See related article, pages 453–460

Vectorial Sodium Transport Across the Mammalian Alveolar Epithelium

It Occurs but Through Which Cells?

Sadis Matalon, Ian C. Davis

The fluid that fills the alveolar spaces in the fetal lung is cleared shortly after birth, mainly as a consequence of active transport of sodium ions (Na^+) across the alveolar epithelium. This transport establishes an osmotic gradient that favors reabsorption of intra-alveolar fluid.[1] Studies that demonstrate both the reabsorption of intratracheally instilled isotonic fluid or plasma from the alveolar spaces of adult anesthetized animals and resected human lungs, and the partial inhibition of this process by amiloride and ouabain, indicate that adult alveolar epithelial cells are also capable of actively transporting Na^+ ions (see reviews[2,3]). Although it remains unclear whether active Na^+ transport plays an important role in keeping alveolar spaces free of fluid in the normal lung, a variety of studies have clearly established that active Na^+ transport limits the degree of alveolar edema under pathological conditions in which the alveolar epithelium has been damaged. For example, intratracheal instillation of a Na^+ channel blocker in rats exposed to hyperoxia increased the amount of extravascular lung water.[4] Conversely, intratracheal instillation of adenoviral vectors expressing the Na^+,K^+-ATPase genes increased survival of rats exposed to hyperoxia.[5] Moreover, patients with acute lung injury who are still able to concentrate alveolar protein (as a result of active Na^+ reabsorption) have a better prognosis than those who cannot.[6,7]

Insight into the nature and regulation of transport pathways has come from electrophysiological studies of freshly isolated and cultured alveolar type II (ATII) cells. These cells, which make up 67% of the alveolar epithelial cell population, but which constitute only 3% of the alveolar surface area in the adult lung, can be isolated at high purity and cultured to form confluent monolayers.[8] The results of a variety of electrophysiological studies, of both confluent monolayers of ATII cells mounted in Ussing chambers, and individual cells by patch-clamp analysis, indicate that Na^+ ions diffuse passively into ATII cells through apically located amiloride-sensitive cation and sodium selective channels[9–11] and are extruded across the basolateral cell membranes by the ouabain-sensitive Na^+,K^+-ATPase.[12] The cation channels on the apical surface usually constitute the rate-limiting step in this process, offering more than 90% of the resistance to transcellular Na^+ transport.

Over the last 20 years, there have been only a handful of publications on the transport properties of alveolar type I (ATI) cells. There are two major reasons for this relative dearth of studies: first, in their original immunocytochemical study of the distribution of Na^+,K^+-ATPase subunit proteins in lungs, Schneeberger and McCarthy[13] reported that the ATPase was present in the basolateral membranes of ATII but not of ATI cells; similar findings were reported by Ingbar et al[14] in rat fetal lungs. Second, isolation of a pure population of ATI cells has been difficult and accomplished only in a couple of laboratories. Thus, until recently, the prevailing wisdom has been that ATII cells are metabolically active and responsible for surfactant secretion and ion transport, whereas the squamous ATI cells are merely a "passive" component of the alveolar epithelial barrier.

However, in a recent study, Johnson et al[15] clearly demonstrated that ATI cells isolated from rat lungs exhibit amiloride- and ouabain-sensitive Na^+ and K^+ transport, at considerable higher levels than in ATII cells. Furthermore, these cells immunostained for the amiloride-sensitive sodium channel (ENaC) and Na^+,K^+-ATPase proteins. In a study published in this issue of *Circulation Research*, Ridge et al[16] used immunofluorescent and electron microscopy techniques to determine the distribution of two subunits of the Na^+,K^+-ATPase (α_1 and α_2) in alveolar epithelial cells in vivo. Interestingly, they found that while both subunits were present in ATI cells, only the α_1 subunit of Na^+,K^+-ATPase was detected in ATII cells. Most importantly, the authors ingeniously exploited the differential sensitivity to ouabain of the two Na^+,K^+-ATPase subunits (α_1 and α_2) to demonstrate the importance of vectorial sodium transport across ATI cells to normal lung fluid balance: specifically perfusion of isolated lungs with solutions containing 100 nmol/L ouabain resulted in 60% inhibition of basal alveolar fluid clearance (AFC) (secondary to an osmotic gradient generated by the active transport of sodium) and totally abolished the increase in AFC that follows intratracheal instillation of isoproterenol, a β-agonist. The authors argued that because ouabain at these low concentrations blocks the α_2 subunit (which is expressed only in ATI cells) but not the α_1 (which is expressed in both ATI and ATII cells), active vectorial transport of sodium across ATI cells must play a major role in both basal- and β-adrenergic–stimulated fluid clearance across the alveolar epithelium.

