Review

Bicarbonate in cystic fibrosis

Karl Kunzelmann a,⁎, Rainer Schreiber a, Hans Beat Hadorn b

a Physiological Institute, University of Regensburg, University Street 31, 93053, Germany
b Professor Emeritus, Department of Pediatrics, Dr. V. Hauner Childrens Hospital, Ludwig-Maximilian-University, Munich, Germany

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Abstract

Background: Cystic fibrosis (CF, mucoviscidosis) is caused by mutations in the gene encoding CF transmembrane conductance regulator (CFTR), which is a chloride and bicarbonate channel necessary for fluid secretion and extracellular alkalization. For a long time, research concentrated on abnormal Cl− and Na+ transport, but neglected bicarbonate as a crucial factor in CF.

Methods: The present short review reports early findings as well as recent insights into the role of CFTR for bicarbonate transport and its defects in CF.

Results: The available data indicate impaired bicarbonate transport not only in pancreas, intestine, airways, and reproductive organs, but also in salivary glands, sweat duct and renal tubular epithelial cells. Defective bicarbonate transport is closely related to the impaired mucus properties and mucus blocking in secretory organs of CF patients, causing the life threatening lung disease.

Conclusions: Apart from the devastating lung disease, abrogated bicarbonate transport also leads to many other organ dysfunctions, which are outlined in the present review.

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Keywords: Mucoviscidosis; Cystic fibrosis; Bicarbonate; Mucus; Cystic fibrosis transmembrane conductance regulator; CFTR

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1. Mucoviscidosis, a historical perspective

Clinical, biochemical and physiological findings on mucoviscidosis (cystic fibrosis) were obtained long before the discovery of the disease causing gene cystic fibrosis transmembrane conductance regulator (CFTR) in 1989 [1–4]. In 1936 the
Swiss pediatricians Fanconi and his colleagues described the "coeliac syndrome", with congenital fibrosis of the pancreas and lung bronchiectasis [5]. This was followed by a more precise description of the disease, coined the term cystic fibrosis [6]. In a cooperative study, the clinician Harry Shwachman and pathologist Sydney Farber described the abnormally high viscosity of intestinal contents which they called mucoviscidosis [7].

Di Sant Agnese’s description of sweat abnormalities in cystic fibrosis (CF) in 1953 pointed to a membrane transport defect for anions, which they confirmed later in technically more sophisticated experiments [8]. It took another 15 years for a better characterization of the transport defect in sweat glands by micropuncture of single human glands. Unfortunately, these results were not published in common scientific journals and therefore received little attention [9,10]. In the same year, deficient pancreatic secretion upon stimulation with secretin was observed by Johansen, Hadorn and Anderson, defining a primary generalized disturbance of fluid and bicarbonate secretion in CF [10]. Paul Quinton’s groundbreaking work on the sweat duct secretion had not been further studied until Quinton and others [14,15] finally brought the anion transport defect to everybody’s attention. Its importance was recognized early by Shwachman and colleagues, but it took another 15 years until Rick detected strongly compromised single channel chloride currents in cells from CF patients, although with some confusion regarding the biophysical properties of the channel [15–18]. These impressive scientific achievements were crowned by identification of CFTR as a CAMP regulated chloride channel, which unleashed a powerful research activity that lasts until today [2–4].

Yet it was still unclear how dysfunctional CFTR leads to abnormal mucus in lungs, intestine, and reproductive organs of CF patients. CFTR was shown to inhibit epithelial Na⁺ channels (ENaC) ex vivo and to decrease transport by electroneutral Na⁺/H⁺-exchangers (NHE3) in airways and intestine, which explained enhanced Na⁺ absorption in the absence of functional CFTR [19,20]. Hyperabsorption of NaCl with thinning of the airway surface liquid (ASL) layer due to hyperactive epithelial Na⁺ channels (ENaC) was blamed as the cause of dehydrated mucus with poor rheological properties [21–24]. However, there was no evidence for Na⁺ hyperabsorption in CF sweat ducts or pancreas, while other findings argued against Na⁺ hyperabsorption in CF airways and found enhanced salt content in CF ASL [25–27]. Moreover chronic inflammation, either intrinsic or due to infection, was discussed as major cause for mucus hypersecretion [28–32]. In contrast a possible impact of defective bicarbonate secretion had not been further studied until Quinton and others provided evidence that bicarbonate was essential for proper mucus release and viscosity [33,34].

