Commentary

Amiloride-sensitive sodium channels contribute to the woes of the flu

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Major discomforts with influenza infections and other types of respiratory infection are runny nose, airway congestion, and accumulation of fluid within the eustachian tubes and middle ear (1). The amount of fluid in the airway is determined by a combination of ion channels that promote the secretion of salt and water, such as the cystic fibrosis (CF) transmembrane regulator (CFTR), and those that promote the absorption of fluid, such as the amiloride-sensitive epithelial sodium channel (ENaC). The fetal lung is filled with fluid, but the epithelium of the adult airways normally creates and maintains only a thin layer of periciliary fluid that lines the surface epithelial cells (2). This periciliary fluid provides a watery microenvironment that enables cilia to function to clear mucus from the airway, a process vital for the lung to defend itself against infections. Too much fluid can cause respiratory distress, as noted by Barket et al. in premature infants with reduced sodium absorption (2). On the other hand, too little fluid can cause dry airways and reduced mucociliary clearance, such as in CF, a disease associated with impaired mucociliary clearance, abnormal mucous, and chronic infections and inflammation (3).

In this issue of PNAS, Kunzelmann et al. show that influenza virus inhibits amiloride-sensitive epithelial sodium currents across mouse tracheal epithelium with a half-time of about 60 min (4). Significantly, the inhibitory effect of influenza virus is receptor mediated, occurring by direct binding of viral hemagglutinin to a cell-surface receptor and subsequent activation of protein kinase C (PKC). It is well known that diarrhea-causing pathogens often enhance fluid secretion in the gut by directly stimulating secretory pathways (5). For example, Escherichia coli heat-stable toxin causes fluid loss in the intestinal tract by binding and activating cell-surface guanylate cyclase receptors that stimulate guanylate cyclase activity, enhancing chloride-mediated fluid secretion (6) and blocking sodium-mediated fluid absorption via sodium, proton exchanger-3 (NHE3). The Kunzelmann report is significant because it is the first demonstration that pathogens can alter fluid balance in the airway by inhibiting fluid absorption via an amiloride-sensitive transport pathway.

ENaC plays a key role in regulating the amount of periciliary fluid that bathes the surface epithelium (7, 8). Airway surface fluid secretion is driven by salt secretion that depends on CFTR. On the other hand, fluid absorption depends on the action of the ENaCs (8). The effect of inhibiting ENaC function on fluid balance in lung has been demonstrated in knockout mouse models. ENaC is comprised of three subunits, α, β, and γ, which have varying roles in the airway (9). Inactivation of αENaC results in failure to clear fetal lung liquid at birth and in early neonatal death (10). In contrast, neonatal βENaC-deficient mice have respiratory failure and exhibit only a small increase in wet lung weight at birth (11). Newborn mice lacking the γENaC subunit clear lung liquid more slowly than normal mice, but lung water at 12 h is nearly normal (12). These studies suggest that the α subunit is most critical for the absorption of lung fluid at birth, but that β- and γENaC may only enhance neonatal lung liquid clearance in mice. This is in contrast to the kidney, where all subunits are necessary for normal body sodium balance (10–13).

In humans, autosomal recessive mutations of either α-, β-, or γENaC subunits cause pseudohypoaldosteronism type 1 (11). This renal salt-wasting disorder is associated with severe hypovolemia, high plasma aldosterone, hypokalemia, life-threatening hypokalemia, and metabolic acidosis. Kerem et al. (14) tested respiratory fluid transport in patients with pseudohypoaldosteronism. These patients have no sodium absorption from airway surfaces and a volume of airway surface liquid that is more than twice the normal value. Patients 5 years of age or less have recurrent episodes of chest congestion, coughing, and wheezing. Interestingly, pa-
sorptive (15). The importance of these channel, cyclic nucleotide-gated cation channels, is also abundant in lung and plays a role in transepithelial sodium absorption (15). The importance of these channels was shown in sheep lungs artificially perfused in situ (16). The airspace of the lungs were filled with liquid containing an impermeant tracer to allow measurement of the rate of net transepithelial liquid movement under various conditions. In the lungs of sheep aged 6 months, $10^{-4}$ M amiloride inhibits the resting absorption of liquid by about 70%, and the addition of either dichlorobenzamil or pimozone, blockers of cyclic nucleotide-gated channels, inhibits the remaining fluid absorption to cause net fluid secretion. However, in the lung of late gestational lambs, $1.5 \times 10^{-5}$ M dichlorobenzamil does not exert an additive effect to that of amiloride. Therefore, cyclic nucleotide-gated cation channels drive a component of lung liquid absorption in older sheep but not in neonatal animals, implying further that amiloride-sensitive channels are the crucial step in the early stages of adaptation to air breathing. All of the studies discussed above point out that inhibition of amiloride-sensitive sodium channels leads to increased fluid in airways.