Needless to say, the scientific community is reluctant to accept a shift in paradigm without solid, incontrovertible evidence. Thus, it behooves us to carefully examine potential limitations of this study, which by no means detract from its

---

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

From the Department of Anesthesiology, University of Alabama at Birmingham, Birmingham, Ala.

Correspondence to Sadis Matalon, PhD, Alice McNeal Professor of Anesthesiology, University of Alabama at Birmingham, UAB Dept of Anesthesiology, 1530 3rd Ave South, Birmingham, AL 35294-2172. E-mail sadis.matalon@ccc.uab.edu

(*Circ Res.* 2003;92:348-349.)

***Circulation Research* is available at http://www.circresaha.org**
DOI: 10.1161/01.RES.0000061793.14540.2B

overall significance. First of all, no evidence was provided that these subunits are expressed in the basolateral membranes of ATI cells. Instead, these authors argue that these subunits mainly exist in the cytoplasm and traffic to the basolateral compartment after β-adrenergic stimulation. Although this may be true, one would still expect to find higher levels of either subunit in the basolateral membranes of epithelial cells as shown previously.[13,17] Second, no studies were conducted with isolated ATI cells. Instead, the authors used "senescent" ATII cells, which express some markers of ATI cells after 7 to 10 days in culture. It is interesting to note that immunocytochemical studies in freshly purified rat ATI cells showed the existence of the α_1 but not of the α_2 protein and mRNA Na^+,K^+-ATPase.[15,18] Possible reasons for these differences are not discussed in this manuscript. Third, previous studies of either freshly isolated ATII cells or ATII cells in short-term culture have clearly shown that agents that increase intracellular cAMP (such as β-agonists) also increase the open probability and number of amiloride-sensitive ENaC channels in ATII cells.[11,19] Because no comparable data exist for ATI cells, the contribution of ATII cells to vectorial Na^+ transport after increases in cAMP should not be discounted without additional evidence. It should be remembered that sodium transport across epithelial sodium channels is the rate-limiting step in vectorial sodium transport across epithelial cells, and so only limited conclusions can be drawn from studies confined to examining only the role of the ATPase. Finally, because of well-documented differences in rates of transport among various species, it will be important to repeat these studies in human lung preparations. Clearly, some of these questions need to be addressed in future studies. Development of inducible transgenic mice may help us unravel the contributions of each subunit.

What are the physiological implications of these findings? ATI cells are more susceptible to oxidant injury than ATII cells. If vectorial Na^+ transport is dependent on transporters located predominantly on these cells, then the likelihood of developing pulmonary edema after lung injury will be greater, because these cells will be the first to be damaged. It is thus critical to know whether ATII cells that differentiate to form ATI cells after injury will also express α_2 Na^+,K^+-ATPase. Also, measurements of vectorial Na^+ transport across confluent monolayers of ATI cells are clearly needed.

Finally, the authors report that overexpression of the α_2 but not of the α_1 Na^+,K^+-ATPase in the alveolar epithelium after intratracheal instillation of adenoviral vectors resulted in considerable enhancement of AFC. These results are very interesting and potentially important. However, in previous studies, these authors reported that overexpression of the β_1 but not of the α_1 Na^+,K^+-ATPase in rats and fetal type II cells increased AFC and vectorial Na^+ transport, respectively, by enhancing expression of both β_1 and α_1 Na^+,K^+-ATPase in the basolateral membranes.[20,21] The mechanisms by which overexpression of the α_2 Na^+,K^+-ATPase increases AFC were not elucidated.

In summary, this very important study proposes that ATI cells are not simple passive barriers but are metabolically active and play an important role in vectorial Na^+ transport across the alveolar epithelium. This is an exciting development, but certainly many more studies are needed.

