Long-term stimulation of alveolar epithelial cells by β-adrenergic agonists: increased Na⁺ transport and modulation of cell growth?

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CONSIDERABLE PROGRESS HAS BEEN MADE IN THE past 20 years regarding our understanding of pulmonary edema resolution. It is now relatively well accepted that this process, which might contribute to the prognosis of lung injury patients (41), is dependent on active transepithelial Na⁺ transport (25). Insights into the cellular mechanism governing transepithelial Na⁺ transport from the alveolus to the interstitial space have come from a multitude of cellular and physiological studies. Na⁺ enters the cells by amiloride-sensitive Na⁺ channels or other cation channels in the apical membrane (24) and is extruded by Na⁺-K⁺-ATPase on the basolateral membrane (40). The process is believed to occur mainly in alveolar type II cells (23), although there is accumulating evidence that alveolar type I cells might also play an important role (17, 32).

β-Adrenergic agonists were the first agents shown to modulate Na⁺ transport and alveolar liquid clearance. More than 20 years ago, terbutaline, the agent used most often, was demonstrated to stimulate transepithelial Na⁺ transport and dome formation in cultured alveolar type II cells (11). β-Adrenergic agonists have also been reported to augment unidirectional Na⁺ transport across the alveolar epithelial barrier in vivo (12, 35). Terbutaline increases alveolar and lung liquid clearance in the normal lung (2) and in mild lung injury (1). In fact, it was recently proposed that even inhaled β-adrenergic agonists could stimulate alveolar liquid clearance (10, 34). Furthermore, salmeterol, a long-acting β-adrenergic agonist, has been deployed to prevent high altitude pulmonary edema (36).

Although β-adrenergic agonists are now well known to modulate Na⁺ transport in the lung, their mechanism of action is still poorly understood. Because Ussing chamber experiments clearly showed that β-adrenergic agonists increased amiloride-sensitive short-circuit current in cultured alveolar type II cells (4), it was initially believed that their effect was due to direct modulation of Na⁺ channels at the apical surface of the cells. In fact, protein kinase A (PKA) was found to directly alter the activity of a cation channel (24, 43). The increased activity of this channel was postulated to heighten Na⁺ entry into the cell, leading to elevated [Na⁺]; concentration, which would activate Na⁺-K⁺-ATPase (Fig. 1). Overall, the outcome would be enhanced transepithelial Na⁺ transport.

However, recent experimental data have challenged this hypothesis. First, it is noteworthy that the expression of Na⁺ and cation channels on the cell surface is highly dependent on the culture conditions of alveolar type II cells (15). Furthermore, some data suggest that different combinations of the various epithelial Na⁺ channel (ENaC) subunits could produce channels with different electrophysiological properties (selectivity, conductance, etc.). The nonselective cation channels identified in cultured alveolar type II cells might be composed of only α-ENaC (24). Although PKA can modulate the activity of a cation channel in lung epithelial cells, we cannot be certain that this channel is responsible for transepithelial Na⁺ transport in alveolar epithelial cells.

Second, recent experimental data indicate that increased Na⁺ transport is secondary to augmented Cl⁻ transport at the apical surface of the cells (16, 24). The increment of Cl⁻ transport would follow cystic fibrosis transmembrane conductance regulator activation by cAMP (24). Augmented Cl⁻ influx in the cells would create an electrical gradient that would then favor Na⁺ absorption (Fig. 1). This hypothesis is supported by the fact that the cystic fibrosis mouse cannot increase lung liquid clearance in response to β-adrenergic agonist treatment (9). Another postulated mechanism is that changes in [Cl⁻] concentration could lead to altered sensitivity of the nonselective cation channel to calcium. This activation of the nonselective cation channel would enhance Na⁺ absorption (22). This response would also be linked to modifications in alveolar type II cell volume (14, 27).

