Commentary

Surprises from the airway epithelium

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The epithelium of the pulmonary airways subserves a wide range of functions, from providing a barrier against inhaled particles and pathogens to transmitting signals to subepithelial cells. Given the central place of the epithelium in respiratory tract pathophysiology, the molecular determinants of epithelial function are appropriately the focus of intense scrutiny. In a recent issue of PNAS, two groups present important and surprising findings arising from primary observations in the airway epithelium. Krane and colleagues (1) demonstrate that mice with a deletion of the aquaporin-5 (AQP5) gene manifest greater airway constriction to cholinergic stimulation than wild-type animals. Huang and colleagues (2) describe compartmentalized signaling at the apical membrane of airway epithelial cells that regulates activity of the cystic fibrosis transmembrane conductance regulator (CFTR). Each of these observations will likely prove to be of great interest across a range of scientific disciplines.

AQPs are water-specific membrane channel proteins. Of the 11 mammalian AQPs identified to date, four (AQP1, AQP3, AQP4, and AQP5) are expressed in the respiratory tract, with predominantly nonoverlapping cellular and subcellular distributions. In the rat respiratory tract, AQP5 is present in the apical membrane of type I pneumocytes and secretory cells of submucosal glands in nasopharynx and proximal airways (3). This distribution is similar in the human respiratory tract, but in contrast to rat, AQP5 is also present in the surface epithelium of human nasopharynx and proximal airways (4). Krane and colleagues (1) now report that the mouse lung exhibits yet another pattern of AQP5 distribution. In mouse, AQP5 was localized to both the apical and basolateral membrane of ciliated and secretory cells in the trachea; the apical membrane of ciliated cells in the bronchi; basal cells of the bronchi and lobar bronchioles; and both type I and type II pneumocytes in the alveolus. Several of these differences are of note. Previously, AQP5 has been described almost exclusively as an apical membrane protein in the lung, as well as in corneal epithelium and secretory cells of salivary and lacrimal glands (3–5). Type II pneumocytes in rat clearly do not express AQP5 (3, 6) and likely do not in humans (4). Finally, basal cells in proximal airway epithelium of the rat and human respiratory tract express AQP3, not AQP5 (3, 4). These species differences in AQP5 distribution in the respiratory tract raise interesting questions from the perspective of comparative physiology. Do lung epithelial water transport requirements differ between species, and if so, why? What targeting mechanisms dictate AQP5 distribution in different cells? The potential relevance of the latter is emphasized by recent descriptions of an apparent AQP5 trafficking defect in lacrimal and salivary glands of humans with Sjögren's syndrome, who suffer from dry eyes and dry mouth (7, 8). And what are the implications of species differences in AQP distribution for modeling human pathophysiology? Although provocative, these histologic findings are not the most significant aspect of this study.

Krane and colleagues (1) examined airway responsiveness to cholinergic stimulation. In anesthetized, mechanically ventilated animals, total lung resistance and dynamic compliance—measures of airway function—were similar at baseline between wild-type and AQP5-null mice. Both groups responded to i.v. acetylcholine with bronchoconstriction, but the magnitude of airway constriction was significantly greater in the AQP5-null mice (Fig. 1). Airway responsiveness also was examined in awake, spontaneously breathing animals using whole-body barometric plethysmography. The enhanced pause (Penh) is a unitless measure that correlates with changes in airway resistance in response to methacholine challenge (9). Consistent with the findings in anesthetized animals, the Penh was similar in the two groups at baseline. In response to inhaled methacholine, AQP5-null mice had a significantly greater increase in Penh than wild-type animals, reflecting enhanced bronchoconstriction. Therefore, as assessed by two technically different measures, AQP5-null mice were found to have greater airway constriction to cholinergic stimulation than wild-type animals. This finding is very surprising.

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The mechanisms underlying increased bronchoconstriction in AQP5-null mice are not defined in this study. Although the magnitude of the airway responses to acetylcholine and methacholine were clearly greater in AQP5-null mice, the dose–response curve was at most only subtly shifted leftward compared with wild-type mice, suggesting that the observed differences are not explained by changes in receptor function or sensitivity. Additionally, both constriction and relaxation in isolated tracheal rings were similar in the two groups, suggesting that the smooth muscle was not intrinsically different between the groups.