2. Exocrine pancreas

Altered viscosity of the duodenal content in mucoviscidosis had been recognized early by Shwachman and colleagues, but it took another 15 years until Rick detected strongly compromised pancreatic bicarbonate secretion during stimulation with pancreozymin-secretin [31,35]. We adapted the test to measure pancreatic output of enzymes and bicarbonate upon hormonal stimulation in children, and applied the test to study the output of enzymes, volume and bicarbonate in adolescent CF patients with residual exocrine function [36]. Typical results from a CF patient and a healthy volunteer are shown in Fig. 1. In addition to a severe volume reduction, the concentration of bicarbonate was extremely low in the pancreatic juice, while the chloride concentration remained high. Moreover, the apparent viscosity was high in the patient but decreased rapidly even with small amounts of bicarbonate being excreted following stimulation. Fig. 2 demonstrates the discrepancy between chymotrypsin and bicarbonate in patients with residual function [36,37]. Due to the small volume of the pancreatic juice, enzymes were found to be very concentrated. These results were confirmed in subsequent studies [38–40].

After identification of CFTR and invention of the patch clamp technique, secretin, i.e. CAMP-regulated chloride channels were identified in epithelial cells of normal pancreatic ducts that were absent in cells lacking expression of functional CFTR [41,42]. Subsequent studies recognized the importance of functional CFTR in driving basolateral HCO₃⁻ uptake and luminal HCO₃⁻ secretion in the pancreatic duct [43–45] (Fig. 8A). Bicarbonate accumulates in the distal pancreatic duct at very high concentrations, which is due to the high rate of uptake by basolateral transporters and luminal exit through CFTR. After identifying pancreatic HCO₃⁻ transport through CFTR in the groundbreaking work by Muallem and coworkers, abnormal HCO₃⁻ transport in CF finally received the well-deserved attention [33,34]. CFTR
was shown to transport HCO$_3^-$ and to control the activity of other HCO$_3^-$ transporters in the pancreatic duct, such as SLC26A6, thereby achieving large luminal HCO$_3^-$ concentrations [46–52]. After these initial findings subsequent work further analyzed in detail the complex intracellular regulation and coordination of fluid and bicarbonate transport which requires signaling molecules such as SPAK, WNK, and IRBIT [52–54].

3. Salivary glands

After discovering bicarbonate secretion by salivary glands through the work of Yoshimura and coworkers, Kaiser found that saliva pH was lower in CF patients at maximal flow rates, suggesting a defect in bicarbonate secretion [55,56] (Fig. 3). Bicarbonate secretion in salivary ducts is similar to that in the pancreatic duct and requires coordinated action of different anion exchangers and CFTR [57–61] (Fig. 8B). Similar to the pancreatic duct, CFTR dependent regulation of luminal anion exchangers was also found in mouse submandibular ducts [62,63]. However, although similar transporters are used for bicarbonate transport in salivary gland and pancreas, the rate of bicarbonate transport in the pancreatic duct is probably much higher, which explains the milder pH differences between CF and non-CF saliva compared to the large pH differences of the pancreatic juice. Moreover, salivary ducts express apical Na$^+/\text{HCO}_3^-$ transporters which keep luminal HCO$_3^-$ lower, particularly under resting conditions [64]. A strongly reduced saliva pH due to absent bicarbonate secretion was reported in mice carrying the common F508del-CFTR mutation, leading to severe dental caries and contributing to change in tooth color [65].