Another potential mechanism by which β-adrenergic agonists could modulate Na⁺ transport is by membrane insertion of ENaC (5, 24), the highly selective Na⁺ channel present in the cells (Fig. 1). It is widely accepted that this channel is regulated by its membrane trafficking (33, 37). In fact, dysregulation in channel trafficking and from the membrane can evoke a genetic form of hypertension (Liddle’s syndrome) or a metabolic disorder (pseudohypoaldosteronism type I) (37). In Liddle’s syndrome, mutations in the COOH terminus of either β- or γ-ENaC elicit defective channel internalization and degradation. This abnormality is due to disruption of the interaction between Nedd4, a molecule involved in protein ubiquitination, and ENaC (37). Defective channel degradation leads to an increased number of channels at the cell surface.
with augmented Na⁺ transport. In contrast, in pseudohypoaldosteronism type I, mutations in the channel culminate in its decreased expression at the cell surface and reduced Na⁺ absorption (37). Although very little is known about membrane trafficking of ENaC to the cell surface of alveolar epithelial cells, hypoxia has recently been shown to depress the membrane expression of ENaC (30). Furthermore, β-adrenergic agonists can correct this hypoxia-induced decline in ENaC expression (30). Although the mechanism by which β-adrenergic agonists modulate the surface expression of ENaC is not known, it could be related to the potential interaction among cAMP, PKA, and cytoskeleton proteins (24). In addition, other molecules involved in the membrane trafficking of ENaC, such as Sgk (19), could be modulated by cAMP and PKA (29).

Vectorial Na⁺ transport is also highly dependent on Na⁺-K⁺-ATPase activity (1, 40). In fact, Na⁺-K⁺-ATPase dysfunction has been detected in some lung injury models (1, 40). Furthermore, although the rate-limiting step in Na⁺ absorption is said to be the apical entry of Na⁺ into the cells, there is growing evidence that the coregulation of both systems is important for coordinated Na⁺ absorption (6). It then becomes important to determine whether β-adrenergic agonists modulate not only the apical entry of Na⁺ into the cells but also its extrusion at the basolateral surface. In fact, β-adrenergic agonists augment Na⁺-K⁺-ATPase activity, and this effect is not related to increased apical transport of Na⁺, indicating direct activation of Na⁺-K⁺-ATPase by cAMP (39). Recent studies have demonstrated that the effect is potentially mediated, as for ENaC, by membrane trafficking of the molecule (3). This mechanism is dependent on dynamic interaction between Na⁺-K⁺-ATPase and the cytoskeleton machinery (40).

β-Adrenergic agonists not only modulate Na⁺ transport via a short-term regulatory mechanism (activity or membrane insertion) but they also have a long-term regulatory impact on Na⁺ transport (transcriptional regulation). It has been shown that β-adrenergic agonists can heighten the expression of ENaC and Na⁺-K⁺-ATPase (Fig. 1) (26). Unlike steroids, where the action on ENaC and Na⁺-K⁺-ATPase transcription has been relatively well characterized, the mechanism leading to increased mRNA or protein expression after long-term β-adrenergic stimulation is not as clearly defined (6). It has been suggested recently that the increase in Na⁺-K⁺-ATPase protein after long-term stimulation by β-adrenergic agonists might be related to posttranscriptional regulation involving the mTOR pathway (40).

