JOP. J. Pancreas (Online) 2001; 2(4 Suppl):237-242.

Impaired Pancreatic Ductal Bicarbonate Secretion in Cystic Fibrosis

Manoocher Soleimani

Department of Medicine, University of Cincinnati, and Veterans Affairs Medical Center at Cincinnati. Cincinnati, OH, USA

Summary

Patients with cystic fibrosis demonstrate a defect in HCO_3^- secretion by their pancreatic duct cells. However, attempts toward understanding or correcting this defect have been hampered by a lack of knowledge regarding the cellular and molecular mechanisms mediating HCO_3^- transport in these cells. Recent functional and molecular studies indicate a major role for a basolateral electrogenically-driven Na^+:HCO_3^- cotransporter (NBC1) in mediating the transport of HCO_3^- into the duct cells. The HCO_3^- exits at the lumen predominantly via two recently discovered apical HCO_3^- transporters. cAMP, which mediates the stimulatory effect of secretin on pancreatic ductal HCO_3^- secretion, potentiates the basolateral Na^+:HCO_3^- cotransporter due to generation of a favorable electrogenic gradient as a result of membrane depolarization by Cl^--secreting cystic fibrosis transmembrane conductance regulator (CFTR). Two apical HCO_3^- transporters drive the secretion of bicarbonate into the pancreatic duct lumen. Molecular and functional studies indicate that CFTR upregulates the expression of these two apical HCO_3^- transporters. In addition, CFTR may also upregulate the expression of certain water channels and facilitate the secretion of fluid into the duct lumen. In brief, current research suggests that the defect in pancreatic HCO_3^- secretion in patients with cystic fibrosis is multifactorial and involves the alteration in the function/expression of transporters at the basolateral and luminal membrane domains of the duct cells.

The excretory duct system of the pancreas serves as a conduit for delivery of an alkaline, bicarbonate rich fluid to the duodenum [1, 2]. This HCO_3^- rich fluid is secreted in response to the release of secretin by S cells in proximal duodenum in response to the acidic chyme emptying from the stomach [1, 2, 3], with subsequent delivery to the pancreas (via blood) where it stimulates HCO_3^- secretion. Studies examining the mechanism(s) of HCO_3^- transport in pancreatic ducts demonstrate that stimulation of the pancreas with secretin increases both the volume and the HCO_3^- concentration of pancreatic juice [1, 2, 3]. Given the fact that more than 90% of the HCO_3^- in the pancreatic juice is derived from the plasma [1, 2], it becomes evident that specialized and high capacity acid-base transporters are responsible for active HCO_3^- secretion into the pancreatic lumen.

Cystic fibrosis (CF) which is an autosomal recessive disease, and results from mutational inactivation of a cAMP-sensitive Cl^- channel, manifests itself with impairments in the respiratory, pancreatic, hepatobiliary, and genitourinary systems. The pancreatic

dysfunction is felt to result primarily from impairment of secretin-stimulated ductal Cl^- and HCO_3^- secretion [4, 5]. Based on histopathologic evidence, it has been postulated that the reduction in secretin-stimulated HCO_3^- secretion from pancreatic duct epithelial cells alters intraductal pH sufficiently to precipitate proteins secreted from acinar cells. This should result in protein plugs and the disruption of vesicular trafficking in the acinar cell's apical domain [5]. These alterations would lead to pancreatic fibrosis and insufficiency in majority of CF patients [5].

The pathways mediating the uptake and secretion of bicarbonate in pancreatic duct cells have been examined by several investigators. Further, attempts have been made to identify the mechanisms of decreased ductal bicarbonate secretion in CF patients. In this studies, we examined the molecular and functional regulation of apical Cl^-/HCO_3^- exchangers by CFTR in cultured pancreatic duct epithelial cells exhibiting physiologic features prototypical of cystic fibrosis (CFPAC-1 cells, lacking a functional CFTR) or normal duct cells (cystic fibrosis pancreatic adenocarcinoma (CFPAC)-1 cells transfected with functional wild type CFTR, termed CFPAC-WT).

Results

In the first series of experiments, total Cl^-/HCO_3^- exchange activity (apical and basolateral) was assayed in cultured CFPAC-1 and CFPAC-WT cells grown on coverslips. The summary of 6 separate experiments demonstrated that the 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid-(DIDS)-sensitive Cl^-/HCO_3^- exchange is increased (by 220%) in cells transfected with functional CFTR (CFPAC-WT). The Cl^-/HCO_3^- exchange activity was inhibited by about 90% in either cell line in the presence of 500 µM DIDS.

To determine the contribution of apical Cl^-/HCO_3^- exchange activity to total exchange activity measured in cells on coverslips, DIDS-sensitive $^{36}Cl^-$ influx across the luminal surface of the cells grown on permeable support was measured. The results indicated that the DIDS-sensitive, luminal $^{36}Cl^-$ influx was increased by about 400% in cells transfected with functional CFTR ($P<0.01$ *vs.* CFPAC-1 cells).