4. Sweat gland

Abnormal ion composition in the sweat of CF patients had already been described by Di Sant’Agnese [8]. Moreover, early micropuncture studies on sweat glands in vivo by Schulz and Frömter suggested a defect in epithelial ion transport. This study, however, remained largely unrecognized since it was published in German [8,9]. A subsequent paper by Kaiser and Drack
described enhanced HCO$_3^-$ excretion in CF sweat [66]. Unluckily this information was masked due to a mistake in the title, reporting “diminished” instead of “enhanced” HCO$_3^-$ excretion (Fig. 4). Enhanced HCO$_3^-$ excretion by CF sweat glands was not detected in a subsequent study by Bijman and Quinton, possibly due to different experimental techniques [67]. The groundbreaking work by Quinton and a subsequent paper by Bijman and Frömter finally established the Cl$^-$ transport defect in CF sweat ducts [12,68]. In a later study, Reddy and Quinton showed that HCO$_3^-$ transport in the sweat duct requires CFTR and was therefore absent in ducts expressing F508del-CFTR [69] (Fig. 8C).

5. Intestine

Many of the studies that looked into the role of CFTR for intestinal transport were performed in HCO$_3^-$ free Ringer solution, thus demonstrating the role of CFTR for cAMP-dependent chloride secretion but overlooking its impact on bicarbonate transport [70]. Johansen and coworkers found a pronounced discharge of mucus from normal human rectum upon stimulation with secretin, which was attenuated in CF patients [71] (Fig. 5). These results provided hints to a better understanding of the pathogenesis of the intestinal obstruction in CF like meconium ileus and distal intestinal obstructive syndrome (DIOS). Subsequent work demonstrated the role of CFTR for intestinal HCO$_3^-$ secretion activated by intracellular cAMP, cGMP, or calcium [72]. CFTR is required to drive electroneutral HCO$_3^-$ secretion by anion exchangers, while a fraction will be transported to the apical surface by CFTR [51,73,74] (Fig. 8D, E). Reduced intestinal HCO$_3^-$ secretion was directly demonstrated in tissues of CF patients [75]. Moreover, the work by the teams of Quinton and Hansson showed that CFTR-dependent HCO$_3^-$ secretion is essential for normal intestinal mucus release [76–78]. Quinton proposed that HCO$_3^-$ is crucial to proper mucin unfolding and expansion, which in turn is essential to maintain its normal rheological properties to avoid adhesion to the mucosal surface [79].

6. Reproductive organs

Cervical mucus abnormalities, azoospermia, and congenital bilateral absence of the vas deferens are the reasons for the high rate (95%) of male infertility and reduced fertility in females with CF [80,81]. cAMP-regulated anion currents are vital to spermatogenesis and sperm fertilizing capacity and were shown to be absent in epididymis, Sertoli cells and seminal vesicles of CF mice [82,83]. In the female reproductive tract sperms undergo activation, a process called capacitation. It was shown that capacitation requires sperm CFTR to support directly or indirectly the uptake of extracellular HCO$_3^-$ [84]. HCO$_3^-$ is secreted by uterus endometrium and the oviduct epithelium to provide extracellular HCO$_3^-$ for sperm capacitation [85,86]. Moreover, HCO$_3^-$ uptake by CFTR may be also required during early embryonic development [87] (Fig. 8G). The abnormal cervical mucus in CF was shown to be caused by a lack of HCO$_3^-$ secretion [88]. Through this study and subsequent reports, it became clear that bicarbonate is essential for normal mucus secretion and viscoelastic properties, which are determined not only by mucus hydration [89]. After all, defective HCO$_3^-$ transport appears as the major reason for infertility in CF.

7. Airways

Inflammation, remodeling, and mucus accumulation are three features that characterize airways of CF patients. While disruption of anion secretion is a common feature of epithelial
organs affected by CF, most patients succumb to the pulmonary complications of the disease. CF airway epithelia were also shown to absorb airway surface liquid at abnormally high rates at least in ex vivo planar and cylindrical airway cell culture models. This was explained by an enhanced Na⁺ transport which depletes the periciliary liquid layer thereby impairing mucus transport [90]. Enhanced fluid absorption, however, was not detected in newborn porcine CF airway epithelia [91]. Further studies should examine if enhanced Na⁺ and fluid absorption in CF develops over time. This may be due to the lack of HCO₃⁻ secretion and acidification of the ASL, which may affect pH sensitive regulators of ENaC like SPLUNC1 [92].

