J Korean Med Sci 2000; 15(Suppl): S17-20
ISSN 1011-8934

CFTR, a Rectifying, Non-Rectifying Anion Channel?

Paul M. Quinton*, M. M. Reddy

Department of Pediatrics*, UCSD School of Medicine, La Jolla, U.S.A.
Division of Biomedical Sciences, University of California, Riverside, U.S.A.

CFTR is an anion channel with high conductance for Cl^- and other halides. Abnormalities in this channel which decrease plasma membrane Cl^- conductance result in the inherited disease, cystic fibrosis (CF). The fact that this disease is also characterized clinically by significant abnormalities in HCO_3^- secretion in the pancreas (1, 2). provides a priori evidence that this anion channel is critical to HCO_3^- transport in some, possibly all, exocrine epithelia where CFTR is expressed. If CFTR is significantly permeable to HCO_3^- and if HCO_3^- is physiologically transported through CFTR, the defect in HCO_3^- in CF might be self-evident. Thus, it is of fundamental importance to determine the HCO_3^- permeability characteristics of this channel in defining its role in epithelial HCO_3^- management and its impact on the disease process. Moreover, since there are no other anion channels characterized as "HCO_3^- selective", the possibility that CFTR may function as such makes the question academically intriguing. Recently, a renewed interest in defining the role of CFTR in HCO_3^- transport has arisen (3-14). Most studies of the function of CFTR in this regard have been performed in cultured cells or tissues using whole cell or membrane patch techniques and have reported that in these ex vivo systems the permeability of HCO_3^- relative to Cl^- is between 0.1 to 0.2. In early studies using the intact, native, microperfused human sweat duct, which abundantly expresses CFTR in its apical membrane, we were unable to detect differences between HCO_3^- and gluconate (15) (we took gluconate as a standard for impermeant anions). In the present study, we undertook to determine the reason for this discrepancy, if possible, and to further define the relevance of CFTR to HCO_3^- movement.

Key Words: *CFTR; Sweat Duct; Anion Channel; Bicarbonate; Chloride*

Address for correspondence: Paul M. Quinton
Department of Pediatrics, UCSD School of Medicine, La Jolla, CA 92103-0831, U.S.A., Tel: +1.619-543-2884, Fax: +1.619-543-5642, E-mail: pquinton@ucsd.edu

Methods

We isolated single sweat ducts from fresh biopsies of human skin. Segments of ducts greater than 500 μM were microperfused with a double barrel luminal micropipette that served to perfuse and record transepithelial voltage on one side and to pass constant current pulses on the other. This arrangement allowed estimation of the specific membrane conductance from the cable equation (16). After confirming the integrity of the perfused tubule, we applied 1,000-5,000 units/mL of α-toxin from *Staphylococcus aureus* to the bath solution in order to selectively permeabilize the basilateral membrane (17). This procedure leaves the epithelium with an intact and functional apical membrane and a non-selective basal membrane permeable to molecules of up to about 5,000 mwu. Since activation of CFTR is exquisitely sensitive to ATP and cAMP, its activity can be readily controlled in this preparation by controlling the presence of either of these nucleotides in the cytosolic bathing solution. That is, addition of 10 μM cAMP plus 5 mM ATP activates CFTR in seconds whereas removal of either cAMP or ATP from the cytosolic bath deactivates the channel. We then sought to determine the permeability of the apical membrane to HCO_3^- and Cl^- relative to the impermeant gluconate anion by measuring the trans apical membrane diffusion potential generated by chemical gradients for these anions and the simultaneous changes in membrane conductances.