Given that AQP5 is a membrane water channel protein, it is natural to speculate about potential roles for altered water homeostasis in this process. Debate continues as to whether edema alone can precipitate bronchoconstriction, but several lines of evidence suggest that regional changes in lung water can exacerbate airway constriction. Airway smooth muscle contraction produces buckling of the underlying mucosa, with extension of mucosal ridges into the lumen and loss of cross-sectional area (10, 11). Fluid either entering or retained in the airway can fill the intraluminal interstices and ridges, further reducing airway caliber and increasing resistance (12). Fluid accumulation in the peribronchiolar space has been postulated to uncouple the smooth muscle from the elastic forces of the surrounding lung parenchyma, forces that might otherwise tether the smooth muscle and limit constriction. Finally, increased fluid in the airway, epithelium, or interstitium may

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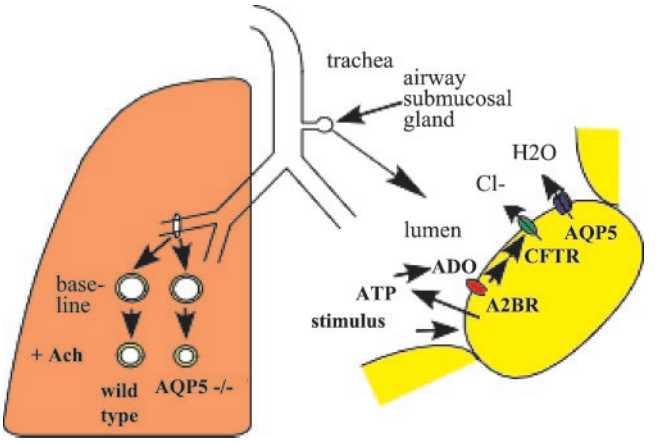

Fig. 1. Schematic of the findings from Krane *et al*. (1) and Huang *et al*. (2). In AQP5-null mice (*Left*), airway resistance was similar at baseline between wild-type and AQP5-null animals. After stimulation with i.v. acetylcholine (Ach), AQP5-null mice exhibited greater airway constriction than wild type. Huang *et al*. examined the mechanism of chloride secretion in response to a physical stimulus in Calu-3 cells, a model of human airway submucosal gland cells (*Right*). After stimulation, cells release ATP that is converted to adenosine (ADO) and binds to the adenosine receptor (A_2BR). Subsequent signaling events are restricted to the apical membrane, leading to chloride secretion through CFTR (2). Although Huang *et al*. do not present data on AQP5, it is known to be present in airway submucosal glands (3, 4), and likely contributes to water secretion from the gland.

amplify the airway obstruction produced by any degree of smooth muscle constriction (11, 13–15). The wet-to-dry weight ratios of the lungs in AQP5-null mice were not different from wild-type animals, suggesting no gross differences in total lung water between the groups. Nonetheless, given that airway resistance increases by $1/\text{radius}^4$ (a 10% reduction in airway radius leads to a 50% increase in resistance), subtle decreases in airway luminal caliber caused by edema anywhere between the smooth muscle and airspace might be sufficient to provide a mechanical basis for the enhanced responses to stimulated bronchoconstriction observed in these studies.

Changes in surface layer osmolality and regulatory volume changes in surface epithelial cells have been postulated as key events in the pathogenesis of some forms of asthma, for example exercise- or hyperpnea-induced asthma (16). In this model, increased airway surface layer osmolality resulting from evaporative water losses triggers mediator release and airway constriction. Based on both function and distribution, it is highly likely that AQP5 participates in generation or modulation of the airway surface layer. Consistent with such a role, AQP5-null mice have reduced pilocarpine-stimulated secretion from airway submucosal glands compared with wild-type animals (17). Although it is appealing to speculate that AQP5-mediated changes in surface liquid composition or volume might contribute to the observed differences in Krane *et al*.'s studies (1), the precise role of AQP5 in acute, cholinergic-stimulated bronchoconstriction remains unclear. Elucidation of the relevant mechanisms in this model should provide novel insights into the pathogenesis of airway constriction.