In search of the identity of the upregulated apical chloride/base exchanger in CFPAC-WT cells we examined the expression of DRA in pancreatic duct cells. Northern hybridizations indicated that down-regulated in adenoma (DRA) mRNA was expressed in functional CFTR-bearing duct epithelial cells, but could not be detected in CFPAC-1 cells expressing a mutant CFTR.

To determine whether DRA is expressed in native pancreatic tissue, Poly (A+) RNA from mouse pancreas (Clonetech; Palo Alto, CA, USA) was used for Northern hybridization. The results demonstrated that mouse pancreas expresses high levels of DRA mRNA. Next we examined the localization of DRA in mouse pancreas by immunohistochemical staining. The DRA immune serum strongly labelled the apical membrane of the duct cells. In addition to the apical labelling, cytoplasmic staining was also detected in the pancreatic duct cells. No staining was detected in acinar cells. Staining with a non immune serum did not show any labelling in the duct cells.

Recent cloning experiments have identified a new transporter with high homology to DRA and Pendrin [6]. This transporter is named putative anion transporter (PAT1 or SLC26A6) and is expressed in pancreas and kidney [6]. Immunocytochemical studies localized PAT1 to the apical membranes of the pancreatic duct cells [6]. Northern hybridization studies in our laboratory indicated that the expression of PAT1 is enhanced by about 5 fold in CF duct cells transfected with functional CFTR. Enhanced expression of PAT1 was associated

with increased protein abundance (data not shown).

Discussion

The currently accepted model of pancreatic ductal HCO_3^- secretion suggests that the intracellular HCO_3^- is accumulated in response to the action of cytosolic carbonic anhydrase on the CO_2 that diffuses from the basolateral membrane. The G protein-coupled receptors (e.g. secretin, vasoactive intestinal peptide) activate cAMP-sensitive CFTR which secrets Cl^- into the lumen. The resultant increases in luminal Cl^- then drives an apical Cl^-/HCO_3^- exchanger (reviewed in [1, 2, 7]). However, recent reports in mammalians indicate that HCO_3^- uptake at the basolateral membrane of pancreatic duct cells is Na^+-dependent [8] and mediated via Na^+:$nHCO_3^-$ cotransporter [9].

Molecular cloning studies have identified several NBC isoforms (reviewed in [10]). We have examined the expression of NBC isoforms in cultured pancreatic duct epithelium, in order to achieve a better insight into the mechanism of pancreatic HCO_3^- secretion defect in cystic fibrosis. The results demonstrate the expression of three NBC isoforms (NBC1, NBC2 and NBC3) in cultured duct cells. Functional studies indicate the localization of NBC isoforms to the basolateral membrane. Given the intra and extracellular Na and HCO_3^- concentrations and the membrane potential measurements across the basolateral membrane it is presumed that all NBC isoforms work in the influx mode and transport bicarbonate to the duct cell.

Apical bicarbonate transporters are responsible for the secretion of bicarbonate into the duct lumen [1, 2]. However, the identity of apical bicarbonate transporters in the pancreatic duct remains unknown. Several studies suggest that a Cl^-/HCO_3^- exchanger and or a bicarbonate conductive pathway likely mediate bicarbonate secretion. A new family of anion exchangers has been recently identified. Several members of this family are down-regulated in adenoma (DRA), Pendrin, and PAT1 or SLC26A6 [6, 11, 12]. DRA, which was originally cloned from colon and found to mediate sulfate, oxalate and chloride transport in Xenopus oocytes [11, 13], was recently shown to be a Cl^-/HCO_3^- exchanger [14]. Coupled to immunolocalization studies, it has been proposed that DRA is an apical anion exchanger in the colon. We have examined the expression of DRA in cultured pancreatic duct epithelial cells exhibiting physiologic features prototypical of cystic fibrosis (CFPAC-1 cells, lacking a functional CFTR) or CF duct cells transfected with functional wild type CFTR (CFPAC-WT). These studies indicated that DRA is heavily upregulated by functional CFTR.

Very recently a new protein with high homology to DRA was cloned [6]. This transporter which is named putative anion transporter (PAT-1) is expressed in pancreas and kidney [6]. Immunocytochemical studies localized PAT1 to the apical domain of the pancreatic duct cells [6]. We have examined the expression of PAT-1 in cultured pancreatic duct cells. The results indicate that the expression of PAT-1 is significantly increased in cells transfected with functional CFTR. Studies are currently underway to examine the functional identity of PAT1. Pendrin and AE4, the other known apical Cl^-/HCO_3^- exchangers, are not expressed in cultured pancreatic duct cells.