Smith and Welsh reported stimulation of bicarbonate secretion by cAMP in normal airway epithelia which was absent in CF [93], while Poulsen and coworkers demonstrated a permeability by cAMP in normal airway epithelia which was absent in CF of CFTR for HCO₃⁻ [51]. Inhibition of fluid/HCO₃⁻ secretion in CF airways was shown to have adverse effects on the physical properties of airway mucus [94]. Expression of CFTR was also detected in mucin granules from airway epithelial cells, which may play a role for the mucus phenotype found in CF [95]. Small airways secrete mucus and also express highest levels of CFTR, which drives electrolyte secretion and alkalization of the ASL required for antimicrobial defense and maintaining low mucus viscosity [96,97]. Airway epithelial cells are equipped with basolateral Na⁺ coupled HCO₃⁻ transporters (NBC) to take up HCO₃⁻ for apical release through SLC26A4 (pendrin). As in other tissues, CFTR serves as a Cl⁻ recycling pathway to drive HCO₃⁻ secretion through pendrin, and properly secretes HCO₃⁻ directly. Airway epithelial cells are able to switch between a CF- and a HCO₃⁻ secretory mode [98,99], which was also found for the sweat duct epithelium [69,100] (Fig. 8C,F). In a remarkable series of excellent studies the team by Michael Welsh reproduced human CF pathology in a CF pig model [91,101]. These results were supported by an independent CF pig model [102]. CF pigs demonstrate reduced airway surface pH, impaired bacterial killing and adhesive mucus that disrupts mucociliary transport [103–106]. Noteworthy, Hoegger et al. showed abnormal mucociliary transport in CF under submerged conditions, questioning the role of surface dehydration [106]. A number of additional reports by the teams of Ballard, Inglis, Wine and others supported the role of Cl⁻ and HCO₃⁻ transport for proper mucociliary clearance [107–111]. These and other studies unmasked a tight correlation between the level of CFTR, HCO₃⁻ secretion and host defense [112].

8. Kidney

CFTR is well expressed in the kidney, mainly in proximal tubules and in the collecting duct (Fig. 6). Lack of CFTR function in human and mouse CF kidneys was shown to cause low molecular weight proteinuria and possibly enhanced Na⁺ absorption under salt restriction [114–116]. While primary renal disease is an unusual feature in CF, secondary renal dysfunctions may cause problems in the near future, due to the ever-increasing life expectancy of CF patients [117]. Despite the above findings, the role of CFTR in proximal and distal tubules remains largely elusive. Notably, Oetliker and coworkers found that the gastrointestinal hormone secretin induces loss of bicarbonate in the normal kidney [118]. Secretin receptors have been shown to be expressed all along the renal tubules [119]. A bicarbonate titration method was used that simultaneously infuses small amounts of bicarbonate along with secretin, to continuously measure urinary bicarbonate excretion at different serum bicarbonate levels, thus permitting an assessment of the reabsorptive capacity [120]. Renal HCO₃⁻ excretion was induced in healthy volunteers upon application of secretin and elevation of serum HCO₃⁻, while no response to secretin was found in CF patients [120] (Fig. 7). The data suggest a role of CFTR for renal tubular HCO₃⁻ transport. Unfortunately, these results were only reported as an abstract, while a later study used secretin without simultaneous infusion of bicarbonate and thus did not report a

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<td>E_{HCO₃⁻}</td>
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<td>E_{H⁺}</td>
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<td>7.6 ± 7.5</td>
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<td>F_{Na⁺}</td>
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<td>2.3 ± 0.6</td>
<td>&lt;0.02</td>
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Fig. 6. Renal expression of CFTR. Immunoperoxidase labeling of CFTR in mouse kidney in proximal and distal tubules, using a polyclonal anti-mouse CFTR antibody and a peroxidase coupled secondary antibody. Note the dark precipitations within the brush boarder and along the apical membrane. No precipitation was detected in the absence of primary antibody. Bar indicates 30 μm.

