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Bicarbonate Secretion in the Murine Gallbladder - Lessons for the Treatment of Cystic Fibrosis

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Summary

The epithelium lining the gallbladder of mammalian species has absorptive and secretory functions. An important function is the secretion of a bicarbonate rich fluid that helps neutralise stomach acid and provides an appropriate environment for intestinal enzymes. In cystic fibrosis (CF) this secretory function is lost. This study concerns the bicarbonate secreting activity of murine gallbladders *in vitro* using wild type and CF mice and four main questions are considered as follows: a) Does the murine gallbladder secrete bicarbonate electrogenically and is this prevented in CF? b) Can the secretory activity in CF gallbladders be restored by gene therapy or pharmacologically? c) How is the cystic fibrosis transmembrane conductance regulator (CFTR) involved in bicarbonate secretion? d) Does the data offer prospects for the treatment of CF?. Work from both the author's laboratory and the literature will be reviewed. Consideration of the currently available data indicates that the wild type murine gallbladder does secrete bicarbonate electrogenically and that this is absent in CF mice. Further it has been demonstrated that bicarbonate secretory activity can be restored by both gene therapy and by the use of drugs. The role of CFTR in bicarbonate secretion remains equivocal. Much evidence suggests that CFTR can act as a channel for HCO_3^- ions as well as Cl^- ions, while others propose a parallel arrangement of CFTR with a Cl^-/HCO_3^- exchanger is necessary. The matter is further complicated by the regulatory role of CFTR on other transporting activities. Opportunities for possible application to man are discussed.

Secretion of Bicarbonate by the Mouse Gallbladder

Mouse bile contains around 40 mEq/L of bicarbonate that is within the normal range of most mammals. Agents that increase cAMP either by activating adenylate cyclase, such as forskolin and vasoactive intestinal polypeptide (VIP), or in other ways, such as isobutylmethylxanthine (IBMX) and di-butyryl cAMP, cause an increase in short circuit current (SCC) when applied to isolated gallbladders mounted in Ussing chambers and voltage clamped at zero potential. The basal SCC in gallbladders is 7.2±4.3 $\mu A/cm^2$ and increased by 48.2±6.1$\mu A/cm^2$ (n=21) following treatment with forskolin (Figure 1). The direction of the current and its insensitivity to amiloride suggests it is due to the secretion of anions [1]. Several pieces of evidence indicate that the current is generated by the transport of bicarbonate in the serosal to apical direction. First the secretory current is significantly

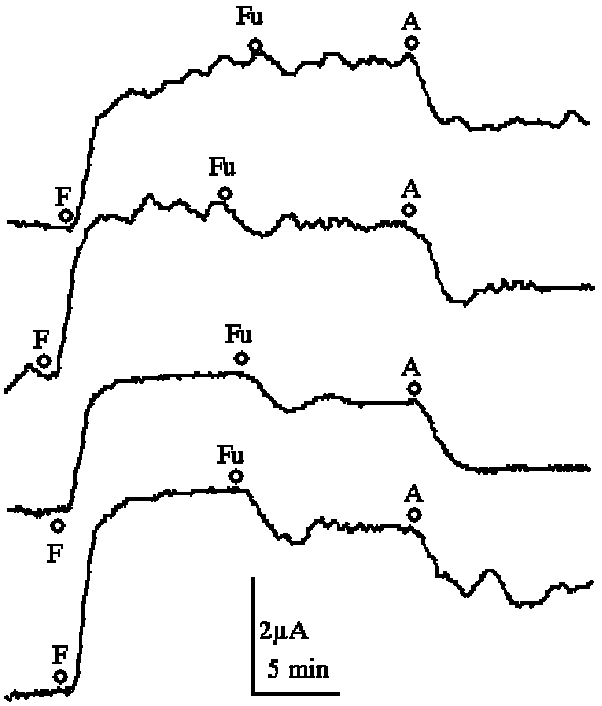

Figure 1. SCC recordings from a mouse gallbladder over 8 hours. Sequential additions of forskolin 10 μM (F), furosemide 1 mM (Fu), and acetazolamide 100 μM (A) were made as indicated. Redrawn from [1].

reduced when HCO_3^- and CO_2 are removed from the bathing fluid (Figure 2). This procedure reduced the current by 84% ($P<0.05$, n=5). Removal of bicarbonate alone caused a reduction in the response to forskolin of 78% ($P<0.05$, n=5), suggesting the bicarbonate ion is more important than the hydration of CO_2.