References

1. Hummler E, Barker P, Gatzy J, Beermann F, Verdumo C, Schmidt A, Boucher R, Rossier BC. Early death due to defective neonatal lung liquid clearance in *αENaC*-deficient mice. *Nat Genet*. 1996;12:325–328.
2. Matalon S, O'Brodovich H. Sodium channels in alveolar epithelial cells: molecular characterization, biophysical properties, and physiological significance. *Annu Rev Physiol*. 1999;61:627–661.
3. Matthay MA, Folkesson HG, Clerici C. Lung epithelial fluid transport and the resolution of pulmonary edema. *Physiol Rev*. 2002;82:569–600.
4. Yue G, Matalon S. Mechanisms and sequelae of increased alveolar fluid clearance in hyperoxic rats. *Am J Physiol*. 1997;272(3 pt 1):L407–L412.
5. Factor P, Dumasius V, Saldias F, Brown LA, Sznajder JI. Adenovirus-mediated transfer of an N^a/K^+-ATPase β1 subunit gene improves alveolar fluid clearance and survival in hyperoxic rats. *Hum Gene Ther*. 2000;11: 2231–2242.
6. Ware LB, Matthay MA. Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. *Am J Respir Crit Care Med*. 2001;163:1376–1383.
7. Matthay MA, Wiener-Kronish JP. Intact epithelial barrier function is critical for the resolution of alveolar edema in humans. *Am Rev Respir Dis*. 1990; 142:1250–1257.
8. Dobbs LG. Isolation and culture of alveolar type II cells. *Am J Physiol*. 1990;258:L134–L147.
9. Cheek JM, Kim KJ, Crandall ED. Tight monolayers of rat alveolar epithelial cells: bioelectric properties and active sodium transport. *Am J Physiol*. 1989; 256:C688–C693.
10. Yue G, Russell WJ, Benos DJ, Jackson RM, Olman MA, Matalon S. Increased expression and activity of sodium channels in alveolar type II cells of hyperoxic rats. *Proc Natl Acad Sci U S A*. 1995;92:8418–8422.
11. Jain L, Chen XJ, Ramosevac S, Brown LA, Eaton DC. Expression of highly selective sodium channels in alveolar type II cells is determined by culture conditions. *Am J Physiol Lung Cell Mol Physiol*. 2001;280:L646–L658.
12. Factor P, Senne C, Dumasius V, Ridge K, Jaffe HA, Uhal B, Gao Z, Sznajder JI. Overexpression of the Na^+,K^+-ATPase α1 subunit increases Na^+,K^+-ATPase function in A549 cells. *Am J Respir Cell Mol Biol*. 1998;18:741–749.
13. Schneeberger EE, McCarthy KM. Cytochemical localization of Na^+-K^+-ATPase in rat type II pneumocytes. *J Appl Physiol*. 1986;60:1584–1589.
14. Ingbar DH, Duvick S, Savick SK, Schellhase DE, Detterding R, Jamieson JD, Shannon JM. Developmental changes of fetal rat lung Na-K-ATPase after maternal treatment with dexamethasone. *Am J Physiol*. 1997;272(4 pt 1):L665–L672.
15. Johnson MD, Widdicombe JH, Allen L, Barbry P, Dobbs LG. Alveolar epithelial type I cells contain transport proteins and transport sodium, supporting an active role for type I cells in regulation of lung liquid homeostasis. *Proc Natl Acad Sci U S A*. 2002;99:1966–1971.
16. Ridge KM, Olivera WG, Saldias F, Azzam S, Horowitz S, Rutschman DH, Dumasius V, Factor P, Sznajder JI. Alveolar type 1 cells express the α2 Na,K-ATPase, which contributes to lung liquid clearance. *Circ Res*. 2003; 92:453–460.
17. Nici L, Dowin R, Gilmore-Hebert M, Jamieson JD, Ingbar DH. Upregulation of rat lung Na-K-ATPase during hyperoxic injury. *Am J Physiol*. 1991;261: L307–L314.
18. Borok Z, Liebler JM, Lubman RL, Foster MJ, Zhou B, Li X, Zabski SM, Kim KJ, Crandall ED. Na transport proteins are expressed by rat alveolar epithelial type I cells. *Am J Physiol Lung Cell Mol Physiol*. 2002;282: L599–L608.
19. Yue G, Shoemaker RL, Matalon S. Regulation of low-amiloride-affinity sodium channels in alveolar type II cells. *Am J Physiol*. 1994;267:L94–L100.
20. Factor P, Saldias F, Ridge K, Dumasius V, Zabner J, Jaffe HA, Blanco G, Barnard M, Mercer R, Perrin R, Sznajder JI. Augmentation of lung liquid clearance via adenovirus-mediated transfer of a Na,K-ATPase β1 subunit gene. *J Clin Invest*. 1998;102:1421–1430.
21. Thome U, Chen L, Factor P, Dumasius V, Freeman B, Sznajder JI, Matalon S, Na,K-ATPase gene transfer mitigates an oxidant-induced decrease of active sodium transport in rat fetal ATII cells. *Am J Respir Cell Mol Biol*. 2001;24:245–252.

KEY WORDS: alveolar type I/II cells ■ Na^+,K^+-ATPase isoforms ■ sodium channels ■ alveolar fluid clearance ■ pulmonary edema

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

Vectorial Sodium Transport Across the Mammalian Alveolar Epithelium: It Occurs but Through Which Cells?

Sadis Matalon and Ian C. Davis

Circ Res. 2003;92:348-349
doi: 10.1161/01.RES.0000061793.14540.2B
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571