Understanding the cellular mechanism for inhaled hyperosmotic saline therapy for patients with cystic fibrosis. Focus on “Effect of apical hyperosmotic sodium challenge and amiloride on sodium transport in human bronchial epithelial cells from cystic fibrosis donors”

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Since the first clinical trials on inhaled hyperosmotic saline therapy (HS) in cystic fibrosis (CF) patients (3, 9), HS has proven to substantially improve a number of critical measures of lung function and, thus, is now considered a first-line therapy for these patients (2).

One mechanism believed to link CF genotype to phenotype is abnormalities in properties of the airway surface liquid (ASL). Understanding how important ASL is to airway hydration and mucociliary clearance has constituted a driver for extensive study of airway epithelia. Airway surfaces are covered with ASL, a thin layer of fluid consisting of periciliary liquid or sol layer with a viscous gel layer above. ASL functions to trap particulate matter in the mucus layer and move the contents cephalad by ciliary activity so that ultimately the contents are swallowed or expectorated. In normal airways, the surface epithelium and submucosal glands generate ASL, which contains, in addition to ions, antimicrobial and immune defense molecules (Fig. 1). In normal airways, the volume of ASL is a function of the highly water-permeable airway epithelia and active transport processes that control NaCl mass on airway surfaces. Isotonic ASL is maintained by absorbing Na⁺ through sodium channels (ENaC) and Cl⁻ via the paracellular pathway with water moving passively by osmosis through airway epithelial water channels. Application of HS to normal airway surfaces results in an electrochemical driving force favorable for Cl⁻ absorption across the apical membrane. In this new environment, Na⁺ and Cl⁻ are absorbed rapidly via ENaC and CF transmembrane conductance regulator (CFTR) or Ca-regulated Cl channels, respectively, with a small movement of water to the lumen.

In cystic fibrosis, mutations in the CFTR impact cAMP-mediated Cl⁻ secretion and also increase ENaC-mediated ion absorption in airway epithelium (1). One consequence is increased Na⁺ and Cl⁻ absorption generated by a high rate of transcellular Na⁺ absorption, which increases osmotic water absorption from the airway lumen to the submucosa via water channels. This leads to severe dehydration of ASL in which the sol layer is significantly diminished and the interaction of the gel phase with cilia becomes ineffective, leading to an environment favorable for infection, notably by Pseudomonas aeruginosa or Burkholderia cepacia (1, 8). Application of HS to CF airway surfaces increases the electrochemical gradient for Na⁺ absorption via ENaC but, due to lack of CFTR ion channel function, leaves the paracellular pathway as the only path to absorb Cl⁻. This results in a slower rate of NaCl absorption, retention of more NaCl on the surface epithelium in CF compared with normal epithelium, and thus a protracted osmotic gradient favoring water flow to the lumen. For the CF patient, the net effect is an increase in ASL volume leading to improved mucociliary clearance, lung function, and inflammatory responses (10).

The success of HS therapy hinges on the passive permeability properties of the epithelia, i.e., independent of physiologic active ion transport. Attempts to improve HS therapy by...
use of amiloride to inhibit ENaC failed; instead, amiloride blunted the responses in CF epithelium (3). This contradictory observation generated questions regarding the cellular mechanism for HS therapy and on actions of amiloride, leading to numerous studies on fluid movement in surface airway epithelia (1).

In this issue of American Journal of Physiology-Cell Physiology, Rasgado-Flores et al. (7) report the effects of HS on $\text{Na}^+$ transport ($I_{\text{Na}}$), conductance ($G_T$), and capacitance ($C_T$) with a focus on amiloride-sensitive short circuit current ($I_{sc}$) in human bronchial epithelial cells. Unique to this study is the application of three experimental approaches: conventional Ussing chambers to measure $I_{Na}$, continuously perfused Ussing chambers, and near “thin-film” experiments in Transwell inserts. The experimental approach for these studies is technically challenging, yet impressive for yielding compelling results that increase our understanding of HS therapy in cystic fibrosis and also in other airway disorders characterized by dehydration of ASL, such as asthma and exercise-induced bronchoconstriction. Experiments with Ussing chamber and thin-film approaches yielded similar outcomes favoring the conclusion of inhibition of $I_{Na}$ by HS. Equally important is the finding that this inhibition outlives the exposure to HS, thus offering an explanation for the long-term beneficial effects of HS therapy in CF patients. Inclusion of amiloride with HS led to an increased recovery of $I_{Na}$, thus paradoxically protecting the epithelium from the inhibitory effects of HS, as reported by others (3). Amiloride, however, did not affect recovery of osmolality at the apical surface, thus arguing against inhibition of water channels, which corroborates previous reports (5).

Many questions remain regarding the mechanism of HS therapy. Of particular interest is regulation of membrane expression and activity of ENaC. HS is thought to shrink cells, thus increasing intracellular sodium concentration [Na$^+$]. Does the elevated [Na$^+$]$_i$ provide feedback on ENaC activity and/or its membrane expression? The decrease in $C_T$ induced by HS would support this possibility, yet there are other explanations. One is membrane expression of ENaC; HS may increase ENaC endocytosis or prevent insertion of inactive ENaC channels stored in intracellular vesicles. How amiloride produces its effects on ENaC is still incompletely understood. The authors propose a model that can be tested using the three experimental approaches in the current study so as to probe additional regulatory factors, such as SPLUNC1 (4) and prostatin (6), for their effects on ENaC activity and membrane trafficking. Further studies can assess HS therapy combined with other therapeutics that improve CF lung function. In conclusion, HS therapy gains further support as front-line therapy in cystic fibrosis patients.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author.

**AUTHOR CONTRIBUTIONS**

C.M.L. prepared the figure; drafted, edited, revised, and approved the final version of manuscript.

**REFERENCES**