Removal of HCO_3^-/CO_2 from the basolateral side only causes a significant reduction in the response to forskolin (58% compared to controls, $P<0.003$, n=9), while HCO_3^-/CO_2 removal from the apical side had no effect (103% compared to controls, NS, n=4). Thus removal of bicarbonate from the side from which it is transported reduces the response to the secretagogue, while no effect is apparent when it is absent from the receiving side [1].

Furosemide, an inhibitor of electrogenic chloride secretion has no significant effect on the forskolin induced current, while acetazolamide, a carbonic anhydrase inhibitor, significantly reduced the current (from 48.2±6.1μA/cm^2 by 18.2±2.1μA/cm^2, $P<0.05$, n=21). Even though significant effects of furosemide are not demonstrable the distinct reductions in current sometimes seen following its addition, plus other data argue for a minor component of chloride secretion.

While the experimental set up did not allow the measurement of serosal to mucosal bicarbonate flux, others using small bath volumes, were able to determine J_{s-m} for bicarbonate by titration [2]. The increased flux in response to forskolin in wild type gallbladders was 1.76±0.42 μEq/cm^2/h. This corresponds to a steady state increase in current of 47.2±11.3 μA/cm^2, virtually identical to the value given above.

The presence of CFTR is essential for the response of gallbladders to forskolin (Figure 3) [3]. Neither CF null (*Cftr*^{tm1Cam}) or CFΔF508 (*Cftr*^{tm2Cam}) gallbladders showed significant responses to forskolin. Thus CFTR is essential

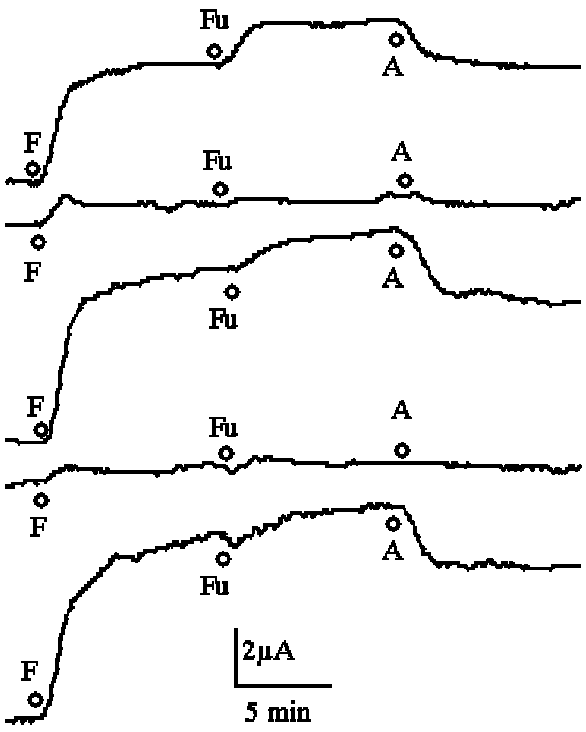

Figure 2. Effect of HCO_3^-/CO_2 removal on gallbladder responses. Sequential additions of forskolin 10 μM (F), furosemide 1 mM (Fu), and acetazolamide 100μM (A) were made as indicated. Redrawn from [1].

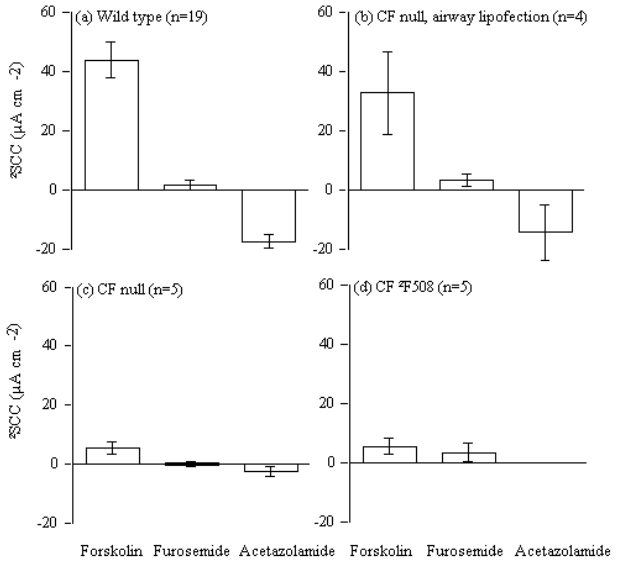

Figure 3. Responses to mouse gallbladders to forskolin (10 μM), furosemide (1 mM) and acetazolamide (100 μM). In (a) are the data for wild type while in (c) and (d) the responses are for CF null and CF ΔF508 gallbladders respectively. In (b) are the data for CF null gallbladders where the mice had been lipofected through the airways two days before the measurements were made.

for HCO_3^- secretion in the murine gallbladder when stimulated by agonists which increase cAMP, which includes endogenous ones.

