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Cl^--Dependent HCO_3^- Transport by Cystic Fibrosis Transmembrane Conductance Regulator

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Summary

Cystic fibrosis (CF) affects the function of multiple organs. The inability to maintain luminal hydration of ducts leads to their plugging and destruction of the affected organs. An exacerbating problem is the acidic pH of the fluid produced by CF patients' secretory glands. This is best documented for pancreatic secretion. Alkaline fluid secretion requires vectorial transport of electrolytes and of HCO_3^-. The mechanism of HCO_3^- secretion by cystic fibrosis transmembrane conductance regulator (CFTR) expressing cells is not well understood. In the present communication we discuss results suggesting that CFTR itself can transport large amounts of HCO_3^- and that HCO_3^- transport by CFTR is mediated by a coupled, Cl^--dependent process that is different from a simple HCO_3^- conductance.

Cystic fibrosis (CF) affects the function of multiple organs, including the pancreas, intestine and the respiratory tract [1]. In CF, the inability to maintain luminal hydration of ducts that contain or secrete large molecules leads to plugging of ducts and destruction of the affected organs. The exocrine pancreas and the Wolffian ducts are most susceptible to the disease and are affected earliest [2], probably because of their extensive and branched ductal systems. Hydration of the ducts requires fluid secretion, which is driven by vectorial electrolyte transport. Vectorial electrolyte and fluid transport are defective in CF [3, 4, 5, 6, 7, 8]. Identification of the gene responsible for CF [9, 10] led to elucidation of its protein product, the cystic fibrosis transmembrane conductance regulator (CFTR), and its function as a cAMP regulated Cl^- channel [8]. Mutations in CFTR can cause CF with variable phenotype from very severe with pancreatic insufficiency (PI) to very mild with pancreatic sufficiency (PS), depending on the specific mutation in CFTR [2]. Mutations that cause abnormal Cl^- transport by CFTR are believed to be responsible for the pathogenesis of the disease. However, we argue that in tissues like the pancreas, CFTR-supported HCO_3^- transport is as critical as Cl^- transport for the pathogenesis of CF.

Most CFTR expressing cells secret alkaline fluid that is poor in Cl^- and rich in HCO_3^- [4, 5, 6, 7]. The mechanism of fluid and electrolyte secretion by secretory epithelia is best understood in the pancreas [6] and salivary glands [7]. In the case of the pancreas, the acinar cells secret digestive enzymes and a small volume of isotonic fluid containing about 140 mM NaCl and 25 mM HCO_3^- [6]. The CFTR-expressing duct secretes most of the fluid, absorbs the Cl^- and secretes HCO_3^- to determine the final volume and electrolyte composition of the pancreatic juice [6]. In

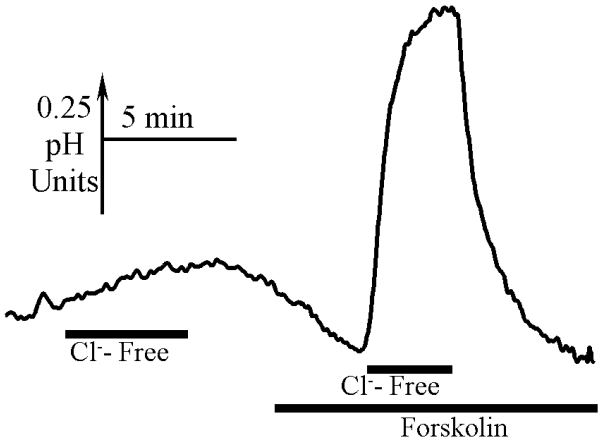

Figure 1. Measurement of intracellular pH (pH_i) in HEK293 cell transfected with wild type- (WT)-CFTR.

humans, the pancreatic juice contains about 20 mM Cl and as much as 140 mM HCO_3^- [6].

HCO_3^- secretion by the duct requires mechanisms for transductal HCO_3^- transport. Little is known about the pathways mediating HCO_3^- influx across the basolateral membrane (BLM) of the duct. The best studies available suggest that the bulk of HCO_3^- influx in mediated by a Na^+-HCO_3^- co-transporter (NBC) [11, 12, 13]. Recent molecular cloning identified the pNBC1 isoform, a member of the electrogenic NBC family as the isoform expressed in pancreatic acinar and duct cells [14]. HCO_3^- efflux across the BLM and its regulation are poorly understood. Most models assume that the substantial electroneutral component of HCO_3^- efflux is mediated by a Cl /HCO_3^- exchange activity [6, 13]. This function is also believed to mediate part of the Cl absorption by the duct. The electrogenic component of ductal HCO_3^- secretion can be directly mediated by CFTR or a CFTR-associated mechanism. Several investigators reported that CFTR could conduct HCO_3^- [15]. In a recent finding, Gray and co-workers used isolated duct cells from the guinea pig pancreas to report a HCO_3^-/Cl permeability ratio of about 0.4 [16].