In the report from Pesce et al., one of the current articles in focus (Ref. 29a, see p. L802 in this issue), the authors provide further evidence for this concept of posttranslational regulation of Na⁺-K⁺-ATPase expression. They have shown that stimulation of alveolar epithelial cells with β-adrenergic agonists for 3 days regulates Na⁺-K⁺-ATPase expression via activation of the MAPK and rapamycin-sensitive pathways [mammalian target of rapamycin (mTOR)], since pretreatment with a MAPK inhibitor (U-0126) or rapamycin (mTOR) suppresses the response. Because mTOR has been implicated in the regulation of translation through activation of the protein S6 kinase (p70S6k) enzymatic complex (8), its potential role in the response was investigated. Stimulation with β-adrenergic agonists induced p70S6k phosphorylation via MAPK and rapamycin-sensitive pathways. To establish a link between p70S6k phosphorylation and increased Na⁺-K⁺-ATPase, these authors transfected a dominant negative form of p70S6k and a rapamycin-resistant form of p70S6k. In cells transfected by the dominant negative form of p70S6k stimulation of Na⁺-K⁺-ATPase expression was inhibited. Furthermore, in the presence of the rapamycin-resistant form of p70S6k, the increased ex-
pression of Na⁺-K⁺-ATPase by β-adrenergic agonists could not be suppressed by rapamycin. These data strongly suggest that ribosomal S6 kinases are essential for the modulation of Na⁺-K⁺-ATPase expression after β-adrenergic agonist stimulation. Their paper is one of the first to investigate in depth the intracellular signaling pathway leading to changes in Na⁺-K⁺-ATPase expression after long-term β-adrenergic agonist stimulation. Pesce et al. have dissected, in great detail, the potential pathways involved. However, they did not determine whether p70s6k activation really leads to increased Na⁺-K⁺-ATPase activity or transepithelial Na⁺ transport. Thus although there is inhibition of the increase in Na⁺-K⁺-ATPase expression with suppression of p70s6k, no evidence is provided to show that it elicits a decreased response in Na⁺ transport across the monolayer. Experiments are required to establish the importance of this mechanism in the modulation of Na⁺ transport by long-term treatment with β-adrenergic agonists. Nevertheless, their work clearly demonstrates that researchers need to consider translation as an important regulatory mechanism of expression of ion transport molecules in alveolar epithelial cells and most probably airway epithelial cells (Fig. 1). Seventy-kilodalton ribosomal S6 kinase is an ubiquitously expressed serine/threonine kinase that phosphorolyses 40S ribosomal protein S6 (8). This phosphorylation of S6 upregulates the translation of mRNAs with 5’-terminal oligopyrimidine tracts, many of which encode for proteins that can enhance the translational capacity of the cells (8). The system responds to mitogen stimulation and seems to be directly linked to cell growth, differentiation, and survival (8). Overall, these observations suggest that β-adrenergic agonists might be crucial for lung epithelial repair and that modulation of ion transport might be important in this process. In fact, there is now experimental evidence that Na⁺-K⁺-ATPase can act as a signal transducer to regulate genes and cell growth (42). Furthermore, it has been shown that Na⁺-K⁺-ATPase inhibition can modulate apoptosis in vascular smooth muscle cells by changing the ratio of [Na⁺]/i and [K⁺]/i (28).

The possibility that β-adrenergic agonists are involved in cell growth has been the subject of intense investigation in recent years, especially in the cardiovascular system (20). These studies have revealed that the initiation of proliferation by G protein-coupled receptors (GPCRs), as with β-adrenergic receptors, appears to involve the phosphorylation of receptor tyrosine kinases via a transactivation mechanism (13). Although the exact mechanism remains to be defined, it is probably related to autocrine activation of the epidermal growth factor receptor (EGFR). In fact, it is now suggested that on GPCR activation, heparin-binding epidermal growth factor-like growth factor (HB-EGF) is cleaved from the cell surface by a metalloprotease, with soluble HB-EGF then activating EGFR (13). This would lead to phosphatidylinositol 3-kinase (PI 3-kinase) activation, a system that mediates the critical processes of cell growth, proliferation, and survival (18). Although it is not the main focus of the paper by Pesce et al. (29a), there is some evidence that EGFR transactivation is involved in p70s6k activation by β-adrenergic agonists (Fig. 1). They have shown that MAPK activation, which is necessary for p70s6k phosphorylation, is inhibited by PD-153035 (an EGFR inhibitor) and wortmannin (a PI 3-kinase inhibitor). Although their observations are quite fascinating and open a new field of investigation into the role of β-adrenergic agonists in epithelial cells, we nevertheless have to be careful in interpreting the data and their potential extrapolation to the in vivo condition. One of the key aspects of their experimental protocol, as with many experimental protocols studying the cellular response to growth factors, is that it was performed under serum-free conditions. Serum was removed 24 h before the cells were exposed to β-adrenergic agonists. This is a common methodological approach taken by many laboratories to avoid possible interference by serum factors. However, serum starvation can induce the apoptosis machinery (21, 38), which could change the physiological status of the cells (31). Furthermore, it has been suggested recently that β-adrenergic agonists do not elicit cell growth but rather apoptosis of alveolar epithelial cells (7). Obviously, these experiments should stimulate scientists to further investigate the role of β-adrenergic agonists in alveolar epithelial cell growth and differentiation.

In summary, the study by Pesce et al. (29a) proposes that translation might be as important as transcription in modulating the expression of ion transport molecules. Furthermore, although it was thought that β-adrenergic agonists were mainly involved in ion transport modulation and surfactant secretion, their experiments demonstrate EGFR transactivation in alveolar epithelial cells by β-adrenergic agonists and raise the possibility that they might also have a role in epithelial repair (Fig. 1). Obviously, more work is needed to better define their potential in the pharmacological arsenal to treat pulmonary edema and lung injury.

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REFERENCES