Restoration of Bicarbonate Secreting Activity in Murine CF Gallbladder

In vivo gene transfer in CF mice by instilling a plasmid containing the cDNA for CFTR complexed with liposomes either into the trachea or the nose restored the forskolin bicarbonate secretory activity to the gallbladder, measured *in vitro*. Briefly the plasmid *pTrial10-CFTR2* was mixed with DC-Chol/dioleoyl phosphatidylethanolamine (DOPE) liposomes as detailed in Curtis *et al*. [3], and the mice sacrificed two days after transfection. It is important to realise that hCFTR was reintroduced rather than the murine version. Figure 3 shows the profile of responses in transfected mice gallbladders, which is similar to that of wild type and unlike those in CF gallbladders. The procedure used also restored the responses of the mouse tracheal epithelium [4] and that in the nose [5] to forskolin, but had no effect on the epithelia of the intestine.

The route taken by the genetic material from the airways to the gallbladder is still unclear. We were able to detect mRNA for human CFTR in the gallbladders of transfected mice. Furthermore using either pGL-3 *luc* or pCMV-*luc* (containing the cDNA coding for luciferase) lipoplex we were able to detect luciferase activity in gallbladders when given by the nasal or intratracheal route, but not when given orally, or by intramuscular, intraperitoneal or subcutaneous routes. When these lipoplex were given intravenously some luciferase activity was found in gallbladders, but only 3% of that found with the airway route [3]. When the plasmid *pTrial10-CFTR2* was given alone into the airways no transfer of genetic material to the gallbladder occurred, yet it was possible to transfect the gallbladder *in vitro* by exposure to plasmid to restore bicarbonate secretion. Viral vectors with or without cationic amphiphiles can be used to transfect gallbladders *in vitro* or *in vivo* (retrograde perfusion of the bile duct) [6] and provide a further way to restore CFTR function to the gallbladder.

Bicarbonate secretion in CF gallbladders does not necessarily require the restoration of CFTR function. Agents that increase Ca^{2+}_i also increase bicarbonate secretion by activating Ca^{2+} sensitive Cl^- channels. For example, ionomycin increased SCC by 14.8±3.9μA/cm^2 (n=10) in wild type gallbladders and in CF null and CF ΔF508 gallbladders by 25.4±13.4 μA/cm^2 (n=9) and 12.3±3.5 μA/cm^2 (n=11) respectively, none of these values being significantly different from each other. Uridine triphosphate (UTP), via activation of $P2Y_2$ receptors also stimulates bicarbonate secretion in CF mice (*Cftr*$^{tm/UNC}$) measured both as SCC and as J_{s-m} for bicarbonate [2]. In conclusion, gene transfer to gallbladders correlates with the restoration of cAMP-dependent HCO_3^- secretion, but alternative pathways support bicarbonate secretion without CFTR.

CFTR Involvement in Bicarbonate Secretion

Various mechanisms have been proposed for bicarbonate secretion in epithelia as follows: a) HCO_3^- leaves the cell via CFTR channels; b) a parallel arrangement of CFTR with a chloride bicarbonate exchanger; c) complex models dependent on CFTR regulation of other transporting activities.

Certainly CFTR has a bicarbonate conductance although it is lower than for chloride [7]. However it has been argued that the driving force for HCO_3^- exit must be small [8]. Experimental evidence for the electrogenic secretion of bicarbonate via CFTR centres on the failure of chloride removal from the apical face to stop secretion. This opposes the classical view that Cl^-_i lowering, by exit through CFTR, triggers the Cl^-/HCO_3^- exchanger to affect net HCO_3^- transport [9]. Figure 4 shows that chloride removal from the apical surface of mouse gallbladder has only a modest effect on HCO_3^- secretion caused by forskolin. Statistically the reduction is significant, the response to forskolin being reduced from 89.0±14.4 μA/cm^2 to 59.3±14.2 μA/cm^2 ($P<0.02$) in three experiments like the one illustrated. Thus 67% of the current remains when the Cl^-/HCO_3^- exchanger is blocked, suggesting the exit route for HCO_3^- is via CFTR. The 30% reduction in current may mean that this fraction normally uses the exchanger mechanism. Others have made similar arguments for HCO_3^- secretion in the airways [10], intestine [11] and colon [12]. In other situations chloride removal blocks HCO_3^- secretion, and in these tissues the activity of the exchanger and CFTR are tightly linked, as for example in PANC-1 cells derived from the pancreatic duct [13].

