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Controversies surrounding the role of CFTR in airway bicarbonate secretion

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Bicarbonate (HCO_3^-) serves many functions in the human body. It is an important biological buffer required for maintaining intracellular and extracellular acid–base balance, and plays a key role in CO_2 transport by blood. Less well known is that HCO_3^- is secreted onto the mucosal surface of most epithelia where it is involved in a myriad of vital processes including sperm capacitation, innate defence, mucin expansion and mucosal protection. An important challenge in epithelial biology has been to understand the molecular coordination of trans-epithelial HCO_3^- secretion and how this simple anion impacts on such a wide variety of physiological processes. Through studies on the inherited disease cystic fibrosis (CF), it has become clear that the plasma membrane protein encoded by the CF gene, cystic fibrosis transmembrane conductance regulator (CFTR), is essential for epithelial HCO_3^- secretion, but only recently have we begun to fully appreciate the complex role that CFTR plays in this process.

Studies over the last decade on epithelia of the pancreas, small intestine, reproductive tract and salivary glands have shown that CFTR regulates HCO_3^- secretion in at least two ways: (i) directly, by conducting HCO_3^- ions, and (ii) indirectly, through regulating members of the SLC26A family of apically located Cl^-/HCO_3^- exchangers (Ko *et al.* 2004, Garnett *et al.* 2011). Loss of CFTR function therefore leads to aberrant HCO_3^- secretion via reduced Cl^-/HCO_3^- exchange activity, as well as through reduced HCO_3^- efflux via CFTR (Ko *et al.* 2004). The (maximal) HCO_3^- content of the primary fluid secretions from these tissues ranges from ∼60 mM in the salivary glands to ∼150 mM in the pancreas.

Most CF patients die of lung failure due to repeated cycles of infection and inflammation that eventually destroy their airways. CFTR is expressed in the airways of the lung, in both the submucosal glands (SMGs) and surface epithelium. A low pH airway surface liquid (ASL) has long been considered as a causative factor in lung bacterial infection, which is the major problem in CF, and a recent paper by Pezzulo *et al.* (2012) has provided the first substantive evidence for this idea. These authors showed that the acidic ASL in the lungs of transgenic CF pigs (the best model of human CF) did not kill bacteria effectively, probably because anti-bacterial proteins secreted onto the airway surface (e.g. lysozyme, lactoferrin and defensins) do not work properly at low pH. They also found that increasing CF airway ASL pH (from ∼ pH 6.9 to 7.4), using a 100 mM $NaHCO_3^-$ aerosol, restored bacterial killing to the rates seen in wild-type animals. Whilst the fundamental defect leading to the reduced ASL pH in CF pig lungs was not investigated by Pezzulo *et al.* (2012) it is almost certain to be a decrease in airway HCO_3^- secretion. Therefore, understanding the mechanism involved in airway HCO_3^- transport has important implications for CF and is currently a hot topic in epithelial physiology.

In a recent paper in the *Journal of Physiology*, Shan *et al.* (2012) provide a detailed study into the mechanism of CFTR-dependent HCO_3^- secretion in the airways. Using Calu-3 cells as a model of the serous cells in human airway SMGs, Shan and colleagues proposed that HCO_3^- is secreted across the luminal membrane of the epithelium solely through CFTR. They demonstrated that HCO_3^- flux stimulated by forskolin (a cyclic AMP agonist) was substantially reduced by pharmacological inhibition of CFTR and, furthermore, that HCO_3^- flux was much lower across Calu-3 monolayers in which CFTR expression had been knocked down compared to control cells. Shan *et al.* (2012) also reported that the HCO_3^- concentration of forskolin-stimulated Calu-3 fluid secretions was approximately 31 mM (pH 7.55). Studies performed on pig bronchi showed that the maximal HCO_3^- concentration of accumulated fluid on the airway surface was ∼25 mM (pH 7.4) and that HCO_3^- secretion was reduced by CFTR inhibition. Removal of either Cl^- or HCO_3^- from solutions bathing *ex vivo* airway SMGs significantly diminished glandular fluid secretion, consistent with a role for both anions in driving airway fluid secretion (see Garnett *et al.* 2011 for references).