Recent functional and molecular studies indicate the complexity of pancreatic ductal HCO_3^- secretion defect in patients with cystic fibrosis. Based on the current studies and the available literature, we propose that HCO_3^- secretion defect in CF duct cells may involve transporters in both basolateral and apical membrane domains. In cystic fibrosis, the basolateral NBC remains inactive due to the lack of membrane depolarization (which normally results from CFTR activation). Further, the exit of bicarbonate across the apical membrane is decreased due to the downregulation of apical DRA and PAT1.

Methods and Materials

Cell lines CFPAC-1 cells, a pancreatic duct cell line derived from a patient with cystic fibrosis and bearing a delta F 508 mutation, were cultured as previously described [9, 15]. Stably transfected CFPAC-1 cells bearing functional CFTR (termed CFPAC-WT) were generous gifts from Dr. Raymond Frizzell and were cultured in a similar fashion excepting the addition of G418 (1 mg/mL) to the medium [15].

Cell pH measurement Changes in intracellular pH (pH_i) were monitored using the acetoxymethyl ester of the pH-sensitive fluorescent dye 2'7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) in cells grown on glass coverslip, as described [16, 17]. The monolayer was then perfused with the appropriate solutions in a thermostatically controlled holding chamber (37 °C) in a Delta Scan dual excitation spectrofluoremeter (PTI, South Brunswick, NJ).

RNA isolation and Northern Blot hybridization Total cellular RNA was extracted from CFPAC-1 and CFPAC-WT cells according to the established methods [18], quantitated spectrophotometrically, and stored at –80 °C. Hybridization was performed according to Church and Gilbert [19], using [^{32}P] dCTP-(NEN. Boston, MA) labelled cDNA probes.

Immunocytochemistry of DRA in mouse pancreas Pancreas from normal mice were cut into slices and mounted on holders to form tissue blocks. The tissues were fixed in a solution containing 0.1% glutaraldehyde plus 2% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.20, and stored in 0.1 M cacodylate buffer, pH 7.20 at 4 °C. For immunohistochemistry, the tissue blocks were sectioned into 5 µm section. A DRA specific antibody [20] was applied to the slides in 1/100 dilution in PBS + 1% BSA and the presence of saponin, and incubated in a humidified chamber for 2 hours at room temperature. The peroxidase-anti-peroxidase (PAP) conjugate diluted in 1:100 in PBS + 1% BSA was applied to the slides.

PAT-1 antibody generation and immunoblot analysis Polyclonal antibodies were raised in 2 rabbits against mouse PAT1 using a synthetic peptide with amino acid sequence MDLRRRDYHMERPLLNQEHL. The preimmune and immune sera of the 3rd bleed were purified by IgG purification kit (Sigma Co., St. Louis, USA) and used for immunoblot analysis. Microsomes from cultured CFPAC-1 and CFPAC-WT cells were prepared and resolved by SDS-PAGE (30 µg/lane) and transferred to nitrocellulose membrane. The membrane was blocked with 5% milk proteins, and then incubated for 6 hrs with 40 µL of putative anion transporter 1 (PAT1) immune serum diluted at 1:400. The secondary antibody was a donkey anti-rabbit IgG conjugated to horseradish peroxidase (Pierce Chemical, Rockford, IL, USA). The site of antigen-antibody complexation on the nitrocellulose membranes was visualized using chemiluminescence method (SuperSignal Substrate, Pierce Chemical, Rockford, IL, USA) and captured on light sensitive imaging film (Kodak, Rochester, NY, USA).

Statistical analyses Values are expressed as mean±SEM. The significance of difference between mean values were examined using ANOVA. P value less than 0.05 was considered statistically significant.

Key words Bicarbonates; Carrier Proteins; Cystic Fibrosis; Ion Transport; Pancreas

Abbreviations BCECF: 2'7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein; CF: cystic fibrosis; CFPAC: cystic fibrosis pancreatic adenocarcinoma; CFTR: cystic

fibrosis transmembrane conductance regulator; DIDS: 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid; DRA: down-regulated in adenoma; NBC: Na^+:HCO_3^- cotransporter; PAP: peroxidase-anti-peroxidase; PAT: putative anion transporter; PDS: pendred syndrome gene; pH_i: intracellular pH

Acknowledgment These studies were supported by the National Institute of Health Grant RO1 DK54430, a Merit Review grant from the Department of Veterans Affairs, a Cystic Fibrosis Foundation Grant and a grant from Dialysis Clinic Incorporated.

Correspondence
Manoocher Soleimani
Department of Medicine
University of Cincinnati
231 Albert Sabin Way
MSB 5502, Cincinnati, Ohio 45267-0585
USA
Phone: +1-513.558.5463
Fax: +1-513.558.4309
E-mail address: manoocher.soleimani@uc.edu

References

1. Argent BE, Case RM. Pancreatic ducts: cellular mechanism and control of HCO_3^- secretion In: Johnson LR, ed. Physiology of the Gastrointestinal Tract. 3rd ed. New York, NY, USA: Raven Press, 1994: 1473-97.