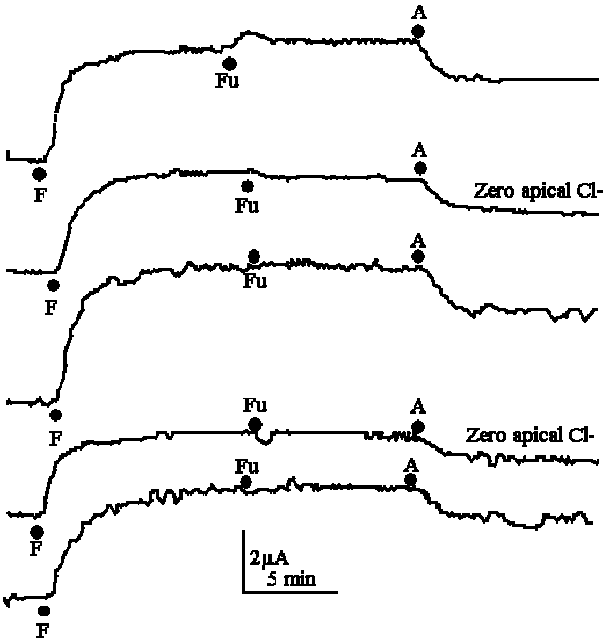

Figure 4. Effect of Cl^- removal from the apical bathing solution. Forskolin (F, 10μM), furosemide (Fu, 1 mM) and acetazolamide (A, 100 μM) were added sequentially as indicated.

Returning to the mouse gallbladder, it is known the UTP can stimulate bicarbonate secretion in CF tissue via activation of $P2Y_2$ receptors. Removal of apical chloride fails to inhibit secretion, as in the normal gallbladder stimulated with forskolin. The conclusion, therefore, is that the Ca^{2+}-activated chloride channel can also conduct bicarbonate ions in the bladder [2].

It is always a mistake to assume that absence of CFTR has no other consequences apart from the removal of a chloride conductance. For example, the presence of CFTR in the membrane, but not its conductance function, has been shown to be necessary for the activation of the Cl^-/HCO_3^- exchanger by cAMP [14, 15]. Consequently in tissues such as submandibular gland ducts and pancreatic ducts from CF mice the exchanger was not activated by forskolin as it is in wild type tissues

Recently, Wheat *et al.* have shown that CFTR is necessary for the expression of down regulated in adenoma (DRA) which in turn is responsible for the upregulation of the Cl^-/HCO_3^- exchanger. It is suggested that the failure of bicarbonate secretion in CF airways may be due in part to the down regulation of the exchanger caused by the absence of DRA [16]. An intriguing finding has been reported by Cremaschi and colleagues [17]. Using patch

clamp analysis, with rabbit gallbladder cells, it was found that inhibition of the Cl^-/HCO_3^- exchanger from the outer surface caused the appearance of a anion selective conductance with a $P_{gluconate}/P_{chloride}$ value of 0.18. As chloride removal from the apical surface also inhibits the exchanger it may well be important to look for this phenomena elsewhere. It may provide an alternative pathway for bicarbonate exit in the absence of external chloride without HCO_3^- ions leaving via CFTR. In conclusion, evidence suggests that bicarbonate ions exit through CFTR in the gallbladder and in other epithelia, but direct proof for this exit route remains an urgent problem.

Lessons for the Treatment of Cystic Fibrosis

It would appear that retrograde perfusion offers a way to specifically target the gallbladder by gene therapy. However the procedure is invasive and unlikely to be useful until long lived benefit without significant risk is available. Consideration might also be given to recruiting the gallbladder as a vehicle for other functions, for example the secretion of pancreatic enzymes. Clinically useful restoration of lung function in CF by gene therapy is the goal of many groups. When this is achieved it would be worthwhile to look at indicators of gallbladder function.The unusual route for gallbladder transfection detailed here might have its counterpart in patients.

key words Cystic Fibrosis Transmembrane Conductance Regulator; Gene therapy; Liposomes; Luciferase; Mice, Inbred CFTR

Abbreviations CF: cystic fibrosis; CFTR: cystic fibrosis transmembrane conductance regulator; DOPE: dioleoyl phosphatidylethanolamine; DRA: down regulated in adenoma; IBMX: isobutylmethylxanthine; SCC: short circuit current; UTP: uridine triphosphate; VIP: vasoactive intestinal polypeptide