In our previous investigations in which we had not seen a HCO_3^- conductance, we had thought it prudent not to exceed the maximum anticipated physiological concentration of 25 mM for HCO_3^- in the sweat duct. Subsequent studies from other laboratories have uniformly used essentially isosmotic concentrations of about 140 mM HCO_3^-. Thus, we felt compelled to examine the behavior of CFTR in the sweat duct at these higher HCO_3^- concentrations as well. Our general experimental protocol was to first inactivate CFTR and perifuse the cytosol with K-gluconate while changing the composition of the luminal perfusate from gluconate to Cl^- to HCO_3^-, not necessarily in that order (we used either the K^+ salt or the Na^+ salt plus 10^{-5} M amiloride to block Na^+ shunt conductance; the K^+ permeability of the duct apical membrane is nil). We then activated CFTR and repeated the luminal perfusate changes. Next, we deactivated

CFTR by withdrawing ATP and/or cAMP and continued to perfuse the lumen of the tubule with 150 mM K-gluconate while we proceeded to change the composition of the cytosolic bath from gluconate to Cl^- to HCO_3^- as before. These maneuvers were repeated again after activating CFTR a second time and the transepithelial diffusion potential differences (ΔVap) and conductances (ΔGap) were recorded after each change on either side of the membrane. The fact that there were no significant changes in electrical parameters until cAMP and ATP were added provides strong evidence that CFTR is the only significant activatable ionic conductance in the membrane under these conditions. After establishing that there were no significant changes in specific conductance or diffusion potential through the apical membrane so long as CFTR remained inactivated, we omitted these steps in the protocol in order to preserve tissue viability.

Results and Discussion

First, after activating CFTR with cAMP and ATP, we kept the cytosol perifused with K gluconate while changing the luminal perfusate. We found that the diffusion potential (Vap) for HCO_3^- vs. gluconate in the lumen did not change (mean ΔVap = -1.6 mV) while that for Cl^- vs. gluconate increased by nearly 40 mV (mean ΔVap = -39.5 mV). Similarly, the conductance (Gap) did not change when HCO_3^- replaced gluconate (mean ΔGap = -0.7 mS/cm^2), but when Cl^- replaced gluconate, Gap increased by more than 31 mS/cm^2 (Table 1a). On the surface, we took these data as confirmation of our initial impression that CFTR is impermeable to HCO_3^- even at these non-physiological concentrations of HCO_3^-. On the other hand, when we kept the lumen perfused with gluconate and changed the composition of the cytosolic perifusion fluid from gluconate to HCO_3^-, the diffusion potential became more negative by 15 mV and the corresponding Gap increased by almost 12 mS/cm^2. The changes to Cl^- in the lumen were even more dramatic. Mean Vap changed by 53 mV with a simultaneous mean increase in Gap of 124 mS/cm^2 (Table 1a).

These combined results suggest that CFTR effectively rectifies HCO_3^- permeation from cell to lumen. To our knowledge, channel behavior that completely rectifies one ion while remaining permeable to another has not been documented and is not easily reconciled from first principles. Further, inspection of the data reveals that both the magnitude of the diffusion p.d. for Cl^- and its associated G_{Cl} are reduced when gluconate is present in the cytosolic fluid. These considerations in addition to a previous report (18) that large organic anions produce a block of CFTR when present on the cytosolic surface suggested that the effect might be due to anomalous rectification induced by our "standard" impermeant anion, gluconate.

Thus, we proceeded to determine the ΔVap and Δ

Table 1. Data show the differences in transapical membrane potentials (ΔVap) and conductances (ΔGap) of the microperfused sweat duct when the bath (...in Cell) or luminal fluids (...in Lumen) are kept at a constant composition of 150 mM Gluconate (Gluconate) or 150 mM Cl (Cl), indicated by headings in bold, while the fluid composition on the contralateral side of the membrane was changed from 150 mM gluconate to 150 mM HCO_3 ($Glu^-\rightarrow HCO_3^-$) and from 150 mM gluconate to 150 mM Cl ($Glu^-\rightarrow Cl^-$), as indicated in the first column. Significant differences from values determined when 150 mM gluconate was present on both sides of the apical membrane were calculated using the Student's T-test for paired values.