Huang and colleagues (2) address fundamental questions relevant to mucociliary clearance in the airway epithelium. Their studies were performed in Calu-3 cells, a commonly used model of human airway submucosal gland serous cells. The investigators examined mechanisms by which physical stimuli initiate intracellular signaling, as well as compartmentalization of that signaling to the apical membrane of the cell. In response to addition of hypotonic medium, Calu-3 cells secrete chloride across the apical membrane. Based on previous work by these and other investigators, a role for cellular nucleotides in this signaling pathway was postulated (18, 19). Consistent with this, ATP, ADP, AMP, and adenosine were detected in luminal fluid secreted by Calu-3 cells. However, because Calu-3 cells do not express $P2Y_2$ nucleotide receptors (20), adenosine was considered a primary candidate to mediate this response. Addition of adenosine deaminase to the luminal fluid eliminated chloride secretion in response to hypotonic medium, and exogenous adenosine analogs could stimulate chloride secretion. Curiously, exogenous adenosine produced only a fraction of the increase in cAMP measured after stimulation of cells with forskolin, despite similar magnitudes of chloride secretion. These findings suggested that in contrast to whole-cell stimulation with forskolin, adenosine-stimulated chloride secretion resulted from localized regulation of CFTR at the apical membrane.

Using patch clamp of single-channel CFTR as the readout for signaling activity, Huang *et al*. (2) demonstrated that inclusion of an inhibitor of adenosine formation (AMPCP) or an adenosine receptor antagonist (8-SPT) in the pipette markedly decreased basal CFTR activity, whereas addition of 100 μM adenosine outside the pipette had no effect on CFTR activity inside the pipette. These findings confirmed local regulation of CFTR activity and strongly suggested that other components of the signaling machinery likely were present in the apical membrane. Using excised outside-out apical membrane patches, the investigators demonstrated that adenosine-stimulated CFTR activity was inhibited by inclusion of a protein kinase A inhibitor in the pipette, as well as by replacement of GTP with GDP. Finally, inhibition of adenosine-stimulated CFTR activity in excised inside-out membrane patches by an adenylyl cyclase inhibitor (SQ22,536) confirmed the presence of adenylyl cyclase in the apical membrane. Taken together, these studies demonstrate the presence of an autocrine pathway for regulation of CFTR that is fully contained in the apical membrane of Calu-3 cells and can thereby markedly limit the spread of signaling events in response to local stimulation (Fig. 1). These findings help us conceptualize how cells can regulate multiple functions, requiring distinct signaling events, in parallel.

Other than that they are both extremely interesting, why comment on these two seemingly unrelated studies together? The answer is the airway fluid. The existence of water-specific membrane channel proteins predicts that in select sites and circumstances, membrane water permeability will be specifically rate-limiting and independently regulated. Although the mechanisms of enhanced bronchoconstriction in AQP5-null mice are not yet defined, the function and distribution of AQP5 portend that disruption of water homeostasis in and/or around the epithelium will be a central component. The downstream result of the signaling events described by Huang *et al.* (2) is secretion across the apical membrane of airway epithelial cells. To consider that mechanisms regulating solute transport and water permeability in the airway epithelium might prove to be related is not such a reach. Examples of this have recently been described. Hypertonic stress up-regulates a variety of transporters and enzymes that increase intracellular organic solutes (21). Hypertonic stress also induces extracellular regulated kinase (ERK)-dependent expression of AQP5 in a

mouse lung epithelial cell line (22) and increases AQP1 abundance in BALB/c fibroblasts by decreasing ubiquitination and increasing stability of the protein (23). The intimate association of water and solute transport may involve complex interactions between several membrane proteins; however, the possibility that these may contribute to important clinical disorders such as asthma or cystic fibrosis is clearly raised by these studies (1, 2).

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