How does cAMP increase active Na absorption across alveolar epithelium?

AMILORIDE-SENSITIVE ACTIVE TRANSPORT of Na across the alveolar epithelium removes lung liquid in the neonate and protects against edema in the adult. Several lines of evidence have established that this process is stimulated by β-adrenergic agents via elevation of cAMP. First, when the lungs are filled with saline in vivo, then β-adrenergic agents or exogenous cAMP stimulates removal of this liquid (2, 3, 12). Furthermore, the increase in liquid clearance induced by terbutaline is inhibited by amiloride (3, 12). Second, in cultured cells, the amiloride-sensitive short-circuit current (Isc) is stimulated by terbutaline (4, 10). Finally, the terbutaline-induced increase in Isc is mirrored by an increase in net Na absorption as determined from the transepithelial fluxes of 22Na (7).

Active Na absorption across the alveolar epithelium presumably occurs by the standard mechanism (8): diffusion of Na across the apical membrane down its electrochemical gradient via amiloride-sensitive channels followed by active extrusion across the basolateral membrane by Na-K-ATPase. The inward Na current across the apical membrane (I(Na)) is given by the product of the membrane conductance to Na (G(Na)) and the driving force for net Na entry (V(a) - E(Na)), where V(a) is the apical membrane potential difference and E(Na) is the equilibrium potential for Na. In this issue of the American Journal of Physiology-Lung Cellular and Molecular Physiology, Lazorak et al. (9) argue that β-adrenergic agents stimulate Na absorption by an effect on G(Na), whereas O'Grady et al. (11) believe that the stimulation is due to a change in V(a) brought about by an increase in Cl conductance.

The overall Na conductance of the alveolar apical membrane (in mS/cm²) is given by G(Na) = g·P(Na)·N, where g is the conductance of individual channels, P(Na) is their average probability of being open, and N is their frequency per unit area of membrane. Patch-clamp studies on cultured type II cells (9) have shown that the predominant Na channel is amiloride sensitive and has a G of ~27 pS. Terbutaline approximately doubles the P(Na) of this channel without affecting G (9). The percent increase in P(Na) of this channel is approximately the same as the percent increase in active Na absorption measured as amiloride-sensitive Isc in Ussing chambers.

However, these experiments were done on single isolated cells, not confluent cell sheets with tight junctions and a measurable transepithelial resistance. It is argued (9) that “ATII cells in primary culture orient themselves so that their basal membranes are attached to the substratum and the apical membranes are pointing upward.” But without tight junctions, one cannot be sure that the upper membrane has the same composition as a true apical membrane. In fact, it is almost certainly different. Structural specializations on the apical membranes of confluent polarized cells (e.g., microvilli and glycocalyx) make them notoriously hard to patch clamp, which is why most studies are performed on single cells or small clumps of cells. Extrapolation of the results from single cells to the function of the intact epithelium should be done with caution; absence of tight junctions may alter the types of ion transport present. In airway epithelium, for instance, the Na-K-2Cl cotransporter of intact epithelium becomes a Na-Cl cotransporter on cell dispersion (5), and nonconfluent cells contain a form of Ca-activated Cl conductance absent from the apical membrane of intact epithelia (1).

In contrast to a direct opening of Na channels, O'Grady et al. (11) suggest an indirect role for Cl in modulating active absorption of Na. Specifically, they propose that the apical membrane of alveolar type II cells has a cAMP-dependent Cl conductance. Also, the electrochemical gradient for Cl is inward across the apical membrane (i.e., E(Cl) is more negative than V(a)). Therefore, the opening of Cl channels by cAMP results in hyperpolarization of the apical membrane and an increased driving force for entry of Na.

This is plausible, but close inspection of the original article by Jiang et al. (6) shows that terbutaline did not in fact increase overall Isc nor are data presented that show that terbutaline increases amiloride-sensitive Isc. In fact, representative traces suggest that terbutaline did not increase active Na absorption (see Fig. 1 in Ref. 11). Nevertheless, others using slightly different alveolar type II cell cultures have reported increases of 40% or 110% (10) in active Na absorption with terbutaline, and it would be worth using such cells to test the mechanism proposed by O'Grady et al. (11). The first step of O'Grady et al.’s hypothesis has been established: cultures of alveolar cells do indeed have an apical membrane Cl conductance. Thus Jiang et al. (6) permeated the basolateral membrane of confluent cell sheets with amphotericin and imposed a Cl gradient across the remaining apical membrane. The addition of terbutaline or cAMP to such preparations increased the Isc. The authors used the same permeabilized preparation to provide evidence against a cAMP-activated apical G(Na). With a Na gradient imposed across the isolated...
apical membrane, cAMP failed to increase amiloride-sensitive transepithelial currents. But given that non-permeabilized cultures showed no increase in amiloride-sensitive I_{sc} in response to terbutaline, this result was to be expected.

The other assumptions in the scheme proposed by O’Grady et al. (11) are that the electrochemical gradient for Cl is directed inward across the apical membrane and that the opening of Cl channels by cAMP results in hyperpolarization of V_{a}. However, there is a difficulty with this part of the proposed mechanism: the driving force for Na entry is already comparatively large and would need to be increased substantially by opening the Cl channels. The driving force for Na entry is E_{Na} - V_{a}. E_{Na} is likely to be approximately +50 mV, and V_{a} in short-circuited tissues (the kind used to measure active Na transport) is probably negative [it is -60 mV in the short-circuited airway epithelium (14)]. Thus the driving force for Na entry should be between 50 and 100 mV. Therefore, to increase active Na absorption by 40–110% solely by altering the driving force will require changes in V_{a} of at least 20 mV and possibly much more. Thus E_{Cl} would have to be considerably more negative than V_{a} (i.e., intracellular Cl activity would be much lower than the equilibrium level predicted for passive distribution according to V_{a}). Clearly, microelectrode measurements of V_{a} and intracellular Cl activity are needed to test these predictions.

In addition to a possible role in regulating active Na absorption, O’Grady et al. (11) suggest that liquid absorption across the alveolar epithelium can be stimulated by raising transepithelial Cl permeability or by increasing active absorption of Cl. The first possibility has recently been demonstrated in primary cultures of bovine airway epithelium (13). The second suggestion is based on the observation by Kim et al. (7) that addition of terbutaline to short-circuited cultures induced a significant net absorption of Cl. This interesting finding can be explained by the downhill movement of Cl through apical membrane Cl channels followed by extrusion across the basolateral membrane by cotransport with K. This would constitute a form of secondary active transport driven by the concentration gradient for K across the basolateral membrane.

In summary, more work needs to be done to test both hypotheses. The apical G_{Na} needs to be studied in confluent cell sheets as well as in single isolated cells. A good approach is to measure V_{a} with microelectrodes and alter the Na concentration in the mucosal bath in the presence and absence of amiloride. Preparations in which the basolateral membrane has been permeated could also be used in Ussing chambers, provided it is first established that the intact tissue does indeed show increases in amiloride-sensitive I_{sc} in response to β-adrenergic agents or cAMP. As to the idea that opening Cl channels increases Na transport by increasing the driving force for Na across the apical membrane, microelectrodes (both ion sensitive and conventional) need to be used to determine the direction of the electrochemical driving force for Cl and to see whether the opening of Cl channels does indeed hyperpolarize V_{a} by the required amount. Finally, it must be noted that stimulating Na absorption by increasing the G_{Na} or the electrochemical driving force for Na entry are not mutually exclusive possibilities, and both sets of workers might be partially right.

REFERENCES