Table 1a	Gluconate in Cell		Gluconate in Lumen	
	ΔVap (mV)	ΔGap (mS/cm^2)	ΔVap (mV)	ΔGap (mS/cm^2)
$Glu^-\rightarrow HCO_3^-$	-1.6	-0.7	15.0*	11.9*
sem =	2.1	2.5	2.5	31.2
$Glu^-\rightarrow Cl^-$	-39.5*	31.2*	53.2†	124.0†
sem =	6.0	9.8	6.7	38.6
	n=7		n=8	

Table 1b	Cl in Cell		Cl in Lumen	
	ΔVap (mV)	ΔGap (mS/cm^2)	ΔVap (mV)	ΔGap (mS/cm^2)
$Glu^-\rightarrow HCO_3^-$	-8.9	14.7*	18.7†	11.1
sem =	4.4	8.5	5.1	7.0
$Glu^-\rightarrow Cl^-$	-41.9*	56.0*	61.7†	75.6†
sem =	10.0	13.7	8.2	23.4
	n=4		n=10	

*Significantly different: $p<0.05$
†Significantly different: $p<0.01$

Gap for HCO_3^- and Cl^- (compared to gluconate) when the contralateral anion was 150 mM Cl^-, instead of gluconate. With 150 mM Cl^- on the luminal side, we changed the cytosolic bath from gluconate to HCO_3^- to Cl^- as before and found that cytosolic HCO_3^- hyperpolarized Vap by about 19 mV and increased Gap by about 11 mS/cm^2 (Table 1b). Changes to cytosolic Cl^- hyperpolarized Vap by 62 mV and increased Gap by about 75 mS/cm^2. When we held KCl on the cytosolic surface, changing the lumen from gluconate to HCO_3^- depolarized Vap by 9 mV and increased Gap by about 15 mS/cm^2 whereas changing to luminal Cl^- depolarized Vap by 42 mV and increased Gap by about 56 mS/cm^2.

These results indicate that at these high concentrations of HCO_3^-, a CFTR dependent HCO_3^- conductance is present, but low, relative to Cl^- conductance. The statistically similar changes in Vap and Gap when the Cl^- and HCO_3^- gradients are reversed suggest that CFTR does not significantly rectify conductance with either anion in the absence of gluconate. However, the data indicate that in the cytosol, gluconate interacts with a cytosolic portion of the CFTR molecule to effectively block HCO_3^- permeation from the luminal surface. Consistent observations of asymmetrical cytosolic blocking have been reported for several organic anions, including gluconate, on single channel CFTR Cl^- conductance in membrane patch-clamp studies (18). These effects raise the question of whether native organic anions normally in the cell might exert similar effects physiologically. Such an effect might explain the lack of HCO_3^- permeability observed in the intact unpermeabilized native sweat duct (15). Whether or not this is the case, the question remains as to whether the apparent, relatively low HCO_3^- permeability of CFTR is adequate to play a physiological role in transporting HCO_3^-. It is tempting to speculate that the channel in its native environment may rectify HCO_3^- and allow movement out of the cell, but block its re-entry by virtue of cytoplasmic anions. Indeed, relative to normal cells, CF cells isolated from the rat[19] and human duodenum have been reported to recover poorly from alkaline load (20).

Lastly, the uncomfortable question remains as to whether explorations of channel function in the presence of such high HCO_3^- concentration are physiologically consonant. The lumen of the pancreatic duct is one of the few, perhaps only site, where concentrations of this magnitude are known to occur. Our data may suggest that at concentrations generally thought to occur in the cell and in the extracellular fluid, CFTR may not be a principal route for HCO_3^- permeation through the plasma membrane, especially if significant amounts of blocking anions are present in the cytoplasm. Nevertheless, if CFTR does not function as a physiological conductive HCO_3^- permeation pathway, we are left without explanation of how it exerts such a pronounced deleterious effect on HCO_3^- transport in CF.