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References

1. Martin LC, Hickman ME, Curtis CM, MacVinish LJ, Cuthbert AW. Electrogenic bicarbonate secretion in the mouse gallbladder. Am J Physiol 1998: 274:G1045-52. [98359064]

2. Clarke LL, Harline MC, Gawenis LR, Walker NM, Turner JT, Weisman GA. Extracellular UTP stimulates electrogenic bicarbonate secretion across CFTR knockout gallbladder epithelium. Am J Physiol 2000; 279:G132-8. [20357578]

3. Curtis CM, Martin LC, Higgins CF, Colledge WH, Hickman ME, Evans MJ, et al. Restoration by intratracheal gene transfer of bicarbonate secretion in cystic fibrosis mouse gallbladder. Am J Physiol 1998: 274:G1053-60. [98359065]

4. MacVinish LJ, Gill DR, Hyde SC, Mofford KA, Evans MJ, Higgins CF, et al. Chloride secretion in the trachea of null cystic fibrosis mice: the effects of transfection with pTrial10-CFTR2. J Physiol 1997; 499:677-87.

5. MacVinish LJ, Goddard C, Colledge WH, Higgins CF, Evans MJ, Cuthbert AW. Normalisation of ion transport in murine cystic fibrosis nasal epithelium using gene transfer. Am J Physiol 1997; 273:C734-40. [97423429]

6. McKay TR, MacVinish LJ, Carpenter B, Themis M, Jezzard S, Goldin R, et al. Selective *in vivo* transfection

of murine biliary epithelia using selctive polycation-enhanced adenovirus. Gene Therapy 2000; 7:644-52.

7. Gray MA, Pollard CE, Harris A, Coleman L, Greenwell JR, Argent BE. Anion selectivity and the block of the small conductance chloride channel on pancreatic duct cells. Am J Physiol 1990: 259:C752-61. [91051856]

8. Kunzelmann K, Gerlach L, Frobe U, Greger R. Bicarbonate permeability of epithelial chloride channels. Pflugers Arch 1991; 417:616-21.

9. Novak I, Greger R. Properties of the luminal membrane of isolated perfused rat pancreatic ducts. Pflugers Arch 1988: 411:546-53. [88262412]

10. Poulsen JH, Machen TE. HCO_3^--dependent pH_I regulation in tracheal epithelial cells. Pflugers Arch 1996; 432:546-54. [96281574]

11. Hogan DL, Crombie DL, Isenberg JI, Svendsen P, Schaffalitzky-de-Muckadell OB, Ainsworth MA. CFTR mediates cAMP- and Ca^{2+}-actiavted duodenal epithelial HCO_3^- secretion. Am J Physiol 1997; 272:G872-8. [97287792]

12. Geibel JP, Singh S, Rajendran VM, Binder HJ. HCO_3^- secretion in rat colonic crypts is closely linked to Cl^- secretion. Gastroenterology 2000; 118:101-7. [20079124]

13. Zsembery A, Strazzabosco M, Graf J. Ca^{2+}-activated Cl^- channels can substitute for CFTR in stimulation of pancreatic duct bicarbonate secretion. FASEB J 2000; 14:2345-56. [20507653]

14. Lee MG, Wigley WC, Zeng W, Noel LE, Marino CR, Thomas PJ, Muallem S. Regulation of Cl^-/HCO_3^- exchange by cystic fibrosis conductance regulator expressed in NIH 3T3 and HEK 293 cells. J Biol Chem 1999; 274:3414-21. [99121077]

15. Lee MG, Choi JY, Luo X, Strickland E, Thomas PJ, Muallem S. Cystic fibrosis transmembrane conductance regulator regulates luminal Cl^-/HCO_3^- exchange in mouse submandibular and pancreatic ducts. J Biol Chem 1999; 274:14670-7. [99262614]

16. Wheat VJ, Shumaker H, Burnham C, Shull G, Yankaskas JR, Soleimani M. CFTR induces the expression of DRA along with Cl^-/HCO_3^- exchange activity in tracheal epithelial cells. Am J Physiol 2000; 279:C62-71. [20357597]

17. Meyer G, Porta C, Garavaglia M, Cremaschi D. Inhibitors of Cl^-/HCO_3^- exchanger activate an anion channel with similar features in the epithelial cells of rabbit gallbladder: patch-clamp analysis. Pflugers Arch 2001; 441:467-73. [21079733]