Although Kunzelmann et al. (4) did not test the effect on cyclic nucleotide-gated channels, influenza virus had no effect on chloride secretion caused by either forskolin or carbachol, which increase intracellular cAMP or intracellular calcium, respectively. Nor was there any effect on the component of the absorption carried by the Na$^+$-glucose carrier. This suggests that the receptor for the influenza virus specifically targets the amiloride-sensitive sodium absorptive pathway. Thus, given the involvement of ENaC in neonatal lung fluid transport, if the effect of influenza virus is restricted to ENaC, as noted by Kunzelmann et al., the impact on lung fluid balance may be more severe in younger individuals.

The receptor-mediated inhibition of amiloride-sensitive currents by influenza virus is mediated through PKC. PKC inhibition of ENaC has been observed in other tissues and in recombinant ENaC expressed in oocytes. For example, amiloride-sensitive Na$^+$ currents generated after coexpression of $\alpha$, $\beta$, and $\gamma$ subunits of renal ENaC are reduced by about 80% by PKC. When studied in bilayers, PKC causes a 70% decrease in channel open probability generated of a combination of $\alpha$, $\beta$, and $\gamma$ subunits of renal ENaC. These studies indicate a direct effect of PKC on ENaC activity (17). Eaton and collaborators (18), by using subunit-specific antibodies, studied the regulation of ENaC subunit protein levels by PKC in A6, a renal epithelial cell line. In this cell line, PKC activation decreases the levels of both $\beta$ and $\gamma$ subunit protein but not that of $\alpha$ENaC. Reduction in protein levels by PKC also results in a decrease in transepithelial sodium absorption in A6 cells for up to 48 h. These results show directly for the first time that PKC differentially regulates ENaC subunit levels. If these studies can be extrapolated to airway tissue, this would suggest that the inhibition of amiloride-sensitive currents in airway cells induced by influenza virus could occur by reducing ENaC channel activity as well as by reducing protein expression of individual subunits. However, contrary to results in kidney, the relatively modest role that the $\beta$ and $\gamma$ subunits play in airway sodium absorption suggests that to have the greatest effect, PKC would have to modulate the activity or protein expression of the $\alpha$ENaC subunit.

It is well known that CFTR acts as conductance regulator, influencing the functional regulation of ENaC (4, 18, 19, 20). For example, when the three ENaC subunits were expressed in Madin–Darby canine kidney cells, they generated amiloride-sensitive sodium currents that were stimulated by cAMP, whereas coexpression of human CFTR with ENaC generates smaller basal sodium currents that are inhibited by cAMP (19). CFTR seems to act as a molecular switch that controls the way cAMP-dependent kinase affects ENaC. In CF airway epithelia, where the regulatory function of CFTR is absent, the result is an up-regulation of amiloride-sensitive currents in the presence of cAMP (19). The overall effect of the abnormal up-regulation of ENaC in patients with CF is enhanced sodium and fluid absorption and drier-than-normal airways.

The significance of the Kunzelmann report is that it provides an explanation of some symptoms of respiratory infections, such as influenza. The work further points to the importance of sodium channels in maintaining salt balance in the airway. Beyond this, it also demonstrates that viral hemagglutinin receptors are expressed in the airway cells that regulate sodium transport in the airway. These receptors may provide new targets for therapeutic strategies to block the hyperabsorption of sodium and fluid in CF. Indeed, a preliminary clinical trial comparing the effects of 5 mM amiloride aerosolized in a volume of 3.5 ml four times daily with that of vehicle alone showed less decrease in lung function associated with CF as well as decreased sputum viscosity and elasticity. These findings suggest that one beneficial clinical effect of amiloride is to increase fluid in the airways (21), and they provide some support for the concept that blocking sodium absorption may be beneficial. On the other hand, these preliminary studies also showed that amiloride action is short lived, thus eliminating it as a reasonable therapeutic agent. Therefore longer-acting drugs must be developed to block sodium absorption in the lung. Thus, the main benefit of this study will be to focus our attention more closely on regulatory pathways, like PKC, that lead to sodium absorption and the mechanisms underlying how these regulatory pathways may alter fluid balance in respiratory infections and in CF.

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