNBC-1 (3). Nevertheless, it is tempting to speculate that the inactivation of pNBC-1 may, at least in some patients, decreases ductal HCO₃⁻ secretion similarly as in CF, leading to pancreatic dysfunction. It would be expected, in particular, that mutations in the unique NH₂-terminal region of pNBC-1 would induce a pancreatic phenotype without severe pRTA, because kNBC-1 seems to be responsible for a majority of HCO₃⁻ reabsorption from renal proximal tubules (1, 8). It is currently unknown whether some environmental factors modify the clinical course of pancreatic dysfunction in patients with NBC-1 mutations.

Nonalcoholic chronic pancreatitis is a potentially life-threatening disease (14). Although idiopathic pancreatitis is frequently associated with CFTR mutations, recent studies suggest that other genetic factors may be also involved (13, 30). To better understand the pathogenesis of idiopathic pancreatitis and to establish the more effective therapeutic strategy, future studies are required, including the genetic testing of pNBC-1 mutations.

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