2. Case RM, Argent BE. Pancreatic duct cell secretion. Control and mechanisms of transport. In: Go, VLW., DiMagno, E. R., Gardner, J. D., Lebenthal, F. P., Reber, H. A., Scheele, G. A. eds. The Pancreas: Biology, Pathobiology, and Disease. 2nd ed. New York, NY, USA: Raven Press, 1993: 301-50.

3. Lebenthal E, Lerner A, Rolston DK. The pancreas in cystic fibrosis. In: Go VLW, DiMagno ER, Gardner J D, Lebenthal F P, Reber HA, Scheele G, eds. The Pancreas: Biology, Pathobiology, and Disease. 2nd ed. New York, NY, USA: Raven Press, 1993: 1041-81.

4. Rosenstein BJ, Zeitlin PL. Cystic fibrosis. Lancet 1998; 351:277-82. [98118296]

5. Scheele GA, Fukuoka SI, Kern HF, Freedman SD. Pancreatic dysfunction in cystic fibrosis occurs as a result of impairments in luminal pH, apical trafficking of zymogen granule membranes, and solubilization of secretory enzymes. Pancreas 1996; 12:1-9. [96402899]

6. Lohi H, Kujala M, Kerkela E, Saarialho-Kere U, Kestila M, Kere J. Mapping of five new putative anion transporter genes in human and characterization of SLC26A6, a candidate gene for pancreatic anion exchanger. Genomics 2000; 70:102-12. [20541715]

7. Sohma Y, Gray MA, Imai Y, Argent BE. A mathematical model of the pancreatic ductal epithelium.J Membr Biol 1996; 154:53-67. [97045048]

8. Ishiguro H, Steward MC, Lindsay AR, Case RM. Accumulation of intracellular HCO3- by Na^+-HCO_3^- cotransport in interlobular ducts from guinea-pig pancreas. J Physiol 1996; 495:169-78. [97019905]

9. Shumaker H, Amlal H, Frizzell R, Ulrich CD 2nd, Soleimani M. CFTR drives Na^+-$nHCO_3^-$ cotransport in pancreatic duct cells: a basis for defective HCO_3^- secretion in CF. Am J Physiol 1999; 276:C16-25. [99103866]

10. Soleimani M, Burnham CE. Physiologic and molecular aspects of the Na^+-HCO_3^- cotransporter in health and disease processes. Kidney Int 2000; 57:371-84. [20117945]

11. Schweinfest CW, Henderson KW, Suster S, Kondoh N, Papas TS. Identification of a colon mucosa gene that is down-regulated in colon adenomas and adenocarcinomas. Proc Natl Acad Sci USA 1993; 90:4166-70. [93248250]

12. Everett LA, Glaser B, Beck JC, Idol JR, Buchs A, Heyman M, et al. Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). Nat Genet 1997; 7:411-22. [98061089]

13. Hoglund P, Haila S, Socha J, Tomaszewski L, Saarialho-Kere U, Karjalainen-Lindsberg ML, et al. Mutations of the Down-regulated in adenoma (DRA) gene cause congenital chloride diarrhoea. Nat Genet 1996; 14:316-9. [97051927]

14. Melvin JE, Park K, Richardson L, Schultheis PJ, Shull GE. Mouse down-regulated in adenoma (DRA) is an intestinal Cl^-/HCO_3^- exchanger and is up-regulated in colon of mice lacking the NHE3 Na^+/H^+ exchanger. J Biol Chem 1999; 274:22855-61. [99357827]

15. Schoumacher RA, Ram J, Iannuzzi MC, Bradbury NA, Wallace RW, Hon CT, et al. A cystic fibrosis pancreatic adenocarcinoma cell line. Proc Natl Acad Sci USA 1990; 87:4012-6. [90251687]

16. Amlal H, Wang Z, Soleimani M. Functional upregulation of H^+-ATPase by lethal acid stress in cultured inner medullary collecting duct cells. Am J Physiol 1997; 273:C1194-205. [98018795]

17. Amlal H, Wang Z, Burnham C, Soleimani M. Functional characterization of a cloned human kidney Na^+:HCO_3^- cotransporter. J Biol Chem 1998; 273:16810-5. [98307915]

18. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162:156-9. [87268451]

19. Church GM, Gilbert W. Genomic sequencing. Proc Natl Acad Sci USA 1984; 81:1991-5. [84193941]

20. Rajendran VM, Black J, Ardito TA, Sangan P, Alper SL, Schweinfest C, et al. Regulation of DRA and AE1 in rat colon by dietary Na depletion. Am J Physiol Gastrointest Liver Physiol 2000; 279:G931-42. [20508345]