Our work suggests that the role of CFTR in ductal Cl absorption and HCO_3^- secretion is more complicated. In the initial part of the work, we showed that stimulation of transiently or stably expressed CFTR with cAMP activated a 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid- (DIDS)-insensitive, Cl-dependent HCO_3^- transport mechanism. Figure 1 shows measurement of intracellular pH (pH_i) in HEK293 cell transfected with wild type- (WT)-CFTR. After equilibration of the cells in a HCO_3^-- buffered solution, the Cl-dependent HCO_3^- transport was measured before and after stimulation of CFTR with cAMP by removal and re-addition of Cl to the incubation medium. It can be seen that stimulation of CFTR with cAMP is needed for activation of HCO_3^- transport that is dependent on removal and addition of Cl to the medium.

The importance of HCO_3^- transport by CFTR was highlighted in recent work demonstrating aberrant HCO_3^- transport by CFTR mutants that retain Cl channel activity, yet cause CF [17]. The discovery that Cl conductance is impaired in CF [18, 19] and that the CFTR gene codes for a cAMP-regulated Cl channel [8] led to the paradigm that mutations in CFTR that inhibit Cl channel activity, lead to inhibition of transepithelial fluid and electrolyte transport and CF [1, 2, 3, 4, 5, 6, 7]. However, a conundrum emerged with the finding that many disease-causing mutants of CFTR retain substantial, Cl channel activity. A possible explanation of this puzzle is that the CFTR mutants that retain Cl channel activity, but cause CF, cannot support HCO_3^- transport. HCO_3^- is an ion of paramount biological importance. Among its other functions, HCO_3^- serves as the biological pH buffer and it enhances the solubility of proteins and ions in biological fluids. HCO_3^- is of particular importance in CF since pH and HCO_3^- affect mucin viscosity [20, 21, 22] and bacterial binding to mucins [23, 24, 25]. This hypothesis was tested by comparing Cl current, net Cl fluxes and net HCO_3^- fluxes by sixteen disease-causing mutants of CFTR that had small, no effect or increased Cl channel activity. It was found that mutants reported to cause CF with

pancreatic insufficiency could not support measurable HCO_3^- transport and mutants reported to cause CF with pancreatic sufficiency showed reduced HCO_3^- transport. These findings indicate that defective CFTR-dependent HCO_3^- transport is critical for the pathology of CF.

The mechanism by which CFTR supports HCO_3^- transport in heterologous systems and in native tissues is not known. However, since mutations in CFTR that cause CF affect HCO_3^- transport it is likely that CFTR itself mediates the transport. This notion is supported by additional mutations that affect both CFTR Cl^- channel activity and HCO_3^- transport. These include mutations in membrane spanning domains and the R domain of CFTR. HCO_3^- transport by CFTR does not conform to conductive transport. Thus, the transport is not affected by membrane depolarization and, more important, Cl^- transport is obligatory for HCO_3^- transport by CFTR. In addition, no HCO_3^- current could be measured under the condition of vigorous Cl^--dependent HCO_3^- transport by CFTR. Calculation of HCO_3^- transport from experiments similar to that reported in Figure 1 in terms of mM/min/cell indicated that the transport should have generated a current of about 220 pA. These findings raise the intriguing possibility that the bulk of HCO_3^- transport by CFTR is note conductive, and that CFTR may function as a Cl^- channel and as a Cl^-/HCO_3^- exchanger. Further probing of the mechanism of HCO_3^- transport by CFTR is needed to test this possibility.

Key words Bicarbonates; Cystic Fibrosis Transmembrane Conductance Regulator; Ion Transport; Pancreatic Ducts; Pancreatic Juice

Abbreviations BLM: basolateral membrane; CF: cystic fibrosis; CFTR: cystic fibrosis transmembrane conductance regulator; DIDS: 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid; NBC: Na^+/HCO_3^- cotransporter; pHi: intracellular pH; WT: wild type

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