References

1. Hadorn B, Johansen PG, Anderson CM. *Pancreozymin secretin test of exocrine pancreatic funtion in cystic fibrosis and the significance of the result for the pathogenesis of the disease. Can Med Assoc J 1968; 98: 377-85.*
2. Kopelman H, Durie P, Gaskin K, Weizman Z, Forstner G. *Pancreatic fluid secretion and protein hyperconcentration in cystic fibrosis. N Engl J Med 1985; 312: 329-34.*
3. Poulsen JH, Fischer H, Illek B, Machen TE. *Bicarbonate conductance and pH regulatory capability of cystic fibrosis transmembrane conductance regulator. Proc Natl Acad Sci USA 1994; 91: 5340-4.*
4. Illek B, Yankaskas JR, Machen TE. *cAMP and genistein stimulate HCO_3^- conductance through CFTR in human airway epithelia. Am J Physiol 1997; 16: L752-61.*
5. Lee MG, Wigley WC, Zeng W, Noel LE, Marino CR, Thomas PJ, Muallem S. *Regulation of Cl^-/ HCO_3^- exchange by cystic fibrosis transmembrane conductance regulator expressed in NIH 3T3 and HEK 293 cells. J Biol Chem 1999; 274: 3414-21.*
6. Zeng W, Lee MG, Yan M, Diaz J, Benjamin I, Marino CR, Kopito R, Freedman S, Cotton C, Muallem S, Thomas P. *Immuno and functional characterization of CFTR in submandibular and pancreatic acinar and duct cells. Am J Physiol 1997; 273: C442-55.*
7. Jiang C, Fang SL, Xiao YF, O'Connor SP, Nadler SG, Lee DW, Jefferson DM, Kaplan JM, Smith AE, Cheng SH. *Partial restoration of cAMP-stimulated CFTR chloride channel activity in DeltaF508 cells by deoxyspergualin. Am J Physiol 1998; 275: C171-8.*
8. Grubman SA, Perrone RD, Lee DW, et al. *Regulation of chloride/bicarbonate exchanger activity by wild type and mutant CFTR. Pediatr Pulmonol 1997; S14: 277.*
9. Poulsen JH, Machen TE. *HCO_3^- dependent pH_i regulation in tracheal epithelial cells. Pflugers Arch 1996; 432: 546-54.*
10. Argent BE, Gray ME. *Bicarbonate secretion by pancreatic duct cells. In: Case RM, ed. The exocrine pancreas. Welwyn Garden City: Hertforeshire, Smith Kline & French Laboratories Ltd., 1989; 24-8.*
11. Sohma Y, Gray MA, Imai Y, Argent BE. *A mathematical model of the pancreatic ductal epithelium. J Membr Biol 1996; 154: 53-67.*
12. Gray MA, Winpenny JP, Verdon B, McAlroy H, Argent BE. *Chloride channels and cystic fibrosis of the pancreas. Biosci Rep 1995; 15: 531-41.*
13. Shumaker H, Amlal H, Frizzell R, Ulrich CD, 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.*
14. Devor DC, Singh AK, Lambert LC, DeLuca A, Frizzell RA,

Bridges RJ. *Bicarbonate and chloride secretion in Calu-3 human airway epithelial cells. J Gen Physiol 1999; 113: 743-60.*

15. Quinton PM, Reddy MM. *Cl^- Conductance and acid secretion in the human sweat duct. In: Durham JH, Hardy MA, eds. Bicarbonate, chloride, and proton transport systems. New York: NYAS Proceedings, 1989; 438-46.*
16. Greger R. *Cation selectivity of the isolated perfused cortical thick ascending limb of Henle's loop of rabbit kidney. Eur J Appl Physiol 1981; 390: 30-7.*
17. Quinton PM, Reddy MM. *Control of CFTR Cl conductance by ATP levels through non-hydrolytic binding. Nature 1992; 360: 79-81.*
18. Linsdell P, Hanrahan JW. *Flickery block of single CFTR chloride channels by intracellular anions and osmolytes. Am J Physiol 1996; 271: C628-34.*
19. Hogan DL, Crombie DL, Isenberg JI, Svendsen P, Schaffalitzky de Muckadell OB, Ainsworth MA. *CFTR mediates cAMP- and Ca^{2+}-activated duodenal epithelial HCO_3^- secretion. Am J Physiol 1997; 272: G872-8.*
20. Pratha V, Hogan D, Martensson B, Conrad D, Light M, Isenberg JI. *Duodenal bicarbonate transport is impaired in cystic fibrosis patients. Pediatr Pulmonol 1999; Suppl 17: 225.*