One puzzling finding from the Shan *et al.* (2012) study is the relatively low HCO_3^- concentration in the secreted fluid (25–31 mM) compared to the primary secretions from other HCO_3^- secretory epithelia. Whether 25–31 mM HCO_3^- is high enough to allow correct expansion of the mucins secreted by the airway epithelium into a functioning mucous gel is questionable. By studying mucus secretion across the ileal mucosa of wild-type and CF mice, Gustafsson *et al.* (2012) were able to show that the dense, adherent, mucus produced by CF mice (which was mimicked in wild-type mice by inhibiting epithelial HCO_3^- secretion) could be expanded by the addition of luminal HCO_3^-. Moreover, they showed that an HCO_3^- concentration of >69 mM close to the apical side of the epithelium was required for normal mucin expansion. Whether similar HCO_3^- concentrations are necessary for effective mucin release and expansion in the airways remains to be seen, but the Gustafsson *et al.* (2012) study certainly raises doubts as to whether 25–31 mM HCO_3^- would be sufficient.

In contrast to Shan *et al.* (2012), our own studies on Calu-3 cells and those of the Wine group in Stanford have shown that the HCO_3^- concentration of forskolin-stimulated secretions can exceed 75 mM (pH 7.9; see Garnett *et al.* 2011 for references). It is worth noting that the Shan *et al.* (2012) study used 'Alter' Calu-3 cells expressing control short hairpin RNA. These cells had reduced CFTR expression compared to parental cells, which could explain why HCO_3^- secretion was lower if HCO_3^- was exiting via CFTR. However, such a finding does not rule out a role for other transporters whose function or expression is CFTR-dependent. Our group has proposed a different mechanism for HCO_3^- secretion in the airways in which an apical anion exchanger, rather than CFTR, is the main exit pathway for the ion (Garnett *et al.* 2011). Intracellular pH measurements on polarised Calu-3 cell monolayers

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revealed Cl^--dependent changes in pH_i, consistent with the existence of an apical anion exchanger. This forskolin-stimulated exchanger remained active in the presence of the CFTR inhibitor GlyH-101 (when applied together with basolateral 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid to inhibit basolateral anion exchange activity), suggesting that apical HCO_3^- secretion can occur independently of CFTR. The pharmacological inhibitor profile, anion selectivity, immunocytochemistry and quantitative real time PCR studies revealed this apical anion exchanger to be SLC26A4 (pendrin). In confirmation, pendrin knockdown in Calu-3 cells caused a reduced rate of apical anion exchange activity and produced a less alkali fluid secretion, compared to wild-type cells. On the other hand, inhibition or knockdown of CFTR reduced the rate of fluid secretion, but had no effect on pH. These observations suggest that pendrin plays a major role in regulating the amount of HCO_3^- secreted by Calu-3 cells (Garnett *et al.* 2011).

If Cl^-/HCO_3^- exchange is important for airway HCO_3^- secretion, why should HCO_3^- secretion be defective in CF resulting in an acidic ASL? Experiments in other epithelial tissues have indicated that CFTR and SLC26 transporters can interact through their R and STAS domains, respectively (Ko *et al.* 2004). This interaction can be enhanced by R domain phosphorylation by protein kinase A and is modulated by PDZ scaffold proteins. These molecular interactions mutually stimulate the transport activities of both CFTR and the anion exchanger, resulting in enhanced HCO_3^- and fluid secretion. If such a structural interaction between CFTR and SLC26 anion exchangers also occurs in the airway SMGs, the absence of functional CFTR in CF would lead to a down-regulation of apical Cl^-/HCO_3^- exchange and HCO_3^- secretion. However, our studies indicate dysregulation of not only apical but also basolateral anion exchange in CF airway cells, where the latter activity would tend to short-circuit luminal HCO_3^- secretion (Garnett *et al.* 2011). In contrast, Shan *et al.* (2012) argue that the activity of localised carbonic anhydrase close to the apical membrane is sufficient to supply HCO_3^- for secretion and, as such, an active basolateral anion exchanger should not affect apical HCO_3^- transport. The reasons for the discrepancies between our own study (Garnett *et al.* 2011) and that of Shan *et al.* (2012) are unclear at the moment, but raise some pertinent questions about the processes involved in airway HCO_3^- secretion and the role of pendrin, a transporter which we have shown is expressed in both submucosal gland serous cells and surface airway epithelia of native human tissue (Garnett *et al.* 2011).

Finally, if the Cl^-/HCO_3^- exchanger pendrin is a key transporter for HCO_3^- secretion in human airways then it is a potential therapeutic target, perhaps for a drug to be used either as an adjunct to gene therapy or in combination with the recently developed Vertex CFTR potentiators and correctors. Increasing the HCO_3^- concentration in the ASL of CF patients is almost certain to be beneficial in terms of treating their lung disease. Therefore, a better understanding of the mechanisms that orchestrate airway HCO_3^- secretion is a priority in CF airway epithelial physiology.

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