Fig. 7. Urinary excretion of bicarbonate after injection of secretin in patients with CF and in non-CF controls. Eight CF patients and 5 non-CF controls were treated with intravenous application of secretin. 200 units of secretin induced changes in urinary excretion of HCO₃⁻ (in μmol/l per 100 ml glomerular filtration), changes in fractional Na⁺ excretion (in %) and changes in excretion of titratable acid (in μmol/l per 100 ml glomerular filtration). Both renal excretion of HCO₃⁻ and Na⁺ were significantly reduced in patients diagnosed with CF.
difference between CF and controls [119]. It is likely that the mild defect in renal HCO₃⁻ excretion in CF is only detected under HCO₃⁻ challenge and/or metabolic alkalosis and therefore remains clinically silent. Thus, subtle attenuation in renal bicarbonate excretion in CF patients may manifest only when the transport capacity for bicarbonate is challenged (Fig. 8H, I).

Fig. 8. Role of CFTR for bicarbonate transport in epithelial tissues. A, B) Acinar cells from pancreas (A) and salivary glands (B) secrete Cl⁻ [125,126] and possibly HCO₃⁻ [127] via ANO1 (TMEM16A) anion channels. CT is exchanged by HCO₃⁻ via SLC26A3 or SLC26A6, with CFTR serving as a CT recycling channel to drive HCO₃⁻ secretion via SLC proteins. High levels of luminal HCO₃⁻ are achieved by direct HCO₃⁻ secretion via CFTR. The contribution of luminal ANO1 channels to HCO₃⁻ secretion in the ducts remains to be determined. Basolateral Na⁺/HCO₃⁻ cotransporter (NBC) take up HCO₃⁻ from the basolateral side. The Na⁺/K⁺-ATPase is required to drive ion transport in all cell types. C) Primary sweat produced by the secretory coil is diluted by reabsorption of NaCl via the epithelial Na⁺ channel (ENaC) and CFTR, which probably also reabsors HCO₃⁻. D, E) Small intestinal (D) and large intestinal (E) epithelial cells engage a number of HCO₃⁻ transporters to secrete HCO₃⁻ to the luminal side. CFTR recycles Cl⁻ and directly secretes HCO₃⁻. DRA, downregulated in adenoma; SCFA, short chain fatty acids. Slc26a9 can function in three distinct modes: as electrogenic Cl⁻/HCO₃⁻ exchanger, as chloride channel, and as Na⁺/Cl⁻ cotransporter. ANO1 is expressed in the basolateral membrane of adult murine but not human colon. F) In airway epithelial cells HCO₃⁻ is secreted to the luminal side via SLC26A4 (pendrin), while the H⁺/K⁺-ATPase acidifies the ASL. CFTR recycles Cl⁻ over the apical membrane and is able to secrete HCO₃⁻ directly. AE2, anion exchanger type 2. G) Epithelial cells of the uterine endometrium secrete HCO₃⁻ via anion exchangers and CFTR. In sperm cells, Sertoli cells and developing embryos, HCO₃⁻ is taken up by anion exchangers and CFTR, H, I) Renal proximal tubular epithelial cells (H) and β-intercalated cells (I) express CFTR, which could serve as channel for HCO₃⁻ secretion. NHE1, NHE3, Na⁺/H⁺ exchanger type 1 or 3; NDCBE, Na⁺ dependent Cl⁻/HCO₃⁻ exchanger (SLC4A8); SLC4A9, Na⁺/HCO₃⁻ cotransporter (NBC). The schemes do not provide information concerning cell type specific localization of transport proteins.
Nevertheless, the moderate defect may be related to the metabolic alkalosis observed in CF patients [121–124].

9. Concluding remarks

This short review emphasizes the role of CFTR for bicarbonate transport and HCO3− homeostasis in different organ compartments. Through CFTR HCO3− is secreted in airways, pancreas, salivary gland, intestine, and reproductive organs, and possibly reabsorbed in the sweat duct and excreted by the kidneys. Very early findings pointed to a defect in bicarbonate transport in CF, but it took many more years to identify the underlying molecular mechanism for bicarbonate secretion. Activation of bicarbonate transport in response to cAMP-dependent stimulation by secretin, isotretinol, adenosine or prostaglandin is impaired in CF. This contributes essentially to the fundamental problems in CF as impaired mucociliary clearance of the lungs, pancreatic insufficiency and compromised intestinal mucus properties, but also explains all other phenotypic changes in CF. After all bicarbonate is no longer a neglected ion [34].

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